RENAL BIOPSY
Clinical and Pathological Significance

Ciba Foundation Symposia

General Volumes

Mammalian Germ Cells	30s.
Preservation and Transplantation of Normal Tissues	25s.
Leukaemia Research	30s.
Chemistry and Biology of Pteridines	42s.
Porphyrin Biosynthesis and Metabolism	30s.
Histamine	50s.
Extrasensory Perception	27s. 6d.
Bone Structure and Metabolism	45s.
Ionizing Radiations and Cell Metabolism	45s.
The Nature of Viruses	42s.
Chemistry and Biology of Purines	48s.
Drug Resistance in Micro-organisms	50s.
The Cerebrospinal Fluid	50s.
Amino Acids and Peptides with Antimetabolic Activity	45s.
Medical Biology and Etruscan Origins	45s.
Biosynthesis of Terpenes and Sterols	45s.
Carcinogenesis: Mechanisms of Action	48s.
Biochemistry of Human Genetics	50s.
Significant Trends in Medical Research	50s.
Congenital Malformations	45s.
Haemopoiesis: Cell Production and its Regulation	60s.
Adrenergic Mechanisms	70s.
Quinones in Electron Transport	60s.
The Nature of Sleep	50s.
Somatic Stability in the Newly Born	50s.
Enzymes and Drug Action	

A leaflet giving details of the Ciba Foundation Colloquia on Endocrinology, Colloquia on Ageing and Study Group volumes is available from the Publishers

CIBA FOUNDATION SYMPOSIUM

ON

RENAL BIOPSY

Clinical and Pathological Significance

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., M.R.C.P.

and

MARGARET P. CAMERON, M.A.

With 134 *Illustrations*

J. & A. CHURCHILL LTD.
104 Gloucester Place, London, W.1
1961

Printed in Great Britain

THE Ciba Foundation, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle. However, being established under British trust law, it enjoys complete independence in practice and policy.

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

PREFACE

IT was Dr. A. M. Joekes who may be said to have needled the Director into the organization of an international gathering to try to assess the usefulness, risks, and potentialities of the procedure of percutaneous renal biopsy. Enough work had been done by 1961 to make such an evaluation practicable, and if much of it had been in the hands of enthusiasts it might well prove all the more salutary or reassuring to attempt a critical estimate of improvements in the treatment of patients through the use of this technique.

Within the very limited membership of one of the Ciba Foundation's symposia, a clinician and a pathologist were invited from each of the major centres where such studies were known to be in progress. To Dr. Joekes and to all who came to take part in this meeting the Foundation is profoundly indebted.

It is obvious that someone out of the ordinary in the way of an impartial, sceptical and widely experienced chairman was required, and every member of the symposium and reader of this book will appreciate our unique good fortune in persuading Professor Arnold Rich to direct the discussions.

The editors hope that this book will enable many other clinicians and pathologists to understand better what information can now be gained from renal biopsy and to appreciate more clearly the importance of studies of this kind if ever treatment of renal disorders is to be placed on a more rational and effective basis than is possible today.

Readers acquainted with early volumes of proceedings of conferences at the Ciba Foundation will understand the pleasure of the senior editor in re-enlisting the conscientious skill of Miss Cameron in this editorial work. They jointly record their

gratitude to Mr. W. Hill for his preparation, as usual in these books, of both subject and author indexes; to Mrs. Margarete Silverman for assisting with the galley proofs; and to Mr. H. A. Thompson, of Cooke, Troughton & Simms Ltd. for the loan of microscopes during the symposium.

CONTENTS

List of those participating in or attending the Symposium on "Clinico-Pathological Significance of Renal Biopsy" 14th–16th March, 1961

A. BERGSTRAND	Institutionen för Allmän Patologi, Karolinska Mediko-Kirurgiska Institutet, Stockholm
D. A. K. BLACK	Dept. of Medicine, The Royal Infirmary, University of Manchester
J. D. BLAINEY	Dept. of Experimental Pathology, Medical School, University of Birmingham
C. BRUN	Central Clinical Laboratory, Kommunehospitalet, Copenhagen
H. BUCHT	St. Erik's Hospital, Stockholm
O. Z. DALGAARD	III Medical Dept., Kommunehospitalet, Copenhagen
E. M. DARMADY	Central Laboratory, Portsmouth
D. P. EARLE	Northwestern University Medical School, Chicago
RENÉE HABIB	Hôpital Necker, Paris
J. HAMBURGER	Hôpital Necker, Paris
J. HARDWICKE	Dept. of Experimental Pathology, Medical School, University of Birmingham
R. H. HEPTINSTALL	Dept. of Pathology, Washington University School of Medicine, St. Louis
M. S. R. HUTT	Louis Jenner Laboratory, St. Thomas' Hospital, London
R. B. JENNINGS	Northwestern University Medical School, Chicago
A. M. JOEKES	Institute of Urology, London
R. M. KARK	Dept. of Medicine, University of Illinois College of Medicine; and Presbyterian-St. Luke's Hospital, Chicago
P. MICHIELSEN	Service de Médecine Interne, Cliniques Universitaires, Louvain
M. D. MILNE	Dept. of Medicine, Westminster Medical School; formerly at Postgraduate Medical School, London
H. Z. MOVAT	Dept. of Pathology, Banting Inst., University of Toronto

C. L. PIRANI	Dept. of Pathology, University of Illinois College of Medicine, Chicago
F. RAASCHOU	III Medical Dept., Kommunehospitalet, Copenhagen
A. R. RICH	Dept. of Pathology, Johns Hopkins University, Baltimore
M. L. ROSENHEIM	Medical Unit, University College Hospita Medical School, London
J. H. ROSS	Hereford; formerly at Medical Unit, The London Hospital, London
R. J. SLATER	Research Inst., Hospital for Sick Children, Toronto
A. C. THOMSEN	III Medical Dept., Kommunehospitalet, Copenhagen
R. L. VERNIER	Dept. of Pediatrics, University of Minnesota Medical School, Minneapolis
H. E. DE WARDENER	Dept. of Medicine, Charing Cross Hospital Medical School, London
C. WILSON	Medical Unit, The London Hospital, London

OPENING REMARKS

ARNOLD R. RICH

I AM sure that all of us warmly appreciate the opportunity provided by the Ciba Foundation to enjoy the advantage of participating in this timely symposium that has been designed, and arranged in these graceful surroundings, by the discerning Director of the Foundation, Dr. Wolstenholme.

Perceptive observation, imagination and insight, unaided by special devices, have often sufficed to produce fundamentally important discoveries, and doubtless will always continue to do so. However, new techniques, which may be mechanically as simple as percussion and auscultation or as complex as cardiac catheterization, have of course also played a rôle of great importance in the advancement of knowledge, either by opening for study new areas which had lacked methods essential for their exploration, or by permitting a deeper penetration into areas that were already under active study, or by serving to reawaken interest in areas in which investigation had become relatively stagnant because the fruitfulness of the older methods seemed to have become exhausted.

In the study of disease, it has often happened that new technical methods have led to a notable expansion of basic knowledge and understanding even when the methods were designed primarily for the practical purposes of diagnosis, treatment or prophylaxis. However, not all clinically useful new techniques have advanced basic knowledge importantly. Some have not been found applicable to the investigation of basic problems, and others have promised more to basic science than they have been able to fulfil when subjected to more critical scrutiny and to the judgment of time. Indeed, numerous promising diagnostic or therapeutic

techniques have not fulfilled even their clinical promise, and have been discarded; and some of those clinically disappointing techniques have turned out to be helpful to the advancement of science. What is the outlook for renal biopsy in these respects?

One of the principal criteria that Dr. Wolstenholme has established as guides for his selection of subjects for these symposia, is that the theme chosen should be scientifically "ready" to be discussed. It is now ten years since Iversen and Brun* published their first 42 successful aspiration biopsies of the kidney, demonstrating the practicality and indicating the reasonable safety of the procedure, both of which have been amply confirmed. During the following two or three years, only a few additional reports appeared; but since then, publications on the subject have steadily increased, and the procedure is now widely known, and has been under intensive study in numerous excellent clinics. During the same decade, improvements have been made in the techniques of electron microscopy and enzyme histochemistry, both of which require the use of fresh tissue for reliable studies. Renal biopsy can, of course, contribute directly to that need in the study of the kidney.

As would be expected, various questions concerning the clinical and pathological advantages and limitations of the procedure, and the interpretation and evaluation of the information that it has yielded, are still not clearly answered. However, in view of the steadily increasing use of this type of renal biopsy, and in view of the widespread interest in a balanced evaluation of the procedure on the part of many others who have refrained from using it because of uncertainty as to its actual clinical value and significance, and since there is now in the hands of thoughtful and competent clinicians and pathologists a considerable accumulated experience derived from its use, it can fairly be said that the clinico-pathological significance of renal biopsy is a subject that is ready for profitable examination in a symposium of this type.

* Iversen, P. and Brun, C. (1951). *Amer. J. Med.*, **11**, 324.

The great value and pleasure of a small international symposium of the character of this one—indeed, the heart of its purpose—lies of course, in the unparalleled opportunity that it provides for investigators from different countries, and with different experiences, to converse together in an informal, leisurely, friendly and frank exchange of observations and opinions relating to a field of mutual interest and concern. The special studies to be presented by the speakers in the present symposium cover a sufficiently broad area to provide a basis for the discussion not only of the special subjects themselves, but also of the advantages and limitations of renal biopsy as an aid to more accurate clinical and pathological diagnosis, and as a possible guide to therapy and to prognosis; and for consideration of the contribution that the procedure may be able to make toward a better understanding of the pathogenesis of renal diseases.

ULTRASTRUCTURE OF THE GLOMERULUS AND CHANGES IN FINE STRUCTURE ASSOCIATED WITH INCREASED PERMEABILITY OF THE GLOMERULUS TO PROTEIN

ROBERT L. VERNIER

Variety Club Heart Hospital Laboratories, Department of Pediatrics, University of Minnesota, Minneapolis and Department of Biophysics, Statens Seruminstitut, Copenhagen

THE classic studies of Wearn and Richards (1924) demonstrated that the glomerular fluid is a filtrate of plasma containing very little protein. Subsequent investigations of the transport of small and large molecules across the glomerulus have indicated many other characteristics of the "filter" and details of the probable structure of the membrane. Pappenheimer, Renkin and Borrero (1951) and others showed that glomerular capillaries are much more permeable to water and small molecules than are the capillaries of muscle and predicted a specialized structure of the glomerular membranes. Studies such as those of Bayliss, Kerridge and Russell (1933) and Wallenius (1959) demonstrated that the glomerulus of mammals is relatively impermeable to molecules with a molecular weight greater than haemoglobin (68,000). According to Pappenheimer (1953) the physiological data suggest the existence of a glomerular membrane containing pores with a radius of about 30 Ångström units (Å), covering 1 to 2 per cent of the membrane surface. However, evidence was advanced that small amounts of rather large molecules may cross the normal glomerular membrane and in the case of haemoglobin about 3 per cent of the infusate was shown to appear in the urine, indicating partial renal clearance of this molecule (Monke and Yule, 1940). Chinard (1952) presented data which predict that the

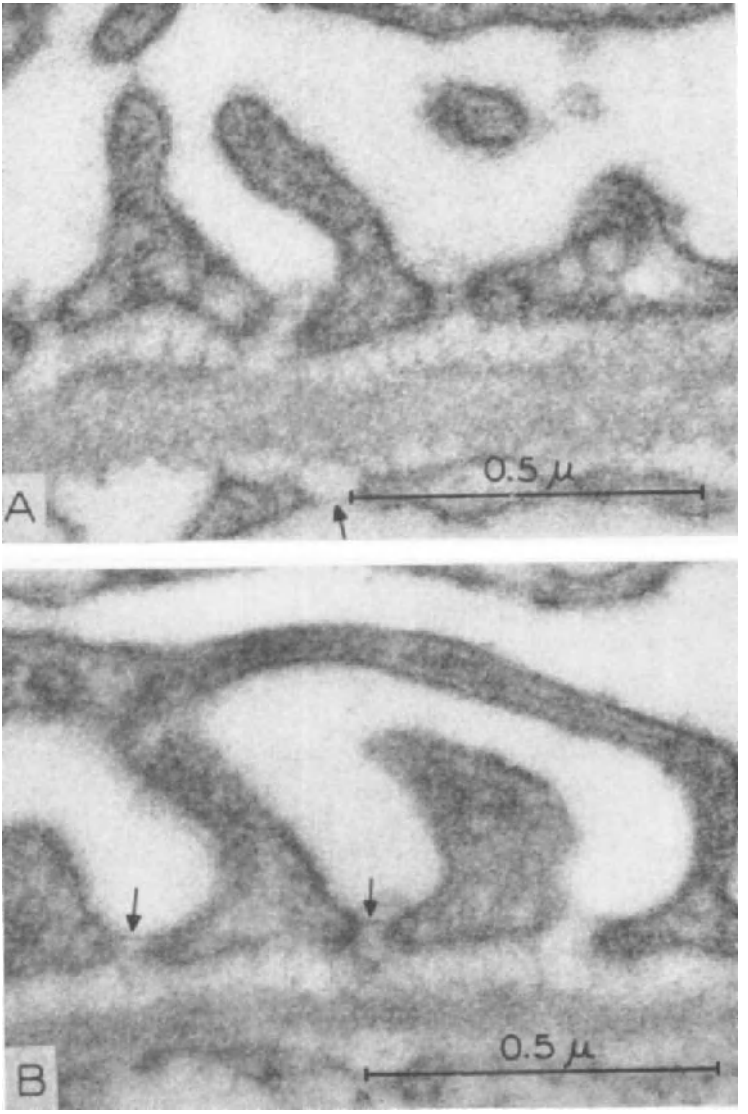
membrane might be composed of a gel-like substance through which the net passage of each molecule is determined by the molecule's diffusion coefficient, which in turn is related to such factors as molecular size, shape and charge. This theory proposes that all substances cross the capillary at a finite rate but that the rate may be negligible in the case of larger molecules (as protein).

Although the protein content of the glomerular fluid in man has not been measured directly, the indirect evidence indicates that very little serum protein is filtered, and it is generally agreed that the presence of more than 100 mg./100 ml. of protein in the urine per day signifies glomerular disease. Perfusion experiments in man by Gregoire, Malmendier and Lambert (1958) and Wallenius (1959) and others have clearly demonstrated an increase in the permeability of the glomerular capillary in patients with proteinuria. Furthermore, studies of this type have shown that increasingly large molecules may cross the glomerulus in patients with increasingly severe proteinuria. These experiments and the bulk of the evidence available suggest that failure of tubular reabsorption of protein is probably not a major factor of the aetiology of proteinuria.

The increased resolution of the electron microscope permits study of biological membranes at the level of large molecules. In conjunction with renal biopsy methods, electron microscopy has led to numerous investigations of the ultrastructure of normal and pathological kidney tissue and has revealed many previously unknown anatomical features. The purpose of this paper is to consider the newer concepts of anatomy of the normal and diseased glomerulus in light of the above physiological observations, with particular attention to the possible relationships between fine structure and glomerular permeability.

Normal glomerular capillary wall

Much of our knowledge of the ultrastructure of the normal mammalian glomerulus has been derived from studies of the rat,


A
0.5 μ
B
0.5 μ

rabbit, and dog. Little difference in the structure of the glomerulus of these laboratory animals and man has thus far been recognized and the following comments, except where specified, may be considered to apply to all mammalian glomeruli.

The normal glomerular capillary wall consists of three components: (1) the endothelium, (2) the basement membrane, and (3) the epithelium. The endothelial cells line the internal surface of the capillary, forming a thin (1000–2000 Å) fenestrated layer of cytoplasm which appears to be uniquely adapted for rapid diffusion or filtration. The pores in the endothelial membrane measure about 1000 Å in diameter and in cross-section appear as interruptions which permit direct contact between the luminal contents and the basement membrane (Fig. 1A). Tangential sections often show the endothelial pores "*en face*" and readily demonstrate their circular outline (Fig. 2). The relatively large size of these pores precludes the possibility that they offer a barrier to the filtration of molecular substances, although it is possible that present techniques of fixation and embedding distort or enlarge the dimensions of structures as they exist in life. The endothelium is intimately attached to the basement membrane and small protrusions of endothelium may appear to be embedded within the basement membrane (Fig. 5). Several studies of ferritin transport, described later in this paper, indicate that one of the functions of endothelium may be phagocytosis of particulate materials.

Cells are often encountered which are very similar to the luminal endothelial cells, but which lie at the sites of branching of capillaries (axially positioned cells) and do not contact the

---

FIG. 1. A: Slightly tangential section of a normal glomerular capillary (dog), showing the three components of the wall. Several pores in the endothelium are demonstrated (arrow). (Osmium-methacrylate. × 94,000.)

B: Cross-section of normal capillary wall; several "filtration slit" membranes are visible (arrows). The total width of the basement membrane in the dog (about 1,800 Å) may be compared with that of the normal human (about 2,800 Å), shown in Fig. 3. (Osmium-methacrylate. × 94,000.)

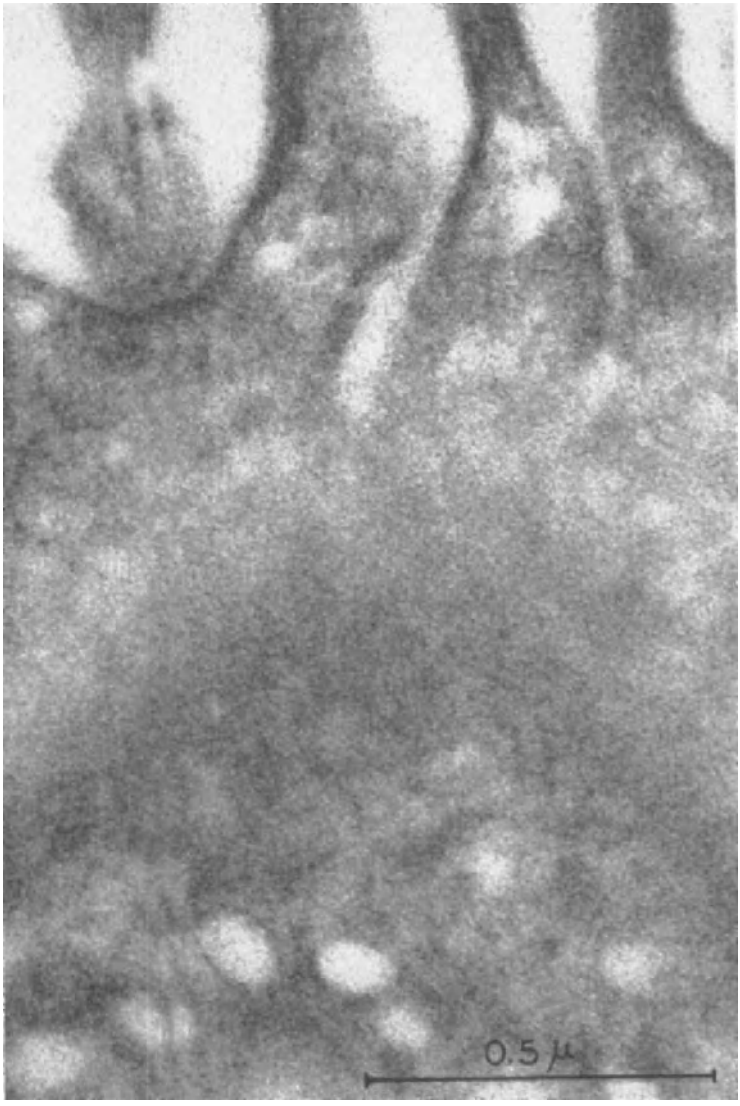
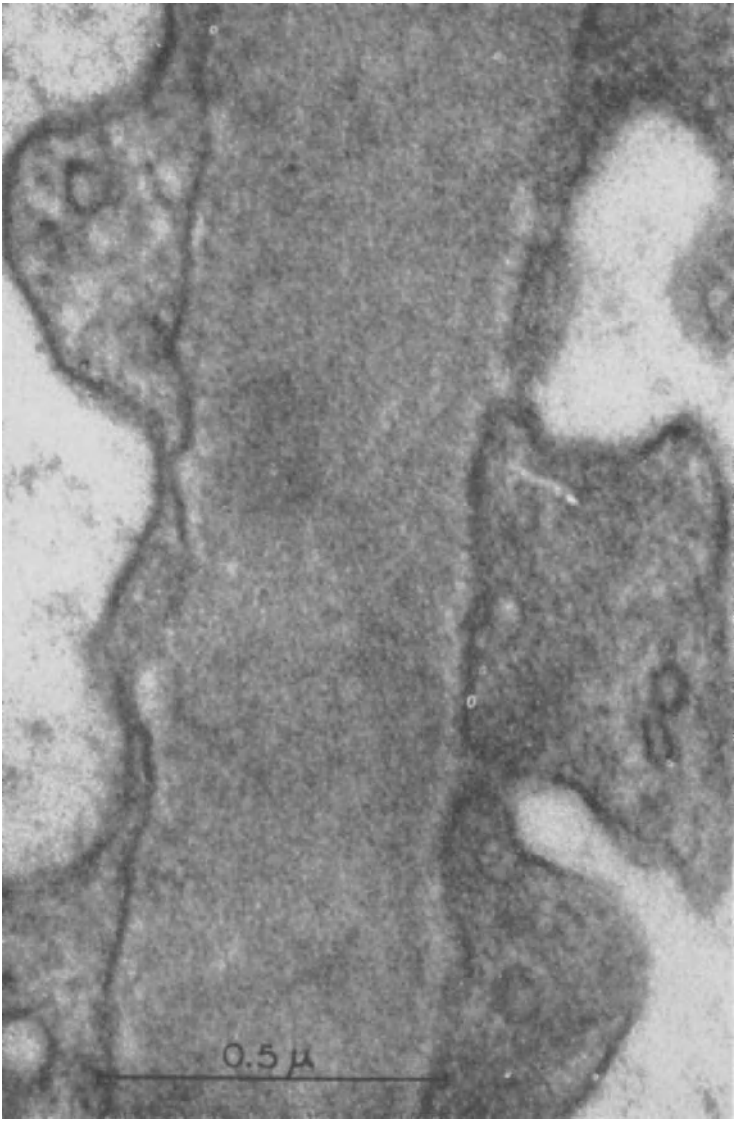


FIG. 2. Tangential section of capillary of normal dog, showing endothelial pores *en face* (bottom). The lamina densa of the basement membrane is homogeneous and finely granular. Filtration slits between adjacent epithelial-cell foot process are shown at the top of the illustration. (Osmium-methacrylate. × 94,000.)

lumen, at least in the plane of the section. Kurtz and McManus (1959) have called such cells *inter*-luminal endothelial cells, in contrast to the luminal type described above. The inter-luminal endothelial cells are probably identical to the mesangial cells of Zimmerman, but solution of the controversy regarding the existence of a specialized function of such cells must await the results of future research.

The basement membrane lies between the endothelium and the epithelium and consists of a dense central layer (the lamina densa) and less dense zones of variable prominence on either side (the lamina rara). The less dense layers of the basement membrane are readily demonstrated in the glomerulus of the rat, but are less regularly seen in human material. Most of the published electron micrographs of the glomerulus have shown tissue embedded in methacrylate, in which case the lamina densa appears as a relatively homogeneous, finely granular layer (Fig. 2). However, in sections of methacrylate-embedded tissue stained with heavy metals (Watson, 1958) or in tissue embedded in the epoxy or polyester resins a fine fibrillar structure within the basement membrane becomes evident. Basement membrane embedded in the polyester Vestopal-W (Ryter and Kellenberger, 1958), and stained as above, reveals interlaced fine filaments (40–60 Å width) separated by irregular less dense zones of about 75 Å (Fig. 3). The filaments are particularly well seen in the lamina densa of the basement membrane of foetuses (Fig. 10). Perhaps these interstices between the fine filaments represent the spaces through which molecules move across the basement membrane: the basis of molecular sieving. This morphological feature, if representative of the situation in life, appears to be entirely compatible with Chinard's concept of membrane structure as based on physiological data.

The fact that the thickness or width of the basement membrane has been shown to increase in certain renal diseases led us to measure the width of the membranes from 25 persons without

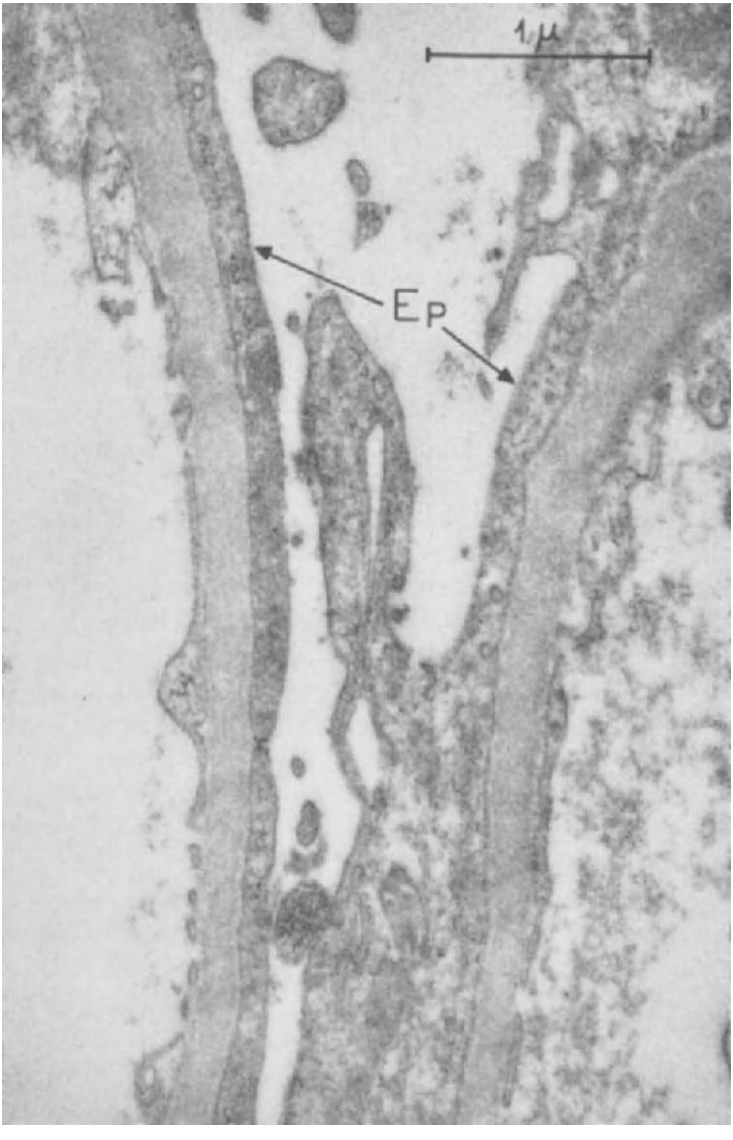

0.5 μ

renal diseases (ages, birth to 80 years) (Bloom, Hartmann and Vernier, 1959). It was found that the membrane is very thin in the infant, with a mean width of 950 Å (S.D.* 150), and that its thickness increases gradually until about the age of 3 years, reaching at that time the adult mean width of 2844 Å (S.D. 570). These observations raise interesting questions regarding the relationship between basement membrane width and the filtration process, since it is known that the filtration rate is low in infants (values corrected for surface area, renal weight, etc.) whereas the membrane is very thin.

The epithelial cells lie external to the basement membrane and have a very complex internal and external structure. The cytoplasm of these cells contains a very highly organized system of endoplasmic reticulum, fine filaments, vacuoles, vesicles, a prominent Golgi zone, and numerous mitochondria (Fig. 9A). The presence of numerous organelles within the cells suggests a highly active and specialized metabolic system. The external structure of the epithelial cell is equally as complex as the interior, consisting of a system of primary processes of cytoplasm which further divide into secondary and terminal, or foot processes (Fig. 1B). The foot processes insert upon the basement membrane to form rather orderly rows and are separated from each other by narrow spaces of about 1000 Å, which Hall (1957) has termed filtration slits. The foot processes are often more dense than the remaining cytoplasm of the epithelial cell, are broad at their base, and taper to join the cytoplasm of the epithelial cell by a thin process (Fig. 1B). Yamada (1955) and others have described in the mouse and rat a thin membrane which extends across the

* Standard Deviation.

FIG. 3. Capillary wall from a renal biopsy in a 6-year-old child who had recovered from the nephrotic syndrome. There are no apparent abnormalities. The lamina densa nearly fills the space between the endothelial and epithelial cells and has a mottled appearance due to less dense zones among the dense fine fibrils. (Osmium-Vestopal. Stained with uranyl acetate. × 94,000.)


1 μ
EP

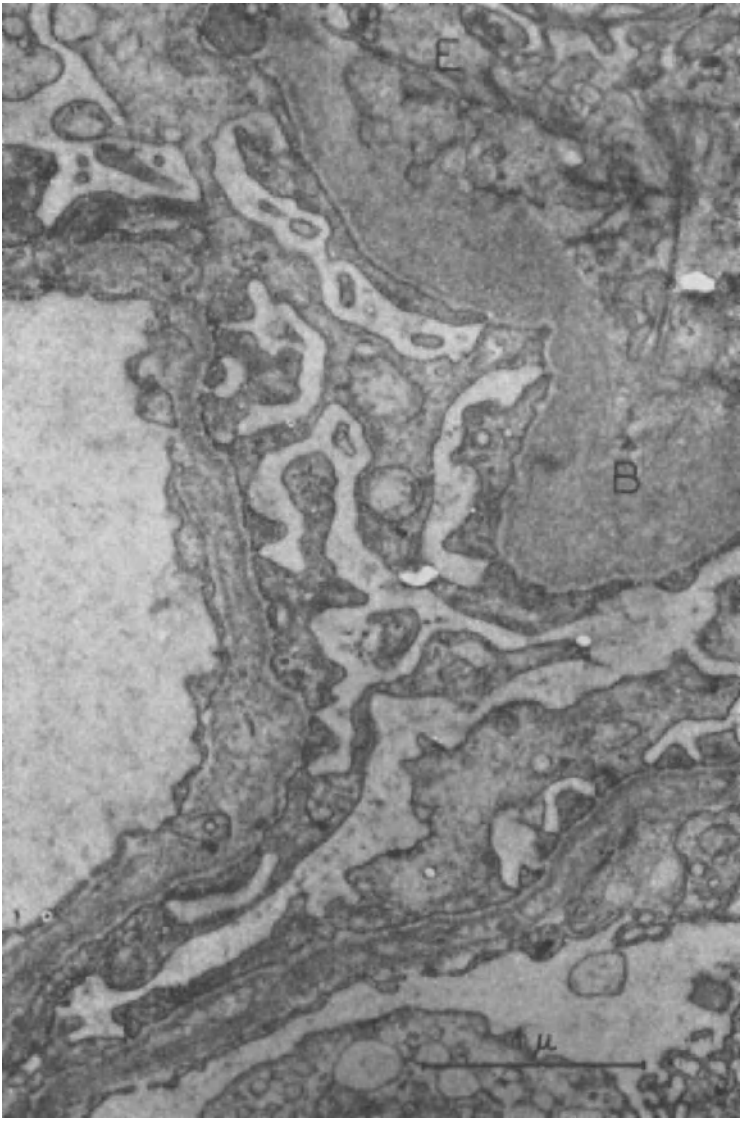
space between adjacent foot processes. This membrane is also visible in the dog (Fig. 1B) and in the human foetus (Fig. 9B). Farquhar, Wissig and Palade (1961) have described a fine filament within the filtration slit membrane and propose that this structure may represent a desmosome-like zone, or point of attachment of the foot processes to one another or to the basement membrane. The function of the epithelial cell is not known. Two hypotheses which have been advanced suggest that this highly specialized cell may serve either to maintain the functional integrity of the membrane through some mechanism as cleaning or débridement (Policard, Collet and Giltaire-Ralyte, 1955) or as a monitor of the basement membrane in regulating the transport of proteins (Farquhar, Wissig and Palade, 1961). Hall's (1957) proposal that the slits between the foot processes may regulate protein transport is difficult to reconcile with the observations made of glomeruli from nephrotic men and animals, and with recent studies of the transport of large molecules.

Human and experimental pathology

Our knowledge of the mechanism of glomerular filtration and insight into the probable function of the various components of the capillary wall have been greatly enhanced by studies of human pathological material and by many investigations in the field of experimental pathology.

The nephrotic syndrome in man is characterized by massive proteinuria and its sequellae. Electron microscopy of glomeruli from patients with the syndrome revealed a prominent abnormality of the epithelial cells (Farquhar, Vernier and Good, 1957*a*)

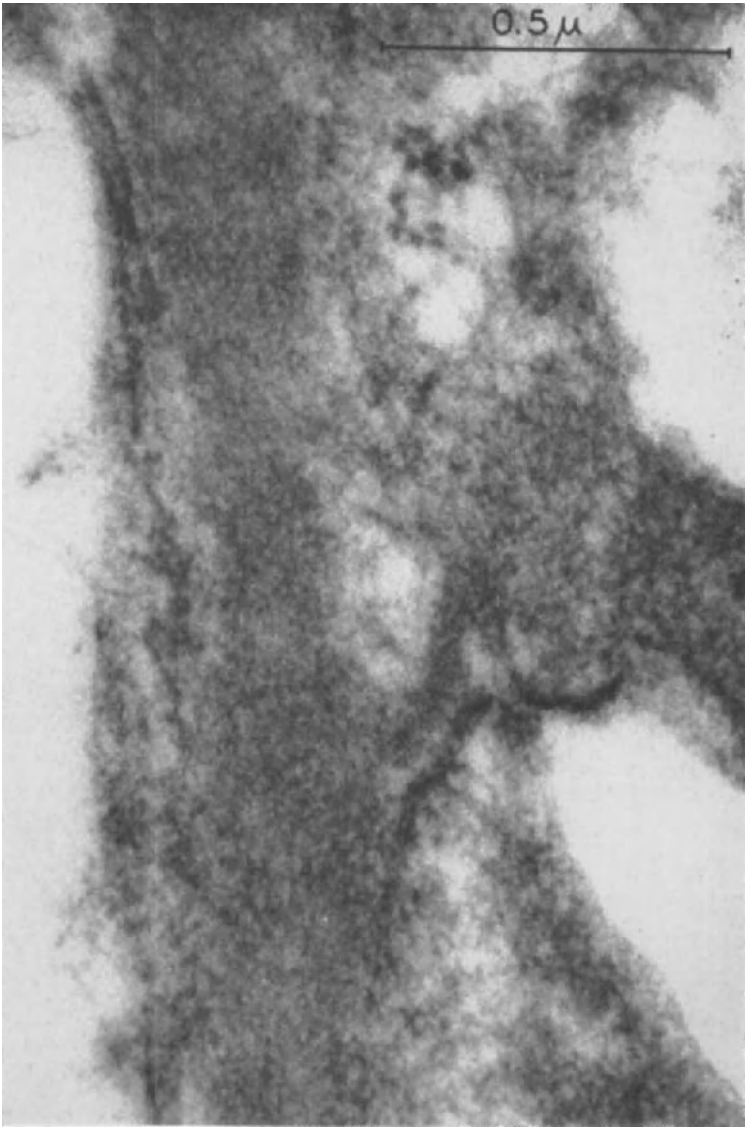
FIG. 4. Portions of two capillaries from a 6-year-old child with nephrotic syndrome. Note the absence of epithelial cell foot processes. The epithelial cytoplasm (Ep) forms a continuous layer about the exterior of both capillaries. The endothelium and basement membrane are normal. (Osmium-Vestopal. Uranyl acetate-stained. × 30,000.)


E
B
1 μ

which has since been described by many investigators. The abnormality consists of fusion of the epithelial cell foot processes into a more or less continuous mass of epithelial cell cytoplasm along the external surface of the basement membrane (Fig. 4). An increase in the size and number of vacuoles within the cytoplasm of the epithelial cell usually accompanies this lesion. Frequently the foot process lesion is the only recognizable abnormality of nephrotic glomeruli which appear entirely normal by light microscopy. Our studies of more than fifty renal biopsy specimens from nephrotic patients (Vernier, Worthen and Good, 1960) show that the extent of the foot process abnormality about the capillaries and the uniformity of the lesion from capillary to capillary are related to the severity of the proteinuria. The accumulated data suggest that the lesion is a morphological expression of proteinuria, and a number of laboratories have been able to demonstrate reversal of the lesion and return of the foot process structure to normal after recovery of the patient and cessation of proteinuria. The foot process lesion is often less diffuse in specimens which show distinct abnormalities of the basement membrane and endothelium (Fig. 5)—usually such specimens also show "basement membrane thickening" by light microscopy—but in our experience a degree of foot process fusion persists in all patients with persistent proteinuria.

Additional abnormalities involving the basement membrane and the endothelial cells are commonly encountered in glomeruli from nephrotic patients. Changes in the basement membrane may take the form of irregular thickening (usually toward the

FIG. 5. Several capillaries from a child with the nephrotic syndrome and early clinical renal insufficiency. The endothelium is thickened and vacuolated in several areas (E, and near micron marker). The basement membrane is irregularly thickened, shows lamination, and numerous irregular areas of decreased density. Most of the epithelial-cell foot processes are distorted and some areas of foot process fusion are shown. Masses and bands of basement membrane material (B) extend toward the endothelium of the capillary at the top of the figure. (Osmium-methacrylate. Uranyl acetate-stained. × 30,000.)


0.5 μ

endothelial cells in children and more often toward the epithelial side of the membrane in adults), stratification into distinct lamellae, or large irregular zones of decreased density may appear within the lamina densa (Fig. 5). The last abnormality, which may result in a "moth-eaten" appearance of the lamina densa (Farquhar, Vernier and Good, 1957*b*) has been described by Spiro (1959) in tissue stained with phosphotungstic acid. He proposes that these areas are the pores which give rise to increased permeability of the nephrotic glomerulus; however, it does not seem likely that these large areas (1000 Å or more) offer a satisfactory explanation of the mechanism of proteinuria. Furthermore, we have not, with similar methods, been able to demonstrate these zones in glomeruli from children with the nephrotic syndrome ("pure nephrosis") even though the patients had severe proteinuria.

Nephrotic basement membrane which has been fixed in acrylic aldehyde (Luft, 1959) and embedded in Vestopal-W demonstrates a fine filamentous structure with about 75-Å spaces between the filaments (Fig. 6). However, the size and shape of these interstices are not apparently different from those seen in normal basement membrane prepared in a similar manner. Further studies of normal and abnormal tissue fixed and embedded by a variety of methods, stained, and examined by high-resolution techniques, will be necessary before the full significance of the fine structure of the membrane is appreciated.

Serial renal biopsies show that proliferation of the glomerular endothelial cells, seen as hypercellularity in the light microscope, may occur early in the clinical course of the nephrotic syndrome, and may precede recognizable changes in the width of the

FIG. 6. Detail of a glomerular capillary wall from a child with severe proteinuria. This tissue was fixed in acrylic aldehyde, and embedded in Vestopal. Lumen to the left. Epithelial cell with large vacuoles to the right. The basement membrane contains numerous irregularly circular zones of lesser density, in a matrix of dense filaments. (Stained with uranyl acetate. ×95,000.)

basement membrane. Electron microscopy of such specimens confirms an increase in number of endothelial cells, and also reveals broadening of the endothelial cytoplasm lining the capillary and a decrease in number and in regularity of the spacings of the endothelial pores (Fig. 5). Usually these abnormalities are accompanied by the appearance of bands and masses of dense material, similar to basement membrane, within the cytoplasm and between the endothelial cells. This basement membrane-like material may be seen in continuity with the original lamina densa and can be shown by studies of adjacent sections with the light and electron microscope to have similar tinctorial characteristics. We believe that this material results in obliteration of the capillary lamina and, eventually, in the "hyalinized", non-functioning glomerulus which is the end stage of the progressive alterations occurring in many renal diseases.

An abnormality of the epithelial-cell foot processes, which is very similar to that described above in the nephrotic syndrome of human beings, has been the predominant electron microscopic feature of a number of forms of experimental nephrotic syndromes in laboratory animals. No definitive abnormalities of the basement membranes have been recognized prior to the development of the foot process lesion. In aminonucleoside nephrosis (Vernier, Papermaster and Good, 1959) the foot processes have been shown to fuse at the onset of the proteinuria, suggesting that the foot process abnormality is either the cause of or the result of proteinuria.

Studies which we have recently completed tend to support the concept that the foot process abnormality is the result of proteinuria (Vernier *et al.*, 1960). We have taken advantage of the principle of threshold proteinuria in producing acute and chronic proteinuria in dogs by elevation of serum protein levels through either constant or intermittent infusions of canine serum proteins. Proteinuria occurs when the serum protein level of the dog reaches 7–9 g./100 ml. Electron microscopy of renal biopsy

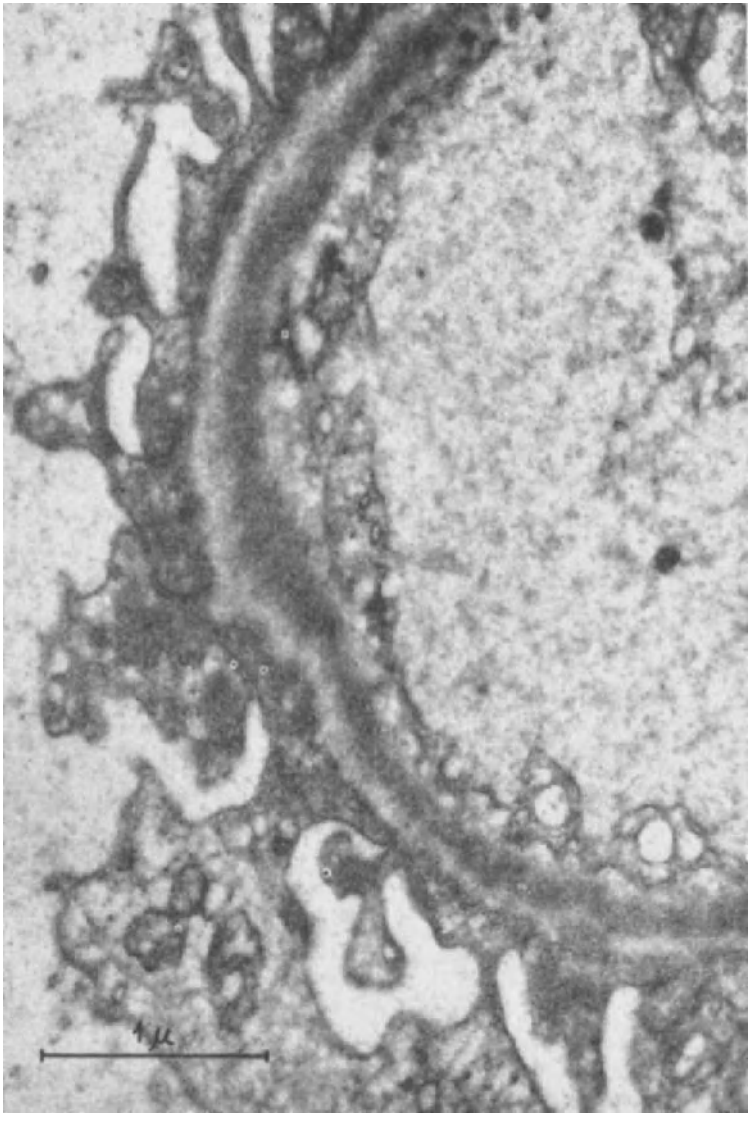


FIG. 7. Tangential section of a capillary from a dog with threshold proteinuria. Many of the epithelial-cell foot processes are fused together to form a more or less continuous layer of cytoplasm about the exterior of the capillary. (Osmium-methacrylate. × 30,000.)

specimens from these animals during threshold proteinuria shows that the foot processes are abnormal as compared to the control biopsy specimens. The foot processes swell near their point of juncture with the basement membrane, resulting in the obliteration of the narrow slits between the adjacent processes, and in many areas this gives rise to a lesion very similar to that seen in human and experimental nephrotic syndromes (Fig. 7). We feel that these experiments justify the conclusion that proteinuria alone, in the absence of renal disease, results in abnormalities of the epithelial cell and that the foot processes probably react to the presence of large amounts of protein to produce the abnormality.

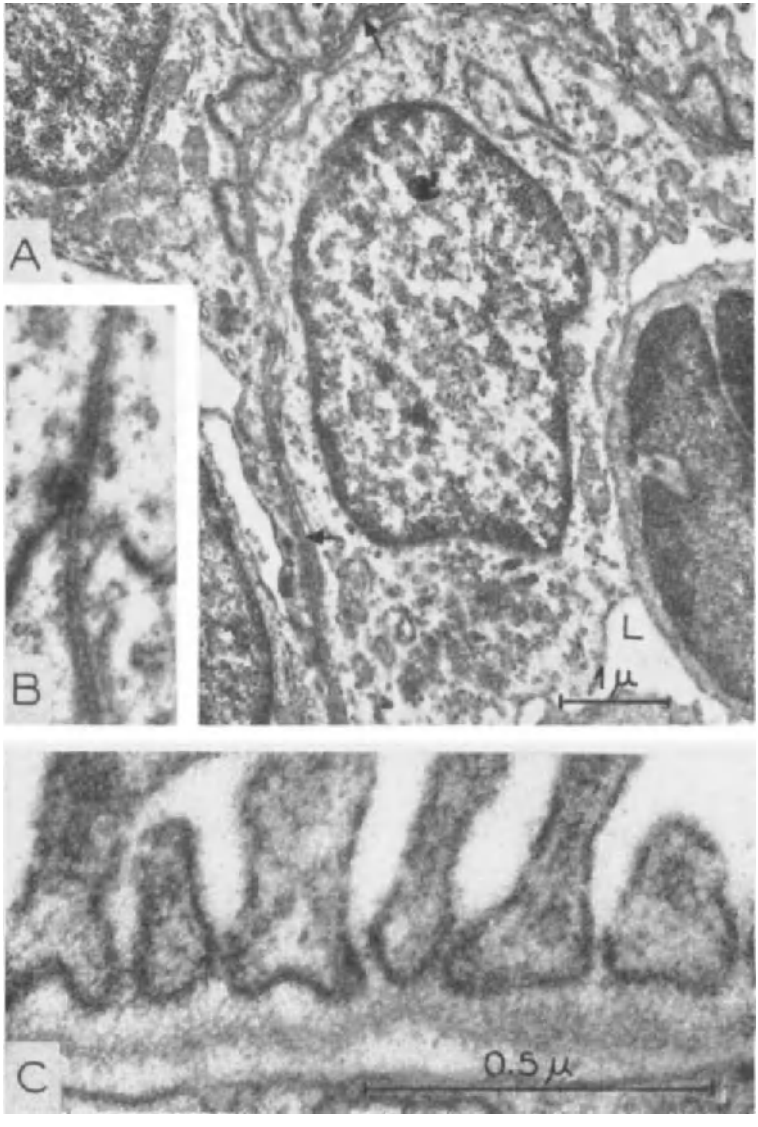
Recently we have investigated the morphology of the glomerulus in human foetuses in an attempt to gain additional information on the relationship between the structure and function of the various components of the capillary wall. Very immature and relatively mature glomeruli from foetuses of various ages have been studied after injection of the foetus with electron-dense materials (Vernier and A. Birch-Andersen). Carbon particles (diameter 200–300 Å) and ferritin molecules (100 Å) have been given into the umbilical artery of the foetus and small blocks of kidney removed within a few minutes after the injection. Electron microscopy of very immature glomeruli reveals no lamina densa in the capillaries but instead the basement membrane is composed of only two plasma membranes separated by a 300–500 Å space of

---

FIG. 8. A: Portion of an immature capillary in a glomerulus from a human foetus. A blood cell lies in the lumen (L) to the lower right. The endothelium has no pores and the epithelial foot processes have not developed. The basement membrane (arrow), which extends vertically and across the top of the picture to the right, is very thin and consists of two plasma membranes, separated by only 300–500 Å. (Osmium-methacrylate. Uranyl acetate-stained. × 15,000.)

B (insert): Detail of a portion of the basement membrane in Fig. 8A. (× 30,000.)

C: Detail of the capillary wall in a more mature foetal glomerulus. The epithelial-cell foot processes are well differentiated. The lamina densa is composed of fine filaments which are rather loosely arranged. (Osmium-Vestopal. Uranyl acetate-stained. × 94,000.)


A
B
L
1 μ
C
0.5 μ

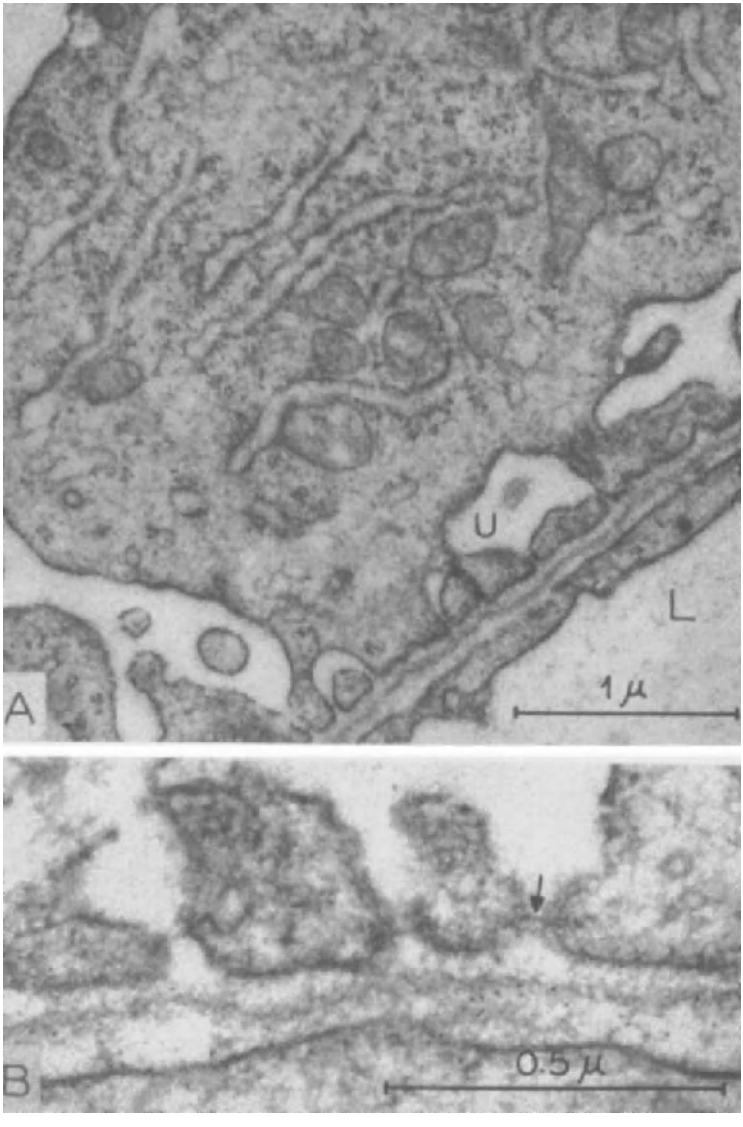
low electron density. The endothelium and epithelium are also poorly differentiated at this time (Fig. 8A, 8B). The earliest lamina densa is extremely thin and is found between the two plasma membranes. It is composed of small filaments which are loosely packed and poorly organized. In the more mature capillaries the filaments remain distinct but are more compactly arranged into a membrane which is often split into two components (Fig. 8C). The lamina densa appears to be a nearly total barrier to the diffusion of carbon particles and a partial barrier to ferritin molecules. Large amounts of ferritin are found within the cytoplasm of the endothelial cell and "piled up" within and beneath the lamina densa. Small amounts of ferritin appear in the cytoplasm of the epithelial cell, both as free particles and within vacuoles. Rare ferritin particles are found free in the urinary space (Figs. 9B, 10).

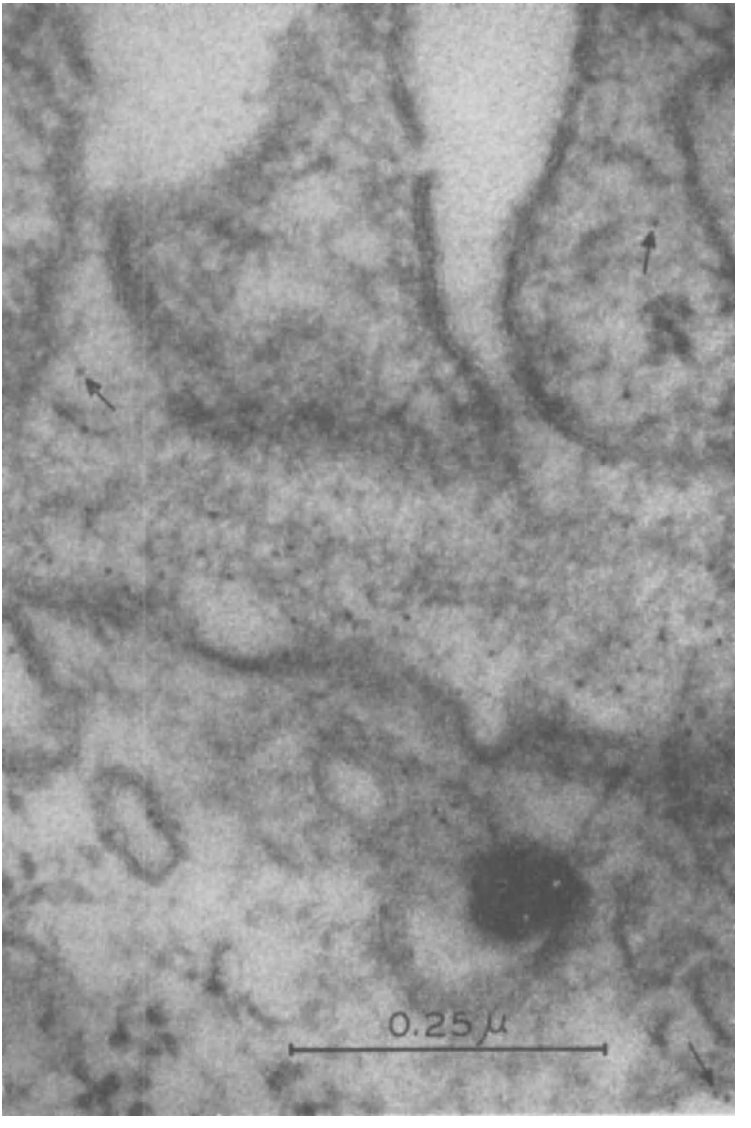
The glomerular transport of ferritin in the normal and nephrotic rat has been described in two fine papers by Farquhar and colleagues (Farquhar and Palade, 1960; Farquhar, Wissig and Palade, 1961). The molecule was observed to enter the epithelial cells of both normal and nephrotic rats where it appeared within vacuoles. In the nephrotic rat, the rate of transport appeared to be increased and more particles were present in the epithelial cells, always within vacuoles. The authors conclude that the particles are taken up by pinocytosis via the epithelial cell foot processes and transported into the epithelial cell as vacuoles. It is significant

---

FIG. 9. A: Portion of a capillary from a foetus given ferritin via the umbilical artery. The lumen (L), to the lower right, contains numerous particles of ferritin among filaments of plasma protein. The urinary space (U) under the epithelial cell, appears to be free of particles at this magnification. (Osmium-Vestopal. Uranyl acetate-stained. × 30,000.)

B: At higher magnification ferritin particles are demonstrated within the basement membrane. Most of the particles lie on the endothelial side of the basement membrane within the lamina densa. Two filtration slit membranes are visible (arrow). (Osmium-Vestopal. Uranyl acetate-stained. × 94,000.)


U
L
A
1 μ
B
0.5 μ


0.25 μ

that no particles were found within the slits between foot processes or free in the urinary space, and the authors express doubt that sizable quantities of protein are filtered via this morphological pathway, previously presumed to be important.

Summary—Correlation of physiology and morphology

The ultrastructure of the normal mammalian glomerulus has been described and some of the changes which occur in the three components of the capillary wall during proteinuria have been discussed. It is apparent that the process of formation of the fluid which ultimately reaches Bowman's space is very complex. The thin fenestrated endothelial cell membrane undoubtedly serves to increase the area of contact between the contents of the capillary lumen and the basement membrane, but the pores are thought to be too large to restrict the transport of proteins. Another demonstrated function of the cell is its active phagocytosis of particulate matter. The lamina densa of the basement membrane of both the rat and the human foetus has been shown to function as at least a partial barrier to the passage of large molecules. The filter-like characteristics of the lamina densa may be related to its demonstrated structure of fine filaments separated by interstices of up to 75 Å in diameter. Although spaces of about these dimensions between filaments in a gel-like matrix would adequately satisfy the physiological data available and would perhaps fulfil the structural requirements expected of a semipermeable glomerular membrane, proof that the structure

FIG. 10. A portion of a capillary from a foetus given both carbon and ferritin is shown. A carbon particle lies within a vacuole in the endothelial cytoplasm. Ferritin particles are visible in the lumen (arrow right corner). The endothelium contains particles both within vacuoles and free in the cytoplasm. Numerous particles appear within the basement membrane. Some particles have penetrated the lamina densa and are found free within the cytoplasm of the epithelial-cell foot processes (right upper arrow). One particle (left arrow) lies within the filtration slit between the epithelial-cell foot processes. (Osmium-Vestopal. Uranyl acetate. × 170,000.)

described serves this purpose is lacking. It is particularly significant that no enlargement of the spaces or other distortion of the arrangement of the filaments and spaces has been found in abnormal membranes believed to be more permeable to protein than normal. The complex organization of the epithelial cell and the changes which develop in this cell during proteinuria suggest that the epithelial cell probably plays an important rôle either in the maintenance of the integrity of the basement membrane or in regulating the rate of transport across the basement membrane. It seems likely that at least the protein portion of the filtrate may enter the epithelial cell and it is thus possible that malfunction of the epithelial cell itself may result in proteinuria. However, most of the evidence seems to indicate that the foot process abnormality which is characteristic of proteinuria, develops as a result of proteinuria. In this case a primary defect of function, and presumably an as yet unrecognized abnormality of structure, of the basement membrane probably exist in most instances of increased glomerular permeability to proteins.

The mechanism of transport of filtrate across the capillary wall has not been satisfactorily elucidated. Although pinocytosis of ferritin molecules by the epithelial cells has been demonstrated there are many reasons, based on physiological data, to doubt that all the filtrate is transported in this manner. Our observations of free ferritin particles (not enclosed in membranes) in the epithelial cells and the urinary space of human foetuses, support the concept that free diffusion also occurs. Probably two (or perhaps more) mechanisms operate: pinocytosis of the bulk of large molecules such as proteins, and free diffusion of small molecules and water. It is attractive to speculate that the pinocytotic activity of the epithelial cells might function as a defensive or protective mechanism, designed to prevent excessive loss of proteins into the urine. Many of the morphological features of the epithelial cell during proteinuria seem compatible with this view.

Acknowledgments

The author is indebted to Aksel Birch-Andersen for assistance and encouragement during completion of phases of this work at the Department of Biophysics, Statens Seruminstitut, Copenhagen.

The technical assistance of Mrs. Helene Ravn and Mrs. Jytte Barslund (Copenhagen) and Miss Joyce Lounberg and Mrs. Ruth Shurr (Minneapolis) is gratefully acknowledged. Miss Anne-Grete Overgård's aid in preparing the illustrations is very much appreciated.

This investigation was conducted during the tenure of an Established Investigatorship of the American Heart Association and a Research Fellowship (1960–61) of the John Simon Guggenheim Memorial Foundation. Support was received from the American Heart Association, the United States Public Health Service, and the Graduate School of the University of Minnesota.

REFERENCES

BAYLISS, L. E., KERRIDGE, P. M. T., and RUSSELL, D. S. (1933). *J. Physiol.* (Lond.), **77,** 386.

BLOOM, P. M., HARTMANN, J. F., and VERNIER, R. L. (1959). *Anat. Rec.*, **133,** 251.

CHINARD, F. P. (1952). *Amer. J. Physiol.*, **171,** 578.

FARQUHAR, M. G., and PALADE, G. E. (1960). *J. biophys. biochem. Cytol.*, **7,** 297.

FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A. (1957*a*). *J. exp. Med.*, **106,** 649.

FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A. (1957*b*). *Amer. J. Path.*, **33,** 791.

FARQUHAR, M. G., WISSIG, S. L., and PALADE, G. E. (1961). *J. exp. Med.*, **113,** 47.

GREGOIRE, F., MALMENDIER, C., and LAMBERT, P. (1958). *Amer. J. Med.*, **25,** 516.

HALL, V. (1957). *Amer. Heart J.*, **54,** 1.

KURTZ, S. M., and MCMANUS, J. F. A. (1959). *Amer. Heart J.*, **58,** 357.

LUFT, J. H. (1959). *Anat. Rec.*, **133,** 256.

MONKE, J. V., and YUILE, C. L. (1940). *J. exp. Med.*, **72,** 149.

PAPPENHEIMER, J. R. (1953). *Physiol. Rev.*, **33,** 387.

PAPPENHEIMER, J. R., RENKIN, E. M., and BORRERO, L. M. (1951). *Amer. J. Physiol.*, **167,** 13.

POLICARD, A., COLLET, A., and GILTAIRE-RALYTE, L. (1955). *Arch. Anat. micr. Morph. exp.*, **44,** 1.

RYTER, A., and KELLENBERGER, E. (1958). *J. Ultrastructure Res.*, **2,** 220.

SPIRO, D. (1959). *Amer. J. Path.*, **35,** 47.

VERNIER, R. L., and BIRCH-ANDERSEN, A. (unpublished observations).

VERNIER, R. L., PAPERMASTER, B. W., and GOOD, R. A. (1959). *J. exp. Med.*, **109,** 115.

VERNIER, R. L., PAPERMASTER, B. W., OLNESS, K., BINET, E., and GOOD, R. A. (1960). *Amer. J. Dis. Child.*, **100**, 476.
VERNIER, R. L., WORTHEN, H. G., and GOOD, R. A. (1961). *J. Pediat.*, **58**, 620.
WALLENIUS, G. (1959). *Acta Soc. Med. upsalien.*, **59**, Suppl. 4.
WATSON, M. L. (1958). *J. biophys. biochem. Cytol.*, **4**, 727.
WEARN, J. T., and RICHARDS, A. N. (1924). *Amer. J. Physiol.*, **71**, 209.
YAMADA, E. (1955). *J. biophys. biochem. Cytol.*, **1**, 551.

DISCUSSION

Bergstrand: I cannot agree with you that there are three layers of the basement membrane: the central lamina densa and two lamina rara, one on the endothelial and one on the epithelial side of the lamina densa. We think that there is only one homogeneous basement membrane and that these zones are dehydration artifacts. Small bridges of a denser substance between the lamina densa and the cell membrane of the endothelial or epithelial side of the basement membrane are highly suggestive of remnants of original substance which has been retracted during the dehydration process. It is also a possibility that the methacrylate embedding produces artifacts, that there is a shrinkage and a subsequent swelling of the tissues during the polymerization of the methacrylates. Vestopal causes fewer artifacts, as can be seen from the mitochondrial structure, the nucleus, the membrane, etc. In human specimens we find an absolutely homogeneous layer between the endothelium and epithelium. Some sections indicate a filamentous structure of the basement membrane, and very thin sections show a foamy appearance. It is more probable, however, that the basement membrane is a gel, without definite structure. It probably consists of very long chains of molecules with strong bonds in the longitudinal direction, and perhaps weak bonds in side chains connecting them, so that they may be easily separated from each other during the preparation of a specimen. These molecules might stretch and become thinner, or they might swell and become shorter; the distance between chains may differ, and this may be the cause of increased or decreased permeability.

Vernier: As I mentioned in my paper, the lamina rara is far more prominent in the basement membrane of the rat and dog than it is in the human, regardless of the way in which the material is prepared.

I agree with you that the width of the lamina rara is exaggerated in methacrylate-embedded tissue, and I think that the contrast between the lamina rara and the lamina densa is far more apparent in stained than in unstained material. In a specimen of a normal human kidney embedded in Vestopal (Fig. 3 of my paper) the lamina rara is very thin; this looks very much like your pictures.

Pirani: I should like to raise the question of whether or not there are always changes in the foot processes in relation to proteinuria. It has been our impression that proteinuria of a quite severe degree, up to the full-blown nephrotic syndrome, may be present with no fusion of foot processes; this has been so in diabetes mellitus, in some cases of systemic lupus nephritis, and even in some cases of membranous glomerulonephritis. On the other hand, fusion of the foot processes has always been present in patients with lipoid nephrosis, in both children and adults. The question is whether there are some diseases which affect the epithelial cells, and the foot processes in particular, more severely and others in which the change is predominantly in the basement membrane.

Jennings: I should like to confirm Dr. Pirani's observations. We have seen the fused foot processes that Dr. Vernier described in all patients with lipoid nephrosis and we have also seen the lesion disappear with reversal of the disease. However, we have not seen fused foot processes in the biopsies of patients with proliferative glomerulonephritis and the nephrotic syndrome. I should also like to state that when one applies new techniques such as electron microscopy to renal biopsy material, one should always also study the material by light microscopy so that one has a way of comparing the new knowledge to the old.

Movat: I believe that lipoid nephrosis (or idiopathic nephrosis) of children and membranous glomerulonephritis in adults are two different conditions. I wonder whether the lesion in the adult is reversible. Dr. Kark and Dr. Pirani published a case in the *Annals of Internal Medicine*—a young man. Has that patient definitely recovered?

Kark: At this time we don't think there is much difference between idiopathic lipoid nephrosis in adults and in children in terms of pathology and pathogenesis; in terms of aetiology, of course, that may be a different thing altogether. The patient you mentioned is perfectly all

right now, although he had a second attack, just as children do. As in children, you get remissions and relapses in this disease, and every time this happens you get the electron microscopic changes which Dr. Vernier has so clearly described.

Vernier: Regarding foot process changes in adults and children, I agree with Dr. Kark. I have studied a few adults with an idiopathic nephrotic syndrome (lipoid nephrosis) and I can't see any difference between the epithelial cell structure in children and adults; the adults may respond as children do, and demonstrate return to normal foot process structure during recovery. The other forms of the nephrotic syndrome are rare in childhood. I have studied only three patients with the nephrotic syndrome associated with diabetes, and in those three patients the foot process lesion has been similar to that seen in our other nephrotic patients. It is conceivable that there are several mechanisms by which proteinuria may occur, that is increased glomerular permeability to protein, decreased tubular reabsorption of protein, and post-glomerular secretion of protein from peritubular capillaries or lymphatics. At least these three mechanisms must be considered, and the structure and function of the various components of the glomerulus examined with these possible mechanisms in mind. I think that the endothelium has at least two functions. One of these functions is phagocytosis of particulate material. Secondly, the endothelium plays a rôle in certain renal diseases, including those associated with proteinuria, in producing hyaline material which leads eventually to obstruction of the capillaries. I think the basement membrane itself, the lamina densa, is the ultimate filter. I believe that in most patients with proteinuria the primary defect is an increased permeability of the lamina densa to protein, and that the epithelial cell changes develop secondarily. It seems likely that one of the epithelial cell's functions is removal of large-molecular-weight materials which pass the filter. When large amounts of these materials are presented to the epithelial cell (due either to an increased concentration in the lumen, such as in the threshold proteinuria experiments, or because of defects in permeability of the lamina densa) the epithelial cell increases its contact with the basement membrane in an attempt to deal with the larger concentrations of material and the foot process fusion lesion results.

Kark: What do you think is the function of the microvilli, which make the epithelial cell look like a porcupine? What I am talking about are the osmiophilic bodies apparently lying free in Bowman's space.

Vernier: I think those are simply the secondary processes of epithelial cells projecting to capillaries in a different plane that are cut either as perfectly round little bodies or as elongated tubes. The epithelial cell processes are probably in motion over the surface of the capillary.

Hamburger: Concerning the significance of the fusion of the foot processes, we do not wholly agree with the statement that it is specific for the nephrotic syndrome. We have also encountered such a picture in biopsies of people who not only do not have the nephrotic syndrome, but do not have even any consistent proteinuria. A recent example is a renal biopsy of a homotransplanted kidney, two months after transplantation, in which there was definite fusion of foot processes in many places; the proteinuria on the day of the biopsy being only 0·1 mg. per minute. This is perhaps relevant to the relationship of this picture with proteinuria.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

GLOMERULAR DAMAGE IN TERMS OF "PORE SIZE"

J. HARDWICKE and J. F. SOOTHILL

Department of Experimental Pathology, Birmingham University Medical School

THIS paper attempts to demonstrate that functional changes in the kidney, as evidenced by loss of serum proteins in the urine, in patients with renal disease can be correlated with histological changes in the glomeruli. From these findings some preliminary inferences can be drawn as to the nature of the damage to the capillary wall in these patients.

It is now recognized that severe proteinuria can occur in a variety of disease processes, in many of which renal involvement is part of a general disease (Squire, Blainey and Hardwicke, 1957; Kark, Pirani, Pollak, Muehrcke and Blainey, 1958). Such conditions are usually amenable to diagnosis on clinical grounds. In this study attention is confined to patients with proteinuria, not associated with such a generalized disease—a group of patients described as "primarily renal". In this group patients have been classified on the basis of histological findings, as "proliferative" or "membranous" and another group in whom no gross histological abnormality could be detected, which we have called "minimal" or "nil" change (Blainey *et al.*, 1960). The numbers studied in each group are shown in Table I.

Light microscopy only has been used on paraffin-embedded material after sectioning and staining with haematoxylin and eosin, haematoxylin and Van Gieson, and periodic acid-Schiff methods.

Assessment of the permeability of the kidney to individual serum proteins is based on the comparison of their "clearances"

Table I

HISTOLOGICAL CLASSIFICATION OF PATIENTS

Diagnosis	*Number*
Membranous glomerulonephritis	11
Atypical membranous glomerulonephritis	2
Proliferative glomerulonephritis	16
Minimal changes	9
	38

from serum. Initially it was shown, using the technique of paper electrophoresis, that the ratio of γ-globulin clearance to albumin clearance was nearly always less than 1·0 (i.e. the kidney preferentially excreted the albumin molecule of mol. wt. 69,000 as compared with the γ-globulin molecule of mol. wt. 150,000). Moreover, the actual ratio varied in different types of renal disease: in some patients the ratio was as low as 0·1 to 0·2, while in others with conditions such as acute glomerulonephritis or disseminated lupus erythematosus, the ratio approached 1 (Hardwicke and Squire, 1955). From this it was inferred that by expressing the γ-globulin clearance as a percentage of albumin clearance (1·0 ratio = 100 per cent) an assessment of the "leakiness" of the glomerulus could be obtained.

This reasoning depends on the relative composition of the proteins in the glomerular filtrate being the same as that in the urine; specific reabsorption in the tubules of any protein being studied would invalidate the above conclusions. However, on following the rise in albumin clearance after intravenous albumin infusion, it was found that the clearances of the globulins also rose in a constant ratio to the albumin clearance. This is interpreted as showing that reabsorption is not selective between the different proteins, and therefore the urine has the same relative composition of individual protein fractions as the glomerular filtrate, though, of course, the total quantity excreted is less by the amount of any protein reabsorption occurring in the tubules (Hardwicke and Squire, 1955).

The paper electrophoretic technique is, however, directly applicable only to albumin and γ-globulin, i.e. to those two fractions which are substantially homogeneous; the α- and β-globulin fractions are mixtures of so many proteins of widely differing molecular weights that clearances can have no real meaning in this context; an immunological technique has therefore been applied to this problem.

Specific antisera have been prepared in rabbits to six serum proteins ranging in molecular weight from 69,000 to 2,500,000 (Table II). By a modification (Soothill, 1961) of the technique of

Table II

PROTEINS ESTIMATED IMMUNOLOGICALLY

	Molecular weight	*Diffusion constant* $D_{20}w$	
Albumin	69,000	6·1	(Gutter, Peterson and Sober, 1957)
Siderophilin	90,000	5·8	(Ellenbogen and Maurer, 1956)
γ-globulin	150,000	3·8	(Phelps and Putnam, 1960)
C-component of complement	?	?	
α_2-glycoprotein	920,000	2·4	(Schönenberger, Schmidtberger and Schultze, 1958)
β-lipoprotein	2,500,000	1·7	(Shore, 1957)

Gell (1957) urine/serum ratios for each of these proteins have been determined over four-hour clearance periods. Initially, these values were obtained in one patient following albumin infusion (Fig. 1) and this more specific technique served to confirm the constant clearance ratios for the different proteins. Subsequently, clearances have been performed on the 38 patients on whom renal biopsies have been performed. All the clearances have then been expressed as percentages of siderophilin clearance. (For technical reasons siderophilin, mol. wt. 90,000, is more accurately estimated than albumin by the immunological method.) Fig. 2 shows the mean values obtained for each protein in the three main groups of histological lesion. Considerable differences are apparent between

the groups. Those with membranous changes show a very marked relative permeability to all the larger proteins, with even β-lipoprotein appearing in the urine and γ-globulin showing a

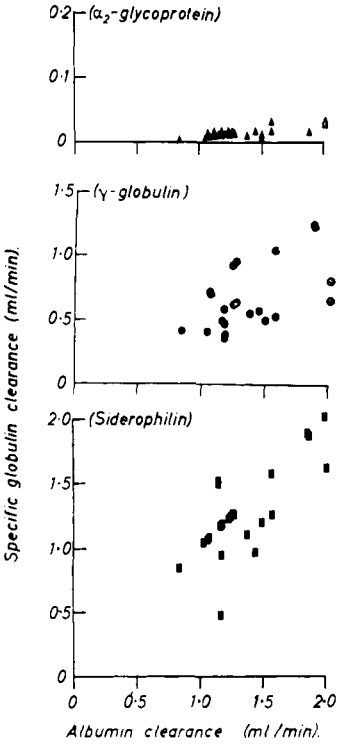


FIG. 1. Relationship between albumin clearances and globulin clearances following albumin infusion.

clearance almost 50 per cent of the siderophilin value. In contrast, the patients with minimal lesions have a very highly selective filter; only proteins below about 200,000 mol. wt. were detected in the urine, and the γ-globulin clearance averaged 10 per cent of

the siderophilin clearance. These results demonstrate a very considerable functional difference among these three histologically differentiable conditions.

Fig. 3 shows in detail the analyses on the patients with membranous and minimal changes. Those with proliferative lesions

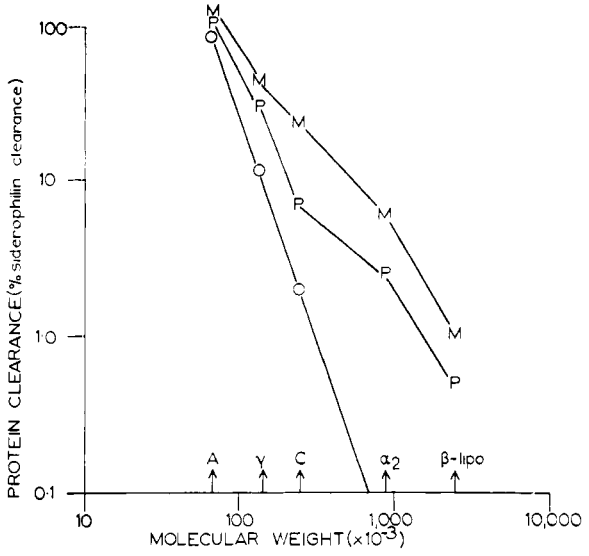


FIG. 2. Differential clearances in "primarily renal disease".
M—M = membranous
P—P = proliferative
O—O = minimal or nil change
Each point represents the mean of the mean value for each patient. For other abbreviations, see Fig. 6.

show intermediate values, and the significance of clearances in this group is being made the subject of further study. The lack of selectivity by all the membranous patients is striking when compared with those with minimal lesions. Three patients with long-standing renal vein thrombosis (one only diagnosed during life) showed typical "membranous" clearances and biopsies (for

further details of one of these see Blainey, Brewer, Hardwicke and Soothill, 1960).

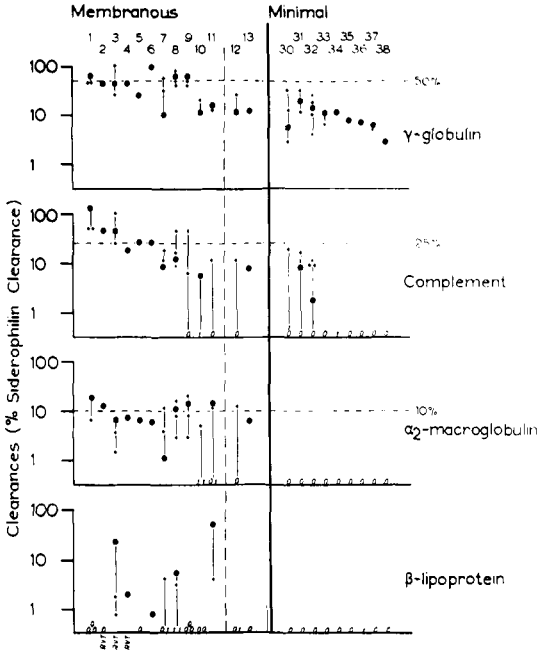


FIG. 3. Differential protein clearances in patients with membranous glomerulonephritis and minimal change. The linked points represent serial analyses on a single patient.

● = first analysis
\+ = subsequent analyses
t = trace
o = o
R.V.T. = renal vein thrombosis

These findings may now be compared with experimental work on the permeability of the kidney to macromolecules. Brewer (1951) in rabbits, and Wallenius (1954) in dogs, have shown that the kidney selectively excretes dextrans of differing molecular

weight; at a molecular weight less than 4,000 the clearance was 100 per cent of the creatinine clearance, while at 28,000 it had fallen to about 10 per cent. This relationship between molecular weight and clearance shows a log-normal type of distribution—a type of distribution frequently found in biological material

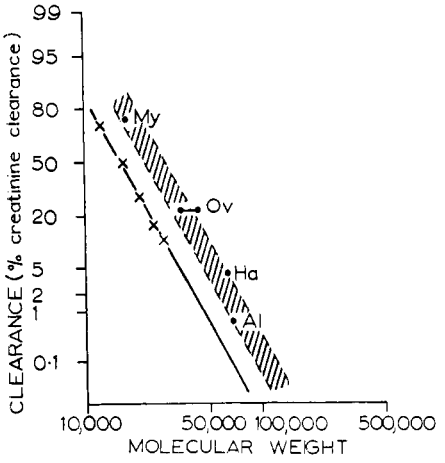


FIG. 4. Normal dog kidney. Relationship between clearance and molecular weight of macromolecules.

X—X = dextran fractions
My = myoglobin
Ov = ovalbumin
Ha = haemoglobin
Al = albumin

(Gaddum, 1945). It is best represented mathematically by a probit analysis. The published data of Wallenius (presented in Table III) are plotted in this manner in Fig. 4; a very satisfactory linear relationship is found. Lambert and his colleagues (Lambert, Gregoire and De Heinzelin de Brancort, 1952; Malmendier and Lambert, 1955) reported the clearances of four other proteins, partly from their own work and partly from the literature, and

TABLE III

RELATION BETWEEN MACROMOLECULE CLEARANCES AND MOLECULAR WEIGHT AND DIFFUSION CONSTANT

Macromolecule	*Molecular weight*	*Diffusion constant* $D_{20}w$	*Clearance* (% *creatinine clearance*)	
Dextran	4,000	14	100	(Wallenius, 1954)
	8,000	10	90·9	
	12,500	8	71·7	
	16,500	7	48·7	
	20,500	6·4	28·7	
	24,500	5·9	16·3	
	28,500	5·45	11·0	
Myoglobin	17,500	1·1	75	(Malmendier, Gregoire and Lambert, 1957)
Ovalbumin	35,000	7·8	22	(Pappenheimer, 1955)
Haemoglobin	68,000	6·9	4	(Monke and Yuile, 1940)
Albumin	69,000	6·1	0·6	(Phelps and Putnam, 1960)

these are included in Fig. 4. Once again the relationship between molecular weight and clearance is demonstrated; the data suggest a regression parallel with that for dextran, but suggesting that protein molecules are excreted slightly more readily than a dextran of equivalent molecular weight; when, however, the same data are plotted on the basis of reciprocal of diffusion constant (a figure which might be expected to be more closely correlated with passage through capillary membranes), an equally good fit is obtained (Fig. 5), with dextran showing clearances slightly greater than those of the proteins. Variations in shape and charge may well account for these differences.

This figure, then, demonstrates the distribution of "pore size" in the normal kidney.

Increase in permeability in disease could occur in two ways: either by an over-all stretching of the capillary wall, which would result in an increase in the size of each "pore" so that the probit regression would simply be moved over to the right; or by actual disruption of the capillary wall, giving a number of "pores" of grossly increased size, which would result in a departure from

linearity of the regression at the lower end of the scale. Fig. 6 shows the results obtained in our patients plotted on the same scale; the membranous patients quite clearly show a grossly abnormal permeability to the high-molecular-weight proteins,

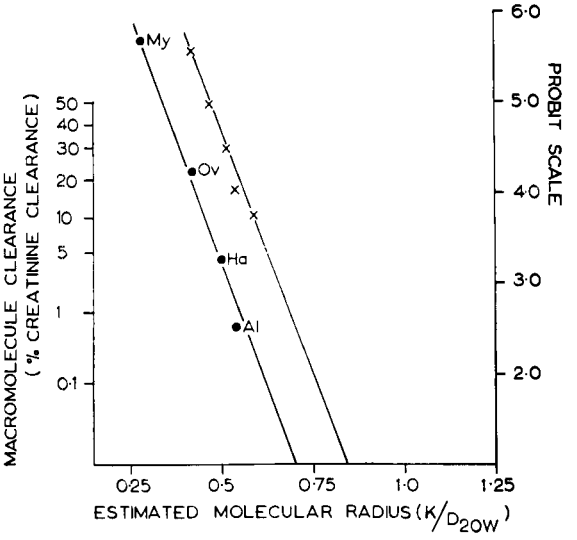


FIG. 5. Normal dog kidney. Relationship between clearance and diffusion constant of macromolecules.

X—X = dextran fractions
My = myoglobin
Ov = ovalbumin
Ha = haemoglobin
Al = albumin

For references see Table III.

suggesting that in this condition the membrane is grossly damaged; in the patients with minimal changes, however, the deviation from the supposed normal line is much less marked, though there is still some suggestion that the permeability of the glomerulus is qualitatively as well as quantitatively disrupted.

It is hoped to extend this work in two ways. Firstly, estimating

relative protein clearances and protein reabsorption concurrently in individual patients after albumin infusion would give a more exact regression line, with values for sieving coefficients (glomerular clearance as percentage of creatinine clearance) for the indivi-

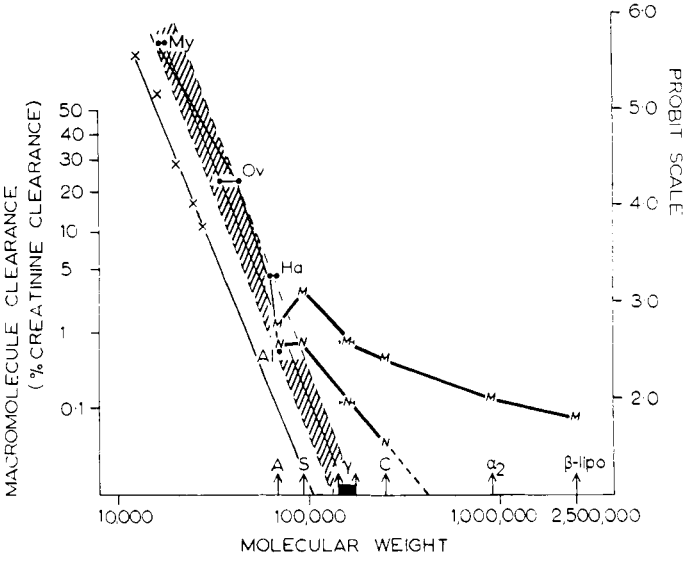


FIG. 6. The permeability of the diseased kidney to proteins compared with the normal permeability to macromolecules.

X—X = dextran
My = myoglobin
Ov = ovalbumin
Ha = haemoglobin
Al = albumin
M—M = membranous glomerulonephritis
N—N = minimal or nil change
A = albumin
S = siderophilin
γ = γ-globulin
C = complement
α_2 = α_2-macroglobulin
β-lipo = β-lipoprotein

dual proteins. Secondly, antisera have been obtained to low-molecular-weight α_1-glycoproteins, clearances of which should extend the analyses to a molecular weight of 40,000, and approximately double the range of analysis (Hardwicke and St. Cyr, 1961).

Summary

The clearances in the urine of six serum proteins, measured immunochemically, have been correlated with renal biopsy findings. Considerable differences have been shown in different histological lesions. The findings are compared with published clearances of macromolecules by normal dogs.

REFERENCES

BLAINEY, J. D., BREWER, D. B., HARDWICKE, J., and SOOTHILL, J. F. (1960). *Quart. J. Med.*, **29**, 235.

BREWER, D. B. (1951). *Proc. roy. Soc. Med.*, **44**, 561.

ELLENBOGEN, E., and MAURER, P. (1956). *Science*, **124**, 266.

GELL, P. G. H. (1957). *J. clin. Path.*, **10**, 67.

GADDUM, J. H. (1945). *Nature (Lond.)*, **156**, 463.

GUTTER, F. J., PETERSON, E. A., and SOBER, H. A. (1957). *Arch. Biochem.*, **72**, 194.

HARDWICKE, J., and ST. CYR, CH. DE VAUX (1961). *Clin. chim. Acta*, **6**, 503.

HARDWICKE, J., and SQUIRE, J. R. (1955). *Clin. Sci.*, **14**, 509.

LAMBERT, P.-P., GRÉGOIRE, F., and DE HEINZELIN DE BRANCORT, C. (1952). *Arch. int. Physiol.*, **60**, 506

KARK, R. M., PIRANI, C. L., POLLAK, V. E., MUEHRCKE, R. C., and BLAINEY, J. D. (1958). *Ann. intern. Med.*, **49**, 751.

MALMENDIER, C., GRÉGOIRE, F., and LAMBERT, P.-P. (1957). *Rev. franç. Ét. clin. biol.*, **2**, 145.

MALMENDIER, C. and LAMBERT, P.-P. (1955). *J. Urol. (Baltimore)*, **61**, 327.

MONKE, J. V. and YUILE, C. L. (1940). *J. exp. Med.*, **72**, 149.

PAPPENHEIMER, J. R. (1955). *Klin. Wschr.*, **33**, 362.

PHELPS, R. A. and PUTNAM, F. W. (1960). *In* The Plasma Proteins, Vol. 1, p. 159. New York and London: Academic Press.

SCHÖNENBERGER, V. M., SCHMIDTBERGER, R. and SCHULTZE, H. E. (1958). *Z. Naturforsch.*, **13b**, 761.

SHORE, B. (1957). *Arch. Biochem.*, **71**, 1.

SOOTHILL, J. F. (1961). *J. Lab. clin. Med.* In press.

SQUIRE, J. R., BLAINEY, J. D., and HARDWICKE, J. (1957). *Brit. med. Bull.*, **13**, 43.

WALLENIUS, G. (1954). *Acta Soc. Med. upsalien*, **59**, Suppl. 4.

DISCUSSION

Earle: Dr. Hardwicke, have you observed reduced serum γ-globulin levels in patients with the nephrotic syndrome who had little or no membranous changes? In some of our patients who had no glomerular lesions by light microscopy the serum γ-globulin and often complement, as well as anti-streptolysin antibodies, were reduced. If these are not lost in the urine, where do they go?

Hardwicke: γ-Globulin is lost in the urine; it has a loss of 10–20 per cent of the albumin clearance, and that appears to be quite sufficient to reduce the serum concentration quite markedly. As far as the complement is concerned, we have found low levels of serum complement, both by the guinea pig complement technique and by an immunological technique (Ellis, H. A., and Walton, K. W. (1958). *Immunology*, **1**, 234), only in acute nephritis or in systemic lupus erythematosus; there the levels were occasionally below 0·5 units/ml.

Earle: We occasionally find it reduced in other forms of renal disease associated with the nephrotic syndrome.

Vernier: Dr. Hardwicke, I think that your results on membranous glomerulonephritis are of great interest in connexion with the changes one sees in the basement membrane in this condition. The basement membrane may present a "moth-eaten" appearance, with large irregular vacuolated areas. Using phosphotungstic-acid-stained tissue from patients who appear to have glomerulonephritis, Dr. D. Spiro describes these areas as "pores" in the membrane. Since these pores are very large (1000 or more Ångström units) I can't see how they could filter proteins selectively, but they might present a morphological track through which large amounts of very high-molecular-weight protein could be transported.

Kark: Dr. Hardwicke, have you studied the occurrence of urinary myeloma globulin in multiple myeloma? We have had a patient recently who was spilling 8 to 10 g. of pure β-globulin (nothing else in the urine at all, except just a trace of albumin) and with a perfectly normal serum electrophoretic pattern and a normal serum γ-globulin. Have you studied any of those patients?

Hardwicke: The myeloma globulins cross-react with γ-globulin and we cannot deal with them immunologically, so all we have been able

to do is estimate the molecular weight of the urine protein, either by osmotic pressure or in the ultracentrifuge. I have the impression that the molecular weight of the urine protein has been low, and that there has also always been a little albumin in the urine. In some patients myeloma protein is only present in the urine in traces, and these presumably are of higher molecular weight.

Hamburger: Have you tried to apply your calculations to physiological proteinuria?

Hardwicke: My colleagues D. S. Rowe and J. F. Soothill (1961. *Clin. Sci.*, **21**, 75, 87) have been working on that. Physiological proteinuria appears at first sight to be relatively non-selective, that is, they get both albumin and γ-globulin out in the same proportions as in serum. However, they have found that the molecular weight of the γ-globulin in the physiological urine protein is much lower than that in the serum. The suggestion is that in physiological proteinuria the γ-globulin has been broken down. But whether this low-molecular-weight γ-globulin comes from the serum, I do not know. The albumin in physiological proteinuria seems to be normal.

Hamburger: Using the method of immuno-electrophoresis developed at the Institut Pasteur, we have found that in the "minimal change" cases, the γ-globulin is similar to that found in physiological proteinuria.

Slater: When the urinary proteins of lordosis are subjected to immuno-electrophoresis with antiserum to serum γ-globulin or to whole serum protein, the precipitin line of the urinary γ-globulin appears to be very similar to that of serum γ-globulin. Quantitative precipitin analyses with antiserum to serum γ-globulin reveal that the height of the equivalence zone is close or identical to that of the homologous serum γ-globulin.

In contrast, the γ-globulin excreted in exercise proteinuria behaves immunologically like that excreted in the normal urine, a reaction only of partial identity. Dr. Eric McKay, in our laboratory, has isolated these altered γ-globulins in a manner similar to previous studies by T. Webb, B. Rose, and A. H. Sehon (1958. *Canad. J. Biochem.*, **36**, 1159, 1167) and E. C. Franklin (1959. *J. clin. Invest.*, **38**, 2159). He has found that a high percentage of the exercise and normal urinary γ-globulin has an estimated molecular weight of approximately 5,000–6,000, one-thirtieth that of the serum γ-globulin.

We do not know whether this difference between the γ-globulin of lordotic proteinuria and exercise proteinuria represents differences in the renal handling of the protein in these two situations, or whether it possibly reflects an increased rate of degradation of γ-globulin in the body during exercise. We do not find similar changes in albumin.

Hardwicke: We seem to be in agreement on this. Another peculiarity is the so-called tubular proteinuria, which shows very little albumin and large quantities of electrophoretically separable α- and β-globulins, and which has been described very well by E. Butler and F. V. Flynn (1958. *Lancet*, **2**, 978). Now there the molecular weights are very small indeed. We are working on that now.

Hamburger: I have had the impression from our work that in the nephrotic syndrome there is a group of cases in which very heavy molecules pass into the urine, and in that group the predominant disease appeared to be amyloidosis. I would like to know if you have the same impression.

Hardwicke: We haven't done much work on amyloidosis. We have had only three cases, in which the clearances were extremely variable, and I think that in diabetic nephropathy, with depositions or with diffuse change, the clearances of high-molecular-weight protein are also extremely variable.

Milne: Am I correct, Dr. Hardwicke, in believing that in nephrotic syndrome from generalized disease, particularly diabetic glomerulosclerosis and lupus erythematosus, you found protein clearances similar to those in membranous glomerulonephritis?

Hardwicke: In lupus erythematosus the clearances are extremely non-selective. In diabetes they vary a good deal and I don't think they follow any very definite pattern.

Blainey: We have found little correlation between clearances of the various serum proteins in the diabetics and the histological changes on biopsy. Usually the clearances are moderately selective, and would fall on the line that Dr. Hardwicke has shown for the proliferatives.

Hardwicke: I didn't want to say much about the proliferatives, because there appear to be at least two types, one of which has highly selective clearances. We haven't sorted this out yet.

Vernier: Dr. Hardwicke, how do you define tubular proteinuria?

I have great difficulty in reconciling what I have seen in the microscope with what Dr. Pirani, Dr. Jennings, and Dr. Slater mentioned; that is, patients with severe proteinuria, in whom the epithelial cell morphology is entirely different from that in our group of patients with severe proteinuria. Do some nephrotic patients have tubular proteinuria? In this case the glomerulus might well look differcnt.

Hardwicke: I carefully avoided saying anything about the aetiology of proteinuria in these cases which we call "primarily renal disease". I was just presenting evidence that the membrane is much more damaged in the membranous cases than it is in those with no change. Tubular proteinuria is a type of proteinuria which is associated with renal tubular acidosis, with Fanconi syndrome (adults or childhood). It occurs also in severe potassium deficiency and one or two other conditions. It seems to be different from normal proteinuria and from the proteinuria of glomerular damage. It only occurs in very small quantities, 100–200 mg. per day.

Milne: It occurs also in Wilson's disease and in galactosaemia, but never in the proximal tubular syndromes of Hartnup disease and cystinuria.

Darmady: Is tubular proteinuria always associated with aminoaciduria?

Milne: Not in renal tubular acidosis.

Blainey: I think that every case that I have seen has been associated with abnormal aminoaciduria, including the patients with acute potassium deficiency. In this instance the functional change is reversible. It is also associated with glycosuria, and phosphaturia.

Slater: I should like to bring up the question of the amount of protein believed to be transferred across the glomerular filtration barrier in normal circumstances. In adults this has been estimated at 30–40 g. per day, with tubular reabsorption occurring almost completely, so that only 80 mg. is excreted. As Dr. Kark has observed, we know of patients with multiple myeloma who may excrete large amounts of Bence-Jones protein, in concentrations up to 2,000 mg./100 ml., according to F. V. Flynn and E. A. Stow (1958. *J. clin. Path.*, **11**, 334). This protein has a small molecular size (approximate mol. wt. 45,000) and may be excreted alone, unaccompanied by other plasma proteins. Now, if there is added to the 40 g. of plasma protein in the glomerular

filtrate a further load of Bence-Jones protein, sufficient to overwhelm tubular reabsorption, how does the Bence-Jones protein appear in the urine as a single entity? If we accept the hypothesis that tubular reabsorption of protein is proportional and non-preferential, why is the Bence-Jones protein not excreted in a mixture with the other proteins of glomerular filtrate? It is possible that Bence-Jones protein is not handled in a proportional manner by the tubules or alternatively that normal glomerular filtration of plasma protein is less than the estimates given above.

Hardwicke: I do not believe that we have seen such a case; more often, of course, you do get an actual nephrotic syndrome in multiple myeloma, with a significant loss of albumin. To return to the normal kidney: the albumin clearances reported by Lambert in dogs and the sort of albumin clearance which you get in nephrotic syndrome in man (the nil-change cases) are about 1–2 per cent of the creatinine clearance. At 2 per cent of the creatinine clearance you would be filtering or clearing 3·6 litres of plasma a day, which is equivalent to 144 g. of albumin alone, with a normal serum albumin. From this sort of physiological study in dogs you are faced with a normal filtrate load of albumin of at least 70 to 80 g. per day. There is also evidence in rats that their tubules can reabsorb a half to a third of their total circulating albumin per day. I think the figures of 70–80 g./day are very open to question, but I feel that one can think big in terms of tubular reabsorption of protein. So that must imply—the morphologists may complain bitterly about this—that the albumin goes straight back through the tubular cells and into the capillaries unchanged, since the daily albumin turnover is only about 15 g.

Joekes: There has been no mention of the thickness of the basement membrane. If one regards the basement membrane as a gel, then taking up water might alter its permeability characteristics and thus explain the increased protein escape. I wonder if Dr. Vernier or Dr. Bergstrand would comment on this.

Vernier: In patients with the idiopathic nephrotic syndrome where the proteinuria is very severe, the basement membrane is usually entirely normal in width. On the other hand, in other conditions associated with marked thickening of the basement membrane (in particular, lupus nephritis and the renal disease of diabetes) the thickening may be

associated with minimal proteinuria. We have not noted a relationship between the extent of the proteinuria and the width of the membrane.

Pirani: However, almost invariably when there is thickening of the basement membrane, there is proteinuria, independently of the degree.

Vernier: I think that you can say, almost as reliably, that whenever there is renal disease there is proteinuria. I don't think that there is any relationship that I can recognize between thickening *per se* and proteinuria.

Wilson: Some of the most severe lesions in the kidney, showing thickening of the basement membrane and deposition of hyaline material, are seen in patients who have never had oedema.

Rich: Of course, there can be massive proteinuria in the early stages of "idiopathic" nephrosis, with practically no thickening of the basement membrane.

Vernier: The big difficulty here is illustrated by Dr. Hardwicke's work, and that is that the patients whose glomeruli, by both light and electron microscopy, show minimal changes in the basement membrane have massive proteinuria, and the proteins in the urine are of low molecular weight. So apparently the capillaries in these patients have neither increased pore size nor membrane thickening. Isn't that more or less what you said?

Hardwicke: That is the suggestion from the functional studies. If one is working on Pappenheimer's diffusion theory, the longer you have the blood in the capillary the more chance there is for complete equilibration of partially filtered colloids across the filter. Thus the longer the blood is in the capillary the more protein is going to diffuse out into Bowman's space. It is striking that in these patients, particularly in children, the capillaries are nearly always grossly dilated and blood-filled. This is unusual in biopsies of other types of renal disease. I wonder if some change in haemodynamics is occurring, giving a grossly increased filtering load without much change in the membrane. This is all I can suggest to fit in with the histological appearances.

Vernier: The other alternative is that these patients have a more or less severe tubular abnormality which prevents the reabsorption of the normally filtered constituents of plasma. But then why do they show such pronounced glomerular change at the epithelial cell level?

Raaschou: In some cases of severe pyelonephritis you do not see proteinuria, or only very slight proteinuria. Does all the filtered protein go back in the damaged tubule? In these cases the biopsies show normal glomeruli but severe tubular atrophy.

Vernier: I can only assume that normal glomeruli filter very little protein and that in these patients significant reabsorption is occurring, even though they have severe interstitial and tubular disease.

Darmady: Surely there is another answer: that either the functional nephrons remaining are normal, or that the dilated tubules seen in pyelonephritis, which appear to be very hypertrophied on microdissection, are able to reabsorb the protein more completely.

Pirani: What is the electrophoretic pattern of the 80 mg. of protein normally present in the urine? Is this different from serum protein?

Hardwicke: On paper electrophoresis it looks almost exactly like serum. When you do immunological studies on it, the clearances of γ-globulin—though not of the high-molecular-weight α_2-glycoprotein—are practically non-selective, that is 100 per cent of the albumin clearance. But the evidence from chemical studies is that the urinary γ-globulin is in some way altered from the circulating γ-globulin. On the other hand, this urinary γ-globulin could represent a very small proportion of the total γ-globulin in the serum (Rowe and Soothill (1961). *Clin. Sci.*, **21**, 75; 87).

Slater: We are concerned by the fact that, although there may be some resemblance between the appearance of the paper electrophoretic patterns of serum and normal urinary proteins, we really do not know how much the individual electrophoretic peaks in the patterns can be compared. Some of the urinary proteins may arise from the walls of the collecting system or from the tubule as suggested by G. H. Grant (1957. *J. clin. Path.*, **10**, 360), and thus not represent material transferred out of the plasma.

Hardwicke: I entirely agree with you about the paper electrophoresis and that is why we dropped it. I only cited the results of electrophoresis at the beginning of my paper as an illustration; we draw no conclusions from paper electrophoresis in terms of individual protein fractions. From the immunological study we do get something which is valid, except in the case of cross-reacting proteins. In tubular proteinuria we have looked into how much of the protein is derived

from the urinary tract. Dr. Grant has prepared an antiserum to whole normal urine protein and then adsorbed that antiserum with normal serum, so that any antibodies that were left were to urinary tract protein. We have checked with his antiserum and found that urinary tract protein is not present in pathological urine protein to any significant degree.

Rich: Have you studied febrile proteinuria, a condition in which "cloudy swelling" of the tubular epithelium occurs and cytoplasmic protein appears to be shed off from the cells into the lumen?

Hardwicke: I don't really know anything about that. I have seen increases in proteinuria in association with fever in subacute nephritis with proteinuria of the order of 4 to 5 g. per day, and on those occasions it has been associated with quite incredible rises in creatinine clearance. We have not done inulin clearances on those cases. But in a child I have seen the creatinine clearance rise from 60 ml./min. to 350 ml./min. in association with a simple streptococcal throat. That of course would increase the filtered load enormously.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

ELECTRON MICROSCOPY OF RENAL GLOMERULAR AMYLOIDOSIS

A. BERGSTRAND

Institutionen för Allmän Patologi, Karolinska Mediko-Kirurgiska Institutet, Stockholm

and

H. BUCHT

St. Erik's Hospital, Stockholm

ELECTRON microscope investigations on renal amyloidosis in man have been published by several authors (Geer *et al.*, 1958; Spiro, 1959; Farquhar, Hopper and Moon, 1959; Meriel *et al.*, 1961; and Movat, 1960). The number of patients examined is very small, however. Similar investigations on experimentally produced renal amyloidosis have also been published by Miller and Bohle (1956), Cohen and Calkins (1959) and Letterer, Caesar and Vogt (1960). These have revealed many important facts about the structure and nature of amyloid. However, there is still disagreement on many essential points such as the exact localization of renal amyloid and its relations to the glomerular capillary basement membrane.

In this paper we will report the clinical and morphological observations made on six patients with a clinical diagnosis of renal amyloidosis verified by renal biopsy. A detailed description of this material has recently been published elsewhere (Bergstrand and Bucht, 1961) and we shall therefore restrict this presentation of our observations to the essential facts. We shall also discuss the importance of renal biopsy for the diagnosis and the conclusion which may be drawn from our observations about the correlations between changes of renal function and morphological lesions.

Material and methods

Inulin clearance was used as a measure of the glomerular filtration rate in all cases except nos. 62 and 143, because they were under the care of other specialists.

Renal vein catheterization was performed in cases 26, 35 and 73 to determine the extraction rate of PAH (*para*-aminohippuric acid).

The pitressin-thirst and water dilution tests were used as measures of the function of the distal parts of the nephrons. Determination of the urinary pH the morning after a load with ammonium chloride (0·1 g./kg. body weight for three days) was also used as a test of tubular function.

The biopsy material was fixed in buffered osmium tetroxide, dehydrated in graded alcohols and embedded in a mixture of methylbutylmethacrylate. No staining was used for electron microscopy. Half of each specimen was fixed in 10 per cent formalin embedded in paraffin and stained with routine methods for light microscopy. The presence of amyloid was determined by the metachromatic staining properties with crystal violet.

Observations

The pertinent clinical data are given in Table I. Five cases are considered as secondary and one as primary amyloidosis. There was no correlation between the severity of the primary disease and renal impairment. The Congo red retention test showed only slightly pathological values, probably because there were no signs of generalized amyloidosis. Thus no extrarenal amyloid was found in liver biopsies (no. 26, 35, 153) or at autopsy (no. 73). None of the patients showed a nephrotic syndrome at the time of the investigation. Patient no. 26, however, died a few months after the last examination from a severe nephrotic syndrome with uraemia. It could not be ascertained if this was due to a renal vein thrombosis or not since autopsy was not permitted. One patient

Table I

CLINICAL DATA IN SIX PATIENTS WITH RENAL AMYLOIDOSIS

Case no.	*Age and sex*	*Primary disease*	*Blood pressure*	*NPN*	*Congo red retention per cent*	*CO_2 combined m-mole/l.*	*Plasma protein g./100 ml.*	*Albumin / Globulin quotient*	*Proteinuria g./24 hours*
26	57 male	none	170/85*	53	50	31	1956 5·2 1957 6·1	1956 1·2 1957 1·2	3·4–5·7
35	34 male	Tuberculosis	105/75	21	43	28	4·1	0·45	5
62	38 male	Tuberculosis	115/80	27	51	—	6·4	—	1·2–5
73	65 male	Tuberculosis	120/70	35	55	28	7·0	0·61	4·7
143	36 male	Tuberculosis	130/90	37	—	26	7·3	1·1	traces
153	34 male	Rheumatoid arthritis	125/80	34	60	30	4·7	0·4	12

* Treated with Hydralazin (1-hydrazinophthalazine).

(153) had a polycythaemia, a finding which has previously been described in cases of amyloidosis. Considerable amounts of

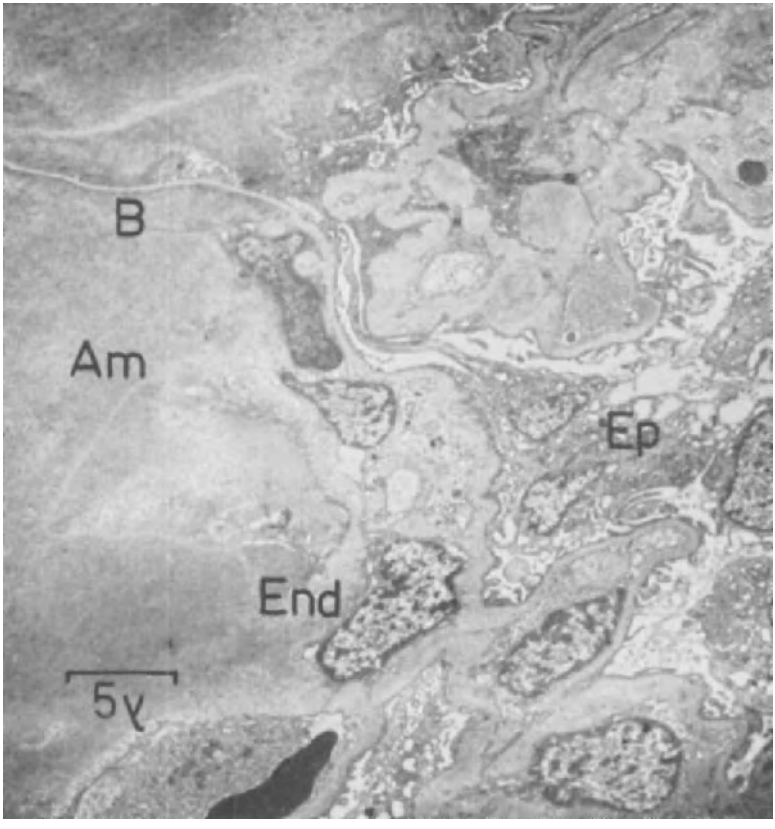


FIG. 1. Survey picture of glomerular capillaries with large amyloid mass (Am) at left. Endothelial cell (End) and epithelial cell (Ep). Basement membrane (B). The scale in this and following figures is in microns (× 2,400).

amyloid were found in the spleen and bone marrow. It is therefore possible that the polycythaemia was due to deposits of amyloid in the entire reticulo-endothelial system with a prolonged

life of the red cells. An unexplained observation is the increased CO_2-combining capacity of the plasma in spite of impaired renal function. The changes in clearance values were quite similar in

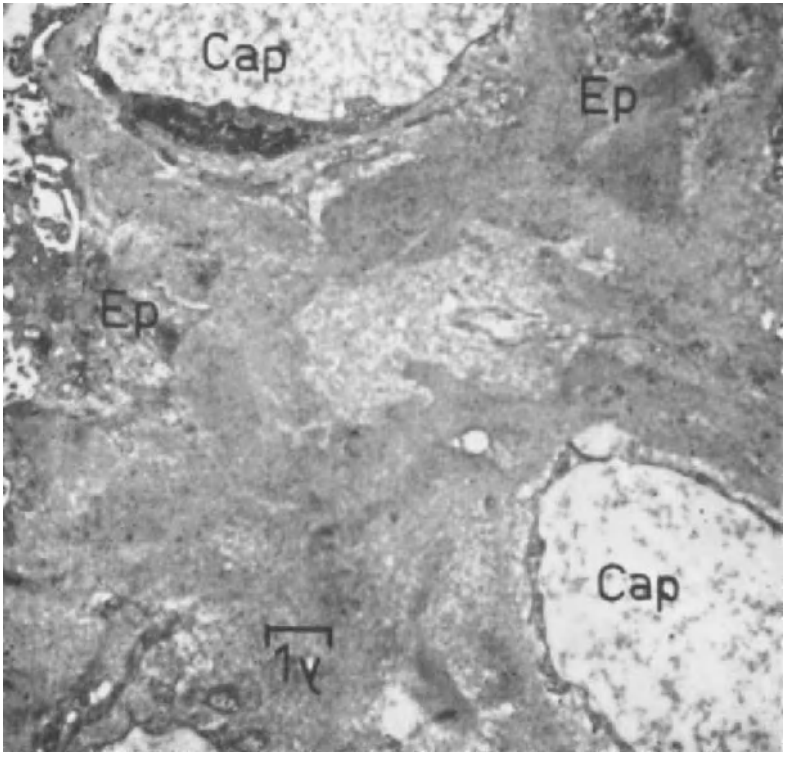


FIG. 2. Two capillaries (Cap) with amyloid between them. Remnants of epithelial cells (Ep). The endothelial cells are well preserved (× 7,200).

all cases, so only the one most thoroughly investigated is presented in Table II.

Light microscopy showed considerable amounts of amyloid in all glomeruli of the biopsy specimens but very small deposits in the walls of the vessels or the interstitial connective tissue. A

Table II

RENAL FUNCTION TESTS—CASE 26

Date	*Inulin clearance ml./min.*	*PAH clearance ml./min.*	*Filtration fraction %*	*PAH Extr. %*	*Urine dilution mosm./l.*	*Urine conc. mosm./l.*	*Urine pH*
31/1/57	72	446	16·1				
15/3/57	75	358	21·0	74	193	606	
		488	18·3				
10/6/57	56	293	19·4				
13/9/57	76	409	18·8		383	595	4·90
10/12/58	29	311	9·5	79·4			
		393	7·3				
16/10/59	8	29	28·0				

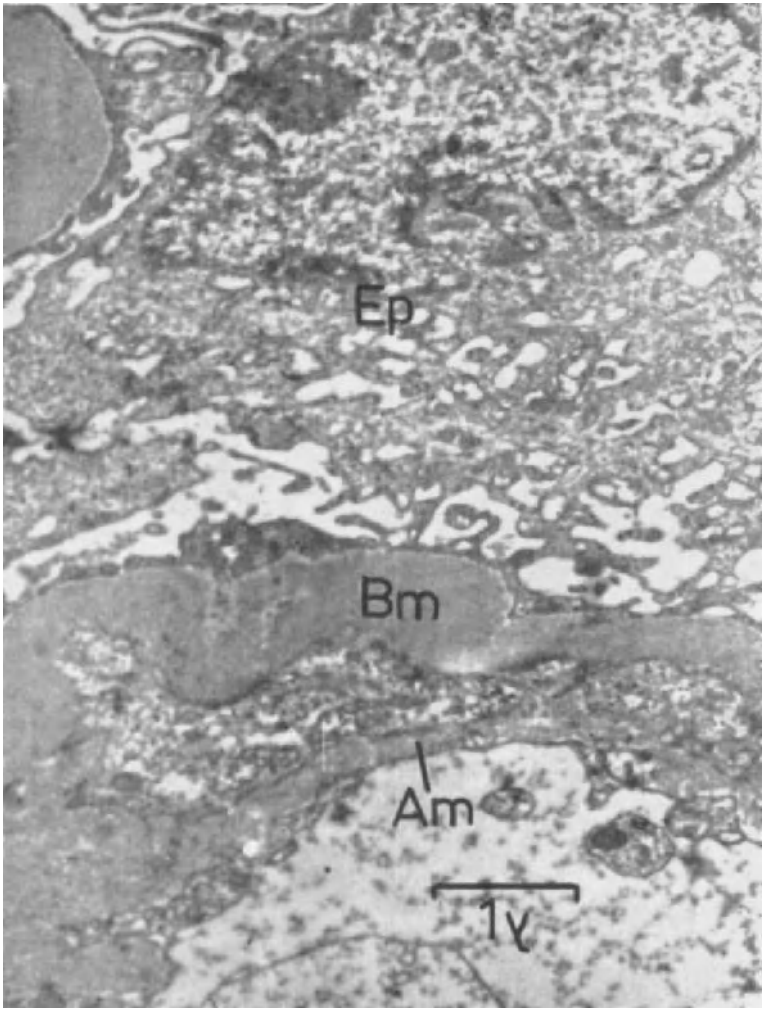


FIG. 3. Glomerular capillary wall with amyloid deposit (Am) inside the endothelial cell cytoplasm. Basement membrane (Bm) tortuous and thickened. Epithelial cell (Ep) with a large number of vacuoles in the cytoplasm (× 17,775).

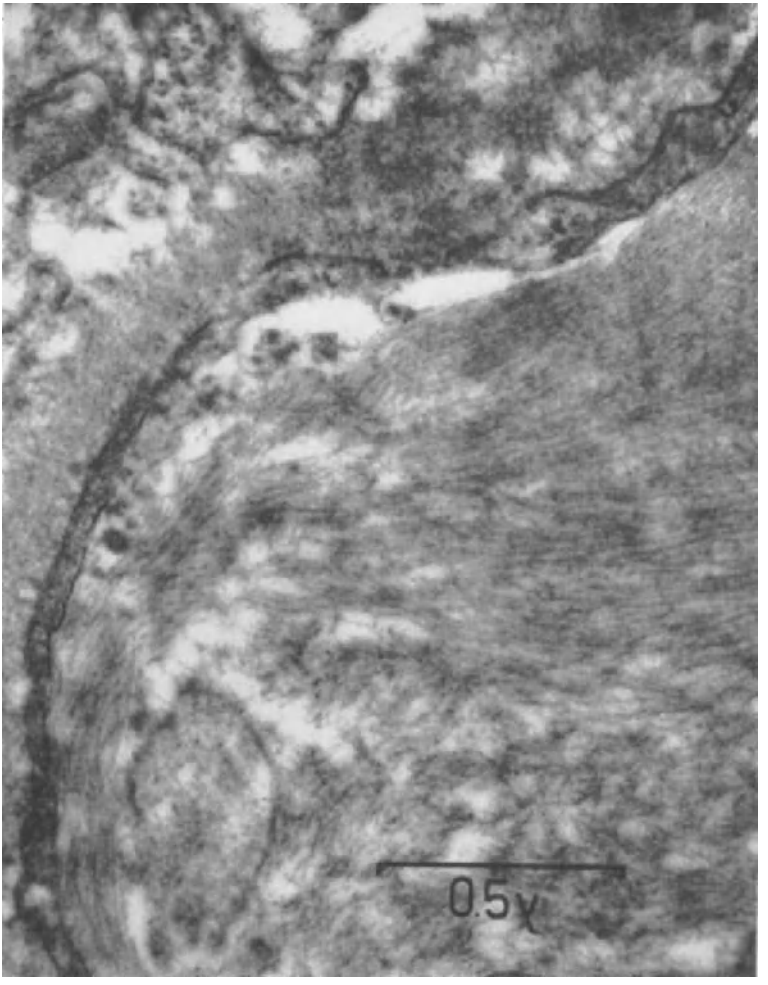


FIG. 4. Partly parallel, partly irregular fibrils in the amyloid (× 66,665).

positive birefringence was observed with the polarization microscope.

The morphological changes as observed in the electron microscope are depicted in the figures. In Fig. 1 a large amyloid mass is seen at left. The lighter tortuous band is the basement membrane. The three nuclei derive from endothelial cells. Evidently the amyloid is located on the *inside* of the basement membrane with

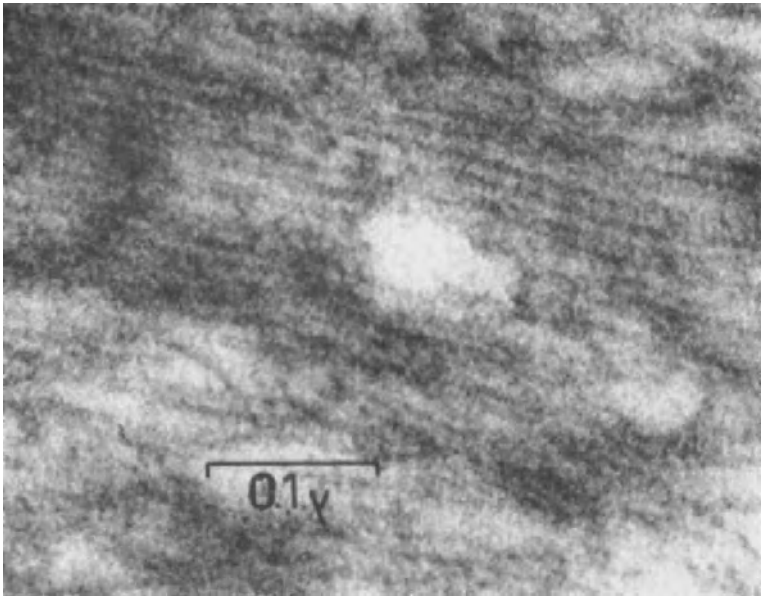


FIG. 5. High magnification of the amyloid with fibrils. The diameter is estimated at 100 Å (× 220,000).

severe destruction of the endothelial cells. In the right half of the picture are several other capillaries with minor changes. The basement membranes are thickened and tortuous, but endothelial and epithelial cells are still visible. The foot processes of the epithelial cells are fused in some areas, preserved in others. In the left upper part of the pictures it may also be seen that amyloid

is present *outside* the basement membrane with destruction of the epithelial cells.

This is shown in more detail in Fig. 2, where a large mass of amyloid is found between two capillaries, with nearly complete destruction of the epithelial cells. The endothelial cells are well preserved in this case. Fig. 3 is a higher magnification of a capillary wall. The amyloid seems to be located inside the endothelial cell cytoplasm. In more advanced cases the entire lumen may be filled by amyloid. Figs. 4 and 5 are high magnifications of the amyloid. A network of fine fibrils is observed, in some areas in irregular, in others in a parallel arrangement. The diameter of the fibrils is about 100 Å.

Discussion

Our observations indicate that renal biopsy is the sole reliable method for obtaining the diagnosis of renal amyloidosis, above all in cases with slight proteinuria and nearly normal renal function.

Amyloid is deposited on the endothelial *or* epithelial side of the basement membrane proper, perhaps simultaneously on both sides. It is probably localized outside the endothelial or epithelial cell membranes in early lesions. In more advanced cases where severe cell destruction takes place it may also be precipitated in the cell cytoplasm.

The relations to the basement membrane proper are more difficult to decide. In early lesions the basement membrane proper shows a more dense and osmiophilic appearance than amyloid. This is even more apparent in sections stained with silver methenamine, as has been shown by Movat (1960). This author concludes that amyloid is not deposited in the basement membrane proper. We do not entirely agree with this conclusion. In those sections where amyloid is deposited and the basement membrane is retained, the latter is often markedly thickened and tortuous, which indicates changes also in the basement mem-

brane proper. Furthermore, in the unstained sections there is a gradual disintegration of the basement membrane proper into the amyloid masses. According to Movat the remnants of the basement membranes stand out against the background of amyloid through their affinity to silver methenamine. We doubt that this is always the case. The observations on unstained sections indicate that the basement membrane is heavily involved in the precipitation of amyloid. It is generally agreed that amyloidosis is a disorder of protein metabolism on an immunological basis with the formation of abnormal protein compounds (Letterer, Caesar and Vogt, 1960; and others). Histochemical and biochemical analyses of amyloid also indicate that it is closely related to the ground substance of connective tissue and probably also to the substance of the different basement membranes of epithelial and endothelial cells in the body.

Our conclusion is, therefore, that the precipitation of amyloid in the renal glomeruli is a process closely related to the capillary basement membrane. It is probably associated with a metabolic change and a change of structure of the basement membrane proper, which slowly disintegrates as the amyloid masses are precipitated in the capillary wall, dislodging and "engulfing" both endothelial and epithelial cells.

Our observations give no information, however, on the much disputed question whether amyloid is formed *in situ* or transported to the renal capillary walls by the blood stream.

The fibrillar structure of amyloid was first described by Miller and Bohle (1956) in experimental amyloidosis and later by others who have studied human amyloidosis. We want to emphasize, however, that we have not been able to demonstrate the presence of fibrils everywhere in the amyloid masses. Our impression is that amyloid does not show any internal structure in the early stages. The formation of fibrils seems to be a "maturing" process as it is in collagen. Letterer, Caesar and Vogt (1960) have measured the diameter and length of the fibrils as 100 and 3600 Å,

respectively. We do not think that it is possible to measure the dimensions of the fibrils with such accuracy. This applies especially to the length of the fibrils since they are tortuous and seldom appear in the whole of their length in one ultra-thin section. The difficulties are clearly demonstrated by the figures of Letterer, Caesar and Vogt, which range from 9650 to 460 Å. Our *estimation* of the diameter, however, is in close agreement with the figures of Letterer, Caesar and Vogt, i.e. 100 Å.

No cross-striation was observed in the fibrils. It may therefore be concluded that the fibrils are neither fibrin nor collagen—the presence of which could also be shown by routine staining methods. There remains the question whether the fibrils observed in amyloid are pre-collagen, a sign of transformation of amyloid into collagen. Our observations on renal amyloidosis both in this material and in autopsy cases indicate, however, that collagen is formed from Bowman's capsule, slowly growing into the amyloid and replacing it. We have found no indication that amyloid is transformed into collagen. Chemical analyses of amyloid show no content of hydroxyproline.

We regard the formation of fibrils as a "maturing" process in amyloid, a possibly reversible change of phase, similar to the formation of collagen fibrils from the connective-tissue ground substance. Obviously the presence of fibrils is responsible for the weakly positive birefringence of amyloid as first described by Missmahl and Hartwig (1953).

Our conclusions about the correlation between changes of renal structure and function must be drawn with extreme care. There are so many factors which may influence the renal filtration rate and blood flow that changes in either or both cannot be correlated with certainty to specific glomerular lesions. The filtration fraction (FF), however, is in our opinion a true measure of the resistance to filtration through the glomerular capillary wall, provided that the extraction of PAH is measured through renal vein catheterization and the renal blood flow thus determined with accur-

acy. This was done in three of our cases which all showed a moderate or marked decrease in FF.

The cause of this increased resistance to filtration is most probably the deposition of a foreign material close to or in the basement membrane proper, which is increased in diameter. The length of the pathway for the fluid through the capillary wall is much increased. The basement membrane proper is the site of changes which probably also include a change of structure. This does not necessarily mean a change of *visible* structure (there is none so far demonstrated in the normal basement membrane) but a change of molecular arrangement, for instance a swelling and shortening or a narrowing and stretching of molecular chains which may give rise to changes in permeability (proteinuria) concomitant with increased resistance to filtration.

Analysis of tubular function shows that this is remarkably well preserved in spite of severe decrease in glomerular filtration. This is what might be expected, considering the distribution of amyloid observed in the light microscope. Even the distal parts of the nephron seem to retain their function almost unimpaired, as shown in Table II. The ability to acidify the urine after a load of ammonium chloride was within normal limits. During the later stages of the disease there was a decrease in ability to dilute the urine but the concentration power was unimpaired throughout the observation time. This observation is of special interest since del Greco and de Wardener (1956) and Berliner and Davidson (1957) in experiments on dogs produced a hypertonic urine during water load through a restriction of glomerular filtration rate.

Thus there seems to be a selective impairment of glomerular function in renal amyloidosis corresponding to the severe morphological changes. Our observations on the anatomical changes of the renal tubules are as yet very few but they seem to confirm the opinion, based on clinical observations, that there is relatively little tubular damage.

Summary

Renal biopsy material from the kidneys of six patients with clinically moderate to severe amyloidosis has been examined with the light microscope and the electron microscope. The clinical examination showed a slight retention of Congo red, proteinuria, decreased inulin and PAH clearance values, and a decreased filtration fraction in those cases where the PAH extraction was measured through renal vein catheterization.

Severe amyloidosis was observed in the glomeruli but there were only slight deposits in the vessels or the interstitial connective tissue. Electron microscopy showed severe destruction of both epithelial and endothelial cells in the capillary walls with deposits of amyloid in close correlation to the basement membrane proper. The basement membrane was increased in thickness and tortuous. It is concluded that amyloid is primarily laid down in the basement membrane with secondary changes in the adjacent cells.

A weak optical birefringence was observed with the polarization microscope. This is due to the fibrillar structure of amyloid. The thickness of the fibrils is estimated at 100 Å. The fibrils have probably no connexion with collagen or fibrin.

The increased resistance to the passage of fluid through the capillary wall, indicated by the low filtration fraction, is explained by the changes in the basement membrane and the deposition of amyloid in the capillary wall.

REFERENCES

BERGSTRAND, A., and BUCHT, H. (1961). *J. Path. Bact.*, **81**, 495.
BERLINER, R. W., and DAVIDSON, D. G. (1957). *J. clin. Invest.*, **36**, 1416.
COHEN, A. S., and CALKINS, E. (1959). *Nature (Lond.)*, **183**, 1202.
FARQUHAR, M. G., HOPPER, J., JR., and MOON, H. D. (1959). *Amer. J. Path.*, **35**, 721.
GEER, J. C., STRONG, J. P., MCGILL, H. C., and MUSLOW, I. (1958). *Lab. Invest.*, **7**, 554.
DEL GRECO, F., and DE WARDENER, H. E. (1956). *J. Physiol. (Lond.)*, **131**, 307.

LETTERER, E., CAESAR, R., and VOGT, A. (1960). *Dtsch. med. Wschr.*, **85**, 1909.
MERIEL, P., MOREAU, G., SUC, J. M., and PUTOIS, J. (1962). In press.
MILLER, F., and BOHLE, A. (1956). *Klin. Wschr.*, **34**, 1204.
MISSMAHL, H. P., and HARTWIG, M. (1953). *Virchows Arch. path. Anat.*, **324**, 489.
MOVAT, H. Z. (1960). *Arch. Path. (Chicago)*, **69**, 1960.
SPIRO, D. (1959). *Amer. J. Path.*, **35**, 47.

DISCUSSION

Milne: I would like to defend the Congo red test, provided it is put in its proper perspective. I agree that it is valueless as a diagnostic test for renal amyloidosis. I think that it measures roughly the total mass of amyloid in the body. In our experience of about 30 Congo red tests in amyloid it has been positive only when there was hepatic amyloid in considerable quantity, except in one case where there was a grossly enlarged spleen, proved at necropsy to be almost full of amyloid material. So that the Congo red test adds to the information given by percutaneous renal biopsy, which I agree is the only certain diagnostic measure of renal amyloidosis.

Bucht: I agree with you that the Congo red test is useful in generalized amyloidosis, and especially if there is amyloid in the liver. However, the cases we reported are almost entirely renal, and in these cases the Congo red test is not diagnostic.

Milne: I should like to bring up again the very important clinical question which was often discussed in the literature before renal biopsy was used: whether in secondary amyloid there ever could be complete clearing. I have seen cases where the primary disease has been effectively treated, where all clinical manifestations of amyloidosis have disappeared as shown by the criteria used by the pre-biopsy investigators (i.e. there has been complete disappearance of proteinuria, renal function tests have become normal and the liver has become impalpable) and yet biopsy both of the liver and kidney has revealed considerable amyloid infiltration. Is there any proof that secondary amyloid can completely disappear and regress, as claimed in the pre-biopsy days, when the primary disease is completely removed, as by amputation of an osteomyelitic limb?

Bucht: About 20 years ago Henning Waldenström described cases with a nephrotic syndrome which he ascribed to renal amyloidosis

and which disappeared completely after the operation of a "rotten leg". We have about 15 patients with renal amyloidosis and in none of those cases has the proteinuria disappeared. I think it is easier to remove amyloid from the liver than from the kidney. There is much evidence from liver biopsy that amyloidosis in the liver can disappear completely. But as far as I know this has never been shown in cases with renal amyloidosis.

Pirani: There is considerable evidence in the experimental animal that amyloidosis can disappear.

Joekes: Has that been shown in the kidneys?

Pirani: I am not sure. Certainly it has been shown for liver and spleen. This was amyloidosis induced by ribonucleic acid treatment in rabbits (Richter, G. W. (1954). *Amer. J. Path.*, **30**, 239).

Dr. Bergstrand, you implied that there were certain similarities between the "hyaline" material of diabetic glomerulosclerosis and amyloid in terms of fibrillar appearance. However, from your pictures it seems to me that the site of deposition in amyloidosis is somewhat different from that in diabetes; amyloid appears not only on the inside of the capillary but in advanced cases also on the outside, whereas in diabetes the hyaline appears only on the inside, with nodules closely related to the basement membrane and to the interluminal space, but no deposition is noted around the epithelial cells.

Bergstrand: I quite agree on that. I said that there was some morphological similarity. However, from the histochemical point of view they are two entirely different substances, and also the localization is different.

Movat: I would like to ask Dr. Bergstrand about the severe lesions that he showed, where there was apparent destruction of the basement membrane. We (Movat, H. Z. (1960). *Arch. Path. (Chicago)*, **69**, 323) have examined only three cases, but whenever we impregnated, particularly with silver methenamine (Movat, H. Z. (1961). *Amer. J. clin. Path.*, **35**, in press), we found the basement membrane to be sometimes irregular in thickness or wavy, but always intact. A. S. Cohen and E. Calkins reported recently (1960. *J. exp. Med.*, **112**, 479) that they found the basement membrane intact in experimental amyloid; in other words, not disrupted. The cases you showed were obviously unstained. Did you do any staining to see whether any basement

membrane can be demonstrated in places where it seemed to be missing?

Bergstrand: I did not want to stain, because then I would not be able to determine the degree of severity of amyloid damage. I had the impression that the basement membrane is retained in the early stages of the disease, and that in more severe changes, with disintegration of the cellular elements on both sides, the basement membrane disappears also.

Movat: I have the experience that the basement membrane is present whenever you stain, even in severe cases.

Wilson: What is the relationship of this severe change in the basement membrane to the appearance of the proliferative changes of nephritis? You didn't talk about the complication of amyloid with glomerulitis which one sees in some cases where renal failure has developed rapidly.

Bergstrand: This is biopsy material from renal patients whose clinical symptoms were not very severe, and we have not seen any proliferative changes that you could designate as proliferative glomerulonephritis. Two of them have died. On one of them we were able to perform an autopsy and we could not see any proliferative changes in the glomeruli.

Wilson: It would be interesting to have information from the electron microscope on the relation between the basement membrane changes and the development of glomerulonephritis—whether they are two distinct processes or different stages of the same kind of process.

de Wardener: I am surprised that the plasma flow is not more reduced in your patients. You explain that the glomerular filtration rate may be higher than you would expect because the basement membrane is increased in size and perhaps the pores are greater. But can you explain how the blood gets through the almost avascular glomeruli?

Bucht: There are vascular changes too; the vessels may be rather rigid. However, in amyloidosis it is difficult to judge the function from the microscopic findings because vessels which look completely destroyed may still permit the passage of fluid through the membranes and blood through the vessels.

Joekes: In a case of amyloidosis we have seen grossly involved glomeruli in three biopsies from one kidney (we haven't done the other

kidney yet) and yet there is a creatinine clearance of 90 ml./min. It is very difficult to understand how one gets these high clearances in kidneys that don't appear to have any glomerular capillaries.

Black: Glomerular by-passes would not contribute to *p*-amino-hippurate clearance, unless of course the blood going through them should perfuse tubules later on.

Pirani: In lupus, which is a disease focal within the kidney and within the glomerular tufts, creatinine clearances are maintained quite well even in advanced cases. It seems to me that there is a difference between the real diffuse glomerular diseases and the ones which are more focal, such as amyloid, where some capillary loops still remain patent in each glomerulus.

Hamburger: Wouldn't you think that the total experience of renal biopsy gives an impression of slight weakness of confidence in classic renal function tests?

Earle: Of course it could be the other way round!

Hutt: Dr. Bergstrand, in these cases, were there any abnormalities in the epithelial cells other than those caused by deposition of amyloid within the cells? Did you see frequently a loss of foot processes as has been described in the nephrotic syndrome due to other causes?

Bergstrand: Yes. Most of our cases (in the earlier stages also) had rather severe changes in the epithelial cells, where one couldn't observe any foot processes.

Bucht: The expression "amyloid nephrosis" has no bearing on these cases. Only one case had a real nephrotic syndrome—a patient with rheumatoid arthritis. He started with nephrotic syndrome and then lost his oedema, and then when he died he had nephrotic syndrome again. One other case died in a severe nephrotic syndrome but we couldn't get an autopsy; he might have had a renal vein thrombosis.

Hutt: I was wondering whether there was any relatoinship between the degree of proteinuria and the finding that the foot processes have disappeared. Many of your cases did not have very great proteinuria and yet you say they lost their foot processes.

Bergstrand: That is a question we cannot answer. The degree of proteinuria can be measured with mathematical exactness, but the disappearance of foot processes is a very subjective observation.

Milne: I think it is unusual for renal vein thrombosis complicating

amyloidosis either to cause or exacerbate a nephrotic syndrome. The lesion usually consists of multiple thromboses in small venules, and this causes an acute oliguric renal failure, presumably because it is very difficult to develop a collateral circulation when so many small venules are occluded. I think that nephrotic syndrome from renal vein thrombosis in renal amyloidosis is a great rarity.

Rich: In experimental amyloidosis, crystals of amyloid may appear. Dr. Bergstrand, do you think these fibrils of yours could be protocrystals, or composite groups of these crystals?

Bergstrand: We see a weak optical birefringence in amyloid, especially when it is stained with Congo red, and we correlate this with a filamentous structure, but we have never seen crystals.

de Wardener: Have you any observations on what happens in the medulla?

Bergstrand: Very few. We see an accumulation of a foreign material on the outside of the basement membrane of the renal tubules, from the borderline between the tubular basement membrane, and collagenous connective tissue in the stroma of the kidney.

de Wardener: Any particular part? Vasa recta, or the loops, or the collecting tubules?

Bergstrand: I can't say. That would require an extensive examination using serial sections.

Rich: But you don't see amyloid between the basement membrane and the epithelial cell of the tubule? It differs in that way from the glomerulus?

Bergstrand: Yes.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

CLINICAL, MICROSCOPIC AND ELECTRON MICROSCOPIC DATA IN THE NEPHROTIC SYNDROME OF UNKNOWN ORIGIN

R. HABIB, P. MICHIELSEN, E. DE MONTERA, N. HINGLAIS, P. GALLE and J. HAMBURGER

Hôpital Necker, Paris

IT is partly due to renal biopsy that the concept of "lipoid nephrosis", an autonomous disorder, has been replaced by the concept of nephrotic syndrome caused by renal changes of various origins and nature. Thus amyloidosis, lupus and diabetic glomerulosclerosis are today listed among the causes of nephrotic syndrome. For the majority of cases, however, no such aetiology has been established, and they are often designated as "primary nephrotic syndrome". In this study we shall refer to them as "nephrotic syndrome of unknown origin". The histological lesions revealed by renal biopsy in these cases are far from being unequivocal (Allen, 1955; Joekes, Heptinstall and Porter, 1958; Blainey *et al.*, 1960). Various morphological types, not precisely defined, have been described during the last few years, all differentiated essentially by the appearance of the glomeruli. Do these different pictures represent different pathological states? Are several autonomous illnesses hidden within the primary nephrotic syndrome group? Do anatomical-clinical correlations support this attempt at differentiation? These are the questions which we shall try to answer.

This study is based on the analysis of 108 cases of nephrotic syndrome of unknown origin, belonging to a series of 127 cases

of nephrotic syndrome (the other 19 cases were: 9 with secondary or primary amyloidosis, 2 with renal vein thrombosis and 8 with lupus). Cases of unknown origin in this series represented, therefore, 85 per cent of all observations.

All cases included in this study were subjected to one or several renal biopsies carried out with a Ducrot-Montera needle, following a short lateral incision to the kidney (Hamburger, Crosnier and Montera, 1960). The 108 cases were examined under the optical microscope. This study is particularly concerned with the glomerular changes and only these will be described in detail in this paper.

This description will concern successively: the elementary lesions which can alter each of the constituent elements of the glomerulus; the main morphological types which these lesions can produce, together with a discussion of the clinical correlations of each type.

I. ELEMENTARY LESIONS

A. Epithelial cells

(1) Foot processes

Since the publications of Farquhar, Vernier and Good (Vernier *et al.*, 1958; Folli *et al.*, 1958), the fusion of the epithelial foot processes has been a well known phenomenon in all types of nephrotic syndrome. It has been a constant finding in all the cases which we have examined under the electron microscope. When it is isolated, it is not detectable under the optical microscope. At the most one could attribute to it capillary loop dilatation; such dilatation can be explained by the disappearance of the foot processes which support the capillary loops.

The fusion of the foot processes is very often diffuse and homogeneous. However, such a lesion (particularly in the "lobular glomerulitis" group) is sometimes focal, and an epithelial cell can exhibit normal foot processes side by side with pathological ones.

Fusion of the foot processes is reversible. A biopsy carried out on one of our patients at the time of a remission due to treatment with corticosteroids enabled us to study the reconstitution of the foot processes. Numerous cytoplasmic extensions can be seen to appear, extending towards the fused foot processes, which begin at the same time to break up.

Fusion of the foot processes is certainly not specific to the nephrotic syndrome. We have observed it, highly localized, it must be admitted, in some biopsies taken from patients with only minimal proteinuria: for instance in a homotransplanted kidney, two months after surgery, when proteinuria was no higher than 0·1 mg. per min.

(2) Other epithelial changes

Besides fusion of the foot processes, we have consistently found a series of other epithelial cytoplasmic changes: a large number of vacuoles with homogeneous content, dilatation of the cytoplasmic vacuoles, formation of microvilli on the surface. Sometimes there is a cytoplasmic atrophy, which gives the illusion of an increase in the number of nuclei and of cellular proliferation. In other cases, there is true proliferation of the glomerular or capsular epithelium, focal or general, eventually exhibiting epithelial crescents.

B. Basement membrane

The normal basement membrane appears to be made up of three distinct zones. The central zone, or lamina densa, is visible by virtue of its greater electron density and its characteristic structure. This central zone is flanked outside and inside, respectively, by the *lamina rara externa* and *lamina rara interna*; these are lighter, and separate the lamina densa from the epithelium externally and from the endothelium internally. These light zones may be only artifacts. The individuality of the lamina densa is much clearer,

so much so that many authors consider it to be the basement membrane. However, deposits which can be seen to accumulate inside or outside the lamina densa are much more difficult to interpret, and it is often difficult to decide whether they belong to the basement membrane itself or are only contiguous to it. This difficulty explains, as will be seen later, some of the confusion in certain earlier descriptions.

(1) Modifications of the lamina densa

We have never seen homogeneous regular thickening of the lamina densa. By using staining techniques which increase the contrast of the basement membrane in electron microscopy (uranyl nitrate) it is found that the lamina densa is practically always either of normal thickness or slightly thin. The irregularities or partial thickening which can be observed are often difficult to interpret and are sometimes due to the sectioning. It seems to us that the so-called regular thickening of the basement membrane described by some authors is an artifact due to confusion between the lamina densa and the contiguous deposits which will be described below.

The internal structure of the lamina densa is frequently altered, with some zones exhibiting a "moth-eaten" appearance. However, we have never found true interruptions such as Spiro (1959) has described.

(2) Basement membrane deposits

(*a*) *Subepithelial deposits* (see Figs. 3, 4 and 5)

On the epithelial side of the lamina densa deposits of high electron density can be observed. They are usually invaginated by irregular extensions of the neighbouring epithelial cells, from which they are separated by a highly visible cytoplasmic membrane. As these extensions are often of a density comparable to that of the deposits, they can be seen to form with them, with

low magnification, a picture of a false regular thickening of the basement membrane. However, certain special stains enable us to suspect from examination under the optical microscope the existence of this extra-membranous deposit. Thus, after silver impregnation according to Wilder's method, it is possible to make out on the external face of the basement membrane striations of the apparently thickened wall of the vessel which give it a rather characteristic appearance.

The extra-membranous deposits are not homogeneous. They are badly defined and often flecked with darker granular elements and separated by light "moth-eaten" zones. Deposits and light zones are often filled, as Farquhar (1959) has observed, with badly defined, vesicular or membranous-looking structures.

The origin of the subepithelial deposits cannot be stated with any certainty. It is possible that they may represent an abnormal passage of pathological material through a focal lesion in the lamina densa, or that they may, on the contrary, be the result of a primary lesion in the epithelium of the corresponding area. Electron microscopic study of one of our cases has shown that these deposits were localized exclusively in certain zones of a capillary loop while the rest of the capillary loop remained normal. The epithelial cells were normal opposite the intact areas, while they exhibited marked lesions opposite the deposits. This picture underlines the close relationship between the extra-membranous deposits and the epithelial lesions, without enabling us to decide which of the two abnormalities is the origin of the other.

(b) Subendothelial deposits

Between the lamina densa and the endothelium we have observed a type of deposit more variable and less well defined than the extra-membranous deposits. We can distinguish:

Fibrinoid deposits: They are characterized by their osmiophilia and their curdled appearance. This is the same picture as the

lupus wire loop. We have seen it in a number of cases of nephrotic syndrome of unknown origin where there was no reason to suspect lupus.

Hyaline deposits: These are observed in varying amounts whenever there is endocapillary cellular proliferation. They have an amorphous and homogeneous appearance and a density similar to that of the lamina densa; their basement membrane-like structure may give the impression of a partial thickening of the basement membrane.

C. Endocapillary cells

Without taking sides in the quarrel concerning the individuality of intercapillary cells as distinct from endothelial cells, we can agree with Latta (1960) in designating as an "endothelial cell" any cell directly on the rim of a capillary lumen and as an "intercapillary cell" any cell within the basement membrane which, in the plane of the slice, is not in contact with the capillary lumen. This latter type of cell has been defined in our preparations by the presence of fine fibrillar bundles in the cytoplasm, pericellular basement membrane-like hyaline deposits, and irregular cellular pseudopods penetrating these deposits.

(1) Endothelial cells

Various alterations can be observed: (a) cellular oedema with swelling and vacuolization of the cytoplasm, sometimes reminiscent of the picture seen in eclampsia; (b) reduplication of the cytoplasmic membrane, which ramifies towards the capillary lumen in a network of microvillous structures; (c) true endothelial degeneration (which occurs less often) with necrotic cellular débris inside the capillary lumina; (d) signs of endothelial cellular proliferation, with cytoplasmic turgidity, disappearance of the perforated layer, abundance of cytoplasmic organelles, large and often multi-lobular nucleus, frequent mitotic figures.

(2) Intercapillary cells

During most glomerulopathies, a degree of intercapillary cell proliferation seems very common. Some capillary loops are totally or partially filled with cells. Such a picture is irregular and focal, free loops being close to diseased ones. Sometimes proliferation is much more massive and reaches entire lobules in irregular fashion. This aspect will be described more fully later in connexion with lobular glomerulitis.

II. MAIN MORPHOLOGICAL TYPES

The elementary lesions which have just been described can be grouped in several ways, to form six main varieties of glomerular alterations. These varieties are given in Table I, together with the number of cases observed.

Table I

Main types of lesions	*Number of cases*
(1) Minimal changes	35
(2) Thickened capillary walls (or "membranous glomerulonephritis")	19
(3) Extracapillary proliferative glomerulitis (focal or diffuse)	9
(4) Endocapillary proliferative glomerulitis	5
(5) Endocapillary proliferative glomerulitis associated with hyaline nodules ("lobular glomerulitis")	15
(6) Complex or unclassifiable forms	
(a) Irregular glomerular hyalinosis	5
(b) Thickened capillary walls with endocapillary proliferation	7
(c) Advanced forms which cannot be interpreted	13

(1) Minimal changes of the capillary walls

In this group, the glomeruli appear almost normal under the optical microscope. At the most, the capillary walls are slightly more taut than usual, giving a picture of a glomerulus which is almost too handsome. Under the electron microscope, however, it is possible to show characteristic structural changes (Fig. 1):

basement membranes which have not thickened, but the inner structure of which may be slightly altered; fusion of the foot pro-

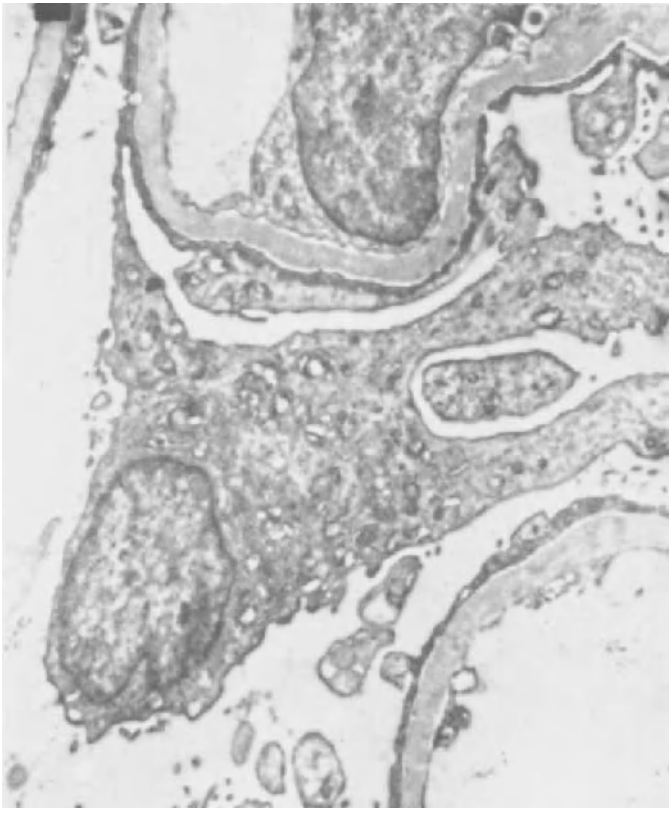


FIG. 1. Minimal changes. In optically normal glomeruli the electron microscope shows a flattening and a complete fusion of the foot processes (podocytes). The basement membrane is of normal thickness. (Electron microscope. × 11,200.)

cesses and signs of damage of the epithelial cells, as described above. In addition to these fundamental lesions, two of our cases showed minor endothelial cell changes (swelling and vacuoles) and

in six cases the intercapillary tissue was slightly more dense than usual.

This picture probably represents the most common form of primary nephrotic syndrome in the child. In our department,

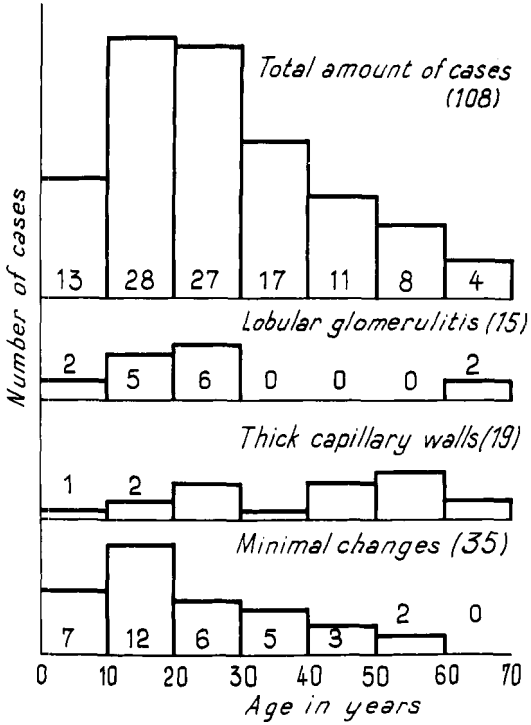


FIG. 2. Various morphological types grouped according to age, compared with 108 cases of unknown origin.

where very few children are admitted, the average age for this variety is still appreciably lower than that for the other cases (Fig. 2) although the range of ages is from six to 56 years old.

The clinical aspect of these cases is generally that of a pure nephrotic syndrome. There is abundant proteinuria—more than

6 mg. per minute at the time of the biopsy in 66 per cent of the cases. Microscopic haematuria, on the other hand, is minimal, zero, or less than 10,000 red blood corpuscles per minute in 80 per cent of the cases. Renal function tests (urea clearance, mannitol clearance, and *p*-aminohippuric acid clearance) were normal at the time of the biopsy in 91 per cent of the cases. Blood pressure was normal in all patients except one, the 56-year-old patient.

Finally, response to cortisone therapy appears to be more satisfactory in this variety than in any other. The frequency of satisfactory response to steroid therapy (according to criteria defined in earlier publications (Crosnier and Josso, 1960)) in all patients in this category who were followed for a year or more after the beginning of treatment is 73 per cent favourable results compared to only 26 per cent for all treated nephrotic syndromes.

(2) Thickened capillary walls

This group is characterized by the fact that on optical microscopy the glomerular capillary walls appear to be regularly thickened (Fig. 3). This thickening is diffuse. It appears with all types of staining, particularly with silver impregnation according to the method of Wilder.

Optical microscopic examination is usually insufficient to enable us to analyse this thickening of the capillary wall. With electron microscopy it is possible to establish that this thickening is not connected with a diffuse increase in the thickness of the lamina densa. This is why we feel that the classic term "membranous glomerulitis" is not justified.

In 11 out of the 19 cases, we were able to establish that the capillary walls were probably thickened by a subepithelial deposit (Berger, Michielsen and Galle, 1960) (Figs. 4 and 5). These subepithelial deposits have been described earlier in the section on elementary lesions.

In four other cases, the thickening appeared to have been produced by subendothelial deposits, but this type of deposit, as we

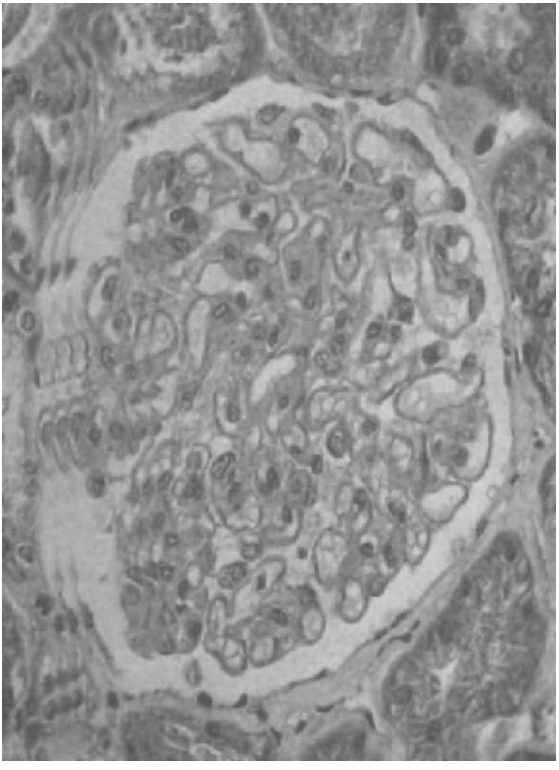


FIG. 3. Thickened capillary walls. Diffuse general thickening of the glomerular capillary walls. (Trichrome Masson. × 400.)

have just said, has a more variable and less easily defined aspect than the extra-membranous deposits.

Since not all our thick-wall cases were examined under the electron microscope, it is not possible to state that the thickening is always due to such deposits.

The clinical characteristics corresponding to this group differ markedly from those of the preceding one. The patients' ages are on an average much higher (Fig. 2): eight patients out of 19 were aged over 50, and one of them over 70, at the onset of the

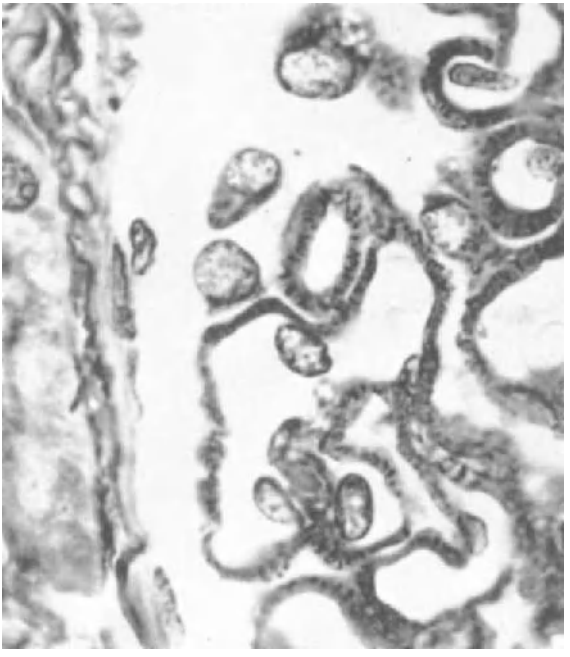


FIG. 4. Thickened capillary walls. Same case as Fig. 3. Silver impregnation makes the capillary walls appear striated. (Jones). (Silver impregnation by Wilder's method. × 1,650.)

illness. Proteinuria was usually lower than in the preceding group: less than 6 mg. per minute in 80 per cent of cases. Microscopic haematuria was greater (over 10,000 red blood corpuscles per minute in 60 per cent of cases). Functional tests showed alterations in 22 per cent of cases and there was an elevated blood pressure in two of the patients.

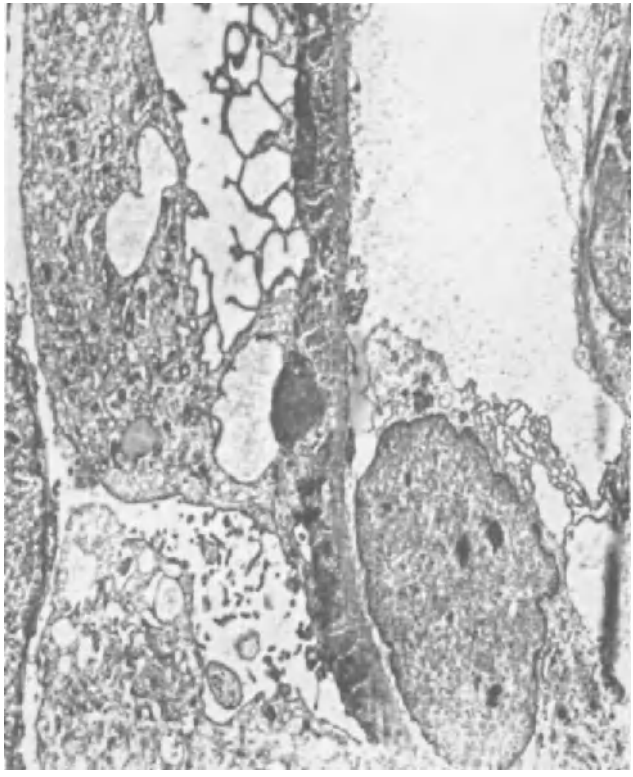


FIG. 5. Thickened capillary walls. Same case as Figs. 3 and 4. Staining with uranyl acetate reveals thickening of the capillary wall due to deposits of an amorphous substance under the epithelium, and not due to thickening of the lamina densa of the basement membrane. (Electron microscope. × 4,350.)

(3) Extracapillary proliferative glomerulitis

Three cases had diffuse lesions of epithelial proliferative nephritis involving all the glomeruli, with extensive crescent formation (Fig. 6). In six other cases, the lesions were more discrete and focal, and in some glomeruli there were partial adhesions between the tuft and Bowman's capsule.

These cases could be expected to represent what is traditionally called the nephrotic stage of an ordinary glomerulonephritis; however, there was paradoxically no evidence of any history of

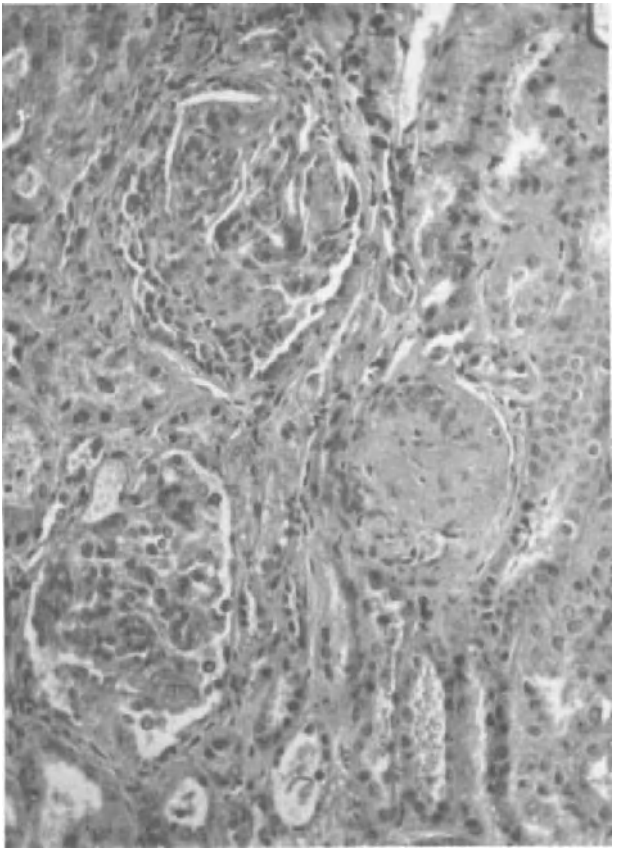


FIG. 6. Extracapillary proliferative glomerulonephritis with epithelial crescents. (Trichrome Masson. × 120.)

acute post-angina glomerulonephritis in any of these nine cases, whereas evidence of previous infectious pharyngitis could be found in other types of nephrotic syndrome, for example in four

of our 14 cases of lobular glomerulitis. It is true that such evidence is always difficult to interpret. One case in this group had developed nephrotic syndrome following an episode of Schönlein-Henoch syndrome.

(4) Endocapillary proliferative glomerulitis

Endocapillary glomerulitis, defined by diffuse endocapillary cell proliferation without marked thickening of the capillary walls, was observed in five cases. This endocapillary glomerulitis sometimes produces a degree of lobulation of the glomerular tuft which can give a false picture of lobular glomerulitis.

Clinical characteristics of this variety are not very different from those of lobular glomerulitis, described below.

(5) Endocapillary proliferative glomerulitis with hyaline nodules ("lobular glomerulitis")

In 15 of our cases we have noted a picture which corresponds to the description by Allen (1955) of "lobular glomerulitis". In optical microscopy of the biopsy, all the glomeruli examined exhibited the same appearance, defined by the following four features: (a) marked segmentation of the tuft into lobules of fairly equal sizes; (b) invasion of the centre of each lobule by a hyaline nodule; (c) rejection of the capillary lumina to the outer periphery of each lobule; (d) endocapillary cellular proliferation of varying degree (Fig. 7).

Electron microscopy applied to three of our lobular glomerulitis biopsies showed just as characteristic a picture as the optical one (Figs. 8 and 9). The nodule in the centre of the lobule consists of a fairly dense hyaline substance within which persist a few atrophic cells and a few retracted lumina. Within the hyaline mass we have been able to observe, in three of our cases, many periodic fibres whose morphological characteristics are comparable to those of collagen fibres; the fibres are of unequal length and may be either isolated and randomly oriented or grouped

in fairly small bundles (Fig. 10). These fibres exhibit a period of about 100 Å, a figure which corresponds to the collagen sub-periods, which are very often the only periods that can be detected in recently formed collagen fibres. The presence of

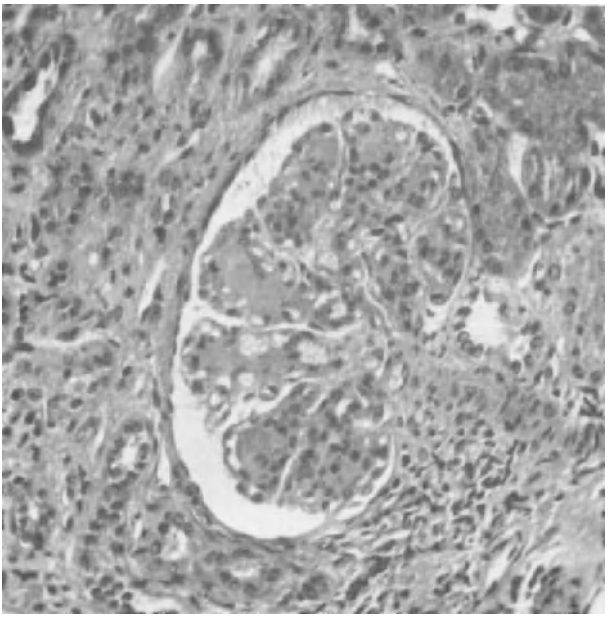


FIG. 7. Lobular glomerulonephritis. Segmentation of the tuft into lobules of equal size with a central hyaline nodule, moderate degree of proliferation, retraction of the capillary walls to the periphery of the lobules. (Trichrome Masson. × 265.)

collagen fibres in the glomerulus has already been noted in other circumstances in man (Spiro, 1959), the rat (Bencosme *et al.*, 1959) and the frog (Yamada, 1960).

The retracted capillaries are seen at the periphery of the hyaline mass. Their walls may be quite normal and the foot processes may even be intact over wide areas. In other cases, the capillary

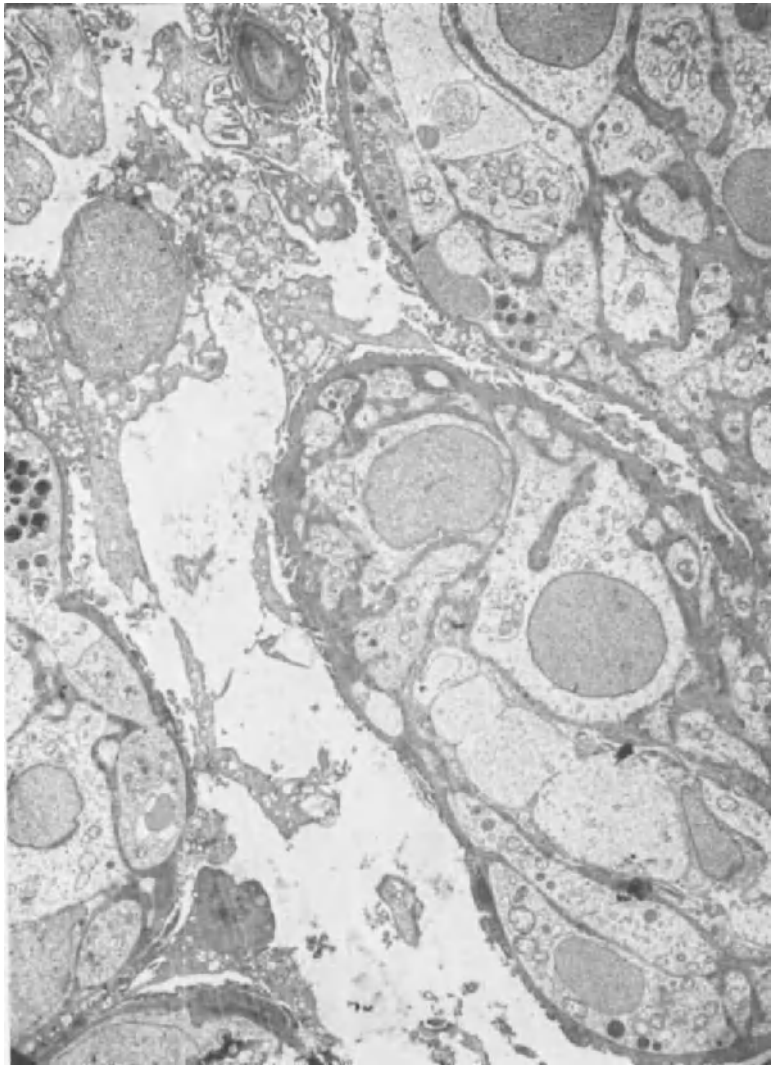


FIG. 8. Lobular glomerulonephritis. Extensive endocapillary cellular proliferation surrounded by an irregularly deposited substance. The basement membrane is normal and entirely surrounds the lobule. The foot process differentiation is preserved in spite of extensive alterations of the epithelial cells. (Electron microscope. × 2,350.)

wall appears to be thickened by subendothelial or sometimes even by subepithelial deposits.

Optical and electron analysis of cases which appear to correspond to relatively early stages enables us to make a few suggestions

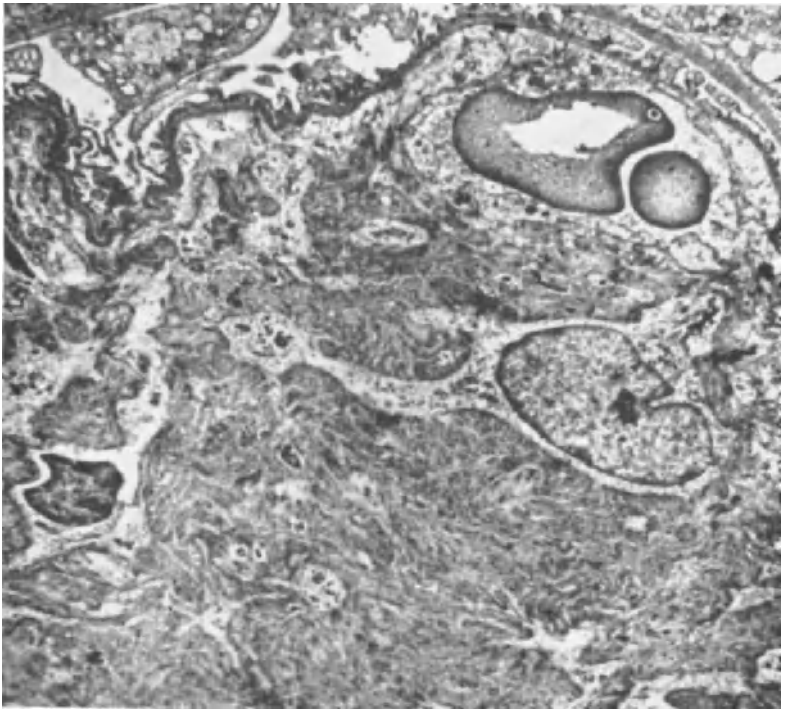


FIG. 9. Lobular glomerulonephritis. Lobule already extensively hyalinized. In the centre of the figure, fibrillar hyaline mass. Upper right, persistence of a narrow lumen. The basement membrane completely surrounds the lobule. (Electron microscope. × 3,750.)

as to the histogenesis of lobular glomerulitis. The initial alteration seems to be an intercapillary cell proliferation, mainly at the base of the capillary loops but also involving the periphery. In these intercapillary zones, there is a gradual accumulation of the

hyaline substance, which divides the cellular groups by means of more or less complete partitions. It is remarkable that in these early stages the capillary wall is still almost always normal and the foot processes preserved, which shows that lobular glomerulitis

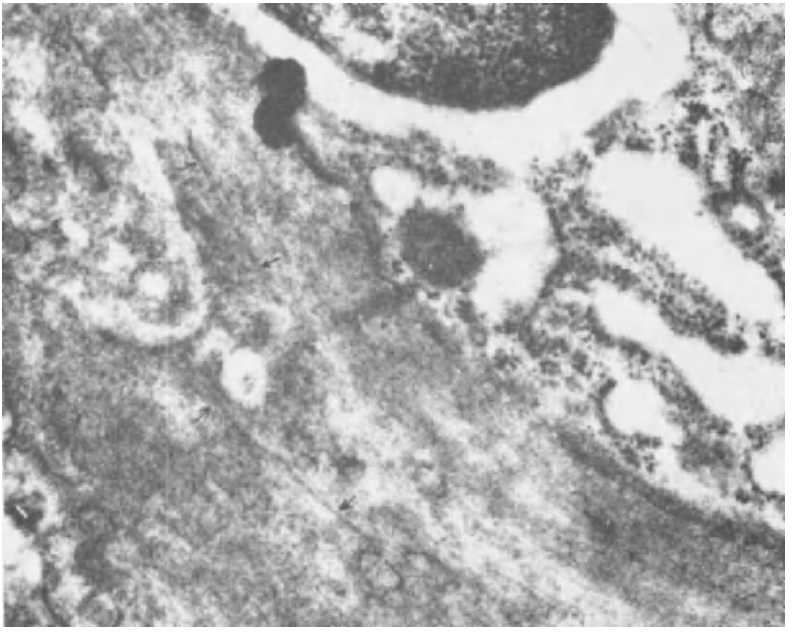


FIG. 10. Lobular glomerulonephritis. After staining with uranyl acetate, numerous fibrils with a periodicity of 100 Å appear in the substance constituting the nodule in the centre of the lobule. (Electron microscope. × 20,000.)

is not, as some have claimed, a stage in the evolution of membranous glomerulitis. As these nodules increase in size, the hyaline mass pushes back the basement membrane to the periphery; the membrane unfolds, surrounding the lobule without penetrating it. If one relates this apparent histogenesis with the possible presence of collagen fibres in the hyaline substance, it can be concluded that lobular glomerulitis could, for those who believe

in the existence of an autonomous intercapillary tissue, be classified as a pathological development of this tissue, as are other pathological conditions such as intercapillary diabetic glomerulosclerosis.

Clinical analysis of these cases shows a few characteristic facts. First the relatively young age of all our patients except one (Fig. 2) should be noted. In the history of this illness, the symptom which is most remarkable, because it is so unusual in other types of nephrotic syndrome, is a macroscopic haematuria; this occurred in four cases, once or several times. Microscopic haematuria is constant and often considerable; it was over 10,000 red blood cells per minute in all of the cases of this group. Some degree of renal functional deficiency is often shown by tests carried out at the time of the biopsy. Finally, the response of the nephrotic syndrome to cortisone therapy is on the whole much poorer than in the group with minimal changes.

(6) Complex or unclassified forms

(a) Glomeruli with irregular hyalinosis

In five cases, the picture was characterized by a rather diffuse hyalinosis of the intercapillary region without cellular proliferation or lobulation. In these cases, deposits of fibrinoid substance irregularly distributed within the hyaline material are sometimes seen. The picture is fairly comparable to what we have observed in 18 cases of isolated chronic proteinuria without nephrotic syndrome or microscopic haematuria and without any apparent aetiology (Antoine, Montera and Tallone, 1959).

(b) Thickened capillary walls associated with endocapillary proliferation

This complex picture, in which capillary wall alterations, hyaline deposits and endocapillary hypercellularity are associated,

was found in seven cases (Fig. 11). It is probable that this is only a stage in the evolution of one of the preceding forms.

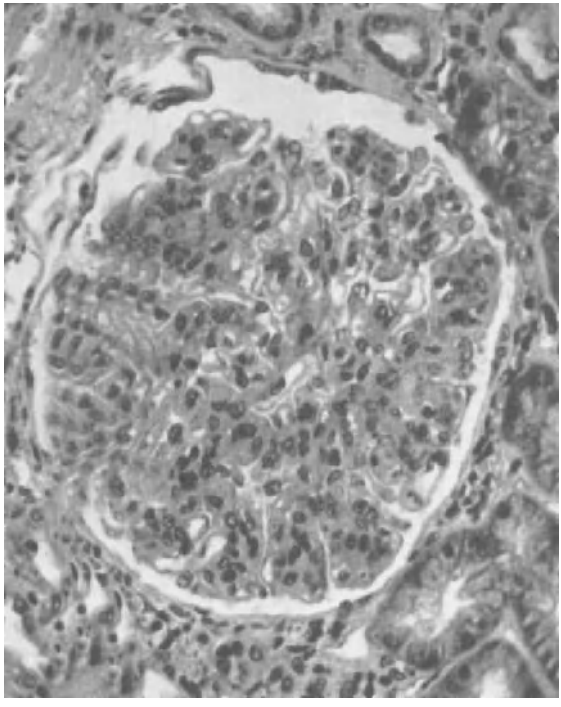


FIG. 11. Thickened capillary walls with endocapillary proliferation. In this type there are no centro-lobular hyaline nodules. (Trichrome Masson. × 4,000.)

(c) Advanced and uninterpretable forms

Thirteen of our 108 cases remained unclassified because they had glomerulonephritic lesions too far advanced to be satisfactorily analysed. This comparatively high number of biopsies which are difficult to interpret morphologically emphasizes the imperfect and provisional aspect of the histological classification which we suggest.

Imperfect though this morphological differentiation may be, it has already supplied us with some data of practical importance. The anatomical-clinical correlations are regular enough in certain forms, for instance, in parietal and in lobular glomerulitis, to provide useful information for the prognosis and the choice of treatment; on the other hand, this classification is open to criticism. It assigns an entity to groups whose true autonomy is far from having been proved. It has the weaknesses possessed by all the attempts at medical classification in which the definition of pathological states is based, not upon precise aetiological data, but only on the often misleading indications of morphology.

Summary

(1) Of 127 cases of nephrotic syndrome (of which 19 were found to be due to specific conditions such as amyloidosis, lupus, etc.), 108 cases were of unknown origin. Among these latter cases, renal biopsy is far from showing a consistently identical morphological picture. Elementary lesions of the glomeruli, epithelial cell alterations, changes in the basement membrane, subepithelial and subendothelial deposits, endothelial and intercapillary changes can be grouped into a few major morphological types.

(2) In 35 cases, lesions could be detected only under the electron microscope, and consisted of fusion of the foot processes, associated with various epithelial cell modifications.

(3) In 19 cases, a marked thickening of the glomerular capillary wall ("membranous glomerulitis") detectable under the optical microscope, was probably due, not to a thickening of the lamina densa, but to subepithelial or subendothelial deposits.

(4) Extracapillary proliferative glomerulitis (9 cases) also had well defined features.

(5) Endocapillary proliferative glomerulitis was either simple (5 cases) or nodular (15 cases). The latter type ("lobular glomerulitis") appears to have morphological autonomy and is not, we feel, a stage in the evolution of a "membranous glomerulitis".

(6) Irregular hyalinosis of the glomeruli (5 cases) and the complex forms in which thickening of the capillary wall is associated with endocapillary proliferation (7 cases) represent a more debatable entity.

(7) In 13 cases, the lesions were too far advanced to be interpretable.

(8) The clinical correlations of each anatomical type have been discussed. They are clear for the form with minimal changes and the lobular form. They are more doubtful for the others. In any case, even if separation between various anatomical-clinical types were satisfactory in practice, it would not allow us to state that we are dealing with autonomous diseases.

REFERENCES

ALLEN, A. C. (1955). *Amer. J. Med.*, **18**, 277.

ANTOINE, B., MONTERA, H., and TALLONE, J. C. (1959). *J. Urol. méd. chir.*, **65**, 623.

BENCOSME, S. A., STONE, S. S., LATTA, H., and MADDEN, S. C. (1959). *J. Ultrastruct. Res.*, **3**, 171.

BERGER, J., MICHIELSEN, P., and GALLE, P. (1961). *J. Urol. méd. chir.*, **67**, 52.

BLAINEY, J. D., BREWER, D. B., HARDWICKE, J., and SOOTHILL, J. F. (1960). *Quart. J. Med.*, **29**, 235.

CROSNIER, J., and JOSSO, F. (1960). *In* Actualités néphrologiques de l'Hôpital Necker, p. 177. Paris: Flammarion.

FARQUHAR, M. G. (1959). *In* Proc. Xth Ann. Conf. on Nephrotic Syndrome, p. 2. Ed. Metcoff, J. New York: National Kidney Foundation.

FOLLI, G., POLLAK, V. E., REID, R. T. W., PIRANI, C. L., and KARK, R. M. (1958). *Ann. intern. Med.*, **49**, 775.

HAMBURGER, J., CROSNIER, J., and MONTERA, H. (1960). *In* Entretiens de Bichat, p. 455. Paris: Expansion Scientifique Française.

JOEKES, A. M., HEPTINSTALL, R. H., and PORTER, K. A. (1958). *Quart. J. Med.*, **108**, 435.

LATTA, H. (1960). *Calif. Med.*, **93**, 272.

MOVAT, H. Z. (1960). *In* Proc. XIth Ann. Conf. on Nephrotic Syndrome, p. 228. Ed. Metcoff, J. New York: National Kidney Foundation.

SPIRO, D. (1959). *Amer. J. Path.*, **35**, 47.

VERNIER, R. L., FARQUHAR, M. G., BRUNSON, J. G., and GOOD, R. A. (1958). *Amer. J. Dis. Child.*, **96**, 306.

YAMADA, E. (1960). *J. biophys. biochem. Cytol.*, **7**, 407.

DISCUSSION

Kark: Frankly, I think that "nephrotic syndrome of unknown origin" should have been called "nephrotic syndrome of unknown origins", because at least 40 aetiologies have been recognized (Kark *et al.* (1958). *Ann. intern. Med.*, **49**, 751).

In your cases, the most interesting ones for us are the group you describe as having no parietal glomerular changes by light microscopy, which we would call "lipoid nephroses" of adults. We have been dividing them into two groups: (1) those in which there is a very rapid and complete response to steroids with complete disappearance of proteinuria, and in which we feel there is a good prognosis; and (2) those in which there is not as good a response, persistent proteinuria being the problem. We have a strong suspicion that the patients who respond well to steroids are suffering from what we call "asthma of the nephron", an allergic response to bee-sting, grass pollens, serum sickness, sea-food, etc.

I was interested in your cases of diffuse hyalinosis. That picture looked to me very much like diabetes. We see an occasional case in which the nephrotic syndrome appears in patients with clear-cut diffuse diabetic glomerulosclerosis, histologically, but no clinical evidence of diabetes. If we go into the family history and do special function tests, we find pre-diabetic abnormalities. I wonder whether this group of cases of hyalinosis are not merely cases of pre-diabetes?

I should also like to point out that among adults with the nephrotic syndrome there are some cases of streptococcal origin. However, I don't want to discuss them at the present time because Dr. Earle and Dr. Jennings will be talking about them later.

In the past, the classification of the nephrotic syndrome into Longcope A and B and Ellis Type I and II served a very useful purpose, but I think we've got long past that stage, and I wonder whether we should not consider dropping these terms altogether?

Hamburger: With regard to pre-diabetes as a possible aetiology, we have no personal data, although we have been interested in this. After a visit of Prof. J. P. Hoet (from Louvain), who is very interested in the problem of pre-diabetes in all diseases, we have spent some time looking for such an origin in our cases, with rather disappointing results.

About the streptococci and the general question of pharyngitis as aetiological factors: we were expecting such an aetiology in the group classified as classic proliferative glomerulonephritis, and we were surprised not to find it. None of our nine cases had a previous history of pharyngitis; the only aetiology found in this group was one case of Schönlein-Henoch syndrome. Conversely, the series in which we had the highest incidence of pharyngitis was lobular glomerulonephritis (four cases out of 14).

Hardwicke: What do you regard as the data of clinical response to steroid therapy? I expect your criteria are much the same as ours, but I would like the information. Secondly, I wonder if you would be prepared to split your parietal group into two—those with minimal or no change, and those with some type of membranous glomerulonephritis, because in our experience the response to steroids of these two groups is strikingly different.

Hamburger: I am afraid that nothing is more difficult than to transform the impression of response to steroid therapy into official data and numbers. I quite agree that in the minimal-change type we certainly get better results with steroids, even if we cannot give exact figures. However, if you actually do want figures and possible criteria, two of my co-workers have published a statistical study on our cases (Crosnier and Josso, 1960, *loc. cit.*), but I must confess that I did not find it entirely satisfactory.

Hardwicke: I am interested in this diffuse change, with core thickening, which you say you have seen in young patients and often in proteinuria of unknown origin. This proteinuria is often a chance finding and is not severe. We don't yet know how these cases progress.

I had been quite unhappy about your entity "lobular glomerulitis" because I knew you were diagnosing it and we had never seen any; however, we have seen three cases in the last six months. We don't have as beautiful preparations as yours, but with silver staining we found disruptions of the basement membrane—it may be completely broken—and fibrils of silver-staining material dispersed throughout the whole of the glomerulus. We see a lot of polymorphs at the periphery of the tuft in all the cases I have recognized.

Michielsen: In lobular glomerulonephritis we also found a large number of polymorphonuclears with an occasional eosinophil even in

advanced cases. We have never seen disruption of the basement membrane. However, the optical appearance of a reduplication of the basement membrane would be easy to understand. In electron microscopy it is an anastomosis between the basement membrane and the ground substance and fibrils surrounding the intercapillary cells.

Vernier: In what you are calling lobular glomerulitis, Dr. Michielsen, you said that the epithelial structure was normal in the presence of proteinuria. In the pictures you showed, I would consider most of those epithelial cells grossly abnormal.

Michielsen: I agree that the epithelial cells are grossly pathological, but there is no coalescence of the foot processes. There is normal differentiation of the foot processes, notwithstanding a massive proteinuria.

Vernier: I understand, Prof. Hamburger, that while you are working with adults for the most part, you have some younger patients in your group. It seems to me your work brings out the overlapping of adult and childhood nephrosis so far as morphological lesions are concerned. Certainly you have a rather large number of patients with minimal changes by light microscopy—which some would call idiopathic lipoid nephrosis of adults. In children occasionally we see the other forms that you describe but I think that the minimal lesion predominates by far. Do you think that you have any evidence of progression of the lesion from minimal to the various other forms? We have observed children to progress from minimal lesion to complete hyalinization of the glomeruli in a short period of time; they may progress through any one or all of the sequences that you have described, in the course of two years perhaps, as shown by serial biopsy. Whether or not they arise as independent aetiologies, I can't say. I doubt that we are justified on morphological data alone to separate them as entities.

Hamburger: That is the crux of the problem. Of course we agree that a single patient can have a minimal-change type and then have a progressive destruction of the glomeruli and finally a totally destroyed kidney, but we have never in all this series seen any pictures suggesting that lobular glomerulitis is a stage in this evolution, and that is why we are wondering whether it is not an original and separate pathological reaction. I believe that there are lines going toward destruction, but the question is whether there is only one line progressing through all

these stages or whether there are several lines. We definitely think that this lobular type is on a different line from the ones beginning with simple epithelial and membranous changes.

Vernier: I would like to further simplify the grouping. It seems to me that you have a good case for a separate line in the lobular group, and that they do perhaps have a different aetiology, but I am doubtful about the separate identity of endothelial accumulations versus epithelial accumulations. Both occur in the course of a disease which originally showed no changes in the basement membrane and they may just be variants in the progressive story. That gives us one less type.

Michielsen: I think the subepithelial deposits are an entity, because in a very early case (two months' evolution), we already found these deposits outside the basement membrane. Secondly, when these cases have a longer evolution we don't find intracapillary proliferative changes associated with this form.

Another reason is that in cases of nephrotic syndrome of known origin such as lupus erythematosus or Kimmelstiel-Wilson disease, we never find such subepithelial deposits although there is a fusion of the foot processes. The subepithelial deposits are thus not in the line of evolution of the fusion of the foot processes.

Hamburger: It seems to me that at the present time two opposite mistakes must be avoided. The first mistake would be to refuse the evident separation between various types of morphological reactions, all capable of inducing a nephrotic syndrome. That some of these aspects correspond to pathological individualities, each having an original evolution, seems to my group reasonably well established in the material we have studied. But another mistake would be to jump to the conclusion that we are facing separate diseases. No one knows whether one or several entirely new diseases will or will not be isolated in the future among nephrotic patients, but there is little doubt that careful description of the different clinical and pathological types is one of the fundamental approaches to the possible discovery of nosologically distinct entities.

Movat: We have found the lobular picture in three different clinical pictures: first of all, in cases which had clinically a definite acute glomerulonephritis and had progressed to this lobular picture (morphologically); secondly, in patients who had a pure nephrosis; and, finally, we

found it in so-called latent nephritis, in patients who merely had haematuria or perhaps slight proteinuria and no other clinical signs. This would suggest that although the morphogenesis may be similar the aetiologies are perhaps different.

Have you seen these extra-membranous deposits (between the basement membrane and the epithelium) in children as well?

Habib: We saw them for the first time about three weeks ago in a 5-year-old child, and our colleagues in Toulouse have seen extra-membranous deposits in a boy of 11.

Movat: We have never seen it in children. Perhaps the epithelial cells of the adult react to the extravasation of plasma proteins differently from those of children—although we have seen an occasional adult with the nephrotic syndrome but without extra-membranous deposits.

Jennings: Tomorrow we will present some data to indicate that a lesion very similar to chronic glomerulonephritis occurs in the subsiding stages of an acute post-streptococcal proliferative glomerulonephritis. This observation has led us to speculate that many cases of chronic diffuse proliferative glomerulonephritis of the lobular type are probably post-streptococcal in aetiology; however, the initial acute post-streptococcal proliferative glomerulonephritis may have occurred so far in the past that it is impossible to establish this diagnosis retrospectively except by implication. Prospective studies should ultimately shed considerable light on this hypothesis.

Pirani: I was disturbed by this rather summary disposal of membranous glomerulonephritis, and by that I mean not the thickening of the entire capillary wall but the thickening of the basement membrane proper. I think there is such an entity both by light microscopy and electron microscopy. This thickening can be due not only to the fusion of the foot processes or to deposition of extracapillary or intracapillary material but to actual thickening of the basement membrane itself, associated with splitting or fibrillation.

The other thing that bothered me is the clear-cut demonstration by electron microscopy of material both on the extracapillary and intracapillary position which your group has identified with fibrin or similar material. Has this material also been demonstrated by light microscopy, by histochemical stains? My impression is that in most

cases of true membranous glomerulonephritis it is difficult to demonstrate any significant amounts of fibrin or fibrinoid. I don't understand the discrepancy between the electron microscopic and the light microscopic findings.

Movat: May I amplify this question? Did you find fibrin, as you have shown it, both within the capillary and on the outside of the basement membrane?

Michielsen: Only within the capillary. What is called fibrinoid degeneration in optical microscopy is not the same as what you see in the electron microscope. Sometimes you find a granular deposit, as in lupus for instance; sometimes you find true fibrin with a typical periodicity. But I think only the electron microscope permits you to say what you are really dealing with.

Movat: What is your definition of fibrinoid in the electron microscope?

Michielsen: Fibrinoid is a term of optical microscopy. It is better not to use it in electron microscopy. I would rather call it a dense granular deposition inside or outside the basement membrane, or fibrin when there is a characteristic periodicity.

Pirani: But was fibrin or fibrinoid seen by light microscopy in those cases where material was demonstrated by electron microscopy?

Habib: Yes, we have seen such deposits along the basement membrane by light microscopy.

Jennings: This is a sign that a patient is in the acute phase of the disease. If you biopsy the patient later the fibrinoid won't be there.

Habib: Of course.

Movat: I can confirm this. We have seen it as well.

Jennings: The same thing is true of intraglomerular polymorphonuclear neutrophils, in so-called lobular glomerulonephritis. These cells are usually common when the patient is clinically at the height of the nephrotic syndrome. They are especially common in glomeruli containing patches of fibrinoid. But when the disease improves these signs of acute inflammation disappear, and you can see patients with lobular glomerulonephritis whose glomeruli show only 0–1 polys per 2-micron section.

Earle: You are referring to the exacerbations in our chronic, originally post-streptococcal, glomerular nephritics. However, in one

patient who had an exacerbation after a virus infection (non-streptococcal) there were no polys.

Heptinstall: Prof. Hamburger, you say that in your lobular glomerulonephritis there is no thickening of the basement membrane. Surely in your picture one of those capillaries was thickened.

Hamburger: I don't know if you consider the lamina densa to be the basement membrane, but in none of our cases except two have we found the lamina densa regularly increased in thickness.

Heptinstall: With a light microscope you are probably seeing the lamina densa of electron microscopy plus stretched-out endothelial cytoplasm.

Movat: No, you can separate them (basement membrane, endo- and epithelial foot processes) with the light microscope as well (Movat, 1959, *loc. cit.*). Has anyone here made systematic measurements on the thickness of basement membranes? One has to measure each case and make quite a number of measurements before one can say that the basement membrane is thickened or not thickened, and if it is thickened it may be focally thickened.

Pirani: I think we know that the capillary wall consists of three layers and in nephrotic syndrome we see by electron microscopy in a fair number of cases both a diffuse and a nodular focal thickening of the basement membrane proper—the lamina densa of electron microscopy. I fully agree with Dr. Movat that evaluation of the thickening of the basement membrane is very difficult. It is difficult to know whether it is a true thickening and, even if we decide it is, we still don't know if this is due to a collapse or a retraction of the capillary itself, so that there is a resulting thickening. On the other hand, I don't feel that the basement membrane is an elastic structure. When there is ischaemia of the glomerulus there is increased folding; the basement membrane tends to fold upon itself rather than to retract as an elastic structure would do, with increased thickening. So when there is what I would judge by electron microscopy to be a true thickening of the basement membrane, I would feel that this is due to real increased deposition of whatever composes the basement membrane.

Vernier: In determining the normal width of the basement membrane, the criteria which we have used (as listed earlier in my paper) are: (1) The capillary measured must be judged to be cut in cross-section,

judged from the appearance of the endothelium and from the orderliness with which the foot processes insert. (2) It must be open, so that one measures capillaries which have a lumen the diameter of two red cells or greater. (3) We measure five or more places randomly around the circumference of five or more capillaries from five or more glomeruli in each patient. From this one can determine the statistical variability of the basement membrane thickness in the normal. We have not yet applied this to a large enough number of cases of various forms of renal disease to be able to state anything about thickening, except in the cases of lupus erythematosus and in about six cases of diabetes mellitus where we think we have evidence of thickening by these criteria. Certainly we ought to have some agreement as to what we mean by thickening.

de Wardener: Could I put it to the meeting that there is no correlation between the histological appearances and the nephrotic syndrome. To work out the correlation between A and B one must include not only all the A's but also all the B's. In this discussion the histological appearances in the nephrotic syndrome have been described; but we all know that these appearances may occur in patients *not* suffering from a nephrotic syndrome. Therefore there is no typical appearance of the glomerulus in the nephrotic syndrome. I do not think that any pathologist here would be prepared to say looking down the microscope whether or not the patient from whom that glomerulus was removed had a nephrotic syndrome.

Earle: Do you liken the nephrotic syndrome in renal disease to congestive heart failure in relation to disease of the heart?

de Wardener: Yes.

Vernier: I think I can say with reasonable accuracy that the patient has proteinuria on the basis of the appearance of the epithelial cells in electron micrographs. This does not tell me that he has the nephrotic syndrome, however.

Bergstrand: I would be very interested to hear what you mean by the "normal" appearance of the glomerulus. There are such wide differences in the appearance of the cells of the glomerulus of a healthy being that you can't use the word "normal". I too have tried to make measurements of the thickness of the basement membrane but I found that there are such great differences in the same glomerulus in the same

patient if one does a sufficient number of measurements that I cannot do a statistical evaluation. I can estimate the thickness of the basement membrane in the healthy adult, but I cannot give an exact figure. I can say that it is somewhere between 3,000 and 3,700 Å, but that is a very wide range. In pathological changes we consider that we must have a three-fold increase in diameter before we can say it is definitely thickened.

Hamburger: I agree with you. With such a definition I think it is rather easy to say that we didn't find this three-fold increase.

Milne: Why does variation invalidate statistics? I thought this was the basis of statistics.

Rich: I gather that in the minds of some there is doubt whether the glomerular lesions of the "idiopathic" nephrotic kidney progress from "nothing to be seen in the light miscroscope" to the thickened basement membrane, and then to the hyaline deposits, and finally to partial and to complete sclerotic obliteration. Is there anyone who thinks that all that cannot happen in sequence?

Earle: We had one patient who did go through the very sequence you have described.

Rich: In the light microscope, in any large series of "idiopathic" nephrotics you can find all these stages in the same kidney in many cases.

Kark: Prof. de Wardener said that you cannot diagnose the nephrotic syndrome from studying the glomerulus. But when a patient has the biochemical lesions of the nephrotic syndrome, one always finds changes in the tubules—fat in tubular cells—which you don't find in other situations, except perhaps in diabetes. When nephrotic oedema appears in the patient, then you frequently find in addition widespread or patchy interstitial oedema in the kidney. When the fatty tubular changes and interstitial oedema coexist, the pathologist should be highly suspicious that he is dealing with the nephrotic syndrome. In answer to the other point, we equate the nephrotic syndrome with the consequence of massive persistent albuminuria.

I also thought that what Prof. Hamburger was trying to do was to find out whether, in a group of patients with the nephrotic syndrome, one could separate them on a pathological basis just as you can cull out lupus nephritis or amyloidosis histologically; perhaps the lobulars

were one entity which would eventually turn out to have a single aetiology, he hoped, and be separated from other causes of the nephrotic syndrome.

Hamburger: I was not so ambitious! I was just trying to answer the question set by our Chairman: Are these various stages of the same lesion or are there several lines going towards the finally destroyed kidney? Our impression is that there are certainly several stages but possibly several lines. This suggests as a possibility that in the so-called idiopathic nephrotic syndrome (or primary nephrotic syndrome or nephrotic syndrome of unknown origins) there are several diseases, but this has of course not been demonstrated.

Rich: Since the aetiology is unknown it is, of course, conceivable that there may be several different causes of the histological sequence that I mentioned, but the difference in the character of the lesions in different glomeruli at different stages of the process (membrane thickening, hyaline deposits, obliterative sclerosis) does not require the assumption of a different aetiology for each type of lesion.

Ross: Our experience suggests that there are separate histological types without any clinical differences. We have made a retrospective study on 30 patients with necropsy specimens, all with "nephrotic syndrome of unknown origin". Of these 10 had a typically membranous picture predominating, seven had your lobular glomerulonephritis and the remainder were mixed. We couldn't correlate any differences in the development of oedema, the development of hypertension, of renal failure or of any other clinical factor. There was no difference at all between those that had the membranous picture and those with the lobular picture.

Hamburger: I agree that the clinical features can be the same. But that was the history of lupus: twenty years ago all the nephrotic syndromes that we call lupus were called lipoid nephrosis. So the question is, have we new "lupus" to discover?

Wilson: Surely lupus is just an example of a collagen disease in which we may get any form of glomerulonephritis. The pathological process in the kidney is the same whether or not other manifestations of collagen disease are present.

THE FINE STRUCTURE OF THE GLOMERULUS IN BRIGHT'S DISEASE: A CLINICO-PATHOLOGICAL STUDY*

HENRY Z. MOVAT and JAN W. STEINER

Department of Pathology, Banting Institute, University of Toronto

and

ROBERT J. SLATER

The Research Institute of The Hospital for Sick Children, Toronto

ABBREVIATIONS

ax—axial region; bm—basement membrane; c—capillary lumen; cm—cell membrane; dep—deposits; en—endothelium; ep—epithelium; er—endoplasmic reticulum; ex—exudate; fp—foot processes; G—Golgi zone; ic—intracapillary cell; ld—lamina densa; lre—lamina rara externa; m—mitochondria; mb—microbody; n—nucleus; ob—osmiophilic bodies; p—polymorphonuclear leucocyte; pad—protein absorption droplets; rbc—red blood cell; rnp—ribonucleoprotein; v—vacuole.

THIS presentation deals with the morphological aspects of Bright's disease and attempts to correlate some aspects of the fine-structural changes with clinical and laboratory findings.

MORPHOLOGICAL CONSIDERATIONS

Acute glomerulonephritis

General

The Malpighian tuft is hypercellular in acute glomerulonephritis. There is hypertrophy and hyperplasia of intracapillary

* The investigations dealing with acute glomerulonephritis were supported by the National Heart Institute, U.S. Public Health Service (H-5657); those dealing with electron microscopy of lipoid nephrosis, membranous and chronic glomerulonephritis were supported by the Medical Research Council of Canada (MA-719); the clinical and laboratory investigations were aided by the Department of National Health and Welfare of Canada (603-13-25) and the Islington Rotary Club.

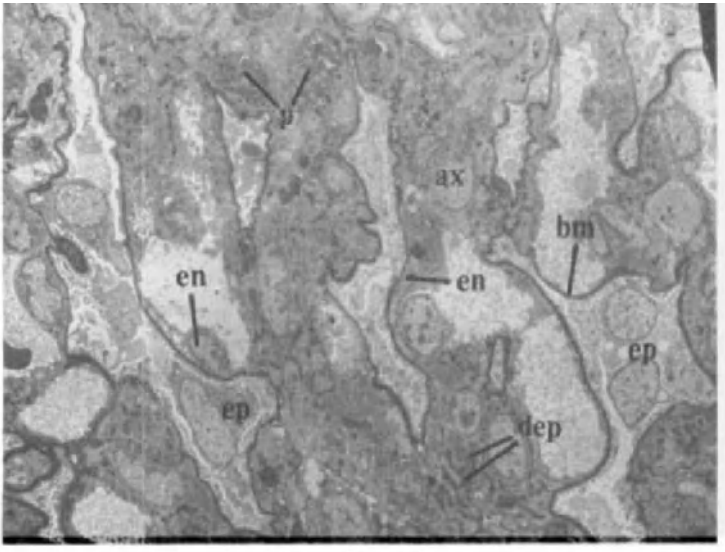


FIG. 1 (× 1,260).

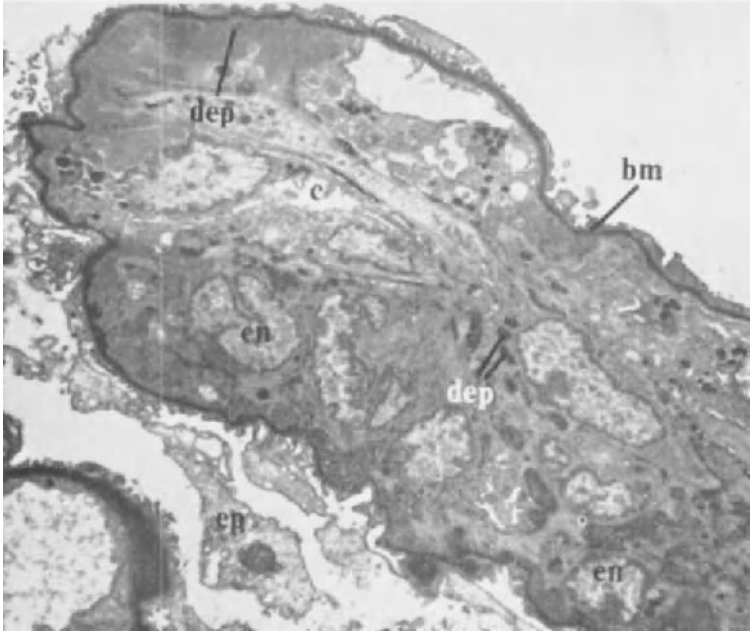


FIG. 2 (× 2,700).

cells (Figs. 1–2). Because of cellular swelling and distortion of capillaries, one cannot separate endothelial and axial cells. (We are inclined to look upon the axial cell as a modified endothelial cell. Its fine structure and its reaction in experimental nephritis are described in other publications (Movat, McGregor and Steiner, 1961).)

Intracapillary deposits

In addition to the changes of intracapillary cells there is an accumulation of electron-dense material within capillaries. This is located between the proliferating cells and between the basement membrane and the cells. It is barely visible in the light microscope, but in electron micrographs impregnated with silver-methenamine after periodate oxidation, one can recognize the precipitates even at low power (Figs. 1 and 2). As shown in Fig. 2, there are variations in density of this material.

Basement membrane

Changes associated with the basement membrane are twofold. Firstly, there is a "Quellung" of the basement membrane. The basement membrane may become several times thicker than normal. Whereas most of the basement membrane shows a diffuse "Quellung", in focal areas there are irregular swellings with addition of an electron-dense material to the basement membrane (Figs. 3 and 6). These focal swellings cause irregular bulges of the basement membrane in both directions, towards the endothelium and towards the epithelium. The bulge towards the epithelium is often well demarcated and is mushroom-shaped (Fig. 6). Such lesions have been described in rabbits given repeated injections of foreign protein (Vazquez *et al.*, 1960).

The second change associated with the basement membrane varies from the former in its appearance, but probably develops in a similar manner. It consists of an accumulation of electron-dense material between intracapillary cells and the basement

membrane. It varies in density: some precipitates have the density and staining properties of basement membrane and some are darker (Figs. 4 and 5). It has been suggested that these might be antigen-antibody complexes or antibody alone and that the

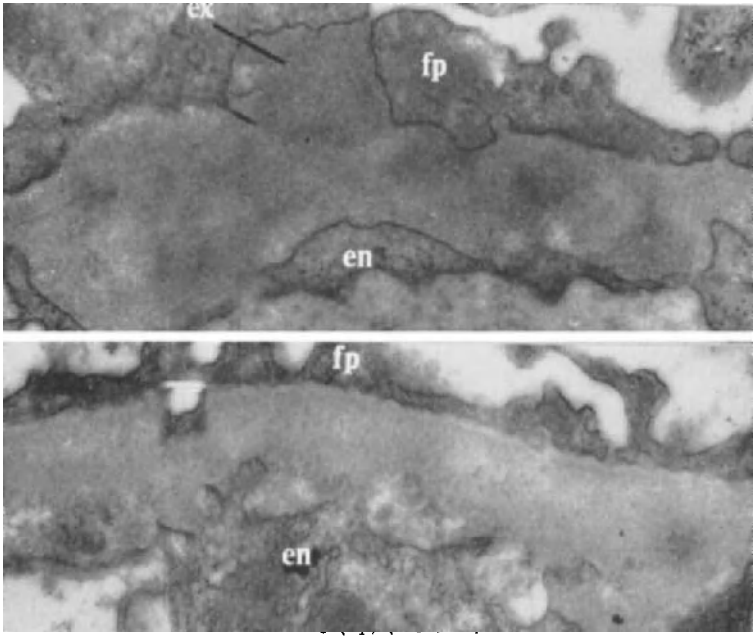


FIG. 4 (*bottom*) (× 36,000).

darker material becomes more basement membrane-like through ageing and polymerization. In cases which do not recover, large quantities of this material accumulate. Fig. 8 is from a case which was biopsied three months after the onset of acute glomerulonephritis, with a clinical picture of mixed nephrosis-nephritis ("nephrotischer Einschlag"). This 13-year-old girl died eight months after the onset of her illness.

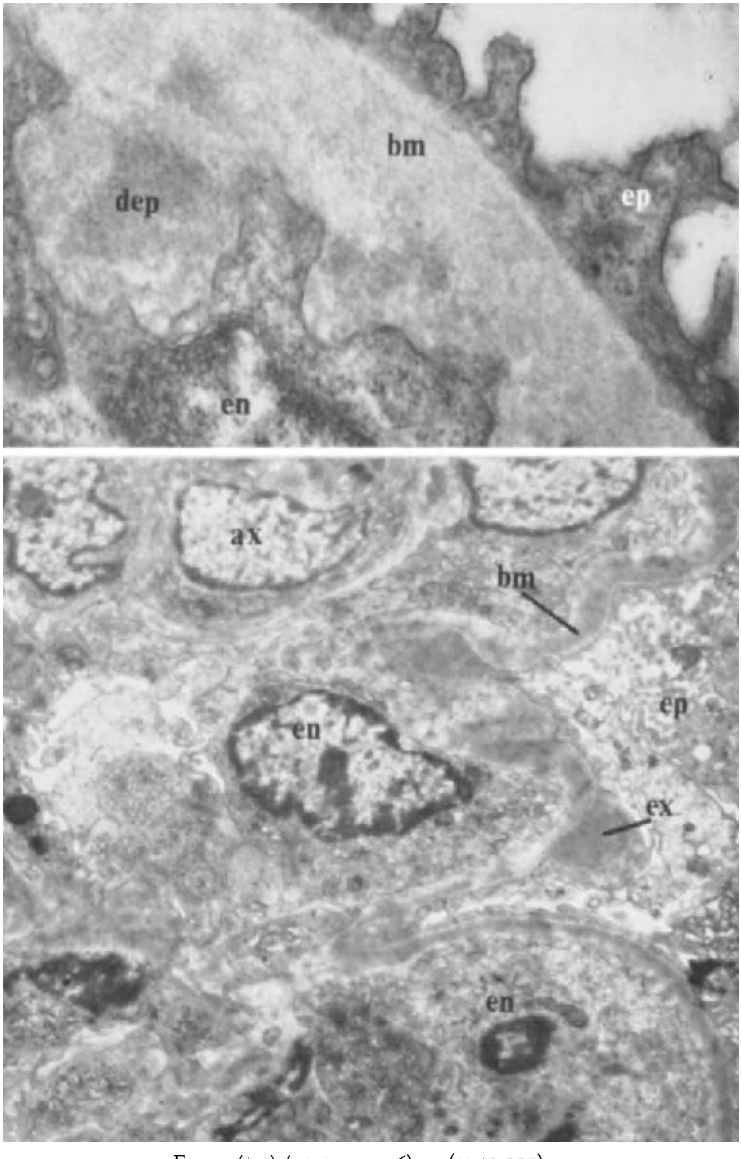


FIG. 5 (*top*) (15,400 × 2·6) = (× 40,000).
FIG. 6 (*bottom*) (2,800 × 3·3) = (× 9,250).

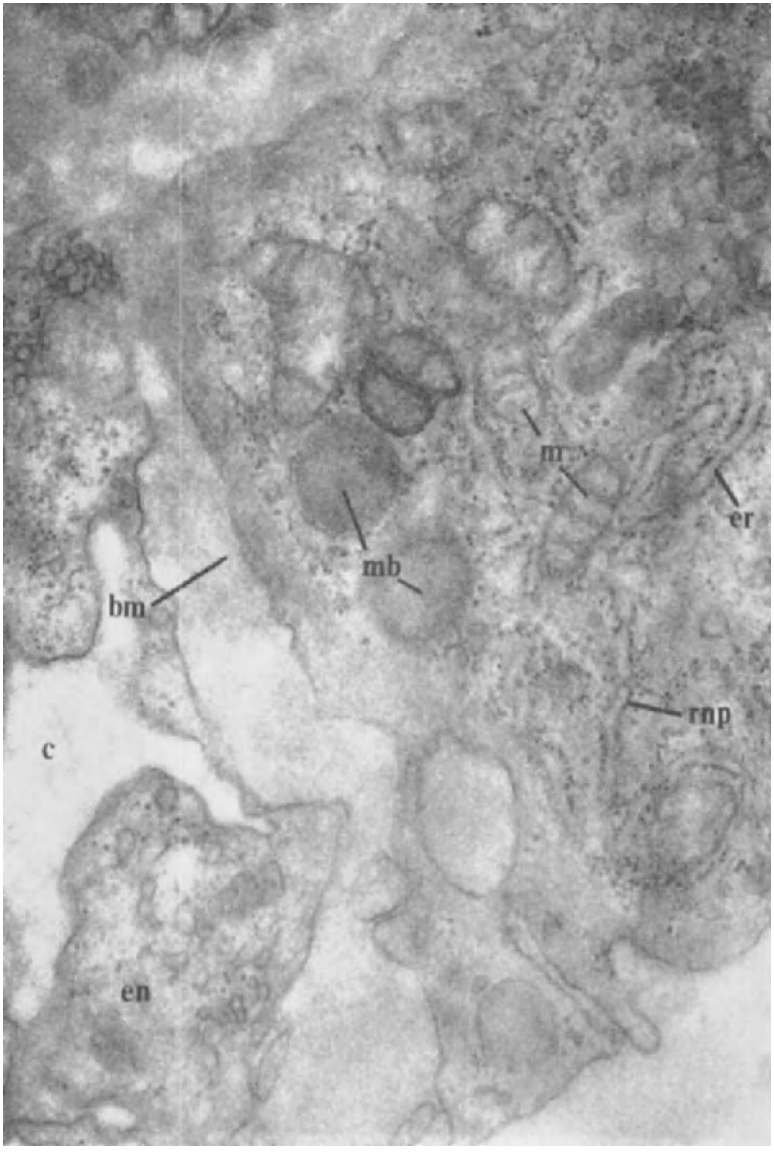


FIG. 7 (× 39,600).

Cellular changes

Intracapillary cells proliferate. They are swollen, often almost occluding the lumen (Figs. 2 and 6). There is increase in the number of mitochondria and of ribonucleoprotein (RNP) granules (Figs. 6 and 7). The latter are found diffusely in the

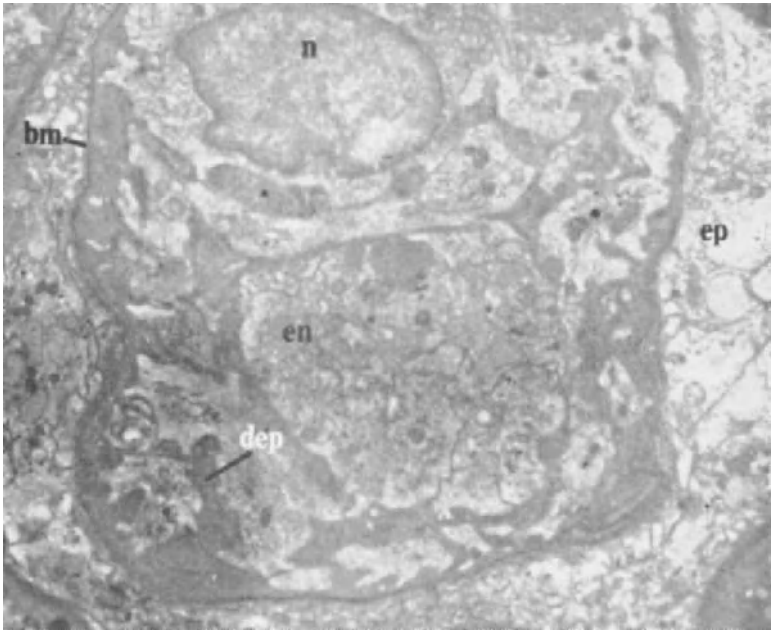


FIG. 8 (4,000 × 2·6) = (× 10,400).

cytoplasm and also cover the vesicles and sacs of the endoplasmic reticulum. Fat droplets occur occasionally and protein absorption (hyaline) droplets are found frequently (Figs. 2 and 7). Haematogenous cells are often encountered among the proliferating fixed cells (Fig. 1).

Epithelial cells show changes which are probably secondary to the injury to the basement membrane. The foot processes may

be plump as if collapsed. There may be some degree of fusion of foot processes (Figs. 3–5), but never as severe as in some cases of lipoid nephrosis. There is always fusion over the mushroom-shaped bulges (Fig. 6). Vacuolization is not as severe as in lipoid nephrosis. Protein absorption droplets may be found. They have no relation to mitochondria. In cases which progress and develop the nephrotic syndrome, epithelial changes are more marked (Fig. 8).

Lipoid nephrosis of children

General

The glomeruli of children with early lipoid nephrosis appear to be normal under the light microscope. Those of children with a history of several years' duration may show a prominence and

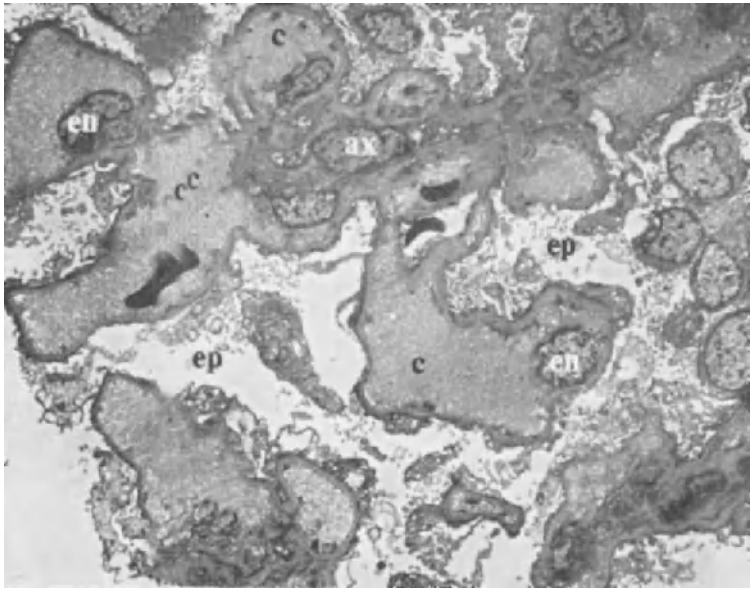


FIG. 9 (1,260 × 2·3) = (× 2,900).

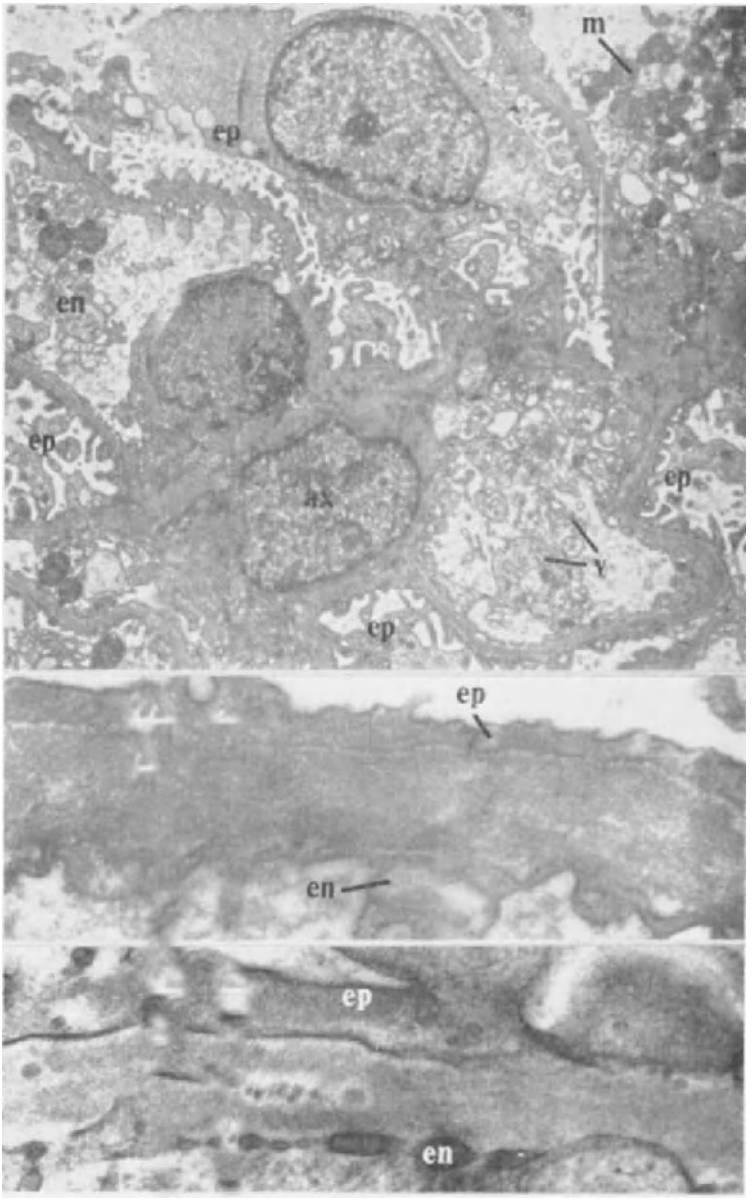
"reticulation" of the axial region. Low-magnification electron micrographs of early cases are characteristic inasmuch as the epithelium shows marked alterations. One has the impression that its cytoplasm is broken up into numerous tiny fragments (Fig. 9). Somewhat similar changes may be encountered, in some cases, in the endothelium (Fig. 10). We have previously referred to this as "villous hypertrophy" of the endothelium (Steiner *et al.*, 1961).

Basement membrane

Unlike other investigators (Farquhar, 1959; Farquhar, Vernier and Good, 1957*a* and *b*) we always encountered slight *diffuse alteration* of the basement membrane in early cases and have referred to these alterations as "Quellung", i.e., swelling with imbibition and irregular rarefaction (Steiner *et al.*, 1961*b*; Movat, 1960*b*). Figs. 11 and 12 show such examples. Fig. 11 is from a section stained with phosphotungstic acid, and Fig. 12 is from material fixed in potassium permanganate. One can observe rarefied areas in the substance of the diffusely swollen basement membrane.

Figs. 13–16 are from cases with lipoid nephrosis of several years' duration who had several remissions as a result of steroid therapy. The basement membrane shown in Fig. 13 was stained with lead hydroxide. Although the inner third is less homogeneous than the outer third, one cannot clearly demarcate the two parts. However, in Fig. 14, from a section stained with phosphotungstic acid, one can separate the lamina densa from a less dense inner band. The inner band is either a swelling of the lamina rara interna or an accumulation of material in that region.

In contrast to the diffuse changes described above, there are also *focal changes* which are found between the endothelium and the lamina densa. These occur in early and, more frequently, in long-standing cases. The changes consist of accumulations of an electron-dense material of approximately the same density as the



10

11

12

FIG. 10 (2,400 × 2·2) = (× 5,300). FIG. 11 (15,400 × 2·5) = (× 38,500).
FIG. 12 (15,400 × 2·5) = (× 38,500).

13

14

15

16

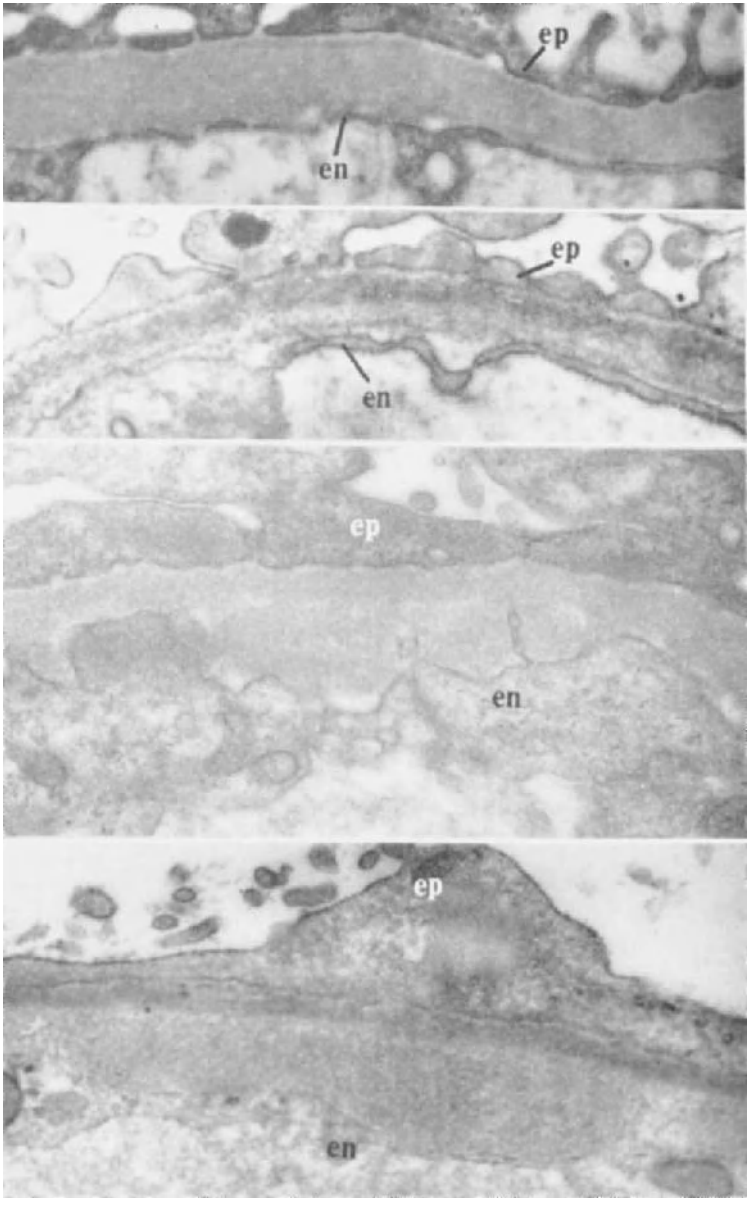


FIG. 13 (12,000 × 2·8) = (× 33,600).
FIG. 14 (12,000 × 3·1) = (× 37,200).
FIG. 15 (9,600 × 3·5) = (× 29,800).
FIG. 16 (11,000 × 3) = (× 33,000).

basement membrane (Figs. 15 and 17), but when stained with phosphotungstic acid they often appear less dense (Fig. 16). (Figs. 15 and 16 are from cases which had progressed after several years to chronic glomerulonephritis. However, they still showed marked fusion of foot processes characteristic of lipoid nephrosis.) The nature of this material is obscure. It may, as we have suggested before, represent antigen-antibody complexes (Steiner *et al.*, 1961*b*). It may represent other normal or abnormal substances derived from the blood, e.g., aggregated platelets, various macroglobulins. It may also represent an abnormal product of endothelial and axial cells. Whatever hypothesis is put forward to elucidate the pathogenesis of idiopathic nephrosis of children, the nature of this material will have to be explained.

Cellular changes

Axial cells are often encroached upon by an electron-dense material (Fig. 17). This may be accumulated material akin to the one described above, or it may be merely an increase of the basement membrane tags which normally anchor these cells to the basement membrane and separate them partly from endothelial cells (Movat and Steiner, 1961).

Endothelial cells may appear normal. They may show a villous hypertrophy (Fig. 10) and they may contain giant mitochondria (Figs. 10 and 18).

Epithelial cells show profound changes, particularly in early untreated cases (Fig. 9). In the main cytoplasmic mass and in trabecula one encounters vacuolization, swelling of mitochondria, homogenization of the peripheral parts of the cytoplasm, accumulation of lipid and of protein absorption droplets (Fig. 19—osmium tetroxide and phosphotungstic acid; Fig. 20—potassium permanganate). Structures which have been referred to as osmiophilic bodies (Folli *et al.*, 1958) are seen at the periphery of the cells (Figs. 9, 19 and 20). These are either true villi, or have developed from ruptured vacuoles. Some of these changes indicate an in-

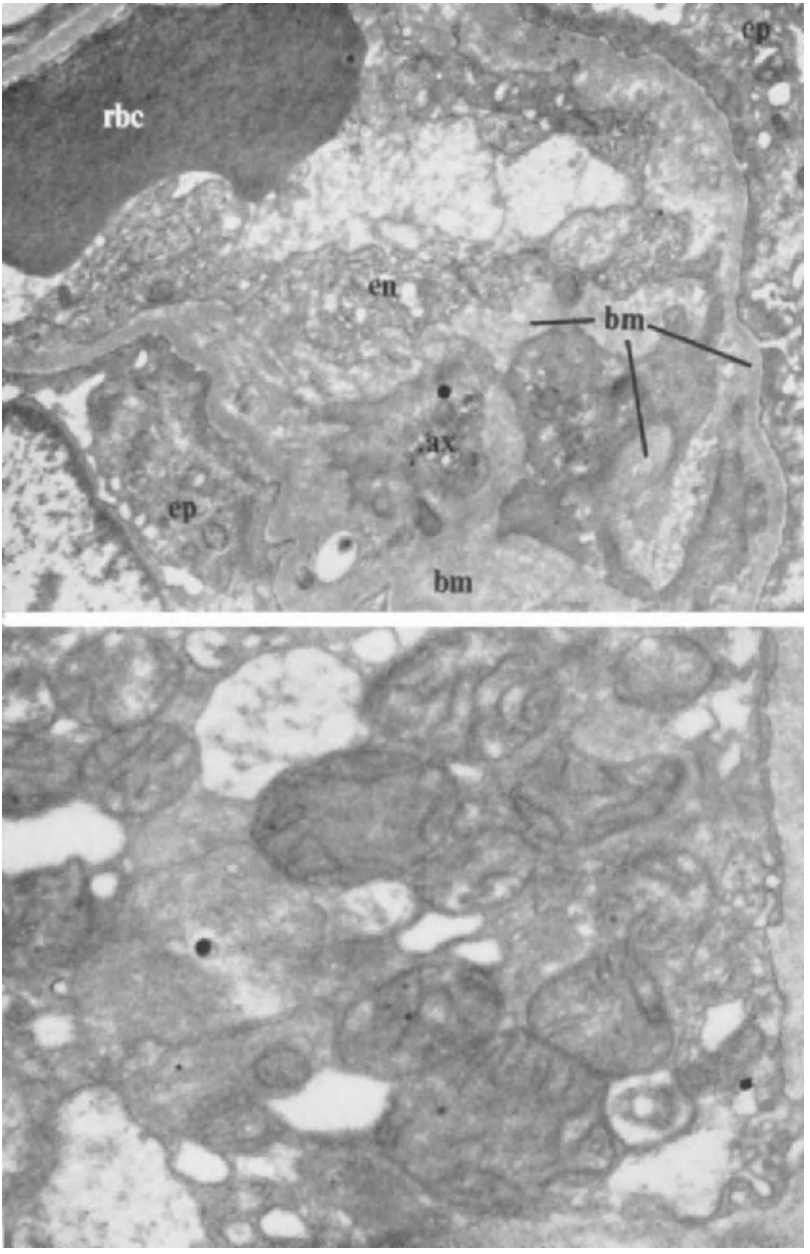


FIG. 17 (*top*) (5,000 × 2·8) = (× 14,000).
FIG. 18 (*bottom*) (7,100 × 4·1) = (× 29,100).

creased hydration of the epithelial cell and represent, in our opinion (Movat, 1960*b*; Steiner *et al.*, 1961*b*), just like the fusion of the foot processes, secondary alterations due to increased permeability of the basement membrane.

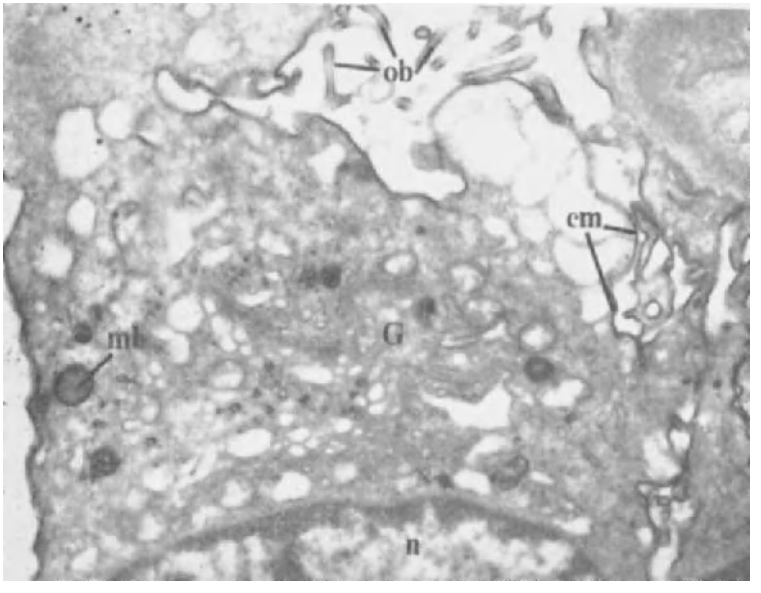


FIG. 19 (9,000 × 2·2) = (× 19,800).

The fusion of the foot processes, first described by Farquhar, Vernier and Good (1957*a*), is characteristic, though not pathognomonic for lipoid nephrosis. We have encountered it to some degree in acute glomerulonephritis, in amyloidosis (Movat, 1960*a*) and in severe lesions of nephrotoxic nephritis (Movat, McGregor and Steiner, 1961). It has also been reported in aminonucleoside nephrosis (Feldman and Fisher, 1959), and quite recently in experimental hypertension (Geer, 1961). It is most severe in early untreated cases. Contrary to what has been stated previously (Movat, 1960*b*; Steiner *et al.*, 1961*b*), if a large enough group is

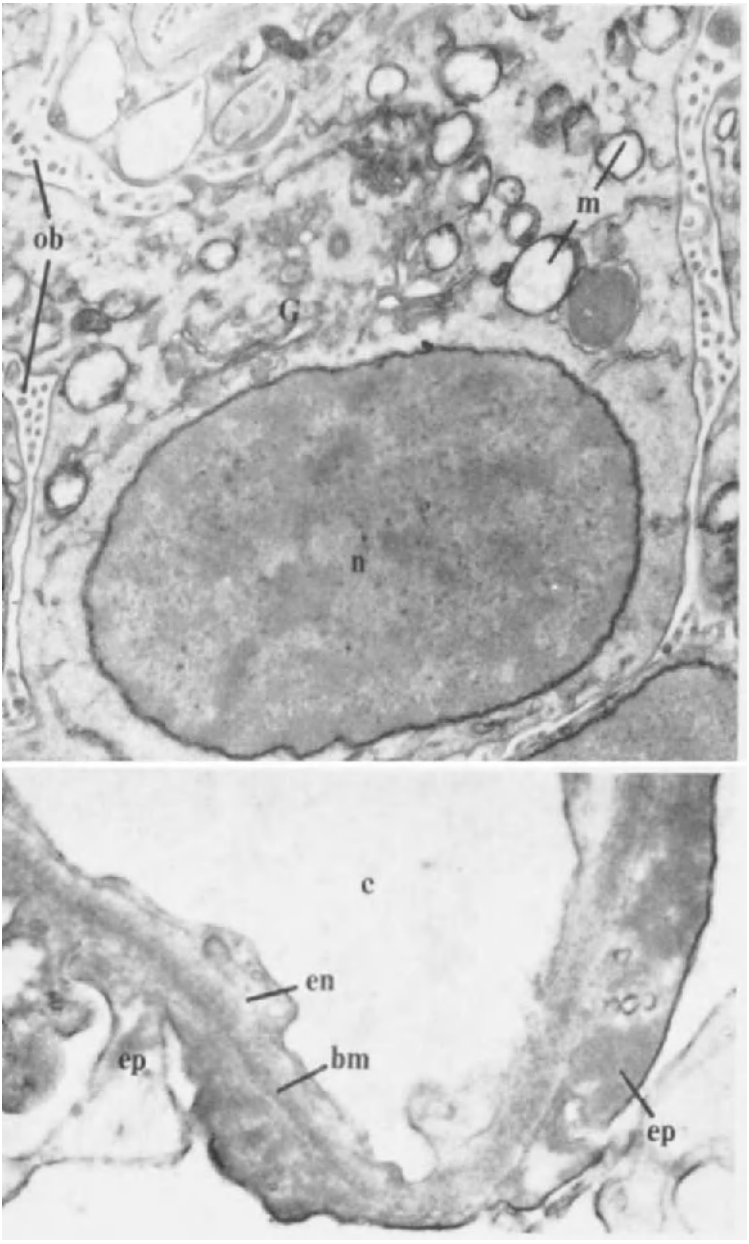


FIG. 20 (*top*) (5,700 × 2·2) = (× 12,500).
FIG. 21 (*bottom*) (9,000 × 3) = (× 2,700).

examined, there is a definite improvement of this change following treatment with steroids. However, certain cases which we examined did not respond well to steroid therapy and we believe that steroids influence the permeability of the basement membrane and only secondarily the foot process alteration. Figs. 11–17 show varying degrees of foot process fusion. Fig. 21 also shows that there may be an increase in density of the cytoplasm over markedly fused foot processes, presumably due to imbibition with protein. (Note that despite the sharp demarcation of the outer cell membrane, the inner cell membrane and the basement membrane are blurred.)

Membranous glomerulonephritis

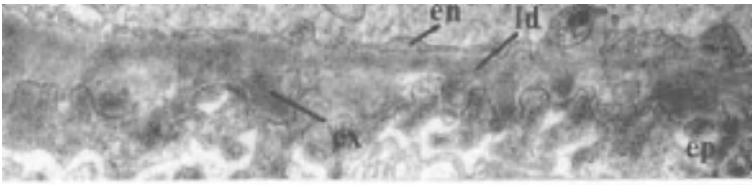
Membranous glomerulonephritis in adults is usually associated with the nephrotic syndrome, and the clinical picture resembles that of children. However, most, if not all, cases progress eventually to chronic glomerulonephritis.

The intracapillary changes are similar to those of lipoid nephrosis in children.

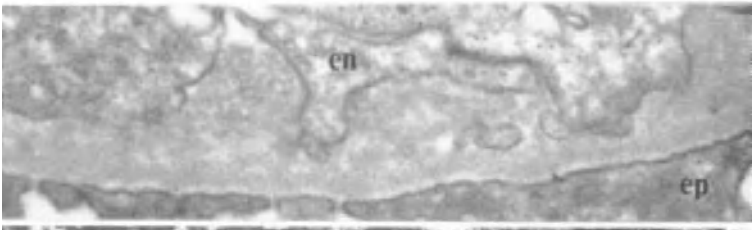
The pathognomonic lesion is the accumulation of an acidophilic material between the basement membrane and the epithelial cells. This material separates the partly or completely fused foot processes from the basement membrane. We have previously stated that this material is probably exuded plasma protein and develops following increase in the permeability of the basement membrane (Movat and McGregor, 1959). We are as yet unable to answer the question as to why this material accumulates in the adult and not in the child. We wonder if the epithelial cell of the adult may be metabolically less well equipped to adapt itself and handle the large quantities of protein passing through the altered basement membrane, which there inspissates and accumulates between the basement membrane and the epithelium.

While it is true that the apparent thickening of the basement membrane in the light microscope is due mainly to accumulations

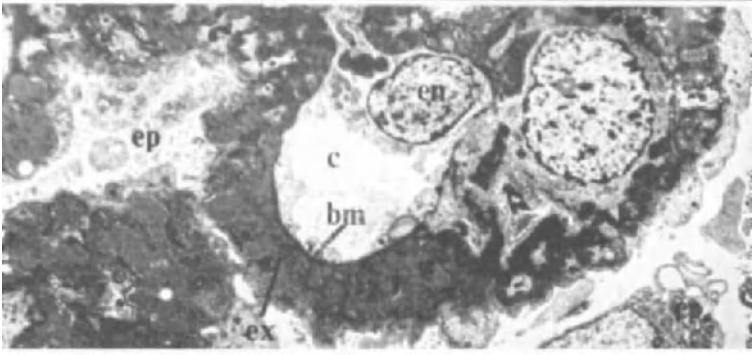
22



23



24



25

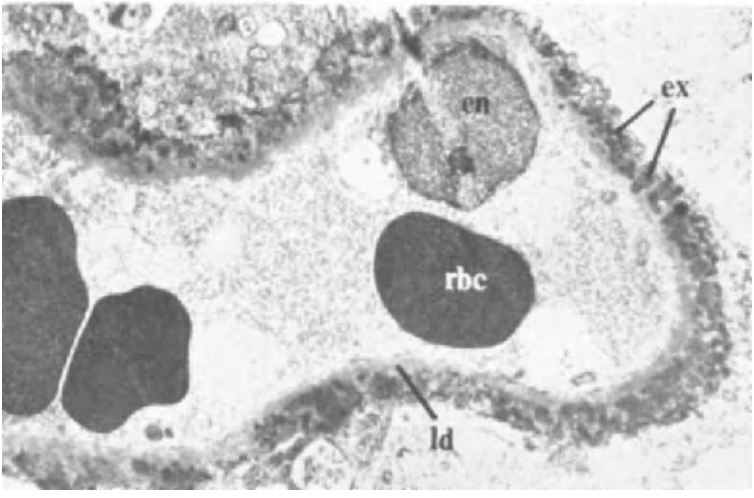


FIG. 22 (× 9,600).
FIG. 23 (× 18,270).
FIG. 24 (× 2,100).
FIG. 25 (× 2,700).

of material outside the basement membrane, contrary to previous statements (Movat and McGregor, 1959), we often find a swollen, i.e., thickened basement membrane in electron micrographs.

Figs. 22 to 25 are examples of membranous glomerulonephritis. Figs. 24 and 25 show large quantities of exuded material between the basement membrane and the epithelium; Fig. 22 shows a milder though similar change. In Figs. 23 and 24 there is accumulation of a dense precipitate inside the basement membrane and between intracapillary cells, similar to what is seen in lipoid nephrosis in children.

Chronic glomerulonephritis

General

The lesions of chronic glomerulonephritis are characterized by an increasing degree of occlusion of glomerular capillaries. In the relatively early phase (Fig. 26) of this process, narrowing and

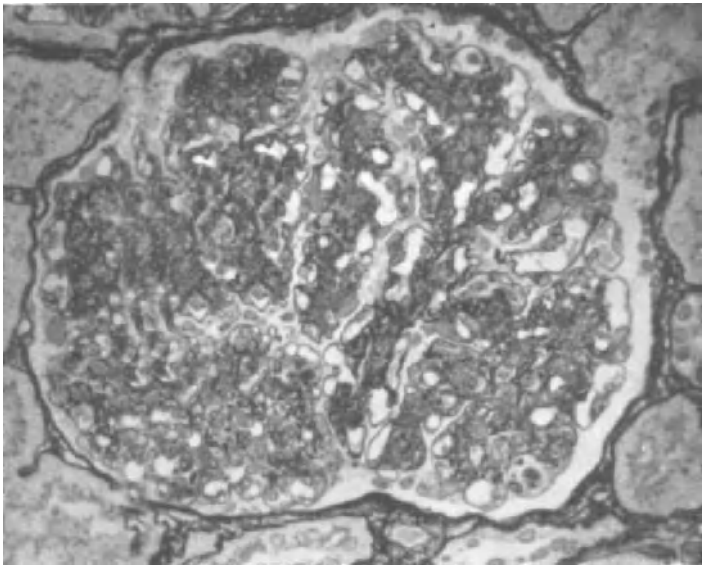


FIG. 26 (× 390).

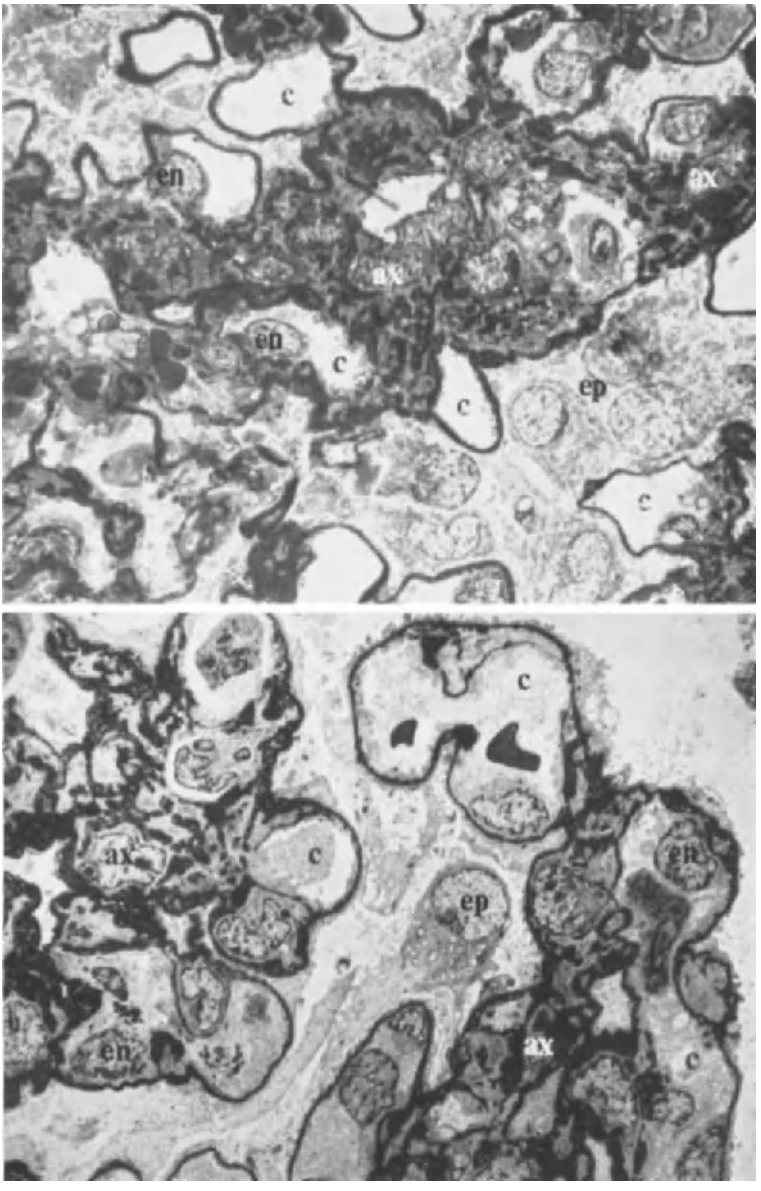


FIG. 27 (*top*) (× 1,200).
FIG. 28 (*bottom*) (400 × 3·2) = (× 12,800)

capillary occlusions occur in those capillaries which lie in close proximity to axial regions. The process at this stage leaves those capillaries which lie in the marginal zones of the axial region relatively intact and patent (Fig. 26, upper right). The low-power electron micrographs (Figs. 27 and 28) illustrate this axial reaction. In the latest phase of development of this disease even the peripherally located capillaries become occluded, producing the picture of an avascular glomerulus, which in conventional haematoxylin and eosin sections is seen as a hyalinized mass with a variable number of cells trapped or enclosed within the amorphous intracapillary eosinophilic material.

It is the nature of this material and the mechanisms which lead to its aggregation in the glomerulus, which form the enigma in attempts to explain the morphogenesis of this disease.

The extracapillary (crescentic) and ischaemic changes are considered to be entirely secondary to the intracapillary happenings and their study offers little reward in attempts to explain the fundamental processes of this disease.

Intracapillary changes

These are interpreted as consisting of two separate processes.

(1) The increased prominence of axial regions is due to an increase of both axial cells and of an electron-dense, argentophilic, basement membrane-like material (Figs. 27 and 28). This cellular proliferation and the accumulation of amorphous material lead to a widening of the normally constricted waist between capillary loops. Further, those capillaries which lie in the midst of axial regions become concentrically narrowed. Those capillaries which lie at the margins of axial regions become narrowed eccentrically, as a result of encroachment of the axial region into this capillary space.

(2) In addition to encroachment by the above process, capillaries become narrowed through hypertrophy and hyperplasia of endothelial cells. To this is added a deposition of an electron-

dense, argentophilic material in areas where evidence of its origin from the axial regions cannot be proven.

These two processes lead ultimately to obliteration of capillary lumina, to the point where the derivation of the material (axial or endothelial) can no longer be determined (Fig. 29). In rapidly

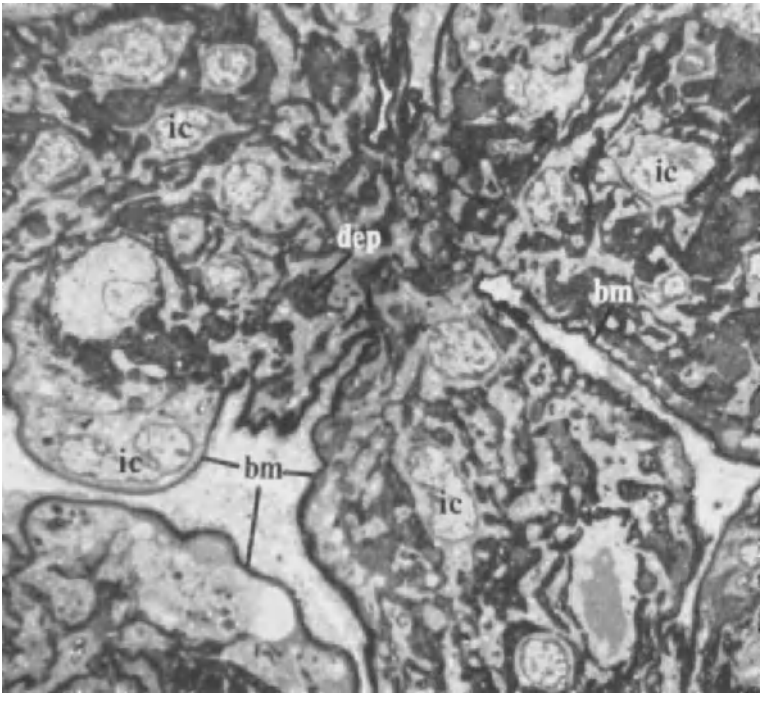


FIG. 29 (400 × 3·2) = (× 12,800).

developing cases the deposition process (between endothelial cells and between these cells and the basement membrane) predominates. The above changes are encountered in all forms of chronic glomerulonephritis, whether they develop insidiously (Fig. 27), from lipoid nephrosis (Fig. 28) or from acute glomerulonephritis (Fig. 29). Thus the crux of the findings reported in this paper lies

in the demonstration of an *additional material* on the luminal side of the basement membrane and between intracapillary cells.

Basement membrane

The basement membrane is thickened both diffusely and focally (Figs. 30 and 31). The diffuse thickening is similar to the one in lipoid nephrosis, but the overlying epithelium shows less fusion of the foot processes. The thickening is at times very marked. In Fig. 30 the basement membrane measures 4800 Å. A possible mechanism

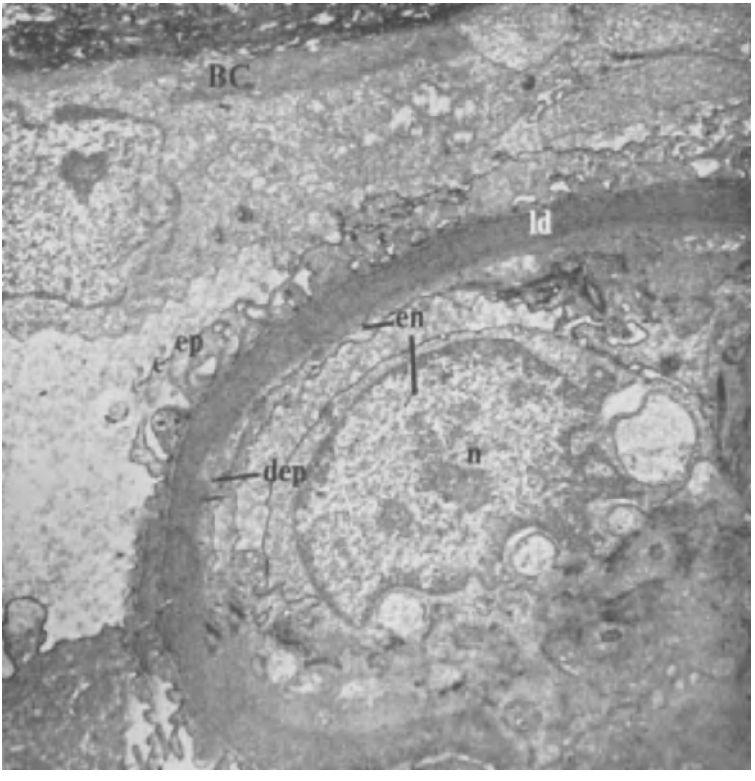


FIG. 30 (5,600 × 1·7) = (× 9,500).

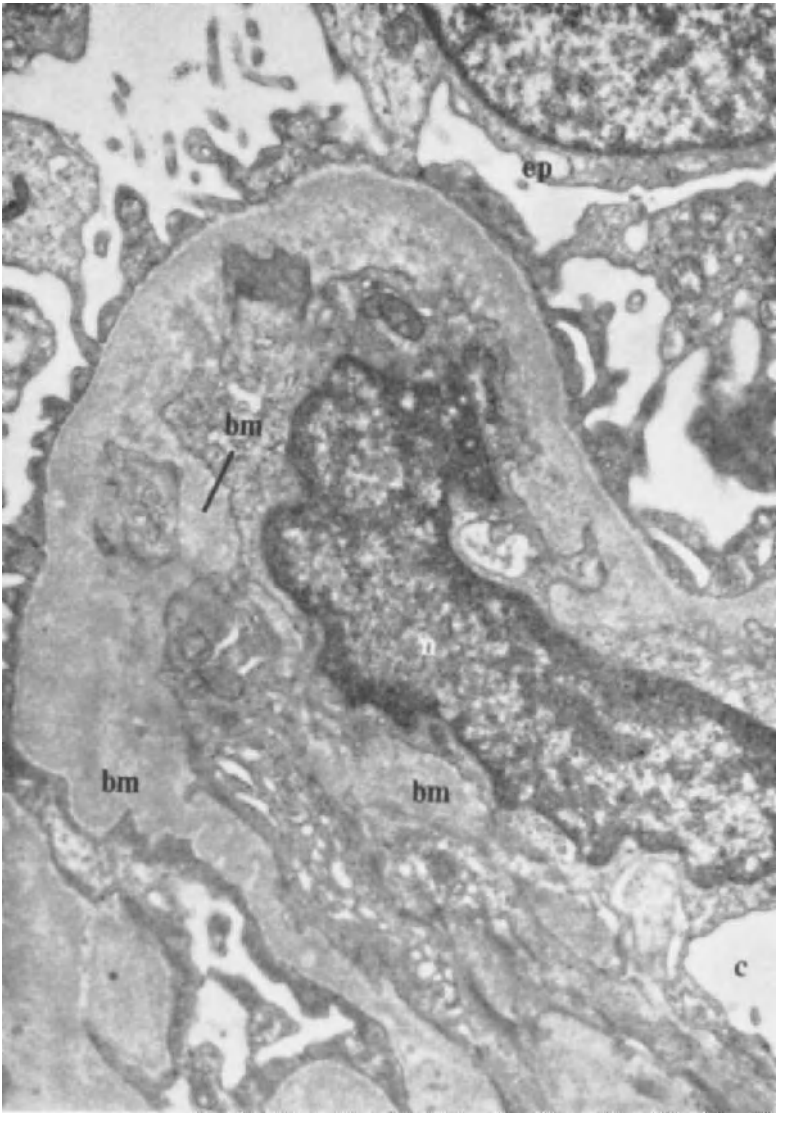


FIG. 31 (× 18,240).

which has to be taken into consideration in the development of a diffuse thickening of the basement membrane in chronic glomerulonephritis is the constant leakage of protein through the altered membrane. Is it possible that some of this protein remains entrapped and becomes eventually incorporated into the basement membrane?

The focal thickening is more likely an addition of material from the luminal side and is often continuous with material in the axial region (Fig. 31).

Cellular changes

We are presently studying the cellular alterations of chronic glomerulonephritis and the results will be described in another publication (Steiner *et al.*, 1961*a*). Axial cells and endothelial cells proliferate as described above. We have often noted that the attenuated portion of endothelial cells becomes continuous and devoid of pores (Fig. 30). Epithelial alterations seem to parallel the degree of altered permeability of the basement membrane.

CLINICO-PATHOLOGICAL CONSIDERATIONS

Our purpose has been to determine, on the basis of 112 biopsies from children with diffuse renal disease, whether any correlation exists between the various alterations in glomerular structure and renal function. Function has been assessed by observing the natural course of the disease, the clearances of creatinine and urea and the quantitative and qualitative characteristics of proteins being excreted in the urine.

Methods

The composition of the urinary proteins, after concentration *in vacuo* to approximately 6 per cent, has been defined by electrophoretic fractionation in a starch gel supporting medium using borate buffer pH 9·15, 0·025 M. The starch gel analysis of Smithies (1959) permits visual identification of proteins with a

spectrum of molecular sizes and weights particularly pertinent to considerations of renal permeability: albumin, mol. wt. 70,000; transferrin, 90,000; γ-globulin, 160,000; ceruloplasmin, 250,000; haptoglobins (type 2), 400,000; and α_2-macroglobulin, 850,000.

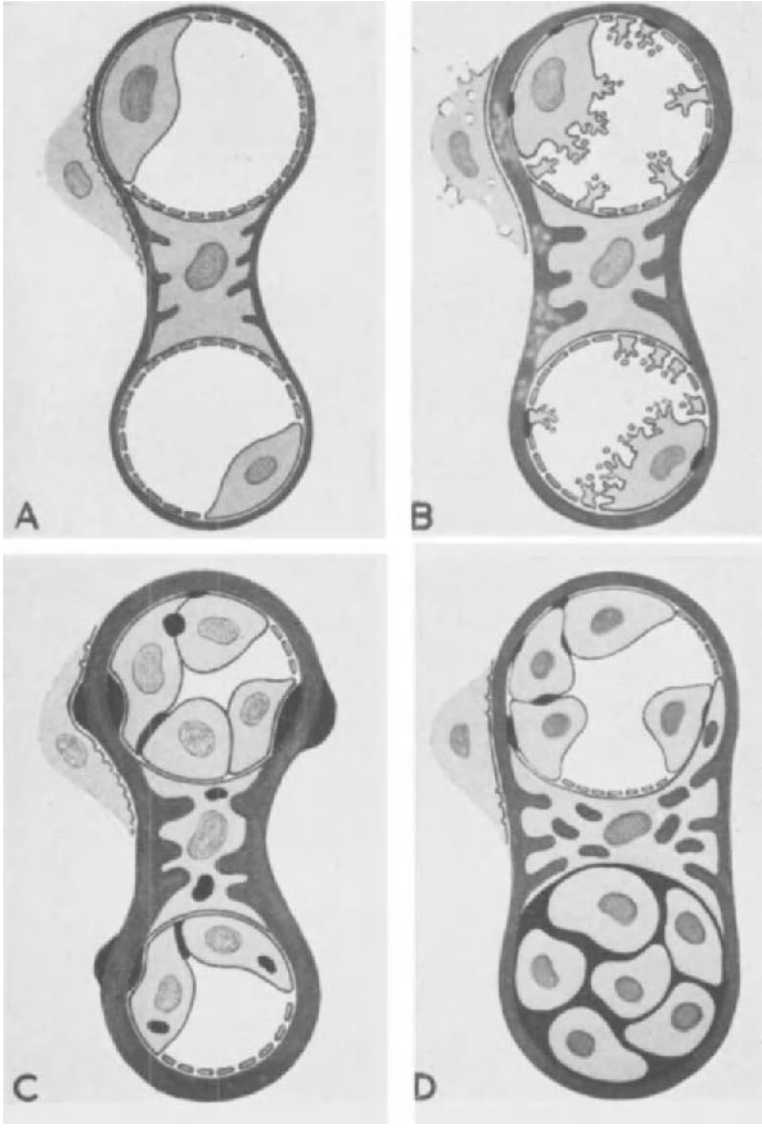
In selected patients, single as well as sequential determinations of the proportion of γ-globulin relative to albumin being cleared from blood to urine have been made by immunological precipitin analyses using specific rabbit antisera.

Interpretation of results

Attempts to correlate glomerular structure and renal function are grossly limited by at least two major factors. Firstly, electron microscopy at high magnification permits assessment of only infinitesimal fractions of the total filtering area. The clinical and biochemical measurements of renal dysfunction, however, reflect a composite of the total derangement of all filtering surfaces. Secondly, the composition of proteinuria is probably controlled by factors affecting tubular reabsorption as well as glomerular filtration. In our interpretations we have made the assumption, based on the results of protein clearances in patients with nephrosis during albumin infusions reported by Hardwicke (1955), and substantiated by us (1961), that glomerular filtration determines the constitution of proteinuria and that tubular reabsorption of the filtered proteins is non-preferential and competitive, being proportional to their concentration in the glomerular filtrate.

It is apparent from the foregoing morphological descriptions that glomerular structures respond in only a limited manner to a wide variety of pathological stimuli. For purposes of interpretation, these limited alterations may be represented in a simplified schematic form.

Children appear unique in that two types of reversible renal disease occur commonly in almost pure form: idiopathic lipoid nephrosis and acute haemorrhagic glomerulonephritis. At the other extreme of the spectrum lies the condition of advanced


A
B
C
D

chronic nephritis, which appears to be the final common pathway of all types of progressive diffuse glomerular disease. In these three areas there appears to be good correlation between structure and function. Between these three extremes of Bright's disease lie many intermediate entities in which correlation, at present, is not attainable. Although these have many morphological, clinical and biochemical features in common with the pure forms, they are nevertheless much less clearly defined as specific pathological entities.

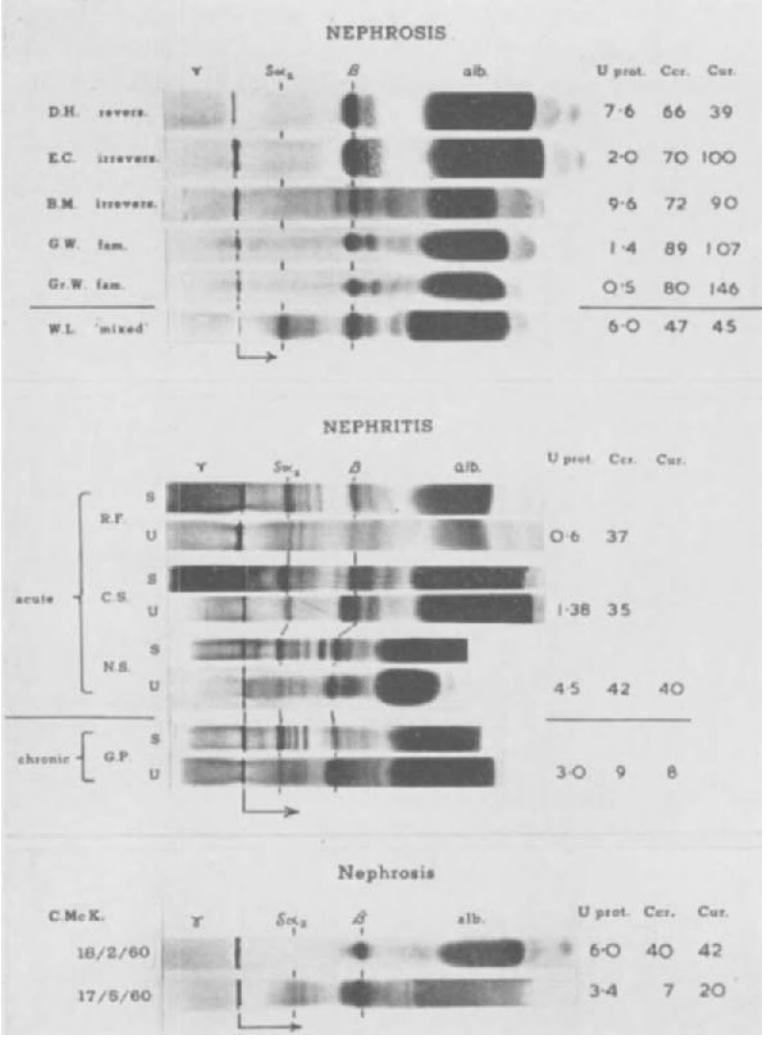
A schematic interpretation of a normal glomerulus is presented in Fig. 32A. Particular attention is directed to (a), the narrow, constricted "waist" of the connecting channel areas and (b) the filtration barrier. This barrier, which determines much of the functional integrity of the kidney, is composed of all three layers

FIG. 32. A: Schematic drawing of normal glomerulus showing endothelial cells with their attenuated fenestrated cytoplasm, epithelial cells with regularly spaced foot processes abutting on to the basement membrane and axial cells located in the waist portion between two capillary loops. The basement membrane is of even thickness and its anchors are seen entering the axial region.

B: Lipoid nephrosis. The basement membrane is diffusely but irregularly thickened. Moth-eaten areas may be seen in this widened structure. Focal deposits are present between the basement membrane and the endothelium (black in diagram). The endothelium shows focal villous hypertrophy. The epithelial cell foot processes are fused.

C: Acute glomerulonephritis. The basement membrane is diffusely thickened. The anchors of the basement membrane in the axial regions participate in this change. On the luminal side of the basement membrane, deposits of a dense nature (black in diagram) are present in juxtaposition to the basement membrane, between and possibly within endothelial cells and in axial regions. The endothelial cells are massively proliferated. Focal exudates are present outside the basement membrane and the epithelium fused over these areas.

D: Chronic glomerulonephritis. The lumina of the capillaries are partially or almost totally occluded by proliferated endothelial and axial cells. The waists between capillary loops are widened. Deposits of a dense material (black in diagram) are present both in juxtaposition to the basement membrane and between endothelial cells. The basement membrane is diffusely thickened, a condition in which the axial-region anchors participate. There is an increase of basement membrane-like material in the axial regions. Epithelial foot processes are partially fused.


A
NEPHROSIS
γ
α2
β
alb.
U prot.
Ccr.
Cur.
D.H. revers.
7·6 66 39
E.C. irrevers.
2·0 70 100
B.M. irrevers.
9·6 72 90
G.W. fam.
1·4 89 107
Gr.W. fam.
0·5 80 146
W.L. 'mixed'
6·0 47 45
B
NEPHRITIS
γ
α2
β
alb.
U prot.
Ccr.
Cur.
acute
R.F.
S
U
0·6 37
C.S.
S
U
1·38 35
N.S.
S
U
4·5 42 40
chronic
G.P.
S
U
3·0 9 8
C
Nephrosis
C.McK.
γ
α2
β
alb.
U prot.
Ccr.
Cur.
18/2/60
6·0 40 42
17/5/60
3·4 7 20

of the capillary wall: the endothelium with its lamina fenestrata, the triple-layered basement membrane and the epithelium. The intracapillary cells are shown subdivided into two groups, (a) the "endothelial" cells, the attenuated portion of which is the lamina fenestrata, and (b) the "axial" cells.

Idiopathic lipoid nephrosis is one extreme of early "pure" renal disease. By conventional light microscopy it may be impossible to define structural alterations in the glomeruli. However, the characteristic changes defined by electron microscopy are depicted schematically in Fig. 32B. Although capillary luminal obstruction is not nearly as marked as in acute glomerulonephritis, some slight increase in the width of the filtration barrier does occur. This is characterized by a variable degree of villous hypertrophy of the endothelial cells. In addition, irregular mild "Quellung" combined with focal loss of electron density, described as a moth-eaten appearance, affect the basement membrane.

Associated with these structural changes are the characteristics of disturbed function depicted in Fig. 33A. Not only are the

---

FIG. 33. This illustrates the pattern of urinary and serum proteins in comparison to other reflections of renal function (S—serum; U—urine; revers.—reversible; irrevers.—irreversible; fam.—familial nephrosis).

The restrictive pattern of the upper 5 patients with nephrosis (group A) is not correlated with reversibility of the disease. The two children with familial nephrosis are asymptomatic but have irreversible biochemical changes. Haptoglobins, type 2, are apparent in pattern B.M. but no slow α_2-macroglobulin. This finding suggests the earliest change toward non-restrictive proteinuria. W.L., with "mixed" nephritis (a combination of nephrosis with nephritis), clearly reveals a non-restrictive pattern.

The non-restrictive patterns of acute and chronic glomerulonephritis (group B) are indistinguishable and give no hint of the stage of the disease. The concentration of γ-globulin relative to albumin is quite variable, as noted also in Table II.

U prot. = protein excretion, g. per 24 hr.
C cr. = creatinine clearance, ml./min./m.^{2} (normal 63–88)
C ur. = urea clearance, per cent (normal 75–125 per cent)

In Fig. 33C the two patterns illustrate the change from a qualitative restrictive to non-restrictive type of proteinuria associated with deterioration of the clinical state in a child with the nephrotic syndrome.

clearances normal to hypernormal, but the quantitative excretion of protein, although variable, is frequently massive. Qualitatively the pattern can be considered restrictive in nature, with the proteins of smaller molecular weight—albumin, ceruloplasmin, transferrin and γ-globulin—being the chief components. No detectable amounts of haptoglobins, α_2-macroglobulin or lipoproteins are excreted.

Two other major points are evident in Fig. 33A. Blainey *et al.* (1960), studying adults with the nephrotic syndrome, have demonstrated a relation between the lack of responsiveness to steroid therapy and the urinary excretion of plasma proteins of greater molecular size. This has not been our experience in the early phases of lipoid nephrosis in children. Even when clinical evidence, e.g., lack of response to steroid therapy, indicates the progress to irreversibility, no early indication that this stage has been reached may be evident from urinalyses, the clearances or the electrophoretic analyses of urinary protein.

Secondly, the restrictive nature of the protein pattern occurs independently of a fairly wide variation in protein excretion. However, while the composition of the proteinuria may remain restrictive, the relative concentration of constituent proteins, as well as their clearances, may change during alteration in renal function from relapse to remission. Thus, as shown in Table I,

Table I

Proteinuria of nephrosis

Date	*U. prot. V.* *mg./min.*	*U. alb.* *mg./ml.*	*U. γ-g.* *mg./ml.*	$\frac{U.\ \gamma\text{-}g.}{U.\ alb.}\%$	$\frac{C.\ \gamma\text{-}g.}{C.\ alb.}\%$
3/5/57	3·83	3·660	0·057	1·6	2
5/5/57	2·36				
7/5/57	0·74	0·250	0·006	2·4	4
10/5/57	0·09	0·060	0·003	5·0	9
13/5/57	0·08				
15/5/57	1·17				
17/5/57	12·12	10·100	0·150	1·5	2

U. prot. V. = protein excretion; U. alb. = albumin excretion; U. γ-g. = γ-globulin excretion; C. γ-g. = γ-globulin clearance; C. alb. = albumin clearance.

patient D.H. progressed from relapse to spontaneous remission and back to relapse of the nephrotic syndrome within a period of 14 days. Decreasing proteinuria was associated with an increase in the proportion of γ-globulin relative to albumin cleared out of the blood into the urine, and vice versa. This experience was borne out in 9 out of 13 studies carried out on 9 patients.

The significance of these variations in the renal handling of discrete proteins is not clear. The changes in the relative permeability of the kidney to the different moieties, which may occur during alterations in the general permeability to protein, could be explained by selective changes in the glomerular filtration barrier, by alterations in the selective tubular reabsorption or possibly even by alterations in the charge or other physical attributes of the specific proteins of the plasma.

Acute glomerulonephritis is the other extreme of early "pure" renal disease. The most significant changes are illustrated in Fig. 32C. The filtration barrier is greatly thickened by reactivity involving chiefly two layers: a diffuse irregular thickening of the lamina densa of the basement membrane and, in particular, a massive hypertrophy and hyperplasia of the intracapillary cells. The significance and identity of the accretions (deposits) of electron-dense material on the inside of the lamina densa and of the exudates outside it have not been defined.

The functional alterations associated with these morphological changes are depicted in Fig. 33B. The decreased capacity of the capillary lumen probably accounts for the decreased renal blood flow and for the decrease in rates of glomerular filtration reflected by the depressed creatinine clearances. Despite the widened filtration barrier, the urinary proteins excreted in the three typical examples shown here contain not only the smaller molecules up to a molecular weight of 160,000, e.g., albumin, transferrin and γ-globulin, but also large moieties, the type 2 haptoglobins and α_2-macroglobulin. In view of the qualitative similarity of the

pattern of urinary proteins to that of serum, the proteinuria of nephritis may be considered non-restrictive in type. In this non-restrictive proteinuria, a considerable variation in the quantity of protein excreted may occur. In certain patients with acute nephritis the amounts may reach proportions which are normally associated with a nephrotic state. However, the data in Table II

Table II

PROTEINURIA OF ACUTE NEPHRITIS

Patient	*U. prot. V.* *g./24 hr.*	*C.* $\frac{\gamma\text{-}g.\%}{C.\ alb.\%}$	*Oedema*	*Haematuria*	*C. creat.* *ml./min./m.²*
R.F.	0·6	47	0	tr.	28
C.S.	1·4	14	face	+	35
T.G.	3·6	30	general	macro.	20
N.S.	4·5	9	0	macro.	42

reveal that the clearance of γ-globulin relative to that of albumin varies from one patient to another during the acute phase of nephritis. These changes do not appear to bear a relationship to the quantity of proteins being transferred across the barrier nor to the creatinine clearance. Indeed, the presence of fairly large amounts of proteinuria was not necessarily associated with oedema.

These wide variations in the relative amounts of γ-globulin and albumin excreted, when compared to the approximate amount of haematuria, indicate that a simple leak of blood plasma along with the red cells does not account for the proteinuria of acute nephritis. If these proteins are of glomerular origin, then increased permeability of the filtration barrier must occur to account for the findings.

The third extreme of renal disease is the picture presented by advanced chronic nephritis. Fig. 32D depicts schematically the widening of the filtration barrier due to a diffuse irregular thickening of the basement membrane, endothelial cell proliferation of a massive nature, and a variable degree of fusion of the epithelial cell foot processes. The reactive increase of the axial cells

produces not only a widening of the "waist" area between the capillary loops but also contributes substantially to the constriction of capillary lumina.

As occurs with the obstructive changes affecting the capillary loops in acute nephritis, the protein excreted in chronic nephritis is non-restrictive in nature as depicted in Fig. 33B and C. Progress of pure lipoid nephrosis to chronic glomerulonephritis is associated with an alteration in the characteristics of the protein composition of the urine. As the clearances of patient C.McK. decreased, the restrictive nature of the protein pattern became altered to the non-restrictive type. In the pattern of W.L. in Fig. 33A a similar change is seen associated with signs of failing renal function.

It will be apparent from the above considerations that the barrier between the lumen and Bowman's space is less in the nephrotic than in the nephritic state, yet there is an obvious limitation in the size of the molecules escaping in nephrosis in contrast to the large quantities of proteins being transferred across the barrier. These findings suggest that the permeability of the filtration barrier is not so much a function of its thickness as of its constitution. Although the filtration barrier has been considered as a unit, it is quite possible that its individual components may each influence both the quantity and quality of the filtrate. Furthermore, the size of the lumen, as well as the rate of plasma flow through the capillary, have a bearing on the rate of diffusion of the constituent macromolecules across the semi-permeable filtration barrier (Pappenheimer, 1953).

From these observations it has not been possible to relate the degree and nature of the proteinuria with the structural alterations of the foot processes of the epithelial cells. The epithelial alterations appear to be secondary to basement membrane injury. The rôle of endothelial cells in the transfer of plasma proteins across the barrier remains obscure. We are inclined to believe that the decisive factor determining the composition of the

proteins in the filtrate is the structural integrity of the basement membrane.

We noted previously that the response of the glomerulus to injury is limited in scope. Electron microscopy has made apparent features which are common, in greater or lesser extent, to all forms of glomerulonephritis. These findings have led us to a unitarian concept of the morphogenesis of Bright's disease, a suggestion which by no means carries any implication of a unitarian aetiology.

The common denominators of all the lesions that we have investigated are a "Quellung" of the basement membrane associated with an accretion of an electron-dense material on its luminal side. These latter changes are associated with a pathological reaction of both types of intracapillary cells.

In the two earliest extremes of Bright's disease, the apparently reversible changes are variable to the extent that the axial reaction is more pronounced in acute glomerulonephritis than in pure lipoid nephrosis. However, when these entities progress toward chronicity, the reaction of axial cells, the production of basement membrane-like material in the axial regions, the massive proliferation of the endothelial cells and the increase of accretions in the free portions of the loop lead to gradual eccentric and concentric occlusion of the capillary lumina. These changes culminate in glomerular obsolescence.

REFERENCES

BLAINEY, J. D., BREWER, D. B., HARDWICKE, J., and SOOTHILL, J. F. (1960). *Quart. J. Med.*, **29**, 235.

FARQUHAR, M. G. (1959). *In* Proc. Xth Ann. Conf. Nephrotic Syndrome, p. 2. Ed. Metcoff, J. New York: The National Kidney Disease Foundation.

FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A. (1957*a*). *Amer. J. Path.*, **33**, 791.

FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A. (1957*b*). *J. exp. Med.*, **106**, 649.

FELDMAN, J. D., and FISHER, E. R. (1959). *Lab. Invest.*, **8**, 371.

FOLLI, G., POLLAK, V. E., REID, R. T. W., PIRANI, C. L., and KARK, R. M. (1958). *Ann. intern. Med.*, **49,** 775.
GEER, J. C. (1961). *Lab. Invest.*, **10,** 51.
HARDWICKE, J., and SQUIRE, J. R. (1955). *Clin. Sci.*, **14,** 509.
MOVAT, H. Z. (1960*a*). *Arch. Path. (Chicago)*, **69,** 323.
MOVAT, H. Z. (1960*b*). *In* Proc. XIth Ann. Conf. Nephrotic Syndrome, p. 228. Ed. Metcoff, J. New York: The National Kidney Disease Foundation.
MOVAT, H. Z., and MCGREGOR, D. D. (1959). *Amer. J. clin. Path.*, **32,** 109.
MOVAT, H. Z., MCGREGOR, D. D., and STEINER, J. W. (1961). *Amer. J. clin. Path.*, **36,** in press.
MOVAT, H. Z., and STEINER, J. W. (1961). *Amer. J. clin. Path.*, **36,** in press.
PAPPENHEIMER, J. (1953). *Physiol. Rev.*, **33,** 387.
SMITHIES, O. (1959). *Biochem. J.*, **71,** 585.
STEINER, J. W., MOVAT, H. Z., YENDT, E. R., and SLATER, R. J. (1961*a*). *Arch. Path. (Chicago)*, in press.
STEINER, J. W., SLATER, R. J., and MOVAT, H. Z. (1961*b*). *Lab. Invest.*, **10,** 763.
VAZQUEZ, J. J., FELDMAN, J. D., DIXON, F. J., and WEIGLE, W. O. (1960). Presentation to the 57th Annual Meeting of the Amer. Ass. Pathol., Memphis.

DISCUSSION

de Wardener: In your Fig. 33C the patient showed a complete change in protein excretion. What were the changes in the biopsy between those two patterns?

Slater: The biopsy taken at the time of the second pattern (17th May, 1960) revealed a mixed picture with some glomeruli showing a minimal increase in cellularity and hyaline change, others showing a variably great degree of both. We have no biopsy taken at the time of the first proteinuria pattern (18th Feb., 1960).

The point I was trying to make is that these children having an irreversible nephrotic syndrome may apparently change from a type of renal dysfunction reflected by restrictive proteinuria to a more advanced stage evidenced by non-restrictive proteinuria. We feel that in some children these changes in the qualitative characteristics of proteinuria may reflect variations in the degree of structural damage, ranging from early minimal glomerular change through to advanced obstructive damage to the capillary loops.

Such were the changes noted in a second child who followed a similar clinical course to the above child. Two renal biopsies taken

seven months apart revealed progressive thickening of the basement membranes and lobular stalk areas of the glomeruli.

However, we need much more experience and feel that one cannot necessarily correlate the qualitative pattern of proteinuria with the observed structural damage in the glomeruli. If all or most of the glomeruli have increased permeability to protein, then the final pattern excreted will be a composite of all the varying degrees of relative permeability to proteins of different size, a function which probably varies from glomerulus to glomerulus or indeed from capillary to capillary as the disease becomes progressively worse

Movat: The only change in the second biopsy was an increase of the electron-dense material between the endothelial cells and between these cells and the basement membrane—"hyaline", as it has been called in light microscopy.

Hardwicke: Did you do light microscopy as well?

Movat: Yes. The first biopsy appeared "normal" by light microscopy. In the electron microscope there were changes characteristic of lipoid nephrosis or idiopathic nephrosis, namely, fusion of the foot processes and patchy accumulation of electron-dense material between the endothelium and the basement membrane. This accumulation was considerably increased at the time of the second biopsy, when it was visible in the light microscope as well.

Rich: What did the second biopsy show under the light microscope?

Movat: Early chronic glomerulonephritis.

Rich: In other words just a gradual progression?

Movat: Yes.

Earle: Were these lesions proliferative?

Movat: Not in the case we are talking about. But there *is* "proliferation" in some cases of lipoid nephrosis, under the electron microscope.

Jennings: Increased numbers of cells?

Movat: Not an increased number of cells, but increase in cytoplasmic mass, that is, increase of endothelial cytoplasm. In some cases we find villous hypertrophy, in other cases a massive hypertrophy of endothelial cells; that is, an increase in size (hypertrophy) rather than numbers (hyperplasia).

Hutt: Could we stick to proliferation as meaning an increase in number of cells? I think most of us agree on that.

Joekes: Surely we shouldn't use the term lipoid nephrosis, which is a clinical term, to describe a histological picture. What the clinician asks of the pathologist is a factual description, whether there is proliferation, whether there is deposition, and so on.

Movat: We seldom make a definite morphological diagnosis on renal biopsies of children with nephrosis, but when we talk to the clinician we may refer to "lipoid nephrosis". I agree it is wrong to do this.

de Wardener: But you are in such a strong position. You are actually looking at something and can describe it. You don't have to make any inferences at all.

Movat: It is at times very difficult to name a condition morphologically. The clinician wants a definite name.

Jennings: We call it "no glomerular disease by light microscopy".

Hamburger: If we are going to communicate with each other we have great need for a term which says in one or two words "optically normal glomeruli with fusion of the foot processes and slight epithelial changes on electron microscopic examination". This could be referred to as "minimal changes".

Wilson: A morphological description only represents the state of affairs when you examine that glomerulus. It does not indicate a pathological process. Different names are given to the same pathological process at different stages, when we really need to know the natural history of the disease.

Hamburger: But we need it for conversation, and there is no dangerous implication in a purely descriptive appellation which summarizes the state of affairs at a particular time.

de Wardener: I think this perpetuates a false assumption—that there is a correlation between histology and what the patient presents.

Jennings: I don't completely agree with that. In general there is a correlation between the objective histological picture that one sees in a biopsy and what patients show, but it may not be the correlation you would like to make.

Bergstrand: Since we are talking about the relationship between morphological changes and clinical symptoms, I would like to say a few words of warning about interpreting what we see under the electron microscope. We have done renal biopsies on healthy subjects

one hour after 1½ litres of water was given *per os*, that is, at the peak of water diuresis. The electron microscope pictures show striking changes in the epithelial cells, swelling and vacuolization, which might easily be interpreted as pathological changes.

I should like also to warn against the use of stains. If a section takes stains, it means that there are free and reactive groups to which we can attach molecules of heavy metals (osmium, uranyl acetate, lead, etc.) up to a certain limit, and after this limit is reached and there are no more free groups that can react, then we get only a deposit of metal on the surface of our structures. I am not quite sure about this staining with silver methenamine. Previous work with silver staining seems to indicate that silver does not react with any reactive groups in the molecules, but is deposited on the surface. Such a deposition of a metal may be quite unequal and may give the appearance of, let us say, a moth-eaten basement membrane. It may be that it clarifies the point sometimes, but it may also obscure the picture to a very great degree.

Jennings: Along this line, Bennett in a paper on the structure of capillaries refers to some experiments done by Luft in his laboratory in which adrenal glands from animals were perfused with different media. There appeared to be extensive changes in the structure of the endothelial cytoplasm, depending on what you perfused the gland with, prior to fixation (Bennett, H. S., Luft, J. H., and Hampton, J. C. (1959). *Amer. J. Physiol.*, **196,** 381). I think that Dr. Bergstrand is correct that the structure of the endothelial cytoplasm is probably a function of the milieu that is present at the time you fix it.

Earle: Dr. Bergstrand, in your diuresis experiment one could point out that the basement membrane, the foot processes and the nuclei were quite normal, whereas in diseases these are the abnormal structures.

Bergstrand: Yes, of course; but the swelling of the epithelial cell is quite "abnormal".

Movat: In what proportion of the glomeruli and in how many capillary loops of glomeruli do you find these changes?

Bergstrand: This investigation is in the preliminary stage. We had two patients with water diuresis and one with mannitol diuresis and have not been able to make an extensive research on many glomeruli. As far as I could see from the first two patients, this is a general phenomenon that you see in all parts of the glomeruli.

Vernier: It doesn't seem quite right to say that a person in mannitol diuresis is perfectly normal.

Bergstrand: I said perfectly "healthy".

Vernier: I am not surprised that you obtain changes in the cells at the time of maximal water diuresis.

Bergstrand: It was not an absolute maximum diuresis, although at the peak for this water load. We could probably have obtained a greater diuresis by giving more water.

Vernier: Are you surprised that this causes some changes in the fine structure?

Bergstrand: Not at all. I just wanted to show that it might be dangerous to interpret similar changes in a patient.

Black: Have you ever given anyone $1\frac{1}{2}$ litres of saline? There would be much more water on board then, and one might get the rest of the changes.

Bergstrand: No, we have not given saline, just tap water.

Rosenheim: Helmholz showed long ago the changes in the tubules which occur during prolonged sucrose diuresis. The tubular cells showed extreme hydropic degeneration, and subsequently returned completely to normal (Helmholz, H. F. and Bollman, J. L. (1940). *J. Lab. clin. Med.*, **25,** 1180).

Kark: May I ask how many glomeruli and how many cuts from each glomerulus should be studied from each individual biopsy before one makes a statement about the pathological changes seen or not seen in electron microscopy? There is a tendency in some places for people to look at one small piece of one small glomerulus and make a diagnosis. I wonder if Dr. Bergstrand, Dr. Vernier, and Dr. Movat could give us some opinion of what should be done before a diagnosis is made?

Vernier: I would remind you of a calculation that D. C. Pease has made (1960. Histological Techniques for Electron Microscopy, p. 13. New York: Academic Press), in which he states that if one works in the electron microscope laboratory at the rate of 50 photographs per day at the lowest magnification of most electron microscopes, about 1500 times, it requires one month of work to cover one square millimetre of surface. Now, if one works at this rather maximum rate for a month and has examined well one square millimetre of surface area,

having a thickness of, let's say, 500 Å, the total cubic area examined is very very small. Since a glomerulus is roughly 80μ in diameter, you could only study a few glomeruli under these conditions, and you would only have studied slices through several glomeruli. So this is obviously always a very very slow and incomplete process. I don't think anybody knows how many is "enough". I attempt to study four glomeruli from each case, and this represents a very small proportion of the two million present in the two kidneys, but even that is a very big job. It is perhaps unwise to emphasize the importance of individual changes in individual glomeruli, and we must stick with observations that are consistent from patient to patient, over multiple glomeruli from these patients, and perhaps in time we will be able to make sense out of this. Although it may be opportune at this time to discuss renal biopsies, I don't believe it is opportune to discuss all of the minor changes in morphology at the electron microscope level—they simply haven't been discovered or adequately described.

Rich: It seems to me what Dr. Bergstrand had in mind was just that —the danger, which is evident in some of the literature dealing with electron microscopy, of saying "Here is an abnormality related to this disease", whereas it might be only an independent functional state, and might happen whenever you drink enough beer.

Bergstrand: We never use the electron microscope for a diagnosis.

Slater: Are we to take a completely defeatist attitude though? Can we hope to interpret any of these changes that we see by electron microscopy associated with fairly well-defined clinical states, or are we going to say that there is no correlation at all?

Pirani: In spite of the extreme limitations of the amount of material that we see, it is remarkable how consistent some of these electron microscopic changes are. My experience has been mainly with three or four areas, toxaemia of pregnancy, diabetic glomerulosclerosis, lupus, and so-called lipoid nephrosis, and the morphological picture, in spite of the limitations, is fairly consistent for each disease. When renal biopsy was first introduced, the same situation existed for light microscopy as well: pathologists felt it was hopeless to even look at such a limited amount of tissue. Still, gradually, we have learned to evaluate these biopsies accurately. Certainly seven or eight years ago I would not have been able to see what I can see now, and I think this

has been the experience of most other pathologists. I don't think it is hopeless at all. I am optimistic.

Rich: What you say about the light microscope in renal biopsy is certainly true. Anyone who has ever worked with it knows that at first it is very difficult; you take so much longer to reach a simple conclusion than you would with a section, because you haven't got the repetition of nephrons in all their variations. However, in time it becomes a sensible procedure.

Darmady: One of the difficulties with biopsies is that you are only examining those nephrons which are nearest the surface. When you dissect them out you find that many of these do not have any loops of Henle at all; they are at the periphery. So that one has a difficulty in interpreting the nephron which does not have a fully developed glomerulus in any case.

Rich: There is an almost insoluble difficulty in dealing adequately with the tubules in a renal biopsy.

Brun: I am afraid I don't agree with you, because in our material we have many biopsies with medullary tissue and occasionally even papillary tissue is seen.

Darmady: It may be that your clinicians are braver than mine. We have been disappointed to find out how few of the nephrons are fully developed.

Joekes: Is it possible that electron microscopy is going to teach us to interpret light microscopy much more accurately, and that perhaps that is the most important added knowledge it is going to bring?

Movat: I think it will come to that. After having seen something in the electron microscope one can in retrospect see it in the light microscope. Before we condemn electron microscopy I would say one thing in favour of it. It has at least contributed to the understanding of the morphogenesis of glomerular disease. While much of it is still speculation, we know more about what is happening in the glomerulus.

Hardwicke: I should like to reinforce the last two speakers on this and say that we should always try to interpret electron microscope studies in terms of light microscopy, not only because that will teach us more but because it will enable the "underprivileged" pathologist without an electron microscope to diagnose renal disease.

I would like to come back to Dr. Slater's functional studies. He

divided his patients into nephrotics and nephritics, and that I regard as being an entirely clinical diagnosis and not a pathological diagnosis. The cases which I described earlier were diagnosed on the basis of a pathological diagnosis, albeit with a light microscope. I was somewhat surprised that he had seen a child who proceeded from what we call nil change on to a chronic glomerulonephritis, because we have not yet seen that progression in the adult. Was there any clinical evidence of a more proliferative lesion, any red cells in the urine? This leads me to my other point, that in my talk I said that in the proliferative group the clearances were very variable and that there is one group in which you can get selective clearances associated with red cells in the urine and other features such as a low serum complement, which suggest that it is a proliferative lesion. So obviously there are a number of parameters to look at.

Dr. Slater showed changes in the γ-globulin clearances as the proteinuria diminished. I can confirm this. In pyelonephritis you find very high γ-globulin clearances and in the late stages of chronic glomerulonephritis the γ-globulin clearance also rises. But I think that the patient he showed was only passing 0·09 mg. per minute of protein, which is getting down to the level of "normal" proteinuria, in which the γ-globulin clearances are very high.

Rich: Dr. Hardwicke, you said that, on the basis of your work with adults, you were surprised that a child with lipoid nephrosis proceeded to "chronic glomerulonephritis". However, in the child, many cases of lipoid nephrosis go on to develop microscopic haematuria, nitrogen retention, high blood pressure, and death in uraemia. Microscopically, the glomeruli show thickened membranes, hyaline deposits, and obliterative sclerosis, not ordinary proliferative glomerulonephritis.

Slater: At least 50 per cent of these children used to die, but the mortality has been reduced by the use of steroid therapy. As I tried to show with the electrophoretic patterns, we cannot define the prognosis in the early stages of childhood nephrosis.

Hardwicke: You mean that on cortisone treatment they will go on from a "minimal change", from an entirely normal glomerulus, to chronic glomerulonephritis?

Slater: I should be cautious about answering this, because we haven't had sufficient experience in following a number of children serially

from the early stages to end stage, but we have certainly seen that in one child.

Rich: You've seen it often clinically, haven't you?

Slater: Yes, we have.

Wilson: There is enough correlation between the clinical course and the histological picture in children with "lipoid nephrosis" to show that eventually, if recovery fails to occur, this condition passes into chronic nephritis. I don't think renal biopsy would show anything in the early stages but "minimal change". One of the great problems is to know whether this development into chronic nephritis is due to a continuous progress of the initial lesion, or whether it is due to a different process from an added infection. Sometimes these patients get an attack of haematuria, or they develop hypertension for the first time following pneumonia (I have seen this happen after ten years of "lipoid nephrosis"); or they may pass insidiously into the chronic stage in which the blood pressure starts to rise, renal function deteriorates, without any evidence of secondary infection. We need to find out whether the chronic course is due to streptococcal infection, virus infection or some other antigen-antibody reaction.

Earle: We had a case that might bear on this. A 34-year-old woman who had been coming to our clinic for eight years had five normal urines up to six months before she suddenly developed the nephrotic syndrome. There had been no preceding infection. The first biopsy, taken eight days after the onset of the nephrotic syndrome, contained 25 glomeruli, all of which were normal by light microscopy. We treated her vigorously with steroids for six months with no improvement. Another biopsy seven months after onset likewise revealed glomeruli which were entirely normal by light microscopy. We discontinued the steroids and treated her with diuretics. She did quite well for the next six months although heavy proteinuria persisted. She suffered several non-streptococcal upper respiratory infections. During the first 24 months 27 urine cultures were sterile. She did occasionally have a few white cells in the urine. But the main symptom was a proteinuria up to forty grams a day. Sixteen months after onset we noticed that she was anaemic and that her blood urea nitrogen had increased. A third biopsy showed membranous glomerulonephritis but no proliferation whatsoever. She was subsequently dialysed several

times on the artificial kidney, and died about a year later. At autopsy, as I recall, the picture was similar to the third biopsy.

Jennings: That is correct: she had granular contracted kidneys.

Earle: In addition, I should like to show briefly our experience in the nephrotic syndrome in adults (Table I). These data are similar to those

Table I (Earle)

ADULTS WITH NEPHROTIC SYNDROME

	No.	*Progressive disease*	*Dead*
No lesion*	9	1	1
Membranous	7	4	2
Proliferative	14	10	4
Miscellaneous	5	5	4
Total	35	20	11

* By light microscopy.

Prof. Hamburger presented. These 35 adult patients had the full-blown classic nephrotic syndrome. Nine of them had no glomerular lesion by light microscopy. The one patient of this group who had progressive disease and died is the one just described. The others all responded promptly to steroid therapy, although two of them still require continuous steroid therapy two years after onset. Seven we have labelled membranous, but this includes some with thickened basement membranes, some with deposits; already four have suffered progressive disease, two are already dead. Fourteen had what we call proliferative glomerular lesions. Ten of these have gone into a progressive decline. In seven of these we have good evidence that their disease followed acute glomerulonephritis, which originally followed streptococcal infections. "Miscellaneous" included such conditions as renal vein thrombosis and Kimmelstiel-Wilson disease.

There were a few clinical differences in addition to the response to therapy. In the "no-lesion" group none had functional impairment in the beginning (the one who died is the only one who has developed renal insufficiency so far) and none had hypertension. Two or three had decreased serum complement, but some of them actually had increased complement. In contrast, the patients in the proliferative group

were the ones who had the most frequent and greatest decreases in serum complement. None in the proliferative group had systemic lupus as far as we could tell. The membranous group likewise did not have much decrease in function nor hypertension at the beginning, but they did progress despite the fact that we treated all of them with steroids; only one had a good response. The most outstanding feature other than the proteinuria in the nephrotic syndrome was that all patients in the first two groups had marked reductions in serum streptococccal antibodies. We tend to separate these diseases rather than lump them together.

Hardwicke: Our experience is very similar to yours, both in numbers and in responses to treatment. We have seen one patient with the progressive form, progressing from a minimal change or nil change to membranous glomerulonephritis, whose clearances progressed from those of minimal change (very selective) to those of membranous glomerulonephritis. He died, after three years, in acute renal failure. He was found to have old thrombosis of both renal veins, a calcified plaque at the opening of one renal vein, constriction in the other, and he had a recent thrombosis in the left spermatic vein. I wondered whether you could be absolutely certain there was no obstruction in the renal veins in your progressive case.

Jennings: I didn't personally observe the autopsy but remember that no such obstruction was recorded.

de Wardener: Dr. Hardwicke, I am surprised you say your series resembles Dr. Earle's, because I thought you found a good response to steroid therapy in the proliferative group in the nephrotic syndrome, whereas Dr. Earle's series shows a high mortality.

Earle: Our mortality in the proliferatives with the nephrotic syndrome is now about 40–50 per cent, and the disease is progressive in most of the remainder. One of our proliferative patients did show a good response to steroid, much to our surprise. It took some six to eight months before he started to improve. Whether it was therapy or whether it was spontaneous we do not know.

Hardwicke: In the proliferative group, we believe we have a response to treatment but, as you say, it takes six to eight months. The only evidence for response is continuing fall in the Addis count of red blood cells in the urine and a fall in the proteinuria, both of which relapse if

you reduce the steroid therapy. Your figure of a mortality of four out of 14 shown in the Table is the sort of early mortality which we get; the late mortality is much reduced in our series, due I think to steroid therapy.

Rich: Before the days of steroids and antibiotics, Nature in effect furnished us with what can be seen in serial renal biopsies. Children would come into the hospital with pure lipoid nephrosis and would die from infections, frequently pneumococcal peritonitis, at all stages of the disease. Any autopsy service in a hospital that had a children's service of any size would see cases of nephrosis with glomeruli in every stage of progression from thickening of basement membranes to hyaline deposits and obliterative sclerosis accompanied by hypertension, nitrogen retention, and ending in death from uraemia. In many cases, as I mentioned earlier, all stages from completely hyalinized glomeruli all the way down to glomeruli that are perfectly normal could be seen in the same kidney. This is by light microscopy of course. It seems to me very difficult to believe that those different stages, from the normal-appearing glomerulus, to the thickened basement membrane, the deposits of hyaline, and the sclerotic obliteration of the glomerulus, were due to different diseases, unless one assumes that the children in whom all of those were present in the same kidney had three or four different diseases at the same time.

Movat: When we are studying the aetiologies of different diseases, we tend to look for different external factors and to forget the response of the host. In this connexion, I should like to mention the work of F. J. Dixon, J. J. Vazquez and J. D. Feldman, presented at the 53rd Annual Meeting of the American Association of Pathologists, Memphis, 1960. They injected foreign protein repeatedly into rabbits. The majority of the animals developed acute nephritis, but only a small proportion developed chronic glomerulonephritis. Immunological studies on these showed that there were three groups: one group of good antibody producers had no chronic glomerulonephritis; the poor antibody producers had no chronic glomerulonephritis; only those that were moderate antibody producers developed chronic glomerulonephritis. The conclusion was that perhaps the antibody-antigen ratio is important for the development and progression of chronic glomerulonephritis.

Earle: I have heard Dr. Dixon present these data several times, and it was quite apparent that the chronic glomerulonephritis was a membranous glomerulonephritis; it was not proliferative or with increased hypercellularity within the capillary loops. When I asked him about this he said that in the early stage some of his rabbits did have transient proliferative lesions, which disappeared in all of them. He thought this was unrelated to his antigen-antibody story with the membranous type, in which there were deposits.

Movat: By fluorescence microscopy they showed deposition of γ-globulin all along the basement membrane, and particularly in those little bulges towards the epithelium which we observed in humans in acute nephritis. They also showed presence of the injected antigen at the same sites. Their lesions in the electron microscope looked exactly like ours in the human.

Earle: I think that we should be careful what we call chronic glomerulonephritis, or at least define which type we are speaking of, because I think that membranous and the post-streptococcal chronic glomerulonephritis are quite distinct.

Movat: Yes, I agree with you.

Hamburger: I think we must go back to Professor de Wardener's excellent remarks. A clinician must use clinical descriptions, pathologists must use pathological descriptions. We must try at the present moment not to make subconscious implications, jumping from clinical observations to pathological ones, or the reverse.

Rich: I think that is very important indeed. If you say only "chronic glomerulonephritis", it doesn't mean anything specific.

Hamburger: That is why I am unhappy about the Toronto group saying that lipoid nephrosis goes on to nephritis. I don't know what this means.

de Wardener: May I go back to the relationship between treatment and the histological appearance? Some of us in this country are rather concerned that the case for steroids in the nephrotic syndrome has not been proven. Everybody will agree that it may produce a diuresis, but from the point of view of extending life we do not think there has been sufficient control evidence. At one end of the scale, there are those who think prednisone is definitely harmful, at the other end those who feel that to withhold prednisone is dangerous. And there

are those of us in the middle who would just like to know and have the evidence. Does anyone here have any views on this?

Rich: May I ask first if you are speaking of childhood nephrosis, adult nephrosis or both?

de Wardener: My own experience is with adults, but I don't think the case has been proven for children either.

Earle: In Table II are results of therapy in the same group of patients

Table II (Earle)

CLINICAL RESPONSE OF 22 ADULT NEPHROTIC PATIENTS TO STEROID THERAPY

	Excellent	*Good*	*Slight or none*
No lesion*	6	2	1
Membranous	1	1	5
Proliferative	0	1	3
Miscellaneous	0	0	2

* By light microscopy.

described earlier, only the numbers are less because not all of them were treated. "Excellent" means that eventually we were able to stop steroid therapy without recurrence of proteinuria. "Good" means that proteinuria has disappeared but we still have to keep up steroid therapy; if the dose is reduced too much proteinuria recurs. In the no-lesion group I would feel that although some of these eventually might have cleared spontaneously I think at least we shortened the course. Eleven of the 22 patients had good or excellent responses, which is high compared to my experience in adults prior to the days of steroids. In the other groups we did not get nearly as good results with steroids. We feel that in the "No lesion by light microscopy" you can make out a reasonably good case for steroid therapy. In most of them you don't have to give very high dosages, or treat them for very long.

Rosenheim: Might not diuretics by themselves be as effective? (See Rosenheim, M. L. and Spencer, A. G. (1956) *Lancet*, **2**, 313.)

Earle: No. Our results refer to the disappearance of proteinuria, not just oedema—although you can clear up the oedema in many of them by diuretics alone.

Rosenheim: Do you believe the disappearance of oedema often precedes the disappearance of the protein?

Earle: It precedes the complete disappearance, but proteinuria usually begins to decrease first.

Wilson: For adults this is an extraordinarily large group with minimal lesions.

Earle: Nine out of 35. It is not unlike some other groups. I believe that Kark's group is similar—perhaps larger.

de Wardener: How many biopsies did you do before steroids were available?

Earle: We didn't do any. But these patients, with one exception, were all biopsied before they were treated.

de Wardener: But you can't compare the results since you started doing biopsies with the results obtained before.

Hardwicke: The only evidence that we can offer on this is failure of reduction of proteinuria without steroids, and the proteinuria starting to fall immediately steroids are given. What are your experiences, Dr. Earle?

Earle: We didn't follow these patients very long before therapy. They averaged about three months of the nephrotic syndrome prior to treatment. In the two "good" results, every time we reduced the dose of steroids, proteinuria came back.

Wilson: But you are producing a change in body water and electrolytes by giving steroids, and this in itself is sufficient to alter the proteinuria.

Earle: An "excellent" result in the table refers to complete disappearance of proteinuria. I don't think we can do this with diuretics.

Wilson: Yes, but what happens without steroids? Unless we have controlled observations, I don't think it can be claimed that this is a specific effect of steroid therapy.

Earle: The evidence is sufficient so that if I had the nephrotic syndrome associated with no glomerular lesion by light microscopy, I personally would like to have steroid therapy. I wonder how Dr. Kark feels.

Kark: I feel exactly the same as you do. However, it will be a long time before we can answer Prof. de Wardener's question about extending life.

de Wardener: How will you know?

Kark: You will know if you compare previous studies which have been done on longevity of patients, such as Dr. Armstrong's and Dr.

Murphy's studies (Armstrong, S. H., and Kushner, D. S. (1960) *Amer. J. Med.*, **29,** 377; Murphy, F. D. (1957). *In* Practice of Medicine, **4,** 549, ed. Tice, F., and Sloan, L. H. Hagerstown: W. F. Prior Co.) with your own patients that have been treated, and with those that haven't

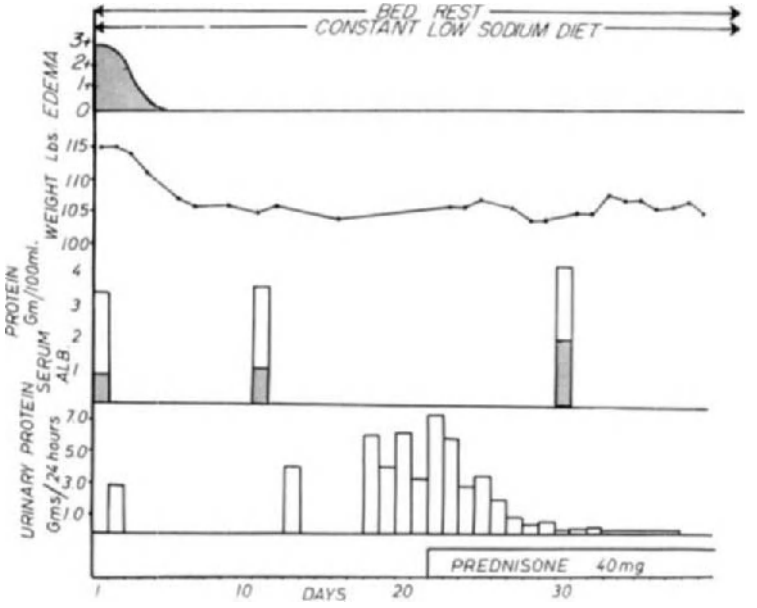


FIG. 1 (Kark). Effects of prednisone on proteinuria in patent J. L., age 23. The patient was ill with oedema and the nephrotic syndrome of 3 months' duration, for which she was treated unsuccessfully with diuretics alone. Light microscopy of the renal biopsy revealed adult-type lipoid nephrosis ("no lesions" in the glomerulus). Oedema rapidly disappeared when treated in hospital on a regimen of bed rest and rigorous low sodium diet, but proteinuria and the biochemical lesions of the nephrotic syndrome persisted. When daily treatment with prednisone was started, proteinuria disappeared completely by the 16th day of treatment. The biochemical abnormalities in the blood returned to normal somewhat later.

been treated—we don't treat all our patients with steroids. I don't want to say much about it now because I have not made a detailed analysis yet, but I have a strong impression, like Dr. Earle, that "no lesions" do very well on steroids. If you treat them with steroids, they get zero proteinuria, which you wouldn't get in patients who've been

given diuretics or a low salt diet (Fig. 1). However, it is true that if they have a spontaneous remission, they may also get zero proteinuria. Now as far as the membranous lesions are concerned, thus far the patients we have treated have been very refractory. As far as proliferative lesions are concerned, we had not treated any until recently because, in the distant past, in a couple of patients we thought the disease had progressed more rapidly on steroids. However, after hearing from Dr. Blainey that some of his patients with proliferative lesions were doing very well, we have treated a few selectively. In some there has been no effect so far; a few are doing well, no proteinuria. I don't think we will really know all the facts on the effects of steroids for ten years.

de Wardener: Do you not think there should be a controlled trial?

Kark: I think that would be very useful, as was the one for rheumatic fever.

Hamburger: Would you be interested in just clinical results? In Paris P. Royer has presented a careful study of the longevity of 123 cases treated with steroids using as a control series 70 patients observed before therapy with corticoids. The control series was done before the extensive use of renal biopsy and therefore the study is on a purely clinical basis. On this basis the longevity of nephrotic patients has been strikingly increased since the discovery of antibiotics, but no further increase is evident after the use of steroids (Royer, P. (1960). *In* Actualités nephrologiques de l'Hôpital Necker. Paris: Flammarion). Slightly more optimistic results of steroid therapy are given by J. P. Méry and F. Josso, (1961. *In* Actualités nephrologiques de l'Hôpital Necker. Paris: Flammarion).

Ross: Were these adults?

Hamburger: This work of Royer was concerned with children.

Black: Some bias could arise if you were selecting the more severe patients to whom to give steroids. This doesn't absolutely show that steroids aren't making any difference.

Hamburger: On the contrary we generally choose the less severe cases, what you call "no optical changes" or "minimal changes" and we very often do not treat with steroids the cases with severe pathological changes.

Milne: I think the evidence as presented is much more satisfactory

for the "no change" group. I am particularly interested in the proliferative group. In the cases in the proliferative group which have been claimed to do well on steroids, has there been clear-cut improvement in the biopsy as well as disappearance of proteinuria? Secondly, has there been a very rapid exacerbation of proteinuria (mentioned by Dr. Earle) on stopping therapy in the proliferative group?

Vernier: At least two observations in children bear on this. One is a report from Havana, by E. Galán and C. Masó (1957. *Pediatrics*, **20,** 610) in which a number of serial biopsies were performed on children with the nephrotic syndrome before and after therapy, and the changes in proliferation, hyalinization and so on were compared in the two biopsies before and after treatment. The principal change in morphology that occurred as a result of successful therapy with steroids was a reduction in the number of glomeruli showing proliferative changes. We had done serial biopsies in about 15 children before and after treatment with steroids, and this is also the only definite change that I think we can recognize by light microscopy—the number of glomeruli showing proliferation of endothelial cells or hypercellularity which may be either endothelial or epithelial is reduced following steroid therapy.

de Wardener: Is this counting nuclei in the glomeruli?

Vernier: No. This is counting the total number of glomeruli and grading each glomerulus for proliferation, for membrane thickening, and a number of pathological features, and then obtaining an index of these various factors.

de Wardener: Does the pathologist know which slide is which?

Vernier: No, these were done blind in our series. The only other change that I am aware of that has been demonstrated in both adults and children is the reversibility of the epithelial cell foot process lesions seen by electron microscopy.

Kark: There is also disappearance of oedema from the kidney, particularly from the interstitial tissue, and restoration of the abnormal lipid-filled tubular cells to normal. I think we are neglecting the tubular cells and interstitial tissue today and have been talking about the glomerulus all the time.

Hamburger: Do you think that these encouraging immediate results imply that the situation of the patients five years later will necessarily be better than similar patients not treated with steroids?

Rich: It is difficult to judge the effect of steroids, because even before steroids were available the rate of progression of nephrosis was so different in different individuals. It would take a study of a large number of carefully documented cases from the pre-steroid and the steroid eras to form more than a general impression—although I don't scorn provisional general impressions of careful observers.

Vernier: The only other work that has been done on this is a combined study of 554 children with nephrotic syndrome that C. M. Riley (1959. *Ann. N.Y. Acad. Sci.*, **82,** 957) has been conducting in co-operation with a number of institutions treating children in the United States. Using the life-table technique for survival (a technique which is criticized by many), there appears to be a decided split between the group collected from these various institutions before steroid and a second group treated with steroids. The steroid group survives far longer than the non-steroid group. But this is not an ideal comparison or controlled study; that hasn't been done.

Earle: In a sense, isn't the group of lesions other than the "no lesion by light microscopy" a sort of control? They don't respond well—the "no lesion" does.

Hamburger: We are all agreed on the immediate response. The question is: are we really doing something more than having good immediate results?

Earle: So far, none of our patients have had recurrence of proteinuria, but presumably some of them will.

Ross: One generally assumes that complete remission is associated with complete structural restoration to normal. I wonder if that is really so. I have been very interested in a boy of eight who had the nephrotic syndrome for four years, had a great variety of treatment, and eventually did remit without proteinuria. A biopsy shortly after remission was normal on light microscopy except for two hyalinized glomeruli, and he remained well for a further three years. I am wondering if others have found complete clinical remission in the absence of a complete structural restoration to normal.

Hamburger: Yes, we have several cases in which there was no more proteinuria, not one single biological or clinical abnormality, and a renal biopsy that was still abnormal.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

POST-STREPTOCOCCAL GLOMERULONEPHRITIS*

DAVID P. EARLE and ROBERT B. JENNINGS

Departments of Medicine and Pathology, Northwestern University Medical School, Chicago

MANY renal diseases are labelled, rather indiscriminately, glomerulonephritis. We believe that recent advances in techniques that can be applied to the study of renal disease demand a reappraisal of our approach to the diagnosis and classification of nephritis. At the very least, students of this important group of diseases should learn to speak a common language. Perhaps even more important, we should try to develop a classification of nephritis on reasonably accepted facts and to reject speculation as a basis of any part of the classification. Nothing is more stultifying than an easily applied diagnostic label that sounds scientific, that superficially seems rational, but which is really a reflection of intellectual laziness. We readily admit that present knowledge and techniques do not permit an entirely satisfactory classification of all types of nephritis, either on aetiological, pathological or pathogenic grounds.

Nevertheless, we believe that a good foundation exists for a first approximation of a reasonable classification. Simple reflection reminds us of several important advances in recent years which have separated off from the amorphous diagnosis of "glomerulonephritis" specific categories such as renal amyloidosis, diabetic glomerulosclerosis and "lupus" nephropathy. We would remind you, however, that we do not know precisely

* Supported in part by Grants from the U.S. Public Health Service (H-1815 and H-1890), and from the Otho S. A. Sprague Foundation.

the pathogenesis of any of these, and that our ability to diagnose some is based on characteristic histological findings, others on associated clinical findings.

We have taken as the keystone of our approach to this problem one generally well recognized diffuse glomerular disease—namely, the glomerulonephritis associated with group A haemolytic streptococcal infections. This type of nephritis in its acute form is readily apparent in the writings of Richard Bright (1827), was shown to be associated with streptococcal infections by Longcope (1936) and by Lyttle *et al.* (1938), and was popularized as Type 1 nephritis by Ellis (1942). The chronic form of glomerulonephritis, however, has been for many years a source of confusion for medical students, practitioners and textbooks, and a source of controversy amongst clinical investigators.

During the past six years we have studied patients with nephritis in a routine fashion. In addition to the usual clinical observations, we have studied (serially whenever possible) histology of percutaneous renal biopsies, bacteriology of associated infection, serum streptococcal antibodies (antistreptolysin, antistreptokinase, antihyaluronidase and specific Type 12), serum complement, C-reactive protein and serum protein fractions (by paper electrophoresis).

From approximately 225 patients so studied, we have collected 37 who had classic clinical and/or laboratory evidence of acute glomerulonephritis following proved group A haemolytic streptococcal infections and from whom adequate renal biopsies were obtained within six months of onset. With rare exceptions these represented sporadic cases in an adult population.

Analysis of the histological findings in the 37 patients with post-streptococcal acute nephritis has shown diffuse glomerular lesions in the great majority, especially when obvious clinical symptoms and signs were present. The characteristic histological change in most of the patients with obvious clinical findings has been endothelial cell proliferation. However, variable amounts of

exudation, as evidenced by polymorphonuclear neutrophil leucocytes within the glomerular capillary loops, was present along with the endothelial proliferation in patients biopsied early. More severe exudative changes such as capillary loop thromboses, lobular necrosis and crescent formation sometimes were noted. In a few patients the exudative changes have been much more marked than the proliferative. These we have labelled "primarily exudative". Several patients, usually with mild clinical symptoms and signs, have not had the characteristic diffuse glomerular lesions.

The later evolution of the proliferative lesions is not yet established firmly. Our data suggest that they regress toward normal in patients with initially mild changes but remain as prominent foci of hypercellularity in the lobular stalks in each unhyalinized glomerulus in those who had marked proliferative glomerulonephritis at the onset. The latter lesion is quite characteristic of post-streptococcal glomerulonephritis in the chronic latent phase. This we usually label as chronic diffuse proliferative glomerulonephritis.

Patients who initially had primarily exudative glomerulonephritis later demonstrated non-specific glomerular lesions such as lobular scars, healed crescents and old adhesions between capillary loops and Bowman's capsule.

We shall analyse data derived from our patients with proved post-streptococcal glomerulonephritis that support the histopathological concepts just described. Variations and exceptions will be noted. The characteristic lesions observed in the chronic phase in patients whose disease was initiated by post-streptococcal acute glomerulonephritis will be used as criteria for selection in an analysis of a group of patients with chronic glomerulonephritis. The incidence of serum antibodies against Type 12 ("nephritogenic") haemolytic streptococci in diffuse proliferative glomerular lesions as compared to other renal lesions will be used as evidence to suggest that our criteria are not unreasonable.

Acute glomerulonephritis

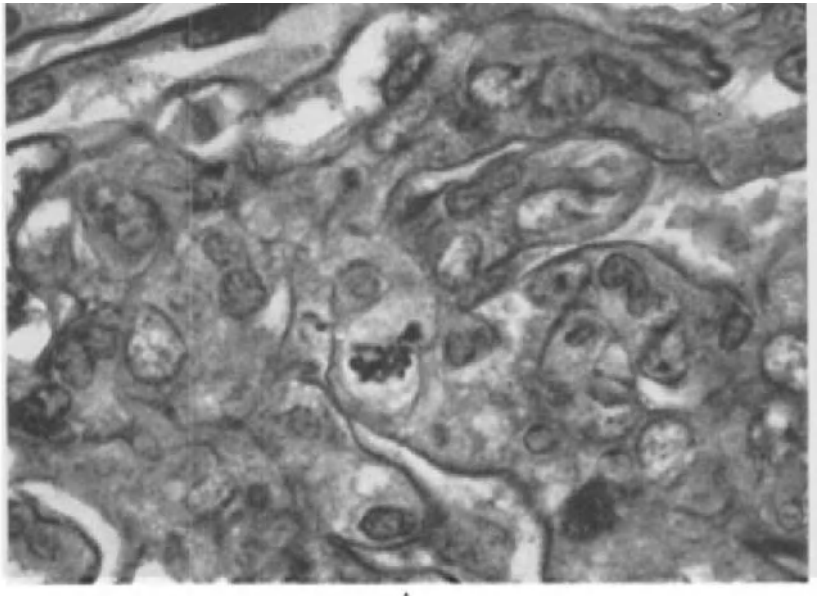
The histological lesions observed in our 37 patients with acute glomerulonephritis following proved group A haemolytic streptococcal infections are summarized in Table I. The predominant glomerular lesion in 27 patients was an increased number of endothelial cells within the glomerular capillary loops (Fig. 1). The extent of endothelial proliferation was variable, although all glomeruli were involved to some degree in all these patients. Twelve of this group, biopsied within six weeks of onset, exhibited some increase in polymorphonuclear leucocytes within capillary loops (Fig. 2). Only two patients had slightly increased numbers of glomerular leucocytes more than six weeks after onset. Evidence of glomerular damage such as lobular necrosis (Fig. 3) or scars, crescents, adhesions or capillary thrombi was noted in five of the 12 patients in the early proliferative group. Few abnormalities of glomerular epithelial cells were observed in this group, while the basement membranes of capillary loops of all unhyalinized or unscarred glomeruli likewise were normal. Small foci of periglomerular oedema and early interstitial fibrosis were present in eight patients. One 59-year-old patient had rather marked interstitial fibrosis, apparently the result of chronic vascular disease.

Table I

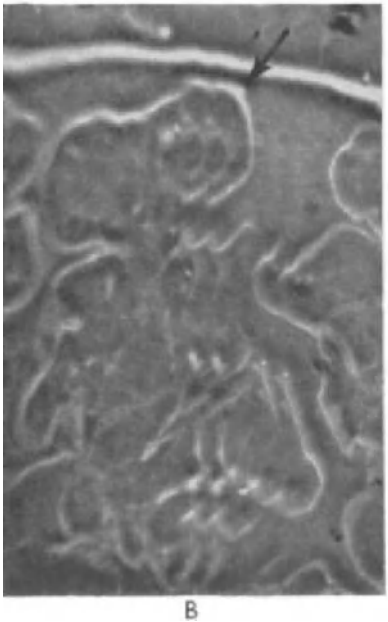
PREDOMINANT LESIONS OBSERVED IN 37 PATIENTS WITH ACUTE GLOMERULONEPHRITIS THAT FOLLOWED PROVED STREPTOCOCCAL INFECTIONS

Lesion	*Number of patients*
Primarily proliferative	27
Primarily exudative	5
Presumed focal glomerulitis	3
Interstitial reaction	2
	37

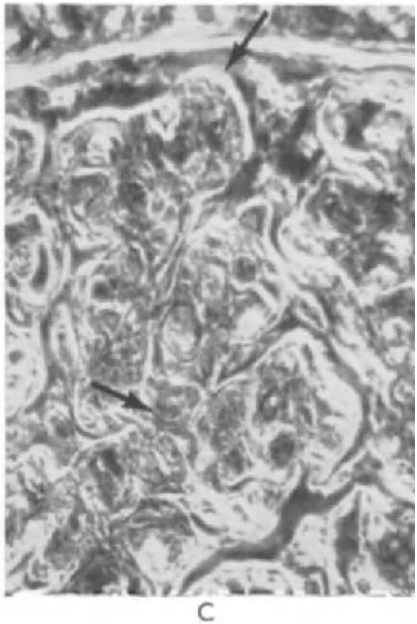
As acute proliferative glomerulonephritis subsided the pattern of glomerular hypercellularity shifted from a generally diffuse



A



B



C

to a focal distribution, with collections of cells in the "stalks" of the lobules (Fig. 4). Nuclei in the hypercellular foci were more basophilic and less vesicular than those of endothelial cells seen in the acute stage, while their cytoplasm was eosinophilic. Cell borders were indistinct and small irregular intracytoplasmic fibres with the staining characteristics of basement membranes were readily seen in thin sections. These fibres exhibited some of the staining characteristics of collagen, but did not stain with silver nor did they show the typical periodicity of collagen in electron micrographs. Again, some glomeruli within the same biopsy and even some lobules in the same glomerulus were more hypercellular than others. In those patients in whom early and late biopsies were obtained, the number of cells within the lobular stalks appeared to be related to the degree of proliferation present during the acute phase.

Several patients who had marked initial proliferative changes also had focal exudative changes which apparently were responsible for subsequent focal changes such as lobular scars, adhesions

---

FIG. 1 (VEG). Early acute diffuse proliferative glomerulonephritis. A: A mitotic figure is present in the centre of this lobule, which shows rather marked endothelial cell proliferation. The acute nephritis healed in this patient. (2 μ-section, periodic acid-Schiff reaction with haematoxylin counterstain. × 1,250.)

B: Photomicrograph made by phase-contrast microscopy of a deparaffinized unstained section of another glomerulus from the same biopsy shown in Fig. 1A. This section was mounted in a mixture of butyl carbitol and α-chloronaphthalene. This solvent mixture has a refractive index of 1·55, which is similar to the refractive index of kidney tissue. The only elements visible are the basement membranes of the capillary loops and Bowman's capsule. Note that the basement membranes of the capillary loops are intact, are of uniform thickness, and are not frayed. The swollen glomerular lobules are clearly outlined by the peripheral basement membranes of the capillary loops of the lobules. (Leitz phase-contrast. × 1,230.)

C: This is another micrograph of the section shown in 1B. A different mounting medium, methylene iodide, which has a refractive index of 1·70, was employed. Use of this medium emphasizes cellular outlines and cytoplasmic detail. Little capillary lumen remains. (Leitz phase-contrast. × 1,230.) (Fig. 1B and 1C were taken by Dr. H. M. Sommers.)

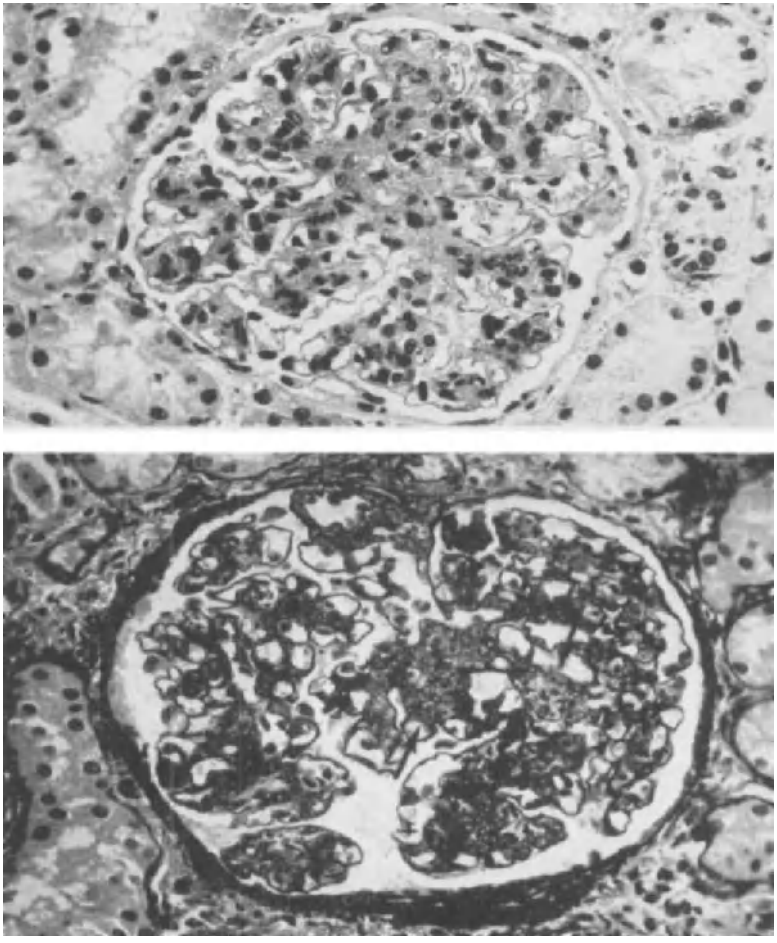


FIG. 2 (VIN). Mild early acute diffuse proliferative glomerulonephritis with some exudation.

A: This glomerulus exhibits a slight increase in the number of endothelial cells in the capillary loops. The new cells have vesicular nuclei. Scattered polymorphonuclear neutrophils are present. The capillary loops are rather widely patent. (Haematoxylin and eosin stain. × 414.)

B: This is another glomerulus from the same patient. It shows considerable numbers of polymorphonuclear neutrophils at the arrows, in addition to a mild increase in the number of endothelial cells. (Periodic acid oxidation, methanamine stain with haematoxylin counterstain. × 445.)

and crescents associated with rather striking lobular stalk hypercellularity (Fig. 5).

A smaller group of patients were considered to have primarily exudative acute glomerulonephritis (Table I). Three patients

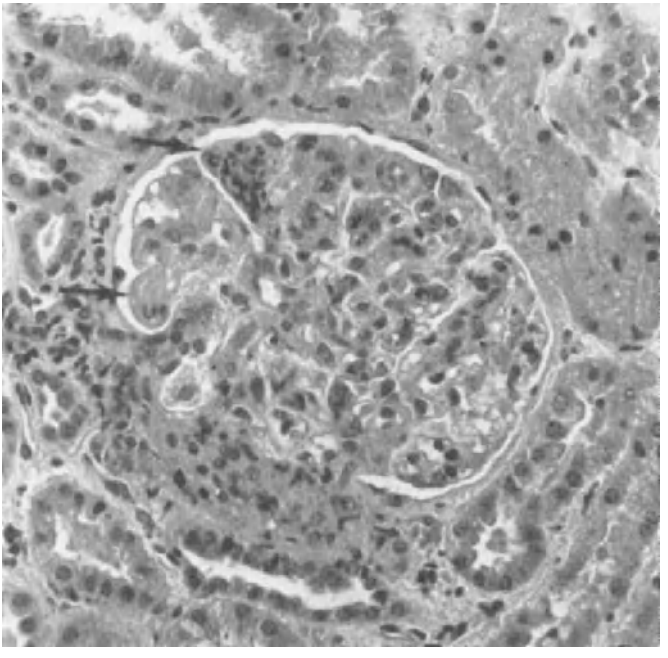
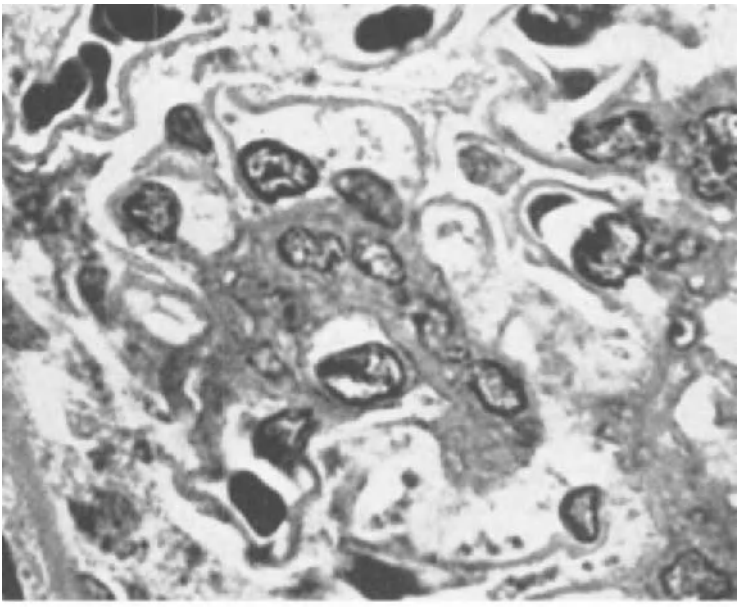


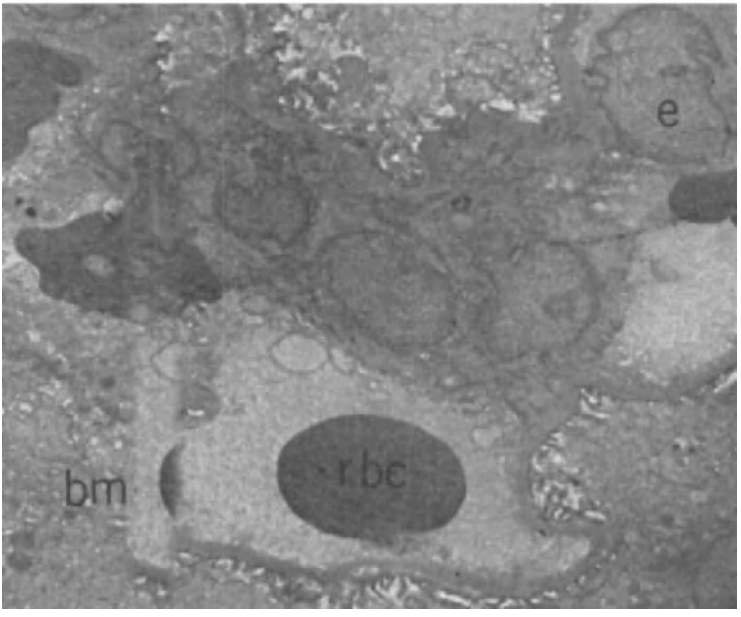
FIG. 3 (MUL). Mild, early, acute diffuse proliferative glomerulonephritis with lobular necrosis. This glomerulus exhibits mild endothelial cell proliferation. It contains a necrotic glomerular lobule at the upper arrow. At the lower arrow there is a thrombus in a capillary loop. The acute nephritis healed in this patient. (2 μ section. Haematoxylin and eosin stain. × 386.)

biopsied within six weeks of onset had marked increases in leucocytes within the glomerular capillary loops and little or no evidence of endothelial cell proliferation (Fig. 6), even in electron micrographs (Fig. 7). These patients also exhibited a considerable

A



B



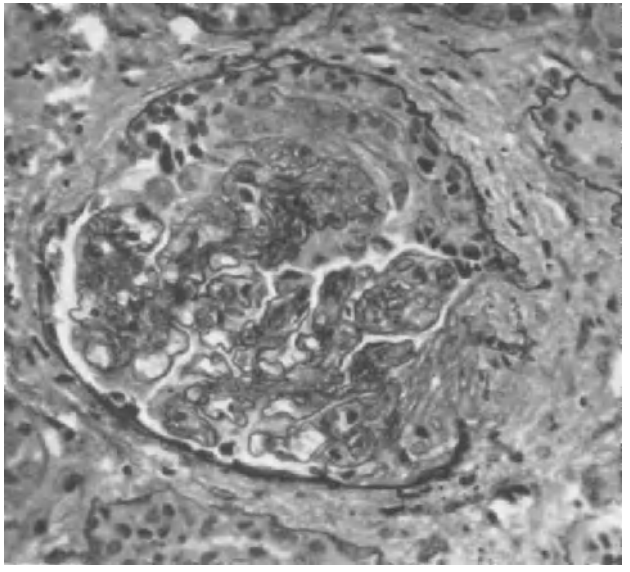


FIG. 5 (TRA). Early chronic latent diffuse proliferative glomerulonephritis. A lobular scar and adhesion to Bowman's capsule are visible in this glomerulus, which also exhibits moderate hypercellularity of all "lobular stalk" areas. This patient's acute nephritis failed to heal. (2 μ section. Periodic acid-Schiff reaction with haematoxylin counterstain. × 510.)(Jennings and Earle, 1961.)

FIG. 4 (MOL). Subsiding acute diffuse proliferative glomerulonephritis.

A: A collection of endothelial cells is present in a "lobular stalk" area. Except in the immediate area of the hypercellular focus the capillary loops and basement membranes are normal. The urine of this patient had become normal before this biopsy was taken. (2 μ section, Heidenhain's connective tissue stain. × 2,370.)

B: Electron micrograph of another glomerulus from the same biopsy. An endothelial cell is at *c*. A small collection of endothelial cells is visible in a "lobular stalk" in the centre of the micrograph. Note that the cells of the lobular stalk show no clear cell membranes and that their cytoplasm contains irregular islands of material with the same density as capillary basement membranes (bm). The foot processes of the epithelial cell are normal. (RCA-EMU3C electron microscope, 100 kv. × 12,500.)(Jennings and Earle, 1961.)

Figs. 4B, 5, 7, 8, 10 and 11 are reproduced by kind permission of the Editor, *Journal of Clinical Investigation.*

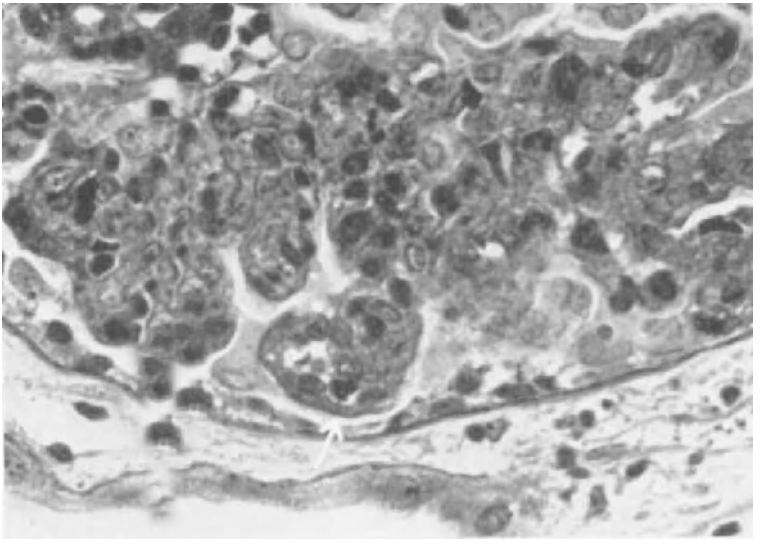


FIG. 6 (APE). Early acute diffuse primarily exudative glomerulonephritis. All glomeruli in this biopsy contained some lobules similar to those illustrated. Note that the glomerulus is hypercellular due mainly to leucocytes within the capillary lumens. Proteinaceous material (fibrin?) is deposited on the inside of the basement membrane of the capillary loop at six o'clock. Most other basement membranes are normal. Although this patient's renal function has gradually returned to normal, proteinuria has persisted for three years. (Heidenhain's connective tissue stain. × 804.)

number of lobular necroses, crescents and adhesions. The cortical tissue was oedematous and many proximal convoluted tubules were lined by regenerating or flattened epithelium. Subsequent biopsies in these and in two other patients first biopsied more than six weeks after onset exhibited interstitial fibrosis, glomerular

FIG. 7 (CAL). Severe acute diffuse primarily exudative glomerulonephritis. Leucocytes (p) within the glomerular capillary loops are very frequent. Endothelial cells (e) are not increased in number. The basement membranes (bm) are of normal thickness. Foot processes are arranged in a generally normal pattern. (Electron micrograph. × 6,120.) (Jennings and Earle, 1961.)


p
rbc
ep
e
p
bm
e

scars, old adhesions and crescents. Lobular stalk hypercellularity was minimal in these patients (Fig. 8).

Three patients who had mild but unequivocal evidence of acute nephritis following proved group A haemolytic streptococcal

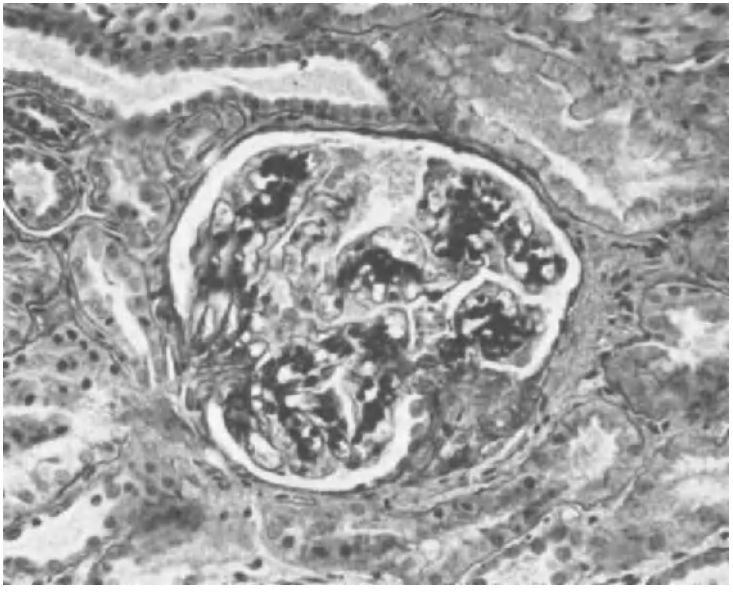


FIG. 8 (APE). Subsiding primarily exudative glomerulonephritis. This glomerulus is from a biopsy obtained 69 days after the onset from the same patient shown in Fig. 6. Note the lobular scar. The unscarred lobules show only slight thickening of stalks. (2 μ section. Periodic acid-Schiff method with hematoxylin counterstain. ×338.) (Jennings and Earle, 1961.)

infections had very little evidence of glomerular lesions on renal biopsy other than erythrocytes in tubule lumina or Bowman's space (Table I). One of these patients had focal and very slight lobular stalk hypercellularity in most glomeruli, one of which also had a lobular scar adherent to a small healing crescent. These patients were considered to have focal glomerulitis. Somewhat

similar observations were made by Bates, Jennings and Earle (1957) in an outbreak of non-streptococcal acute nephritis.

Two additional patients who had definite clinical acute nephritis following streptococcal infections (Type 12 in one) had normal glomeruli but nevertheless exhibited an interstitial reaction. In

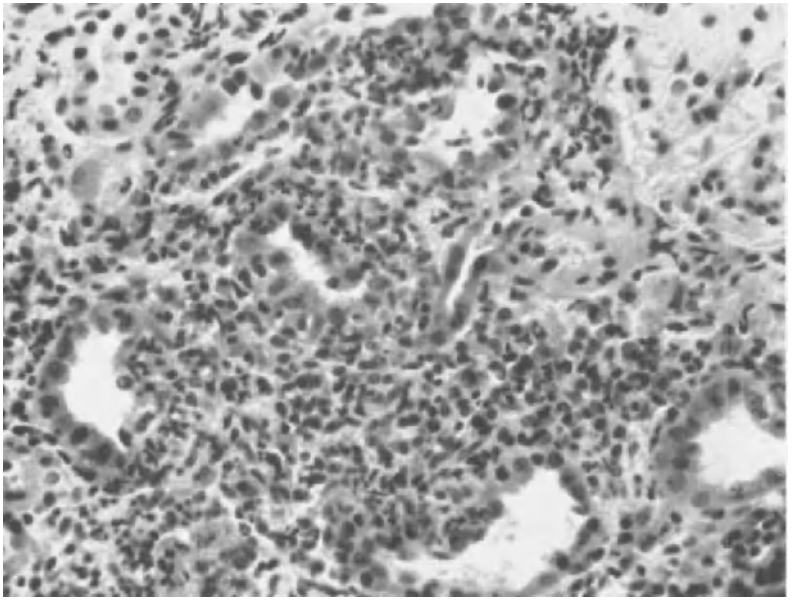


FIG. 9 (BEN). Acute interstitial nephritis. The cortex is swollen and diffusely infiltrated with polymorphonuclear neutrophils. A portion of a normal glomerulus is on the upper right-hand corner. (Hematoxylin and eosin stain. × 418.)

one patient, already described by Earle and Jennings (1960), this was focal in nature but in the other was acute and extensive, characterized by marked infiltration of the interstitium with all types of leucocytes (Fig. 9). Although the latter patient's urine contained a considerable number of white blood cells, cultures were sterile. Careful search for bacteria in sections of the biopsy stained in a variety of ways was unsuccessful. In addition, several

days after the biopsy shown in Fig. 9 was obtained, another large biopsy was ground up and cultured in several media. No organisms were recovered. Despite moderately severe uraemia for more than one month the patient made a complete clinical recovery. The urine became entirely normal. A repeat biopsy two months after onset revealed moderately extensive interstitial fibrosis. The glomeruli were still normal except for a few which exhibited periglomerular fibrosis.

Several other patients with clinical acute nephritis following streptococcal infection deserve special comment. One was a member of a family of six, five of whom developed over a period of 10 days pharyngitis due to Type 12 streptococci. Four of these developed acute glomerulonephritis. The disease healed in all. A biopsy obtained from one revealed typical acute proliferative glomerulonephritis of mild severity.

An 18-year-old male (not included in any of the tables or analyses), 10 days after a streptococcal tonsillitis, suddenly developed definite but transient oedema, hypertension and mild nitrogen retention. Twenty-eight urines and several Addis counts studied during the first month (beginning on the first day of symptoms) were normal. A renal biopsy obtained 14 days after onset unfortunately was inadvertently crushed. In any case, glomerular hypercellularity at best was minimal. Several foci of interstitial fibrosis were present. Many subsequent urines were normal and no evidence of chronic renal disease developed.

Thickening or other evidence of disease of the glomerular capillary basement membranes was not noted during the acute phase of the disease in most glomeruli examined by light microscopy. Membranes sometimes appeared thickened, probably with fibrin, in some patients with exudative glomerulonephritis. Thickening also was not uncommon in areas adjacent to lobular necrosis, hyalinization or scars. Those patients whose disease became chronic (*vide infra*) likewise exhibited practically no basement membrane changes. Except in three patients in the older age

groups who had moderately advanced long-standing nephrosclerosis, vascular changes were infrequent and insignificant.

An attempt, documented elsewhere by Jennings and Earle (1961), was made to correlate the severity of histological changes observed on renal biopsy with the degree of renal-function impairment, hypertension and oedema. In general, a fairly good correlation was apparent. Most of the exceptions had reasonable explanations. However, two patients who had only minimal impairment of function and no hypertension or oedema, on renal biopsy had considerable glomerular hypercellularity. The disease failed to heal in one of these.

Relation between histological lesions and clinical outcome in patients whose acute glomerulonephritis followed proved streptococcal infections

Analysis of histological findings in the patients with post-streptococcal acute glomerulonephritis revealed a reasonably good correlation with the clinical outcome (Table II). We define

Table II

RELATION OF HISTOLOGICAL LESIONS TO OUTCOME OF ACUTE GLOMERULONEPHRITIS

		Clinical outcome	
Lesion	*Severity*	*Healed*	*Chronic*
Endothelial proliferation	0–2+	24	3
	3–4+	1	8
Glomerular damage*	0–25 per cent	22	4
(per cent involved)	>25 per cent	3	7
Interstitial reaction	0–2+	22	4
	3–4+	3	7

* Capillary thromboses, necrosis, adhesions, crescents, scars, hyalinization.

clinical healing as the permanent disappearance of proteinuria. The details of this analysis are documented elsewhere by Jennings and Earle (1961). The clinical outcome appeared to correlate best with the degree of endothelial cell proliferation. The disease in

the three patients without diffuse glomerular changes, and in 21 of 24 patients with only mild to moderate endothelial proliferation healed, as evidenced by disappearance of proteinuria. The three exceptions whose disease failed to heal had a primarily exudative reaction with extensive glomerular damage. Eight of

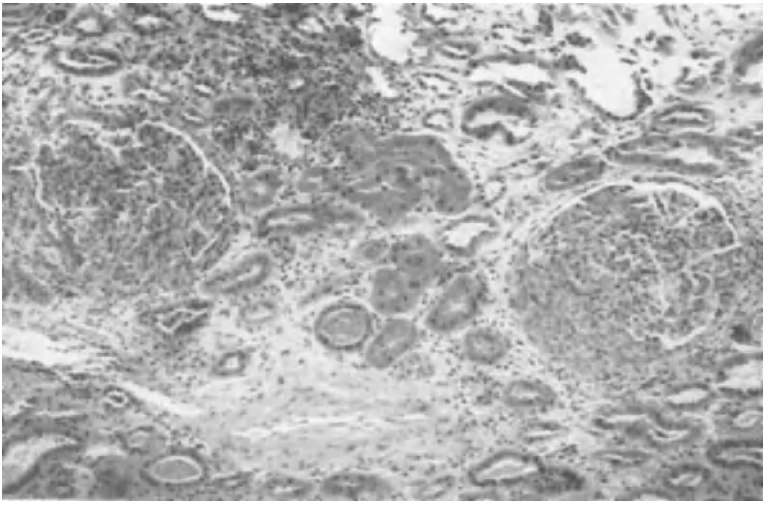


FIG. 10 (CAL). Acute exudative glomerulonephritis. This photomicrograph is from the same biopsy as Fig. 7. The capillary loops of the glomeruli are filled with polymorphonuclear neutrophils. Crescents are present in both glomeruli. Cortical oedema is marked. Extracapillary glomerulonephritis or subacute glomerulonephritis are other names applied to this lesion. (2 μ section. Haematoxylin and eosin stain. × 120.) (Jennings and Earle, 1961.)

the nine patients with 3+ to 4+ hypercellularity still had persistent proteinuria at the last follow-up and were considered to have developed chronic latent glomerulonephritis.

One patient with post-streptococcal acute glomerulonephritis with severe oliguria and hyperkalaemia died one month after onset despite several dialyses on the artificial kidney. On biopsy and at autopsy several days later the circulation in the glomerular capillary loops obviously was completely occluded by polymor-

phonuclear leucocytes within the capillary lumina (Fig. 10). Endothelial proliferation was not present, although fresh crescents and considerable interstitial oedema and early fibrosis were obvious.

This patient and one other who had essential hypertension in addition to acute glomerulonephritis were excluded from the analysis of the relation of histological lesions to outcome.

Chronic glomerulonephritis

Interpretation and consideration of chronic glomerulonephritis are much more difficult. With the exception of some patients studied during the onset of acute glomerulonephritis and whose disease failed to heal, one can only guess as to the aetiology of chronic nephritis. As described in the previous sections, we

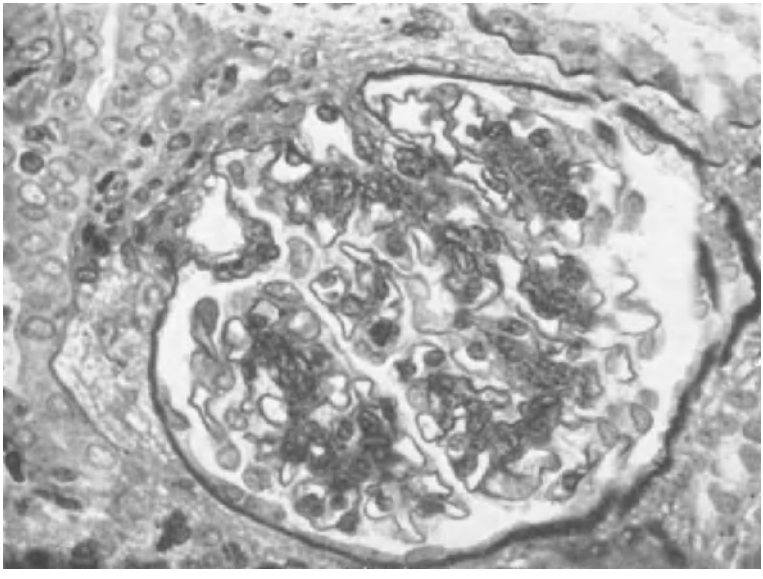


FIG. 11 (TRA). Chronic diffuse proliferative glomerulonephritis, latent phase. This glomerulus is from the same biopsy as Fig. 5. Note the lobular stalk thickening and hypercellularity. (Periodic acid-Schiff reaction with haematoxylin counterstain. × 455.) (Jennings and Earle, 1961.)

observed 12 patients with acute glomerulonephritis following proved haemolytic streptococcal infections who went into the chronic latent phase of glomerulonephritis. Twenty-three additional patients with chronic renal disease were found to have similar chronic diffuse proliferative lesions on renal biopsy (Fig. 11). Evidence relating to the onset or discovery of renal disease in 35 patients with histological chronic proliferative glomerulonephritis is summarized in Table III. Documented or historical evidence of acute nephritis was obtained in 23 of these patients,

Table III

CLINICAL FEATURES IN 35 PATIENTS WITH HISTOLOGICAL CHRONIC DIFFUSE PROLIFERATIVE GLOMERULONEPHRITIS

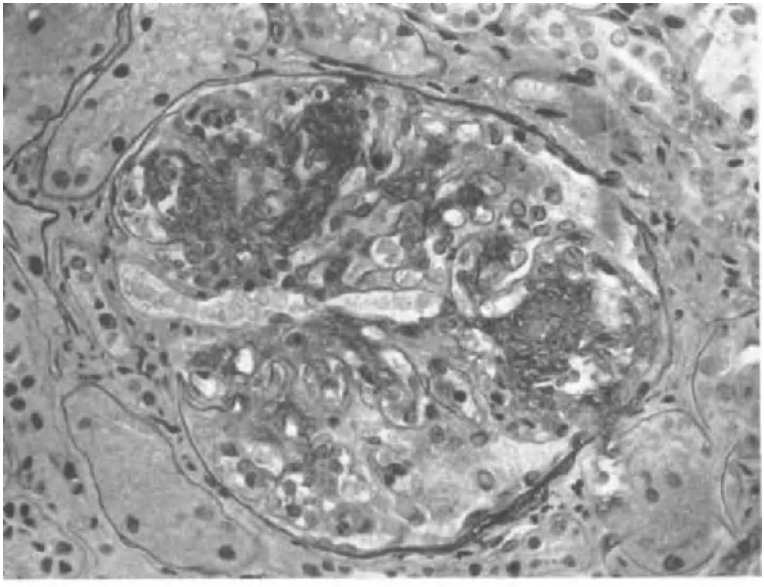
		Number of patients
Typical acute glomerulonephritis at onset		23
Preceding infection proved streptococcal	12	
Preceding infection probably streptococcal	5	
Preceding infection unknown aetiology	4	
No preceding infection	2	
Exacerbation in chronic glomerulonephritis		4
Nephrotic syndrome initial sign of renal disease		4
Routine urinalysis revealed renal disease		4
Total		35
First studied in terminal uraemic phase		3
Mixed proliferative and membranous chronic glomerulonephritis		4
Grand total		42

FIG. 12 (ROS). Chronic diffuse proliferative glomerulonephritis in exacerbation.

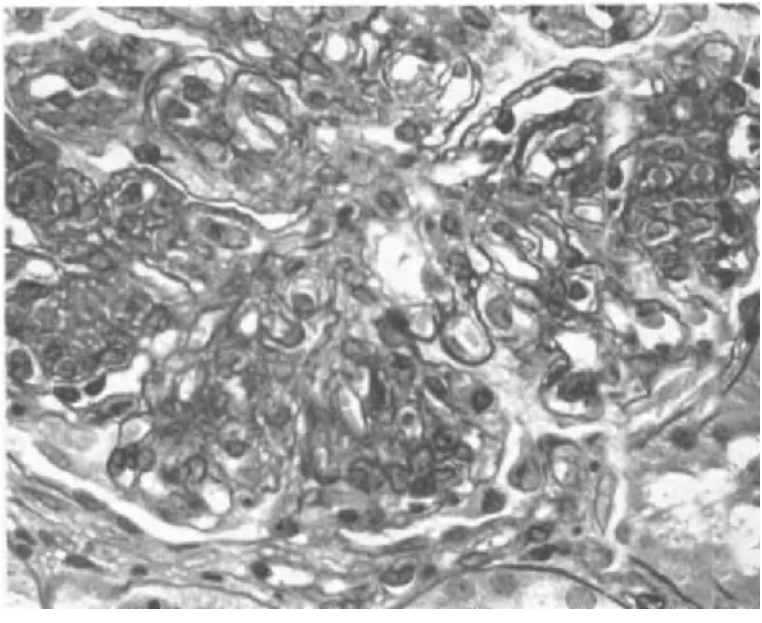
A: This is a representative glomerulus from a biopsy obtained 10 days after an exacerbation which followed a Group A streptococcal infection. There is marked lobular stalk thickening and hypercellularity. Numerous polymorphonuclear neutrophils are present in the capillary loops. (2 μ section. Periodic acid-Schiff reaction with haematoxylin counterstain. × 413.)

B: This is from another biopsy from the same patient obtained 8 months later during an exacerbation which followed a non-streptococcal infection. Note the absence of polymorphonuclear neutrophils and the prominent layer of endothelial cytoplasm (oedema?). (2 μ section. Periodic acid-Schiff reaction with haematoxylin counterstain. × 1,012.)

A



B



12 of whom represent patients studied in the acute phase and in whom we documented the streptococcal aetiology of the preceding infection. The preceding infection was probably of streptococcal aetiology in five and could have been streptoccocal in four others. Evidence of preceding infection was lacking in only two of these patients. Typical exacerbations in chronic glomerulonephritis, as described by Seegal *et al.* (1940), were documented in four patients. Although the onset of glomerulonephritis was not observed in any of these patients, evidence of pre-existing renal disease was documented. At least one observed exacerbation followed a proved streptococcal infection by a day or two in three of these patients. Two exacerbations followed non-streptococcal infections. Renal histological studies in those exacerbations that followed streptococcal infections revealed evidence of acute glomerular changes as well as typical chronic lobular proliferative glomerulonephritis (Fig. 12A), whereas histological studies in clinical exacerbations following non-streptococcal infections did not reveal acute exudative features (Fig. 12B).

Of the 35 patients with established chronic renal disease and histological chronic proliferative glomerulonephritis, in only eight did we fail to obtain evidence of clinical acute glomerulonephritis: in four patients, the renal disease was discovered only after the development of the nephrotic syndrome, while abnormal urine discovered in the course of a routine examination led to the diagnosis of renal disease in the other four patients.

The proliferative lesions in our patients studied within the first few years of onset were very similar to those so ably described by Bell (1938) as being characteristic of chronic latent glomerulonephritis. Typical glomerular lobulation was apparent at low magnification (Fig. 13). At higher magnification significant lobular stalk hypercellularity was obvious (Fig. 11). Adhesions, fibrosis and hyalinization of some glomeruli were common. In general, establishment on histological grounds of the age or duration of chronic glomerulonephritis was rather difficult. As

the disease advanced, interstitial fibrosis, tubular atrophy and hyalinization of glomeruli became prominent features. Diffuse hypercellularity, however, could be observed until the most advanced stages were reached. Indeed, the changes described above were noted in patients whose renal functions were reduced

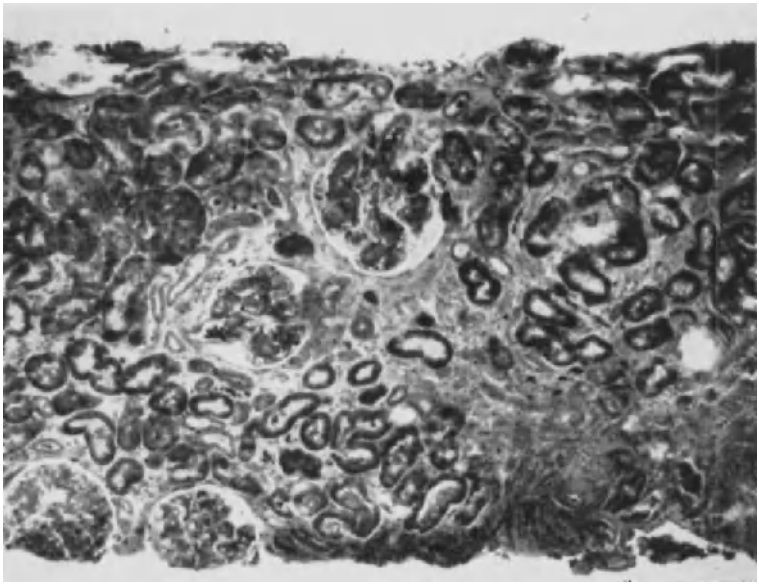


FIG. 13 (RIC). Chronic diffuse proliferative glomerulonephritis. The cortical architecture is disrupted by fibrous tissue. Each glomerulus contains thickened hypercellular lobular stalks. This patient had the nephrotic syndrome at the time of biopsy and died in renal failure several years later. (Heidenhain's connective tissue stain. ×48.)

to as low as 15 per cent of normal. However, renal biopsies obtained in three patients in advanced uraemia with contracted kidneys were not diagnostic, despite clinical histories and findings suggestive of chronic glomerulonephritis. All glomeruli were so scarred and hyalinized in these patients that histological characterization was impossible. At autopsy the diagnosis of chronic

glomerulonephritis was made in each, although not on very secure grounds.

The peripheral glomerular capillary basement membranes in chronic proliferative glomerulonephritis were normal except in

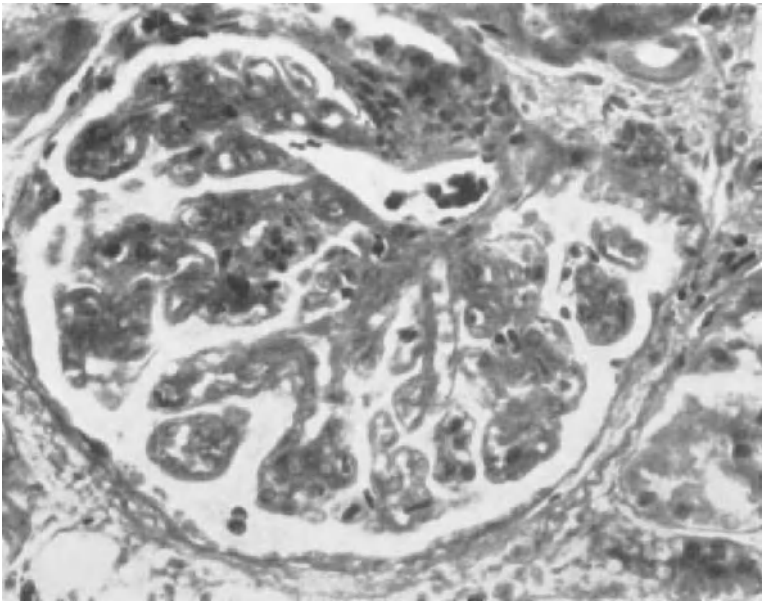


FIG. 14 (SPE). Mixed membranous and proliferative glomerulonephritis. This glomerulus is from a patient who had moderately impaired renal function and the nephrotic syndrome. By light microscopy, the glomeruli showed a mixture of thickened basement membranes and increased cellularity. Some basement membranes were normal in each unhyalinized glomerulus. (2 μ section. Heidenhain's connective tissue stain. × 416.)

areas of extensive glomerular damage. However, mixed membranous and proliferative lesions were observed in an additional four patients with clinical evidence of chronic renal disease. The histological lesions were basically proliferative in nature, with basement membrane thickening in a moderate number of peripheral capillary loops relatively uninvolved by the predominant

proliferative response (Fig. 14). None of these patients had any evidence of acute nephritis. Two had the nephrotic syndrome. We do not know whether these instances represent a variant of the primarily proliferative group so commonly associated originally with streptococcal infections, or whether they represent a different disease. We think that the latter probably is correct, but in any event believe that they should be considered separately until good evidence to the contrary is available.

Type 12 haemolytic streptococcal serum antibodies in glomerulonephritis

Rammelkamp and Weaver (1953) and Wertheim *et al.* (1953) have demonstrated a relationship of acute glomerulonephritis to Type 12 haemolytic streptococcal infections. We present here a preliminary analysis of our data on Type 12 streptococcal antibodies in the sera of patients with glomerulonephritis and of certain control groups (Table IV). We believe that these data

Table IV

INCIDENCE OF TYPE 12 STREPTOCOCCAL ANTIBODIES IN RENAL DISEASE—BIOPSY GROUP

	Number of patients	*Number with T-12*	*Percentage with T-12*
"Normal" population*	38	3	8
Acute glomerulonephritis—proved streptococcal	34	12	35
Chronic proliferative glomerulonephritis	35	11	31
Total	58†	19†	33
Acute proliferative glomerulonephritis—probably streptococcal	5	1	20
Acute nephritis—non-streptococcal	18	1	6
Focal glomerular lesions	28	1	4
Nephrotic syndrome‡	28	3	11
Miscellaneous renal diseases	76	10	13
Total	147†	15	10

* Data of Dr. G. H. Stollerman. † Duplications deleted from totals.
‡ Excluding 7 patients with chronic diffuse proliferative glomerulonephritis.

substantiate, at least indirectly, our approach to the classification of diffuse glomerulonephritis.

It should be recognized, however, that serum type-specific antibodies against streptococci are difficult to measure, that the measurements are subject to error, that they may not achieve detectable levels after promptly and adequately treated streptococcal infections, and that they may decrease below detectable levels in many patients within six to nine months after an infection. In addition, during the period of the present study Type 12 streptococcal infections were found by Siegel, Stollerman and Johnson (1961) to be by far the most common streptococcal infections in the Chicago area, representing approximately 20 per cent of all such infections from which typable strains were recovered. Finally, only a few Type 12 streptococcal infections are followed by acute glomerulonephritis. Stollerman, Siegel and Johnson (1961) found that only one per cent of sporadic proved Type 12 infections in children were followed by acute glomerulonephritis.

Despite these difficulties we have analysed the incidence of Type 12 antibodies in the sera of almost all the patients from whom we obtained renal biopsies. These data are summarized in Table IV. A few patients were studied before we instituted the routine measurement of Type 12 antibodies, and in a few additional instances the tests could not be performed because the patients were receiving antibiotics which interfered with the performance of the assay. Antibodies against Types 6, 30 and Red Lake streptococci also were measured in the majority of patients. Positive reactions against these types were rare (three against Type 6, one against Type 30 and one against Red Lake).

Twelve of 34 patients with proved post-streptococcal acute glomerulonephritis had Type 12 antibodies (including the three patients from whom Type 12 streptococci were recovered on throat culture). Very reassuring to our concept that chronic diffuse proliferative glomerulonephritis is of streptococcal aetiology was the similar incidence of 31 per cent Type 12 antibodies

in patients in whom renal biopsies revealed the characteristic lesions of this condition. It made no difference in this analysis whether or not the patients we observed during their acute phase and who failed to heal were included.

The approximate incidence of one-third Type 12 antibodies is to be contrasted with the 8 per cent incidence in subjects without renal disease. Even more significant is the relatively low incidence of Type 12 antibodies in patients with renal disease which on renal biopsy was not of the diffuse proliferative variety. Only one of 18 patients with acute nephritis which followed non-streptococcal infections had Type 12 antibodies. This result is not surprising since they were all young adults and of necessity studied near the onset of their nephritis to exclude the possibility of associated streptococcal infection. Ten of these have been reported in detail by Bates, Jennings and Earle (1957). Diffuse acute proliferative lesions were observed in five patients who were not studied early enough to determine the aetiology of their associated infections. Type 12 antibodies were found in one. Of 28 patients with focal glomerular lesions, many of which were proliferative in nature, only one had Type 12 antibodies. Likewise, only one of 29 patients with the nephrotic syndrome (excluding seven patients with diffuse proliferative lesions associated with the nephrotic syndrome) had Type 12 antibodies. Finally, of 76 patients with a variety of other non-proliferative lesions, only 10 (13 per cent) had Type 12 antibodies. Thus, the incidence of these antibodies in patients with diffuse proliferative lesions, whether acute or chronic, was significantly greater than in a large group of patients with miscellaneous renal lesions.

Type 12 antibodies were measured in the sera of 132 patients with renal disease but from whom renal biopsies were not obtained. Thirty-six per cent of 36 patients with the clinical diagnosis of acute glomerulonephritis had Type 12 antibodies, while only 10 per cent of the remaining 96 patients with miscellaneous renal disease had these antibodies.

REFERENCES

BATES, R. C., JENNINGS, R. B., and EARLE, D. P. (1957). *Amer. J. Med.*, **23**, 510.
BELL, E. T. (1938). *Amer. J. Path.*, **14**, 691
BRIGHT, R. (1827). Reports of medical cases selected with a view of illustrating the symptoms of and cure of diseases by a reference to morbid anatomy. London: Longman, Rees, Orme, Brown and Greene.
EARLE, D. P., and JENNINGS, R. B. (1960). *Med. Clin. N. Amer.*, **44**, 59.
ELLIS, A. (1942). *Lancet*, **72**, I, 34, 72.
JENNINGS, R. B., and EARLE, D. P. (1961). *J. clin. Invest.*, **40**, in press.
LONGCOPE, W. T. (1936). *J. clin. Invest.*, **15**, 277.
LYTTLE, J. D., SEEGAL, D., LOEB, E. N., and JOST, E. L. (1938). *J. clin. Invest.*, **17**, 631.
RAMMELKAMP, C. H., JR., and WEAVER, R. S. (1953). *J. clin. Invest.*, **32**, 345.
SEEGAL, D., LYTTLE, J. D., LOEB, E. N., JOST, E. L., and DAVIS, G. (1940). *J. clin. Invest.*, **19**, 569.
SIEGEL, A. C., STOLLERMAN, G. H., and JOHNSON, E. E. (1961). Personal Communication.
STOLLERMAN, G. H., SIEGEL, A. C., and JOHNSON, E. E. (1961). Personal Communication.
WERTHEIM, A. R., LYTTLE, J. D., LOEB, E. N., EARLE, D. P., SEEGAL, B. C., and SEEGAL, D. (1953). *J. clin. Invest.*, **32**, 359.

DISCUSSION

Heptinstall: How long is an antistreptolysin O (ASO) titre likely to persist at a high level?

Earle: The duration of the increase in ASO titre is somewhat variable; on the average it would probably be above the individual's normal level for two to three months. If it is very high there is no question about it, but if it is only slightly increased it is important to get serial values. In all our patients we have had serial studies, not only on ASO but on antistreptokinase and antihyaluronidase. ASO will pick up only 85 per cent of the infections, but if you use all three antibodies you get 95 per cent.

Heptinstall: What would you call a significant level? Could you put a number on it?

Earle: I can't be definite about this because it depends on the patient's normal level. A value of 625 units is high, but with 333 to 125 you can't really be sure. We require on serial study more than a 2-tube

increase to represent a significant change. I think that this is standard practice in the streptococcus field.

Heptinstall: When you have done repeat biopsies did you ever see focal glomerulonephritis? Does a proven case of diffuse proliferative glomerulonephritis ever resolve in such a differential way that you may have normal glomeruli but with others still showing some degree of involvement?

Earle: Except for one patient, the original biopsies from all our early patients showed that all the glomeruli were involved. There was considerable variation in the response in different glomeruli, and sometimes in different lobules within the same glomerulus. We believe that it becomes more variable as time goes on, but I can't recall seeing patients with acute nephritis following proved streptococcal infection with some glomeruli involved and others not.

Jennings: I don't think that we have done enough late biopsies to definitely answer that question. By late I mean two or three years or more after the onset.

Heptinstall: In your Table II, you showed that in the group with a 3+ to 4+ endothelial cell proliferation, eight out of nine went into a chronic stage. Did those patients have any other changes in the glomeruli, say crescent formation, apart from purely endothelial proliferative changes?

Earle: Some of these did have crescents although not all of them; it was our impression that the degree of endothelial hypercellularity was the most important finding. In the primarily exudative group, where there was very little endothelial proliferation, the disease in three patients became chronic and all three had a fair number of crescents. However, we have observed crescents as early as eight days after onset in patients whose disease healed, so we cannot put an ominous implication on a few crescents. If there are a great many crescents then it probably is bad.

de Wardener: How many patients with acute glomerulonephritis did you find did not have evidence of streptococcal infection?

Earle: We had 38 post-streptococcal cases and 18 non-streptococcal. Ten of the latter occurred in an outbreak in a naval training station where two more occurred this spring. The rest were instances of sporadic acute nephritis in the Chicago area. In none of these did serial

antibodies or throat cultures reveal evidence of prior streptococcal infection. The latent period between infection and the onset of acute nephritis was short, only one to three days, in contrast to the post-strep patients, where the latent period generally was one to four weeks.

Hutt: We have biopsied a series of patients rather similar to yours; they all had acute glomerulonephritis and there was evidence that this was part of an epidemic with a streptococcal aetiology. In general, our histological findings are in agreement with yours. There is one point, however, that I would like to raise: we found that some of the patients with severe glomerular lesions had in addition marked interstitial and tubular damage and that these were the ones who continued to have proteinuria longer than the others. However, in our series, the two patients who had both gross glomerular and tubular lesions eventually stopped having proteinuria—one patient after ten months and one after fifteen months. There has been no recurrence of proteinuria in either of these cases. These findings make me unhappy about saying that because somebody has proteinuria ten months after an acute attack he should be regarded as entering the chronic phase.

Earle: We shall have to have longer follow-ups, and our figures are subject to revision. We don't know how many of these patients will lose their proteinuria. We had a moderately severe case in whom proteinuria disappeared after two and a half years. However, in our general experience, if proteinuria is going to disappear it usually disappears before a year. Although we are probably wrong in some of our patients, I think we can be fairly confident that most of these will remain chronic. From observations in those whom we have biopsied again in the later stages it would seem unlikely that they are going to heal.

Hutt: Have you any idea how the red cells get into the urine?

Earle: There is some argument about that, but I think that most of them come from damaged glomeruli. In quite a few sections we could see red cells in Bowman's space. If not, they must come from the proximal convoluted tubules, but I don't quite see how this would happen with normal-appearing tubules. As you have commented, many of the severe patients have interstitial reaction, but a number of our patients with marked endothelial proliferation had normal tubules and interstitium.

Jennings: I would like to comment on Dr. Hutt's question about chronicity. I have a strong suspicion, which Dr. Earle only partially shares, that those patients that we have classified as having initial primarily exudative reactions should heal; this is because the end stages of the primarily exudative reaction are either recovery or focal glomerular scarring. If one assumes that such glomerular scars don't lead to the development of hypertension, they would be followed by the development of chronic disease. We haven't seen hypertension develop in these patients, so I think that they have a good chance of healing late; one has healed after 26 months. They might be members of the group Addis labelled on clinical grounds, "healing with a defect".

de Wardener: Did you have any who went into the rapidly progressive stage?

Earle: Yes, we had one who died after 32 days.

Vernier: I believe E. T. Bell called that subacute.

de Wardener: I think the term "subacute" in renal disease has been torpedoed.

Vernier: I am happy to get rid of it. But just to refer to this terminology, would not the lesion that you demonstrated in this woman also fit what is called rapidly progressive glomerulonephritis?

Earle: Yes. Several of the other patients who eventually recovered were terribly ill. One of them had a blood urea nitrogen of 180 mg. per 100 ml. and a pericardial friction rub. We thought he would die, but he recovered and is working as a farmer although he still has proteinuria.

Pirani: What do you think comes first, the exudation of polys or the proliferation of endothelial cells?

Earle: Some patients have just one or the other. Of 12 patients biopsied within six weeks of onset, all had two to three or more polys in each glomerulus. However, proliferation is more characteristic. Large numbers of glomerular polys are uncommon in our experience.

Wilson: I would like to bring up the question of attempting to decide prognosis on the basis of histological lesions (your Table II). It is very difficult not to believe that there was considerable hospital selection in this group of cases. Their division into "slightly proliferative" and "severely proliferative" may represent a different severity of the glomerulitis at the onset or it may represent different durations. I would

like to ask, if you confine yourself to cases admitted within seven days of the first haematuria, what is your proportion of severely proliferative or slightly proliferative, and what is the prognosis of the total group; that is, how many cases became chronic that were admitted in the first week of the disease?

Earle: Of course there is selection; we can't avoid it. We probably get the more severe patients in our hospital practice. However, some of the patients came from the Great Lakes Naval Training Station, where the ward officers would examine the urine after suspected streptococcal infections. So we did get some mild cases. The recovery rate in any series is entirely dependent upon the criteria of selection. In epidemics I am sure that the prognosis is much better than in our sporadic cases of acute nephritis in adults. C. H. Rammelkamp showed in the Bainbridge outbreak, where he studied almost 200 cases, that only one questionably became chronic. I think, however, it is valid to look at the histology of our patients in their first attack of acute nephritis and correlate this with the clinical outcome. Although the correlation between the severely proliferative lesions and chronicity is not 100 per cent, it has been useful in practical prognosis.

Wilson: My suggestion is that in the "severely proliferative" group more cases become chronic because they were chronic when they were admitted.

Earle: Of the twelve that we were able to biopsy within the first six weeks, three or four became chronic.

de Wardener: We have had a small series, 15 adults, without the advantage of the streptococcal evidence that you have. There were no deaths, and at the end of two years we had only one with persistent proteinuria. The lesions were the same as those you have shown; in fact half of them also had tubular necrosis. I find it very strange that there should be this discrepancy in prognosis. In our cases the average biopsy time was five days from the onset of symptoms.

Milne: In this last series did you have any who presented from the onset as acute oliguric renal failure? The majority of my cases of acute nephritis seem to be examples of acute renal failure, but I think this is a very selected and non-representative series.

Earle: The patient who died presented this way. For the entire 32 days that he lived he never put out more than 10–12 ml. per day. Several

of our other patients also were severely oliguric. The one who had a pericardial friction rub had severe oliguria for more than a week. I might say that most of our patients had clinical symptoms and any physician would have diagnosed acute nephritis in these patients without difficulty. Four or five, however, were laboratory diagnoses. They had urinary changes and functional impairment but no symptoms. If the physicians who were taking care of them hadn't been alert and done routine urine examinations after streptococcal infections, they never would have been diagnosed.

Joekes: It seems astonishing from the clinician's point of view to hear that subacute glomerulonephritis has been "shot down". This to me is a very clear-cut entity that Dr. Milne and I seem to see rather frequently. It is a terrifying condition which kills people inside a few weeks, has quite a distinctive histological picture and a quite distinct clinical course. It is quite amazing that one should hear that this has now disappeared.

de Wardener: I was referring to the *term* "subacute glomerular nephritis". It means different things to different people.

Joekes: What are you going to call it?

de Wardener: Some people use subacute nephritis as synonymous with nephrotic syndrome. Now you can't use the same word for two different clinical conditions. I'm just pointing out a semantic confusion, which I feel should be resolved. The best thing to do is to throw the word away and use some other word. "Rapidly progressive" is very good.

Wilson: This confusion was resolved 20 years ago. Why revive it? F. Volhard introduced the term "subacute course of nephritis" and then in this country we used the term "subacute parenchymatous" or "hydropigenous nephritis". The confusion became worse when the pathologists separated "nephrosis" into four or five different entities, according to the nature of the tubular degeneration. The term "proliferative glomerulitis" has been used this morning for a type of Bright's disease entirely different from the kind we were discussing yesterday when we were considering the nephrotic syndrome. It was to avoid this confusion that Ellis introduced the terms Type I and Type II nephritis, and I think what has been said so far in this symposium has entirely justified this classification of nephritis.

Milne: I would like to give more details of the oliguric form of acute glomerulonephritis that I mentioned earlier. Last winter we seemed to have rather an epidemic of this in London. The disease attacked individuals in previous good health as a sudden acute oliguric renal failure, and on percutaneous biopsy the disease was entirely glomerular. The prognosis of this disease is usually bad. Including a few this winter we had a total of 40 cases at The Hammersmith Hospital, and of these 32 died. Of the eight that recovered, none of them have become protein-free; some of them have developed a nephrotic syndrome, but these patients now have a normal blood urea. The patients who recovered were on an average of a lower age group; the oldest was 31 and most of them were children. Renal biopsy from the cases who later recovered, taken when they were clinically severely oliguric, showed the pattern of proliferative glomerulonephritis. In the other patients the disease was rapidly progressive, death occurring in two or three weeks without dialysis and in up to two or three months with dialysis.

We have tried to study the progression of the glomerular lesions, and I think that we can say that the main destruction of the glomerulus was by crescent formation. The glomerulus was progressively invaded from Bowman's capsule, with complete destruction within three weeks, in a patient who was previously perfectly healthy as far as we knew. At necropsy, a low-power view showed complete destruction of all glomeruli, with extensive fibrosis.

This histology was shown in the biopsies of all the fatal cases except one, and this I would have interpreted as a membranous glomerulonephritis with gross thickening of the basement membrane. However, the case was clinically exactly like the others, and was anuric from the onset. Is it possible that this thickened membrane was impermeable, unlike the usual status of the "leaky membrane" of membranous glomerulonephritis, completely sealing off glomerular filtration?

These cases can be kept alive for a considerable time, because statistically the rate of deterioration in the oliguric variety of glomerulonephritis is much slower than in cases of acute tubular necrosis. In acute tubular necrosis of obstetrical or medical cause, the average rate of rise of blood urea in our hands is 33 mg./100 ml. per day, whereas in these cases of glomerulonephritis the average rise is only 21 mg./100 ml. per day.

We have attempted to grade the severity of this disease in relation to urinary volume. The urinary volumes are typical of ordinary acute glomerulonephritis until about the ninth day, and then there is an abrupt and precipitous fall of urinary output, going down to less than 100 ml. per day and then slowly falling off until by the 25th day there is virtually complete anuria. This seems never to improve on dialysis, and I think that one could deduce this from the histology. The critical period for the severe glomerular damage is the tenth day.

Rich: Have you done any biopsies at this critical time?

Milne: We have only just analysed this series, and now we must go back to our biopsies and study that particular time.

While we are very much guided by the results of percutaneous renal biopsy as an indication for continued dialysis in acute glomerulonephritis, I would never be guided by renal biopsy in cases of renal cortical necrosis. We had one example of a percutaneous renal biopsy from a case of renal cortical necrosis occurring after accidental haemorrhage of pregnancy, and to me it looked rather badly damaged. She required two haemodialyses before a diuresis occurred. In spite of this the patient is now well and working, and considers she is completely recovered (although medically she is not perfectly well and has a moderate uraemia). Yet if one had been guided by that biopsy she would have been denied many years of life. This was presumably a patchy renal cortical necrosis. There have been interesting reports of calcification occurring in the late stage of this type of renal cortical necrosis, but I do not think that one can expect this to occur in every case. There was no calcification in her kidney, as demonstrated by radiology with tomography one year after the disease, and there was no increase of calcium in the biopsy removed one month after the onset.

Joekes: I would like to come back to the patient whom you included in your oliguric glomerulonephritis group but who had biopsy changes that appeared membranous. I wonder why you included this case, because histologically this was obviously quite different from the others, and there seemed to be a great deal of tubular damage.

Milne: It was different only in the biopsy appearances; clinically it was exactly the same as the others. I agree that it is presumably a different disease.

Joekes: We have observed that in the history of this type of glomerulonephritis the development is often insidious. If you catch these patients before their urine volume drops off, they just have malaise, and we find that their blood urea is 300; sometimes they have haematuria and sometimes they do not.

Milne: Not in this series. Here you could date the course of the illness. They had a sudden oliguria and they started exactly like less severe examples of acute haemorrhagic glomerulonephritis. On the tenth day after the onset they had a precipitous fall in urinary volume and became cases of acute oliguric renal failure.

Joekes: Did the patient with the atypical biopsy present with haematuria?

Milne: Yes.

Hutt: Was there evidence of a streptococcal aetiology in this group?

Milne: We got them rather late and our studies were not very good in this respect. We had evidence of streptococcal aetiology in only a small proportion, probably less than 20 per cent, both from culture and serology.

Hutt: Do you know whether any of them were Type 12?

Milne: We did not type the organism.

Earle: Did they all have an infection before the onset of this condition?

Milne: About 50 per cent had a history of upper respiratory infection. We do not have exact figures; we are just analysing the series in detail at the moment.

Earle: Was this epidemic or were these sporadic cases over several years?

Milne: Seventy per cent of these cases occurred in the London area, in the winter of last year, but their regional distribution did not indicate an epidemic.

Jennings: Did the other internists here see any similar cases?

Wilson: Yes, we had cases. Very few were anuric. I think this higher incidence of anuric glomerulonephritis was due to the fact that the artificial kidney was available at The Hammersmith Hospital.

Joekes: We have seen them at Halton Hospital quite a lot presumably for the same reason.

Ross: Dr. Milne emphasized the crescent formation in acute, rapidly

progressive glomerulonephritis. One also notices intense necrosis of the glomerular tufts associated with proliferative and exudative lesions around the stalk, around the arteriolar part of the glomerular tuft. I wondered if the development of this particular lesion was perhaps responsible for the rapid downhill course of the patients, rather than the actual crescent formation.

Milne: You mean a strangulation of the blood supply of the glomerulus? Yes, I agree. This is what seems to be indicated from the suddenness of the occurrence of severe oliguria.

Vernier: About five years ago we studied a young girl with what I think is essentially the same syndrome. Morphologically and clinically, this was a rapidly progressing glomerulonephritis. Because of her persisting anuria and because of our tentative conclusion from biopsies of nephrotic patients given steroids that there was some relationship between the decrease in proliferative reaction within the glomerulus and steroid administration, this patient was given enormous doses of hydrocortisone intravenously from the 22nd to the 28th day of anuria, and on the 28th day she had a sudden diuresis. She recovered and is reasonably "well" four years after the treatment. We of course have no idea whether there was any relationship between the administration of steroids in this patient and the recovery. Were your patients given steroids, Dr. Milne and, if so, was there any effect either clinically or morphologically?

Milne: I would have thought none in the group that I have shown you. I have only seen one case of acute oliguric glomerulonephritis where I have thought that steroids had helped the patient, and she was in the milder group where the histology is indistinguishable from the usual run of acute glomerulonephritis.

Joekes: At one time we treated this group of patients with intensive steroid therapy. In about twelve patients, two died from acute gastrointestinal bleeding. In none of the patients did there seem to be any effect at all on the renal lesions when they finally came to post-mortem.

Blainey: We had a similar experience to Dr. Milne's in one patient with acute renal failure, in whom the autopsy revealed typical changes of periarteritis nodosa in the pancreas; I wonder if any of Dr. Milne's cases showed this.

Milne: We have carefully analysed the series for this. During this

period we have had six patients with severe oliguria whom we classified as microscopic polyarteritis nodosa of the type described by Davson and Platt, and two others who historically and clinically were cases of Wegener's granulomatosis. In these cases, the actual clinical course seemed to be very similar to cases of oliguric glomerulonephritis, but we have separated these cases out because they are obviously pathologically a different group.

Rich: In the one case of oliguric membranous nephritis that you described, was that glomerulus characteristic of the other glomeruli?

Milne: Yes. We continued to dialyse that man as long as we could because we thought it looked like a recoverable lesion but it seemed in the end to be as irrecoverable as the rest.

Movat: What did the kidney look like at autopsy? Did the picture change from the biopsy to the autopsy?

Milne: No. I think there was very little change, but I would need to go through the slides again to be absolutely sure of that.

Movat: I was very impressed by the fibrosis that you showed in one case. We saw something similar in experimental nephrotoxic or Masugi nephritis of dogs (Movat, H. Z., McGregor, D. D. and Steiner, J. W. (1961). *Amer. J. clin. Path.*, in press). What seems to happen in Bowman's capsule is that plasma protein, particularly fibrin, is exuded in large quantities, and apparently the organization of this fibrin is what brings about the fibrosis and true collagen development in Bowman's capsule.

Kark: Is it not possible that this one case of yours had this membranous change earlier? Is there any family history of diabetes? Did he have hypertension before? And on top of this has there now developed a streptococcal glomerulonephritis? Have you serial sections through many glomeruli?

Milne: We are obviously going to study this case in more detail. However, there was no clinical history of previous renal disease either from the patient or from relatives. In the other cases one can regard the anuria as simply due to complete disappearance of the space between the tuft and Bowman's capsule, with obliteration of the filtering surface, but this one is still in doubt. I think one has to postulate an impermeable membranous covering to the glomerulus.

Wilson: Patients with various forms of Bright's disease may get

anuria. Sometimes anuria develops in malignant hypertension, and the histological picture is no different from other cases of malignant hypertension. I don't think we can attribute anuria to any specific structural change in the glomerulus.

Hamburger: We have seen 37 cases of anuric acute glomerulonephritis, and in our experience there are two separate types. One type is what you called the progressive and fatal type. However, there are also a number of cases of anuria which are often severe enough to require the artificial kidney but whose prognosis after some months is not worse than in the ordinary glomerulonephritis. Some of them go on to a total cure. We have never treated them with steroids. Perhaps there are two evolutions possible, and the fact that someone is anuric with the picture of glomerulonephritis does not mean that it is necessarily a progressive, acute glomerulonephritis.

Earle: Were the early lesions in these patients exudative or were they proliferative?

Hamburger: Proliferative in all our cases, but I should mention that most of our biopsies were not performed in the very early days of the disease.

Movat: Do I understand, Dr. Milne, that you said that Bowman's space was completely obliterated by crescents?

Milne: In a low-powered view at necropsy we looked over two or three hundred glomeruli and saw no evidence of any Bowman's space whatsoever in any glomerulus.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

FOCAL GLOMERULONEPHRITIS

R. H. HEPTINSTALL*

Department of Pathology, Washington University, St. Louis

and

A. M. JOEKES

Institute of Urology, London

IN 1959 Heptinstall and Joekes described a series of 13 cases of focal glomerulonephritis encountered at renal biopsy in patients with a wide variety of clinical presentations. The present paper is an extension of this study and deals with 31 cases of this condition out of a total of 400 biopsies. The main pathological and clinical features are described and an attempt is made to correlate them.

Pathology

The essential feature of this condition is the way in which only certain glomeruli are affected, with the rest being normal. The proportion affected varies from one-quarter to two-thirds. Further, it is usual for the affected glomeruli to show changes in only a part of the tuft.

In affected glomeruli the tuft may show proliferation of both endothelial and epithelial cells in either the whole tuft (Fig. 1) or more usually in only one or two lobules (Fig. 2). Small foci of necrosis or intracapillary thrombosis are found in a substantial number of cases (Fig. 3), sometimes associated with cellular proliferation in the immediate vicinity. In many cases there are

* Visiting Professor.

eccentric hyaline areas in the tuft, often with adhesion to Bowman's capsule (Fig. 4). Proliferative and necrotizing changes

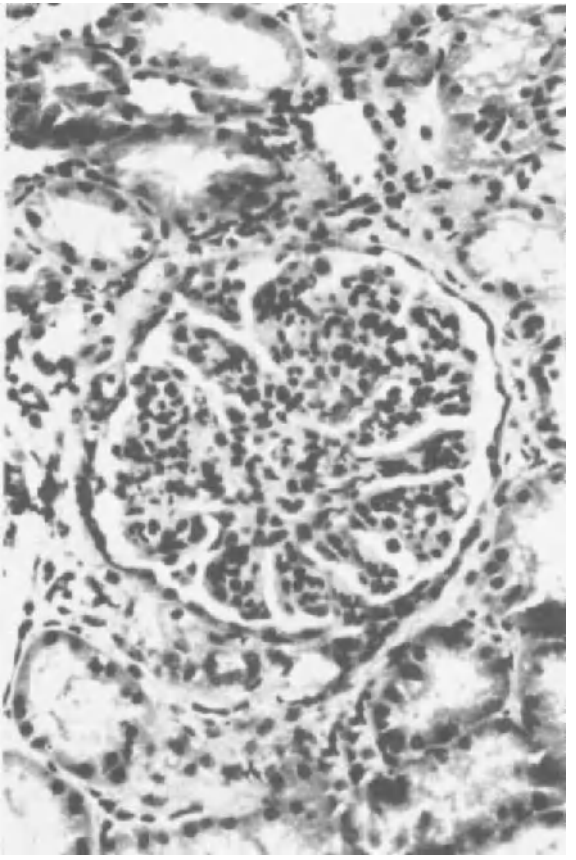


FIG. 1. Diffuse proliferative change in tuft with accentuation of lobular pattern. (Haematoxylin and eosin. × 260.)

may be seen in the same biopsy as the hyaline areas, but often one form of change is seen in the absence of the other. There is sometimes proliferation of cells lining Bowman's capsule, amounting

to crescents in some cases, and organization of these may sometimes be seen. Adhesions between these cells and the tuft are

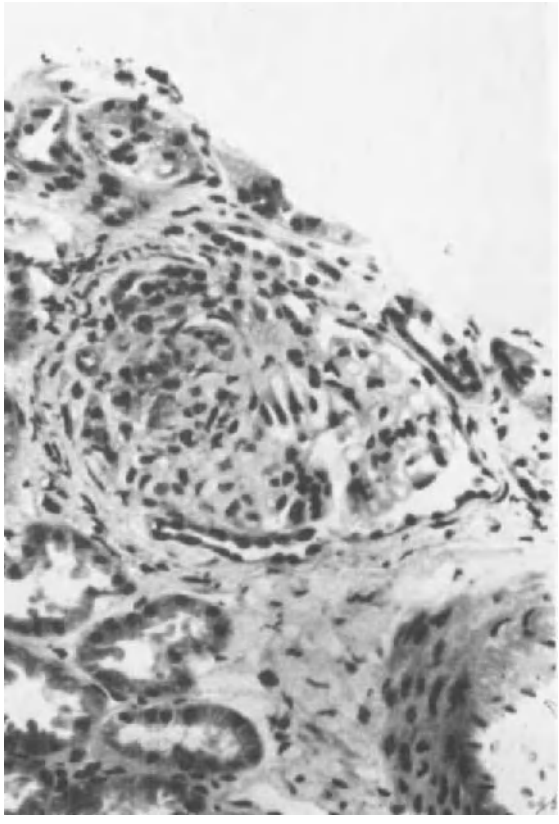


FIG. 2. Area of cellular proliferation confined to one part of glomerulus. There is also some hyalinization in adjacent part of tuft. (Haematoxylin and eosin. × 260.)

common. Localized thickening of the capillary basement membranes may be seen in some cases.

No specific changes are seen in tubules, focal loss being present in the more severely affected cases.

Interstitial tissue may show chronic inflammatory cells, chiefly lymphocytes, in areas of maximal tubular loss.

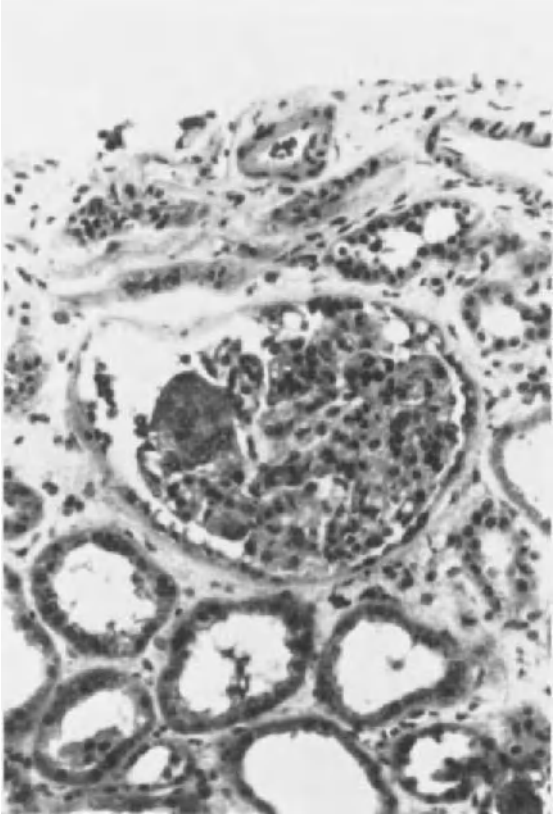


FIG. 3. Tuft with area of fibrinoid necrosis confined to one lobule. (First biopsy from patient M. referred to in Table I.) (Haematoxylin and eosin. × 200.)

Arterial and arteriolar changes are not as a rule conspicuous, but some thickening may be seen in the older patients and in those with raised blood pressure.

Bacterial culture of renal biopsy tissue has invariably been sterile.

Proliferative changes and necrosis are found most often in those cases where biopsy is performed early in the course of the disease, while the eccentric hyaline changes are found most frequently

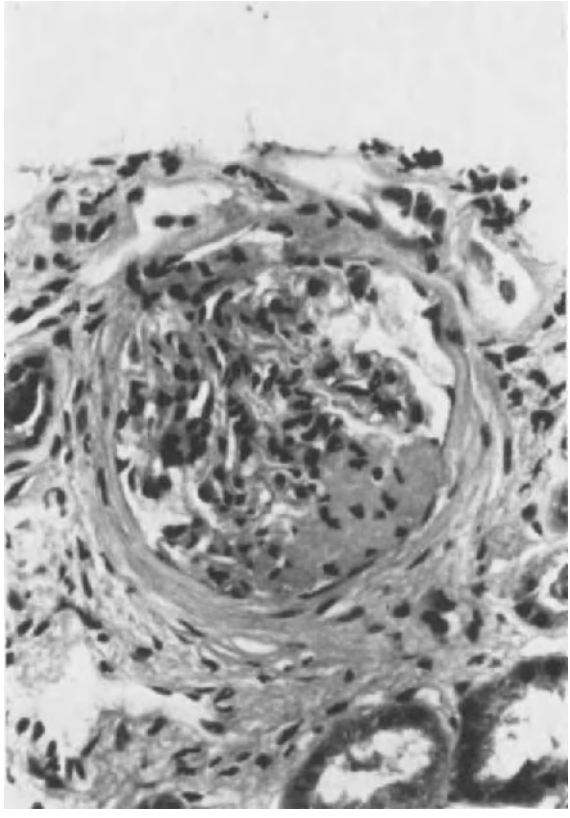


FIG. 4. Eccentric area of hyalinization in glomerular tuft with adhesion to thickened Bowman's capsule. (Second biopsy from patient M. referred to in Table I.) (Haematoxylin and eosin. × 320.)

in those cases with the longest histories. A repeat renal biopsy has been performed in five patients, and the histological changes are summarized in Table I. It will be seen that in the first biopsies the

Table I

CHANGES IN GLOMERULAR TUFTS IN PATIENTS WITH TWO BIOPSIES

Patient 1 = 1st biopsy 2 = 2nd biopsy	*Interval between 1st and 2nd biopsy (months)*	*Number of glomeruli seen*	*Proportion of glomeruli affected*	*Proliferative change* (0 to +++)	*Necrosis* (0 to ++)	*Eccentric hyalinization* (0 to ++)
M 1		14	$\frac{1}{2}$	+++	+	0
M 2	24	20	$\frac{1}{4}$	0	0	++
J 1		30	$\frac{1}{2}$	+++	0	0
J 2	30	22	$\frac{1}{3}$	+	0	++
E 1		27	$\frac{1}{3}$	++	0	+
E 2	9	15	$\frac{1}{5}$	+	0	++
G 1		32	$\frac{1}{2}$	++	++	+
G 2	10	15	$\frac{1}{4}$	+	0	+
K 1		24	$\frac{1}{4}$	+++	+	0
K 2	18	12	$\frac{1}{2}$	+	0	++

Patients G and K had proven systemic lupus erythematosus.

proliferative and necrotizing changes in the glomeruli were abundant, whereas in general the eccentric hyaline areas were sparse. In the second biopsies (nine months to two-and-a-half years later) the proliferative changes were inconspicuous whereas the hyalinized areas were frequent. This provides good evidence for the hyaline areas being the end phase of proliferative or necrotic changes in the tuft.

Histological differential diagnosis

Before a renal lesion can be accepted as being focal glomerulonephritis, certain other pathological processes must be excluded.

The first of these is a resolving acute diffuse glomerulonephritis. In this condition at the height of activity there is a great increase in nuclei in all the glomerular tufts. These nuclei are mainly the endothelial and epithelial cells of the tuft with varying numbers of cells from the blood, chiefly neutrophils. During the process of resolution, which may take several months, there is a gradual decrease in numbers of nuclei until the glomeruli become normal. We have performed serial biopsies in cases of acute diffuse glomerulonephritis and have seen how in general the process of resolution is uniform. Occasionally one or two glomeruli seem to lag behind the rest in their rate of resolution, and such a picture could conceivably be mistaken for focal glomerulonephritis. However, the difference in cellularity between these more slowly resolving glomeruli and the rest of the glomeruli is slight compared with the difference seen in our accepted cases of the focal form. Rich (1956), and Hutt, Pinneger and de Wardener (1958) have shown that in some cases of acute diffuse glomerulonephritis certain glomeruli may show the proliferative lesion to be more pronounced in one lobule of a tuft than in others. We have no information as to how such lesions would behave during the process of resolution, but if these excessively cellular lobules were to resolve more slowly than the others we might have a situation impossible to distinguish from focal glomerulonephritis, for it will

be remembered that in this state the proliferative lesions are predominantly confined to individual lobules in the tuft. The scarred eccentric areas seen in the glomeruli of focal glomerulonephritis could conceivably be the end result of an acute diffuse process in which certain glomeruli had been severely injured, but we have never seen such lesions in cases with a history of acute diffuse glomerulonephritis. The complete absence of a history of post-streptococcal acute glomerulonephritis in all our cases of focal glomerulonephritis is strong evidence for the rejection of the thesis that the latter disease is a stage in the healing of a diffuse process.

The lesions in focal glomerulonephritis are indistinguishable from those which may be seen in the kidneys of patients with subacute bacterial endocarditis. This condition, the so-called focal embolic glomerulonephritis, has been well described by Löhlein (1910), Baehr (1926) and Bell (1932). In this, varying numbers of glomeruli show active necrotizing foci in the tufts, sometimes with associated cellular proliferation and even crescent formation. In addition, healed lesions in the form of eccentric hyaline areas may be found in glomeruli. The way in which focal glomerulonephritis is produced has never been finally settled but, briefly, two separate explanations have been given. The first, championed by the above-mentioned authors, is that all the lesions may be produced by the lodgement in the glomerular tufts of minute infected emboli derived from the infective vegetations. The second explanation, that of Longcope (1916), is that the glomerular changes are the result of some form of sensitization. In the cases under discussion there is no evidence for bacterial endocarditis, but this condition is clearly of importance because it brings up the question of purely mechanical factors in the production of glomerular lesions. A study of the parenchyma around the edges of renal infarcts reveals that some glomeruli are not completely dead and that necrosis confined to only several lobules of a given glomerulus may occur. Proliferation of epithelium lining

Bowman's capsule may also be present in such glomeruli. These areas of necrosis may heal, with the production of an eccentric scar such as is seen in focal glomerulonephritis. A similar glomerular scarring was found experimentally around the periphery of infarcts produced by the introduction of non-infective particulate matter (Alexander, Heptinstall and Pickering, 1961) so it is unnecessary to postulate an infective element. As a further demonstration of the importance of mechanical factors, Muirhead, Booth and Montgomery (1957) produced necrotizing lesions in glomeruli by the injection into the renal artery of autolysed muscle. In no case in the present series was there evidence of a focus from which emboli might become disseminated. Rich (1956), however, has given a timely warning about the possibility of embolization in the production of the glomerular lesions in polyarteritis nodosa from a necrotic vessel in the renal arterial tree. It is clear, therefore, that the possibility of such embolic phenomena should be reduced as far as possible.

The microscopical form of polyarteritis nodosa (Davson, Ball and Platt, 1948) presents very similar changes to those we have described, except that by the time it is seen at autopsy the number of glomeruli involved is very large. Many glomeruli, however, remain uninvolved and it appears that this form of polyarteritis nodosa represents an extensive focal glomerulonephritis. Slow ischaemia such as is present in arteriolosclerosis and arteriosclerosis of the renal vessels is unlikely to be confused with the renal lesion of focal embolic glomerulonephritis. In this situation the changes consist of a shrinkage of the tuft with a surrounding mantle of hyaline material forming inside Bowman's capsule (McManus and Lupton, 1960). The necrotizing changes of malignant hypertension which may be associated with cellular proliferation are unlikely to cause confusion because no necrotizing arteriolitis was found in any case and considerable elevation of diastolic blood pressure was rare.

A wide variety of lesions may be produced in glomeruli in

pyelonephritis and most important among these is what Kimmelstiel and Wilson (1936) called alterative glomerulitis. This change consists of nuclear proliferation, irregular pyknotic nuclei and necrosis occurring in part of the tuft and is almost invariably found in those cases of pyelonephritis which are associated with hypertension and renal insufficiency. Most pyelonephritic lesions are associated with changes in the surrounding tubules and interstitial tissue, whereas such changes are not usually seen in the vicinity of glomeruli affected in focal glomerulonephritis.

We are usually reluctant to diagnose focal glomerulonephritis in the absence of a proliferative element, but in cases referred to before with repeat biopsies we have seen a picture in which only healed lesions are present, whereas in the original biopsy there was a predominantly proliferative lesion. In certain cases, however, with only one biopsy we have seen healed lesions only. In those cases in which we have been able to exclude other pathological processes we have assumed that the eccentric areas of sclerosis in the glomeruli were the healed phase of focal glomerulonephritis. Such a picture was obtained in only four cases, and in a further two cases, although there were no proliferative changes in the tuft, healing crescents were found in several glomeruli.

Clinical course

The clinical presentation of the 31 patients has been extremely varied. It is certainly not possible to correlate the histological diagnosis of focal glomerulonephritis with a characteristic clinical picture. In Table II the clinical presentation of the patients is set out in six arbitrary groups. It is of interest that of the total number of patients 24 have been male and only seven female. The over-all proportion of male to female in our total renal biopsy material is eight to five.

It is similarly not possible to correlate the different histological variants seen within these six clinical groups. Proliferative and hyalinized lesions occur in some of the patients in each of the

Table II

CLINICAL PRESENTATION AND SEX DISTRIBUTION OF SIX ARBITRARY GROUPS

		Male	*Female*
All patients	31	24	7
Nephrotic syndrome	10	8	2
Schoenlein-Henoch syndrome	4*	3	1
Systemic lupus erythematosus	2	0	2
Recurrent haematuria	3	3	0
Chance proteinuria	9	7	2
Miscellaneous	5	5	0

* Two (both male) also included in nephrotic syndrome group.

different clinical groups. The nephrotic syndrome group was the only one in which necrotic changes were consistently absent from the glomeruli.

Only three of the patients presented with recurrent isolated haematuria, which in the past has been accepted as the picture seen with a presumptive histological diagnosis of focal glomerulonephritis. These were three male patients, aged 25, 30 and 33 years at the time of renal biopsy, with respective lengths of history of 18 months, three years and three months. Well marked necrotic and proliferative changes were present in the glomeruli in the patient with the short history, being absent in the other two. The five patients grouped under the miscellaneous heading all presented with a relatively acute illness: one with pneumonia, haemoptysis and haematuria; another with a rash, muscle pains and haematuria; another with recurrent haemoptysis; one with polyarthritis and haematuria; and the last with polyarthritis. All of the patients had at some time had a raised blood pressure and four had impaired renal function, but in only one of these was this permanent. Those patients presenting with a Schoenlein-Henoch syndrome similarly had an acute episode, as did some of the patients with a nephrotic syndrome. In four of the nine patients presenting as chance proteinuria, careful detailed questioning elicited an acute episode in the past with probable renal involvement.

Table III

INCIDENCE OF IMPAIRED RENAL FUNCTION, HYPERTENSION, RASH AND ARTHRITIS ACCORDING TO CLINICAL PRESENTATION

	Total number of patients	*Impaired renal function*		*Hypertension*		*Rash*	*Arthritis*
		Transient	*Permanent*	*Transient*	*Permanent*		
All patients	31	9	4	7	7	10	9
Nephrotic syndrome	10	5	2	3	2	4	3
Schoenlein-Henoch syndrome	4	1	0	0	1	4	2
Systemic lupus erythematosus	2	0	0	0	0	2	1
Recurrent haematuria	3	0	0	1	0	0	0
Chance proteinuria	9	0	1	1	1	1	1
Miscellaneous	5	3	1	2	3	1	2

Apart from evidence of renal involvement, there was hypertension, and some form of rash or arthritis in a significant proportion of the patients. In Table III these features, as well as evidence of renal impairment, are analysed.

Four patients have had permanent or progressive impairment of renal function with follow-up periods from one to two-and-a-half years. Of the patients with hypertension, the blood pressure in seven returned to normal; in three it remained unchanged with only moderately raised diastolic levels, all in elderly patients; in four patients, all males, aged 12, 47 (Fig. 5), 47 and 58 years, the blood pressure has risen since the initial episode, with diastolic values exceeding 120 mm. Hg. Apart from the two patients with systemic lupus erythematosus, the rash associated with the presumed onset of the renal lesion has either been purpura, as in all four cases of Schoenlein-Henoch syndrome, or a papular erythematous rash usually on the legs, fading in about a week, in some instances leaving faint circular brown stains which have gradually disappeared in the following weeks. In only three patients have recurrent arthritic symptoms occurred after the onset of the renal lesion. One of these was a woman of 73 years, who had rheumatoid arthritis presenting with a nephrotic syndrome; another was a woman of 43 years who had systemic lupus erythematosus; and the third was a man of 58 years in whom proteinuria was discovered while he was attending a rheumatology department. In this latter patient, two years after the renal biopsy, the blood pressure had risen to 210/130 mm. Hg, the blood urea was 76 mg./100 ml., and dubious haematological evidence for systemic lupus erythematosus had been found.

The four patients with permanent impairment of renal function all showed a high proportion of involved glomeruli with tubular loss. On the other hand extensive involvement of glomeruli, even including necrotic changes, has not necessarily been associated with evidence of functional renal impairment.

Thirteen patients have recovered completely with regard to

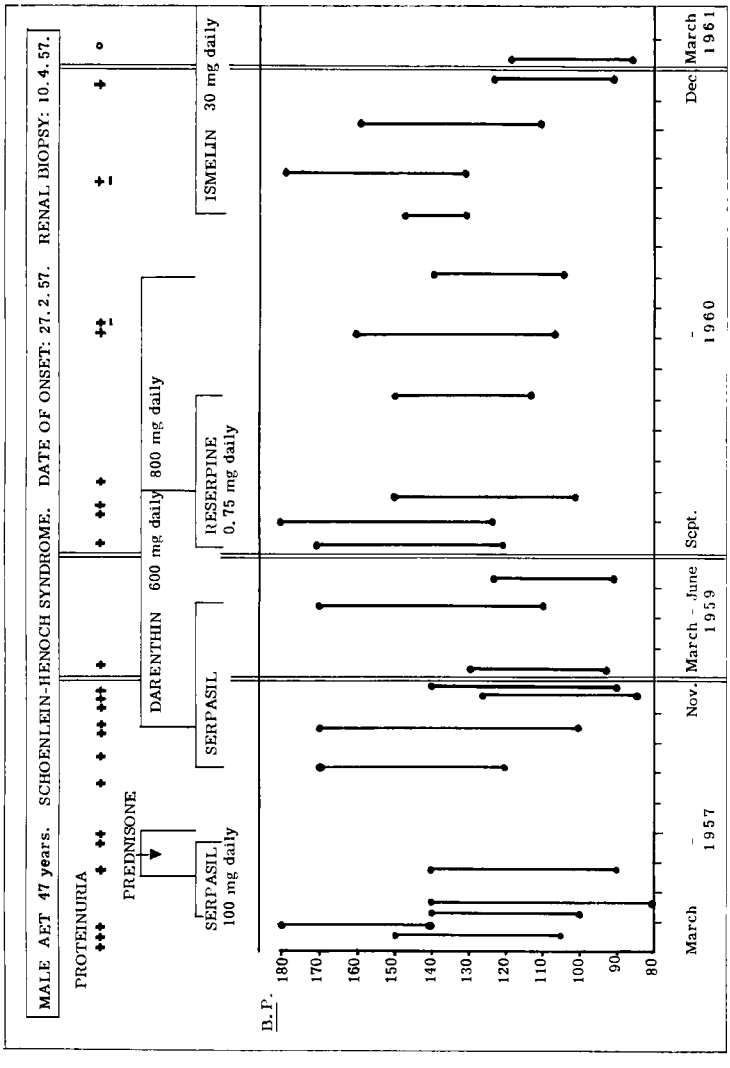


FIG. 5. Male, 47 years, presenting with Schoenlein-Henoch syndrome, showing the hypertension persisting three years after renal biopsy, but having settled with treatment when all urinary abnormality finally disappeared. The blood urea remained normal throughout the course. A second renal biopsy was taken 2.5.59 (M. in Table I.)

Table IV

PATIENTS WITH CLINICAL RECOVERY. DURATION OF STEROID THERAPY WHEN USED

	Total number of patients	*Number of patients with recovery*	*Duration of steroid therapy (months)*			
			<3	<6	>6	*None*
Nephrotic syndrome	10	5	1	1	1	2
Schoenlein-Henoch	4*	4*	2†	0	1†	1
Systemic lupus erythematosus	2	2	0	0	2	0
Recurrent haematuria	3	1	0	0	0	1
Chance proteinuria	9	2	0	0	1	1
Miscellaneous	5	1	0	0	1	0
Totals	31	13	2	1	5	5

* Two included in Nephrotic syndrome.
† One included in Nephrotic syndrome.

evidence of renal involvement, as judged by the disappearance of any urinary abnormality and the presence of a normal blood urea. Table IV shows the number of patients in each clinical group who have recovered. The duration of steroid therapy when used is also shown.

Five patients recovered without steroid therapy. Four of these showed pronounced proliferative lesions and one had necroses in tufts. The fifth patient showed only hyalinized lesions. In all, the disappearance of urinary abnormalities took place many months after the renal biopsy (Fig. 6). In view of the well marked activity of the lesions in those patients recovering completely without steroid therapy the assessment of the value of such treatment is extremely difficult. It must, however, be doubtful whether it may be believed that the two patients with systemic lupus erythematosus would have shown a resolution of the renal lesion if steroids had been withheld. In both patients repeat biopsies showed a progression to the healed form of the lesion, with considerable diminution in proliferative activity and disappearance of necroses. Both however still showed positive LE cell preparations four and three years after the initial biopsy. It is of interest that all the patients with Schoenlein-Henoch syndrome made a full recovery, one without steroid therapy.

Of the 18 patients not shown as recovering, one patient in the nephrotic group could not be followed although known to be living a normal life three years after the biopsy. Two patients in this same group had persistent heavy proteinuria without impairment of renal function, and two had slowly progressive deterioration of renal function. Two patients with recurrent haematuria had persistent proteinuria during the period of follow-up. In three of the patients with chance proteinuria the follow-up period did not exceed six months. One man of 27 years had persistent proteinuria one year after biopsy, but no other abnormality. Another man of 58 years (referred to above) had progressive renal impairment, hypertension and arthritis two and a half years

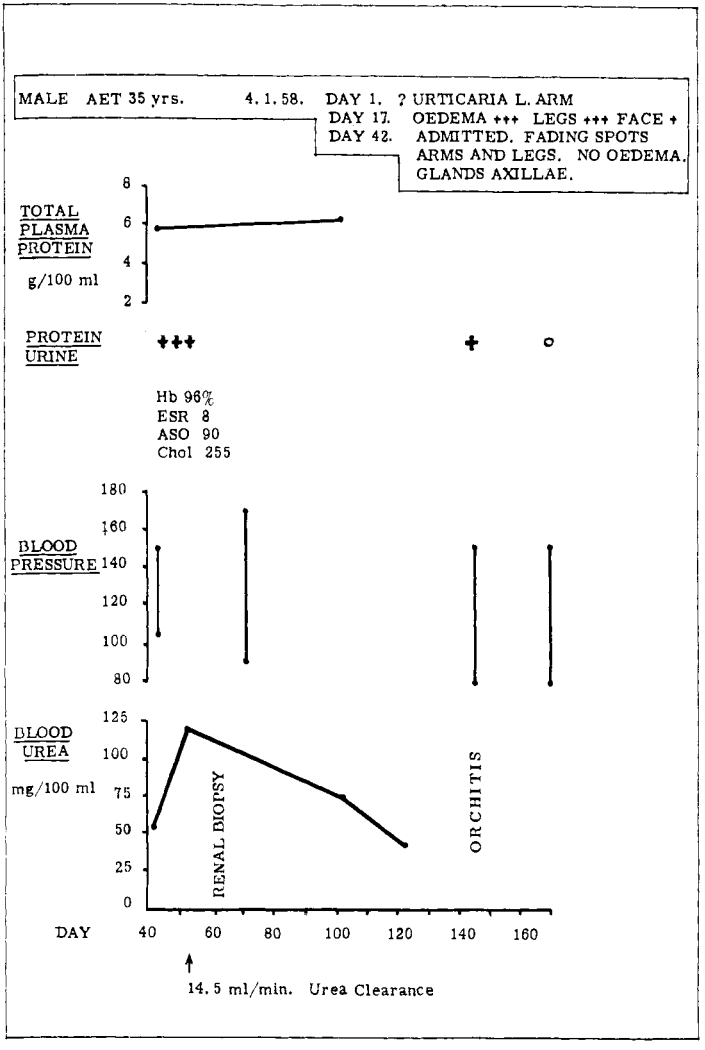


FIG. 6. Male, 35 years, presenting with a nephrotic syndrome. Eventual complete recovery with a three-year follow-up. No steroid therapy.

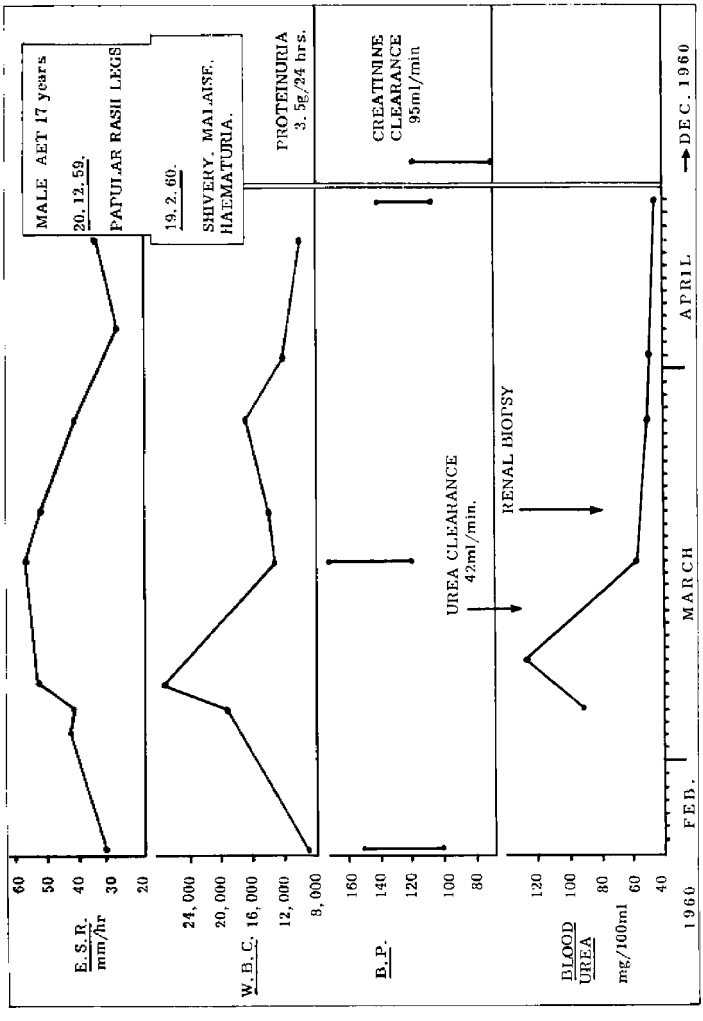


FIG. 7. Male, 17 years, presenting with an acute episode with haematuria two months after a rash on the legs. No steroid therapy.

after biopsy. The other two patients were not accessible for follow-up. Of the five patients in the miscellaneous group presenting with some acute illness, only one had lost all evidence of renal abnormality. Fig. 7 shows a patient in this group with persistent proteinuria.

Conclusions

Although the clinical presentation in these patients has in some cases shown several features of acute diffuse glomerulonephritis, the absence of some typical features has been striking. Only rarely has an upper respiratory infection caused by β-haemolytic streptococci been present and in only one patient has there been a significant increase in the anti-streptolysin O titre. In those patients presenting with either hypertension or oedema or both there has been no history of dyspnoea and clinically the venous pressure has not been raised. It should be emphasized that in none of the patients was there evidence of bacterial endocarditis nor septicaemia.

Recurrent episodes of focal glomerulonephritis are not sufficiently well established in our series to enable us to make a critical comment.

The relatively high proportion of patients in whom there is good evidence of a persistent renal lesion for periods ranging from one to four years (12 out of 31) suggests that at least some cases of so-called latent or chronic glomerulonephritis are derived from this group.

The aetiology of focal glomerulonephritis is not apparent from this study, but this was more fully discussed in our 1959 paper.

Summary

(1) Thirty-one cases of histological focal glomerulonephritis are presented.

(2) The histological features consist of a proportion of the glomeruli showing proliferative, necrotizing or hyaline lesions.

(3) Repeat biopsies in five patients have shown the evolution of the proliferative and necrotizing change to the hyaline.

(4) The clinical presentation is not homogeneous; the patients have been divided into six arbitrary groups according to the mode of presentation.

(5) It was not possible to correlate the clinical presentation with the histological changes.

(6) Thirteen patients showed a complete recovery as judged by a normal blood urea and disappearance of urinary abnormalities.

(7) Twelve patients showed evidence of a persistent renal lesion with either urinary abnormalities, hypertension or renal functional impairment from one to four years after the biopsy. Four of these had persistent or progressive renal functional impairment; biopsy had shown an extensive lesion in these patients.

REFERENCES

ALEXANDER, N., HEPTINSTALL, R. H., and PICKERING, G. W. (1961). *J. Path. Bact.*, **81**, 225.

BAEHR, G. (1926). *J. exp. Med.*, **15**, 330.

BELL, E. T. (1932). *Amer. J. Path.*, **8**, 639.

DAVSON, J., BALL, J., and PLATT, R. (1948). *Quart. J. Med.*, **17**, 175.

HEPTINSTALL, R. H., and JOEKES, A. M. (1959). *Quart. J. Med.*, **28**, 329.

HUTT, M. S. R., PINNIGER, J. L., and DE WARDENER, H. E. (1958). *Quart. J. Med.*, **27**, 265.

KIMMELSTIEL, P., and WILSON, C. (1936). *Amer. J. Path.*, **12**, 99.

LÖHLEIN, M. (1910). *Med. Klin.*, **10**, 375.

LONGCOPE, W. T. (1916). *Amer. J. med. Sci.*, **152**, 625.

MCMANUS, J. F. A., and LUPTON, C. H., JR. (1960). *Lab. Invest.*, **9**, 413.

MUIRHEAD, E. E., BOOTH, E., and MONTGOMERY, P. O. B. (1957). *Arch. Path. (Chicago)*, **63**, 213.

RICH, A. R. (1956). *Bull. Johns Hopk. Hosp.*, **98**, 120.

DISCUSSION

Wilson: I think that the clarification of focal glomerulonephritis is one of the most valuable things that renal biopsy has accomplished. The idea of focal nephritis dates back many years and we should separate out the different disorders in this group. Quite obviously the

collagen diseases must be separated out as a distinct type, and so should the nephrotic syndrome; although there is a superficial similarity in these focal lesions, I should think that electron microscopy will distinguish between them.

I would like to say more about one group which on clinical grounds seems to be a distinct entity. It occurs with recurrent attacks of haematuria, usually without hypertension or oedema. After one or two attacks albuminuria persists. These patients may go on for 10 or 15 years, having recurrent haematuria at frequent intervals, but renal failure is extremely slow in developing. We have observed a group of these cases and Dr. Ross recently reported on them (Ross, J. H. (1960). *Quart. J. Med.*, **29,** 391). I would put in a plea for this as a distinct type of Bright's disease. Renal biopsy has enabled us to characterize it in terms of the focal glomerular lesion. The morbid anatomist previously had difficulty in recognizing this as an entity, because when the patients eventually died with renal failure they usually had diffuse glomerular damage due to recurrent attacks.

In relation to the collagen disorders producing focal nephritis, we have recently published an account of focal glomerulonephritis associated with lung purpura (Rusby, N. L., and Wilson, C. (1960). *Quart. J. Med.*, **29,** 501). In one patient there was a focal glomerulonephritis of the type described here followed by an attack of diffuse nephritis. This was a boy of 16 who had haemoptysis and then developed haematuria after an interval of some months. Biopsy showed a focal glomerulonephritis, and only one or two glomeruli were affected at this time. A second biopsy was taken six weeks later when he had a recurrent attack of haematuria, this time associated with oedema and hypertension. The biopsy showed a diffuse glomerulitis with exudative, proliferative and necrotic changes, characteristic of type I nephritis running a rapidly progressive course. In the same patient then, it is possible to get focal nephritis, followed by diffuse nephritis.

Kark: Is that what one calls Goodpasture's syndrome?

Wilson: Goodpasture described a case of nephritis associated with pneumonia. That is not the same condition as we are describing, because in our cases there is nothing in the lung except haemorrhage —no pneumonia.

Heptinstall: This is the same as the group of seven cases described by T. W. Parkin and his colleagues in 1955 from the Mayo Clinic.

Wilson: There have recently been a number of descriptions but some cases are obviously polyarteritis nodosa. Our cases did not have the vascular lesions nor the clinical features of polyarteritis nodosa.

Pirani: Similar changes have been seen in Wegener's granulomatosis in the kidneys in association with granulomatous lesions in the lungs and perhaps also in primary pulmonary haemosiderosis.

Wilson: There were no granulomata in the lungs in our series. This condition is identical, I am sure, with pulmonary haemosiderosis, with focal or diffuse nephritis.

Ross: We selected the group with recurrent attacks of haematuria that Prof. Wilson mentioned because they seemed to form a homogeneous group of patients. There were nine patients who had recurrent episodes of haematuria which had occurred for up to 31 years, starting in early life. With the exception of one patient who had very slight hypertension, none of them had oedema, hypertension or renal impairment during their attacks of haematuria and the course clinically seemed extremely benign, except that a few years after the onset they did develop persistent proteinuria. The renal lesions found in five biopsies were not really homogeneous but all showed the focal lesions which Dr. Joekes and Dr. Heptinstall have described. One exception was a girl of nine who had almost persistent haematuria for 14 months without any impairment of renal function, no hypertension and no oedema, and all her glomeruli were involved; I think one could almost call it a diffuse glomerulonephritis. One can consider this as a severe example of the focal lesion involving many glomeruli and much of each glomerulus. It was striking that in all these patients there were no exudative lesions, no polymorphs in the glomerular tufts. In one boy of 14 who had almost two years of recurrent episodes of haematuria, again without any other clinical features, there was a predominantly interstitial reaction, but he did also have some of these focal lesions. As Dr. Earle mentioned earlier, we found red cells in Bowman's space and in the convoluted tubules; there were no obvious breaks in the capillary loops.

Joekes: We have taken all our patients with recurrent isolated haematuria and charted them according to the lesions we have found

in the kidney. Although some of the patients with recurrent haematuria have this type of renal lesion, others show quite a different histological picture.

Wilson: This is without oedema, without hypertension—so-called monosymptomatic nephritis?

Joekes: Yes. It is quite surprising how the proteinuria may clear up completely between attacks. Some of these patients have had as many as 20 attacks. Of our 11 cases of this type, three have had diffuse lesions, very similar to those Dr. Ross described, three have shown focal lesions, and in five we were unable to show any change in the kidney.

Hamburger: We had the same experience in at least three cases of recurrent haematuria in which there were more than 35 glomeruli per specimen and all of them were apparently entirely normal. Do you think that this could be consistent with a diagnosis of focal nephritis?

Joekes: Yes, it could. It is interesting that amongst the patients with the "no-change" kidneys there are some that are classically tripped into an attack of haematuria by a cold or a sore throat.

Rosenheim: We have seen eight or nine cases of recurrent haematuria, all in males, and done renal biopsies in six of them. Several of these patients have been in the hands of urologists and had frequent cystoscopies and X-rays. I think one of the important advantages of renal biopsy is that in focal nephritis of this sort, you can at least prevent further investigations being done on the patients.

Vernier: Dr. Joekes, I think that our experience in children would agree well with what you have shown. Although it is difficult to make a specific clinical diagnosis from this lesion I think it is a pathological entity of a sort. A strong thread suggesting Schoenlein-Henoch's disease runs through it, as you have shown, with many patients having rashes and other features such as arthritis that make it difficult to classify them definitely as Schoenlein-Henoch's purpura and others with clear Schoenlein-Henoch's purpura. We had a patient with recurrent haematuria who had four isolated attacks of haematuria and after the fourth attack developed the other symptomatology of Schoenlein-Henoch's purpura. On a biopsy obtained after the second attack, he had a focal lesion which looked like Schoenlein-Henoch's purpura, which he subsequently developed. In addition, I am quite convinced that this

focal pathological lesion can occur in the recovery stages of acute streptococcal-induced glomerulonephritis.

Movat: Electron microscopy may perhaps contribute something further to the understanding of focal glomerulonephritis. I am referring to examination of the unaffected portions of the glomerulus or of the glomeruli completely free of lesions. For example, in a mild case of acute diffuse haemorrhagic nephritis in a child, we found that some glomeruli showed hypercellularity and others appeared to be normal by light microscopy. We were surprised to find in these apparently normal glomeruli definite changes in the basement membrane by electron microscopy. I was wondering if it might not be worth while doing electron microscopy on the unaffected glomeruli of focal glomerulonephritis. Perhaps there are minimal, that is ultramicroscopic, changes in these, and the focal changes would then represent marked accentuation of the disease process, which one might visualize as the focal deposition or accumulation of antigen-antibody complexes.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

LUPUS NEPHRITIS*

CONRAD L. PIRANI

Department of Pathology, University of Illinois College of Medicine, Chicago

and

VICTOR E. POLLAK†, ROBERT C. MUEHRCKE and ROBERT M. KARK

Department of Medicine, University of Illinois College of Medicine, Chicago

THE clinical and pathological characteristics of systemic lupus erythematosus (SLE) have become better understood since the introduction of the LE cell test. With better understanding of the clinical manifestations, it has become apparent that certain conditions are now diagnosed as SLE which in the past were not easily classifiable as SLE (Harvey *et al.*, 1954; Soffer, 1959). In addition, the beneficial effects of treatment have modified both the clinical course and the underlying tissue changes (Muehrcke *et al.*, 1957; Pollack, 1959; Pollak *et al.*, 1961). Consequently the clinical and pathological spectrum of SLE seen today in the clinic and laboratory may differ somewhat from the classic descriptions of the past.

Despite these changing manifestations of SLE, in all patients reported with lupus nephritis there have been acceptable clinical criteria for the diagnosis of SLE. These have been set forth in detail previously (Muehrcke *et al.*, 1957).

Renal involvement in SLE—lupus nephritis—is common and is a most important feature of this disease. Once *significant* renal

* Supported by grants from the U.S. Public Health Service (H-2253) and from the Surgeon General's Office, U.S. Army (Contract DA-49-007-MD-637).

† Established Investigator of the American Heart Association.

involvement occurs, the renal disease is usually relentlessly progressive. Although exacerbations and remissions of SLE may occur, renal insufficiency and death in uraemia result, in almost all cases of severe lupus nephritis (lupus glomerulonephritis).

The introduction of a safe and simple method for percutaneous renal biopsy (Kark and Muehrcke, 1954) made available an excellent investigative tool for the study of renal diseases. For the first time it became possible to determine accurately in the living patient the character and severity of renal lesions, to follow the histological evolution of renal changes and to assess with greater precision the effects of therapy. Clinico-pathological correlations were made meaningful and of immediate practical importance for the care of the patient. Finally, special studies of renal tissue such as electron microscopy, histochemistry and microchemistry were made possible on a large scale in man.

Our interest in lupus nephritis started in 1953. At that time single and serial biopsies from patients ill with SLE became available for study to one of us (Pirani, Muehrcke and Kark, 1954), and it soon became apparent that the histological changes in various stages of this disease differed somewhat from previous descriptions (Klemperer, Pollack and Baehr, 1941). Our first experiences with a series of patients with lupus nephritis studied initially between 1953 and 1955 have been reported in detail (Muehrcke *et al.*, 1955 and 1957). It is the purpose of this report to present new observations, with particular emphasis on electron microscopic studies of the glomerulus and on the effects of two therapeutic regimens on the histological changes of lupus nephritis.

Material and methods

By February 1961, 105 patients ill with SLE had been studied by percutaneous renal biopsies. In 52 patients serial studies were made on two to five biopsies obtained at 2- to 12-monthly intervals. Post-mortem specimens were available in 22 of these cases. Electron microscopic studies were carried out in eight cases.

For light microscopic studies, renal biopsies were fixed in Helly's solution for three hours, washed overnight and embedded in paraffin. Sections were cut at 4–5 microns and stained routinely with haematoxylin-eosin, Masson's trichrome and alcian-blue periodic acid-Schiff. Where indicated in selected cases, additional stains were used. Fifteen to thirty serial sections were obtained from each biopsy. Particular attention was paid to the consistency of thickness of the sections and to the quality of the staining procedures used (Lillie, 1954). This was necessary particularly to facilitate diagnostic interpretation of the early changes, and to permit comparative and semiquantitative observations in serial biopsy studies.

For electron microscopic studies, specimens were fixed in osmium tetroxide and embedded in methacrylate (Palade, 1952). Sections were cut at 0·5 microns and stained with polychrome methylene blue for survey and orientation. The small blocks were trimmed further, sections were cut at 0·025 microns and were observed and photographed in an RCA EM-U2 or EM-U3 electron microscope.

Morphological analysis and quantitative morphology

It is essential to make detailed histological studies of the renal specimens obtained by percutaneous biopsy because it is only in this way that maximum information can be derived from the limited amount of tissue available. In addition, precise characterization of the disease process and assessment of the severity of the renal lesions are necessary to establish clinico-pathological correlations and to provide the clinician with quantitative information on which he can base his therapeutic approach and determine the effects of treatment.

A systematic and careful analysis of the four major components of renal tissue (glomeruli, tubules, vessels, interstitium) must be made. Thus, in lupus nephritis, the following histological features were examined and graded on a scale from 0 to 4 plus: *in the*

glomeruli, increase in number of endothelial and epithelial cells, thickness of the capillary basement membrane, local necrotizing lesions, fibrinoid, karyorrhexis, haematoxylin bodies, "hyaline" thrombi, fibroepithelial crescents, fibrosis and adhesions to Bowman's capsule; *in the interstitium*, the number and type of inflammatory cells and the extent of fibrosis and oedema; *in the small arteries*, the presence of fibrinoid changes, periarterial inflammation and fibrosis; *in the tubules*, the character and degree of tubular degenerative changes and the presence of protein and casts in the lumen. The evaluation and grading of each slide were made by two independent observers.

This type of detailed semiquantitative histological observation made possible consistent and reproducible evaluations of renal biopsies. It enabled us to utilize maximally the information contained in a rather small tissue specimen, and it allowed us to make detailed comparisons of serial biopsies in the same patient.

In electron microscopic studies an effort should be made to examine many sections from five or more glomeruli. This is essential since the problem of adequacy of renal tissue and of random sampling is many times greater than in light microscopic studies, particularly in the case of pleomorphic and focal renal diseases such as lupus nephritis (Arnold and Spargo, 1959).

The lesions of lupus nephritis and their evolution

In many renal biopsies taken from patients ill with SLE in whom there was no clinical or laboratory evidence of renal involvement no histological changes were found. In some cases, however, small local areas of endothelial hypercellularity and occasional swelling of epithelial cells may be observed, particularly at the periphery of the glomerular tufts (Fig. 1). These lesions may even precede the occurrence of abnormal urinary findings. Mild and irregular local and focal thickening of the capillary basement membrane was usually seen in association with these endothelial

cell changes. By electron microscopy the character of the lesions observed by light microscopy has been fully confirmed. In addition, fusion of the foot processes has been observed in isolated capillary loops, at least in kidneys from patients with proteinuria (Fig. 2). The fusion of foot processes cannot be recognized by

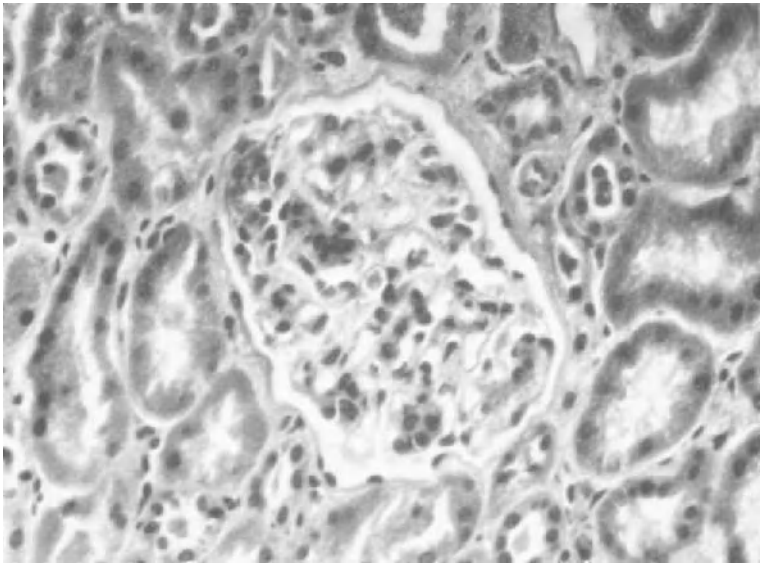


FIG. 1. Local endothelial hypercellularity associated with mild local thickening of the capillary basement membrane in early lupus nephritis (lupus glomerulitis). (Haematoxylin-eosin. × 385.)

light microscopy and might be responsible in part for the greater emphasis on the "membranous" changes in earlier studies (Muehrcke *et al.*, 1957). At this early stage of renal involvement fibrinoid and necrotic changes of the glomeruli were absent, and the tubules, interstitial tissue and small arteries were normal. The mild and predominantly proliferative character of the glomerular changes and the absence of other lesions in the renal parenchyma

justify the term "lupus glomerulitis" for this phase of lupus nephritis. These early lesions, although they strongly suggest the diagnosis of lupus glomerulitis, are not definitely diagnostic. In anaphylactoid purpura and asymptomatic persistent proteinuria, for example, similar changes may be observed (Vernier *et al.*,

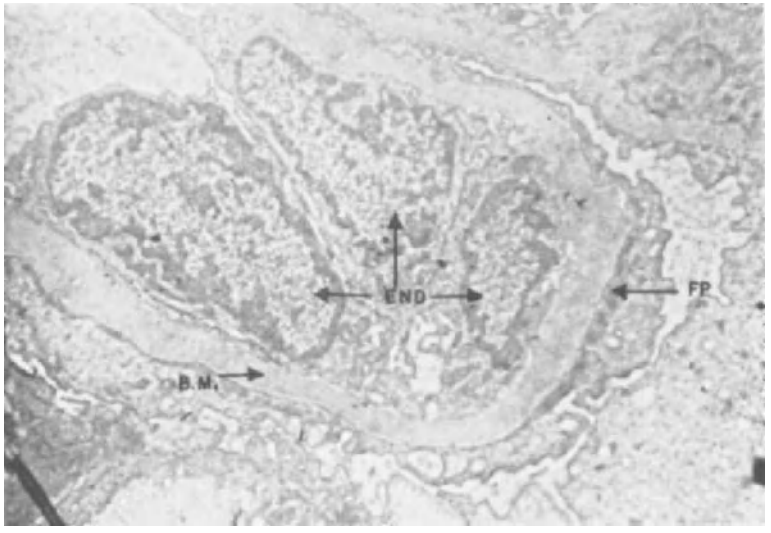


FIG. 2. Endothelial cell proliferation (END) in a capillary loop in early lupus nephritis (lupus glomerulitis). The capillary basement membrane (B.M.) is slightly thickened and there is complete fusion of the foot processes (FP) of the epithelial cells. (Electron microphotograph. × 7,400).

1961; Pollak *et al.*, 1958). Lupus glomerulitis does not necessarily progress to the more advanced phases of lupus glomerulonephritis. In many instances no progression of the renal disease has been observed for many years even when there was other evidence of active SLE.

In other patients lupus glomerulitis is the forerunner of more severe renal lesions, which usually develop within a few months

and progress rapidly unless effective treatment is given. These severe lesions were first seen more frequently, however, without the opportunity to observe the early stage of lupus glomerulitis. Local glomerular necroses usually affected scattered glomeruli

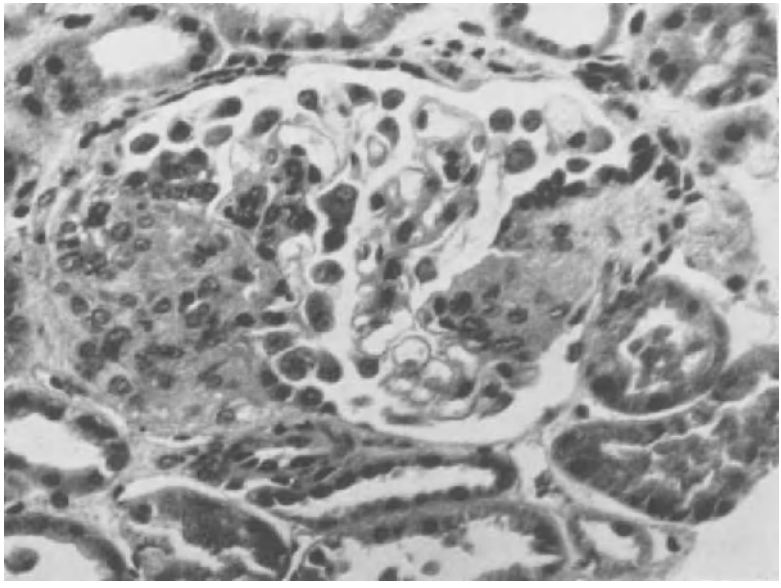


FIG. 3. Loca proliferative and necrotizing lesion in a glomerulus in severe lupus glomerulonephritis. Note the proliferation of endothelial and epithelial cells with karyorrhexis and obliteration of capillary loops. Adhesions to Bowman's capsule are present in these areas. Elsewhere the capillaries are patent but their basement membrane is irregularly thickened. The tubules are well preserved. (Haematoxylin-eosin. × 385.)

and were characterized by obliteration of capillary loops with fibrinoid changes, karyorrhexis and formation of haematoxyphil bodies. Occasional polymorphonuclear leucocytes were also found in these areas (Figs. 3, 4 and 5). Local necroses usually developed in areas of endothelial hypercellularity and were often associated with proliferation of the visceral and later of the parietal

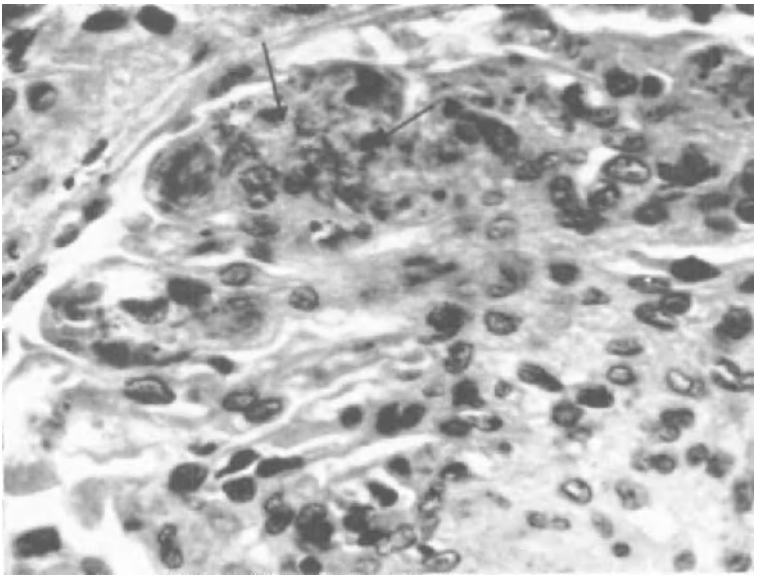


FIG. 4. Local glomerular necrosis in severe acute lupus nephritis. Note the marked karyorrhexis and the numerous haematoxyphil bodies (arrows). (Haematoxylin-eosin. × 665.)

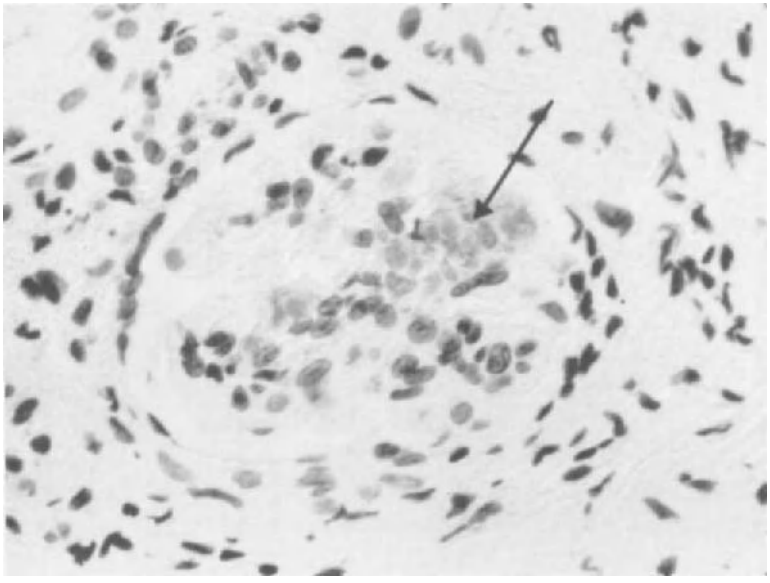


FIG. 5. Clumps of haematoxyphil bodies (arrow) in a glomerulus in severe lupus nephritis. Note the associated proliferative changes, karyorrhexis, and a fibro-epithelial crescent. (Feulgen. × 515.)

epithelial cells of Bowman's capsule, leading eventually to the formation of glomerular adhesions and of fibroepithelial crescents. The frequent presence of fibrin and "hyaline thrombi" (Fig. 6) within the glomerular capillaries suggests the possibility that an ischaemic factor may play a rôle in the histogenesis of local

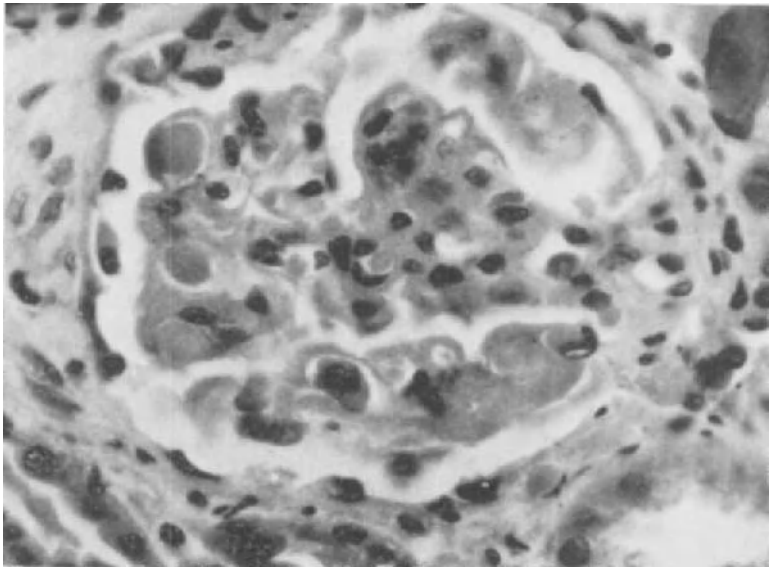


FIG. 6. Irregular basement membrane thickening of the glomerular capillaries in lupus nephritis. Two capillaries on the left contain "hyaline" thrombi. (Haematoxylin-eosin. × 665.)

necrotic lesions. In turn, pyknosis and karyorrhexis of endothelial nuclei and probably also of leucocytes might be related to the formation of haematoxyphil bodies and to the presence of abnormal immune factors including antinuclear globulins. Nuclear material, best shown by the Feulgen's reaction, could also be demonstrated in hyaline thrombi and elsewhere in necrotic areas.

Thickening and other changes in the capillary basement membrane were a consistent and early feature of lupus nephritis. In lupus glomerulitis the thickening was mild, but it is not clear

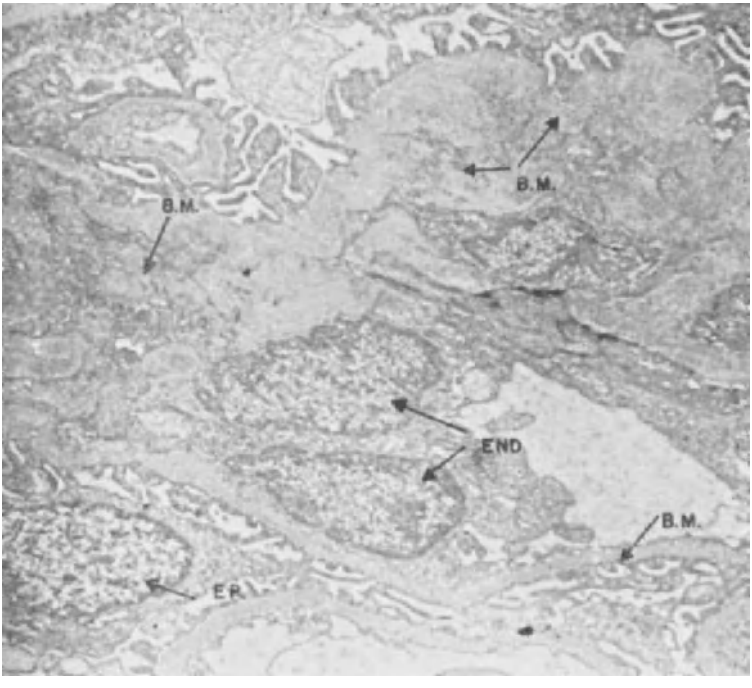


FIG. 7. Abnormal glomerular capillaries in lupus nephritis. The basement membrane (B.M.) is irregularly and markedly thickened in many areas. Basement membrane-like material is present within endothelial cytoplasm. Note the two adjacent endothelial cells (END) in the same capillary. Epithelial cells (EP) and foot processes are essentially normal. (Electron micrograph. × 7,400.)

whether this change precedes or follows the local proliferation of endothelial cells. Basement membrane-like material was noted by electron microscopy within the swollen endothelial cytoplasm in several instances and was probably an indication of an increased

and abnormal reactivity of these cells (Figs. 7 and 8). Whether slight or marked, thickening of the capillary basement membrane

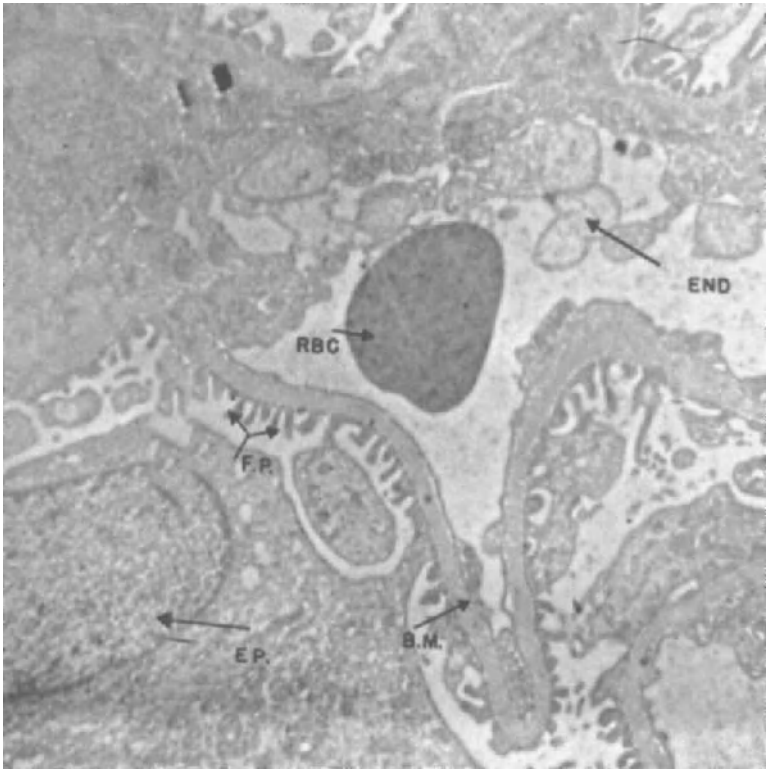


FIG. 8. Glomerular capillary in lupus nephritis with pronounced endothelial cytoplasmic swelling (END). The basement membrane (B.M.) is essentially normal. The epithelial cell and foot processes are not remarkable. (Electron micrograph. × 7,855.)

was characteristically irregular with a local and focal distribution. The degree of basement membrane thickening could be assessed accurately only by special staining procedures. In haematoxylin-eosin preparations it was impossible to resolve the three separate

layers of the capillary wall and the thickness of the basement membrane proper was often over-emphasized. The "wire-loop" lesions were characterized by fibrinoid changes of the thickened capillary wall. In Masson-trichrome preparations fibrinoid material was usually observed inside the capillary basement

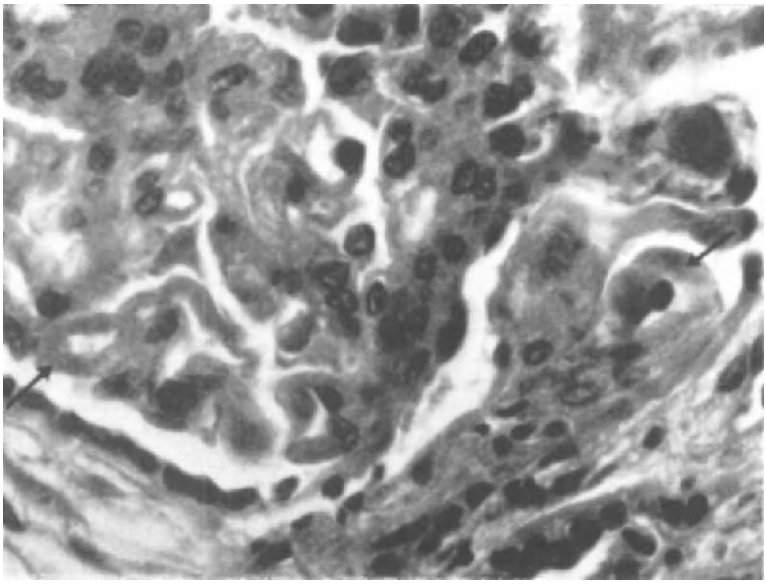


FIG. 9. "Wire-loop" changes (arrows) in a glomerulus in severe lupus nephritis. In this photograph of a Masson trichrome preparation the red fibrinoid appears as a dark-staining material inside the capillary basement membrane. (Masson. × 735.)

membrane (Fig. 9) which, however, may also be involved. By electron microscopy the "wire-loop" lesions were characterized by the presence of dense osmiophilic material between the basement membrane and the endothelial cytoplasm (Figs. 10 and 11) (Farquhar, Vernier and Good, 1957; Farquhar, 1959; Spargo and Arnold, 1960). This material was amorphous and contained no fibrillar structures. Its position and character suggested a trapping

of abnormal blood proteins rather than a local degenerative phenomenon.

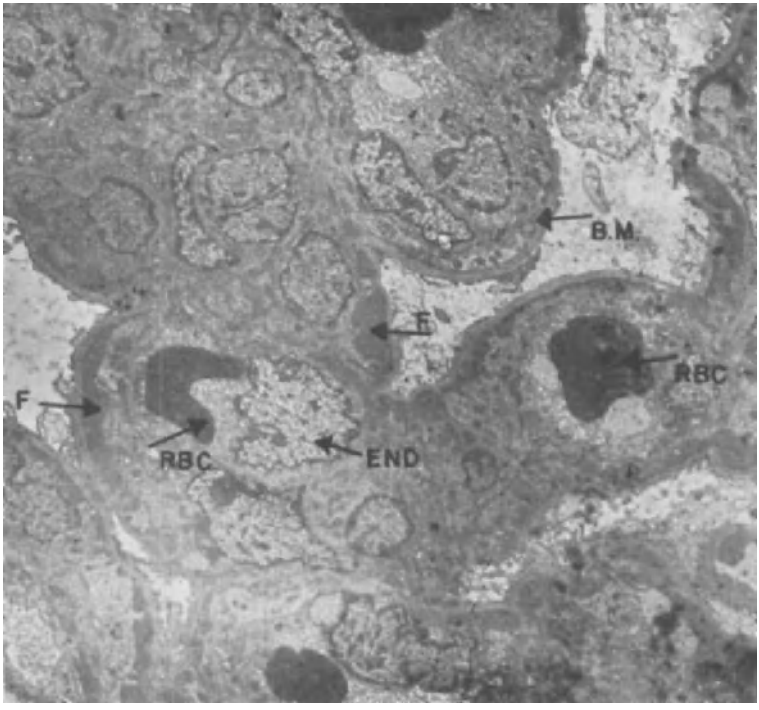


FIG. 10. Abnormal glomerular capillaries in lupus nephritis. Note the narrow capillary lumen with red blood cells (RBC). There is an increased number of endothelial cells (END) with abundant cytoplasm. The basement membrane (B.M.) does not appear abnormal. Between the basement membrane and the endothelial cytoplasm are abundant deposits of osmiophilic material (F) consistent with fibrinoid. The foot processes of the epithelial cells are indistinct and often fused. (Electron micrograph. × 3,000.)

Epithelial cell proliferation was seen commonly in the areas of more severe glomerular damage (Figs. 3 and 12). The epithelial cell cytoplasm was swollen in many places and changes could be observed in the trabeculae, but alterations of the foot processes

were usually minor and were found inconsistently. Epithelial crescents and adhesions occurred frequently. Eventually as a result of the inflammatory and necrotizing lesions glomerular fibrosis ensued. Frequently this process continued to be local and focal in distribution and did not reach the advanced degree and

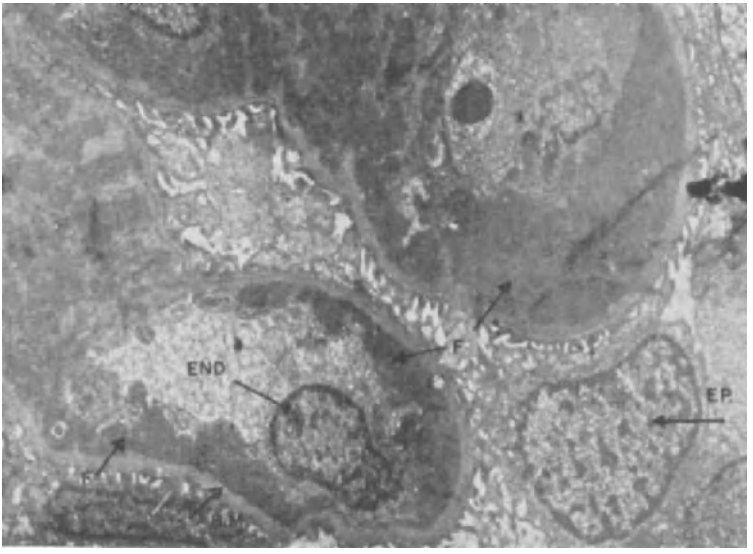


FIG. 11. Two abnormal capillaries in lupus nephritis clearly showing the abundant subendothelial deposits of fibrinoid (F). This appearance corresponds to the "wire-loop" changes of light microscopy. Note that the basement membrane (B.M.), epithelial cells (EP) and foot processes are essentially normal. (Electron micrograph. × 9,550.)

diffuse and generalized distribution seen in post-mortem specimens from other forms of glomerulonephritis.

There were usually no tubular changes in lupus glomerulitis. Even in the more advanced stages of lupus glomerulonephritis the tubular changes were not specific and were less prominent than might be expected from the extent and severity of the glomerular lesions. This fact is probably related to the local and focal distribution of glomerular involvement.

In the interstitium infiltrates of lymphocytes, plasma cells and other mononuclear cells, including mast cells, were not uncommon (Fig. 13). These infiltrates were more abundant around the glomeruli and resembled those found frequently in other tissues in SLE. The cellular composition and distribution of these infil-

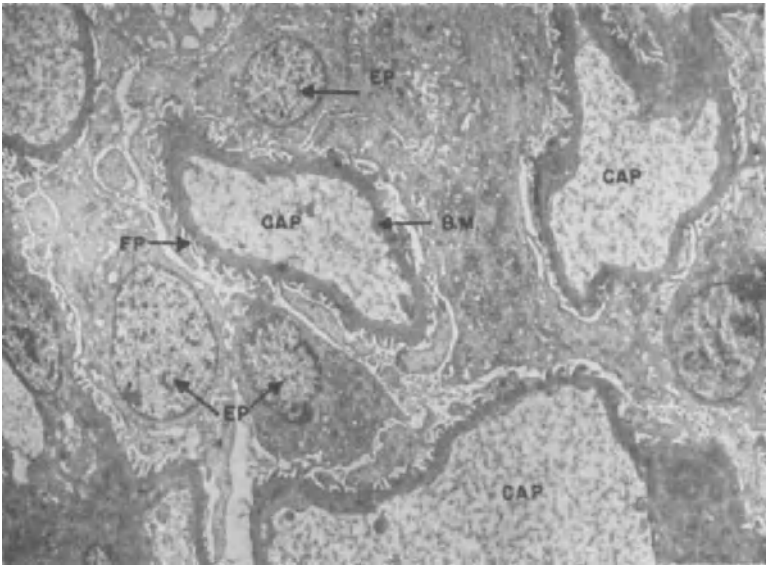


FIG. 12. Glomerular capillaries (CAP) in lupus nephritis. There is an increased number of epithelial cells (EP) with abundant, probably swollen, cytoplasm. The foot processes (FP) are intact, but in Bowman's spaces numerous small osmiophilic structures are recognizable. The basement membrane (B.M.) is thickened in one of the loops. (Electron micrograph. × 2,740.)

trates as well as the lack of supporting clinical data exclude bacterial infection as an aetiological factor. Pyelonephritis, at least in our experience, was an uncommon complication of lupus nephritis even in patients treated with large doses of steroids. Interstitial oedema, patchily distributed, was often seen and was a prominent feature in the biopsies of those patients with the nephrotic syndrome. Interstitial fibrosis, on the other hand,

occurred late and was usually relatively inconspicuous. At autopsy the kidneys of patients dying of lupus nephritis were generally smooth and somewhat enlarged. Contraction of the kidneys was a rare occurrence but might become more common with the prolongation of life resulting from steroid therapy.

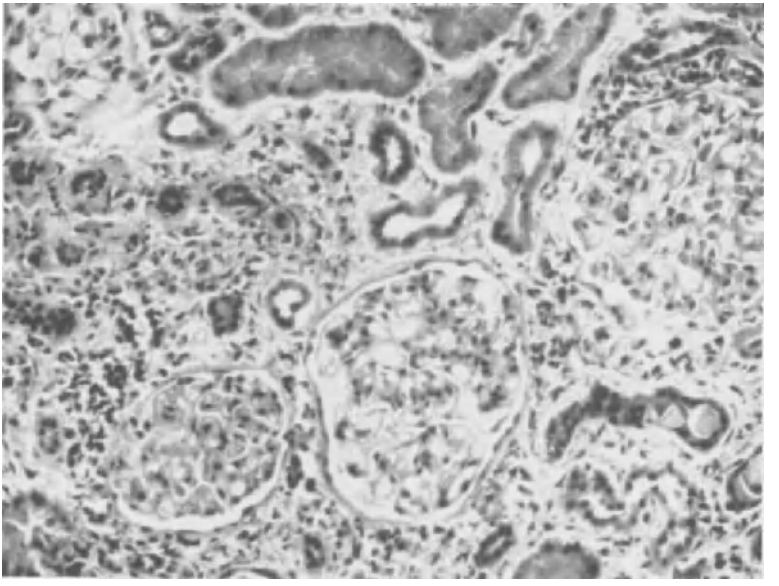


FIG. 13. Interstitial periglomerular infiltrates of lymphocytes, plasma cells and mononuclear cells in lupus nephritis. In the glomeruli irregularly distributed basement membrane thickening can be recognized. (Haematoxylin-eosin. × 220.)

Arteritis was rarely encountered in renal biopsies but fibrinoid changes were commonly observed in the small arteries in patients with lupus glomerulonephritis.

Clinico-pathological correlation

The use of detailed semi-quantitative histological analyses made it possible to correlate renal structural changes with renal function.

In general there was a moderately good correlation between renal structural changes observed in lupus nephritis on the one hand, and the urinary findings and the results of renal function studies on the other (Muehrcke *et al.*, 1957). However, in lupus glomerulitis mild endothelial hypercellularity and local thickening of the capillary basement membrane were often observed in the absence of abnormal urinary findings. In lupus glomerulonephritis there was a good correlation between the degree of capillary basement membrane thickening and the amount of protein excreted in the urine. The presence and degree of hyaline granular changes of the proximal convoluted tubules were also found to be related directly to the amount of protein in the urine. It is interesting to note that there was little correlation between the presence and severity of the histologically more impressive glomerular proliferative and necrotizing changes on the one hand and the degree of proteinuria and the results of renal function tests on the other. In general the severity of renal functional impairment was less than was considered likely from the degree of glomerular damage. The local character of the glomerular lesions and the frequently observed disproportion between the severity of glomerular and tubular lesions probably accounted for this. Only when interstitial and tubular changes became a prominent feature of lupus nephritis was renal function significantly depressed. However, when the results of the specific gravity concentration, urea clearance and phenolsulphonphthalein (PSP) excretion tests were considered together a fair correlation was found with the degree of over-all kidney damage or with the severity of tubular and interstitial changes.

Effect of steroid treatment

Our serial studies on the evolution of the structural changes of lupus nephritis indicate clearly that certain glomerular lesions were more acute and rapidly progressive than others. These lesions appeared to be, at least in part, reversible. However, if

they were sufficiently severe and persistent, irreversible glomerular damage inevitably ensued. Repeated exacerbations of SLE and of lupus nephritis, in particular, led eventually to a more diffuse and generalized process of glomerular destruction with associated tubular and interstitial changes. These "active" lesions included the following: *in the glomeruli*, local necrosis, karyorrhexis, haematoxyphil bodies, fibrinoid changes and hyaline thrombi; *in the interstitium*, infiltrates of plasma cells and mononuclear cells, and oedema; *in the small arteries*, fibrinoid changes. Endothelial-cell and, to a lesser extent, epithelial-cell proliferation in the glomerular tufts did not appear to be manifestations of as active and rapidly progressive lesions as the other changes listed above. Thickening of the glomerular capillary basement membrane, glomerular adhesions, glomerular, interstitial and arterial fibrosis were obviously "irreversible" lesions and were not considered to be evidence of activity of lupus nephritis.

Treatment with low doses of steroids (cortisone, 50 mg. daily), sufficient only to control the general clinical symptoms of SLE, was inadequate either to arrest or to modify significantly the clinical and histological signs of lupus nephritis (Muehrcke *et al.*, 1957). In serial studies it was found that the over-all degree of histological activity persisted and glomerular damage increased (Pollak *et al.*, 1961).

Deliberate prolonged treatment with large doses of steroids for at least six months (cortisone, 160 to 200 mg., or prednisone, 40 to 60 mg. daily) resulted in either a complete disappearance or a marked decrease of the histological changes indicative of activity in the large majority of the patients studied serially. Although the "active" lesions disappeared, "irreversible" lesions persisted and gradually became more severe in some patients. However, the over-all glomerular damage, inclusive of both "active" and "irreversible" glomerular lesions, did not increase in most instances and actually decreased in some.

The initial serum urea nitrogen level was found to be a useful

prognostic criterion in predicting the efficacy of treatment with large doses of steroids in patients with lupus glomerulonephritis (Pollak *et al.*, 1961).

These observations are based on a careful comparative study (Pollak *et al.*, 1961) on two groups of patients with lupus glomerulonephritis which were found to be comparable clinically, functionally and histologically when the first renal biopsy was taken and before treatment was started. The first group of 10 patients, studied initially in 1953 to 1955, was treated with small doses of cortisone and survived on the average 13·8 months. In five the initial serum urea nitrogen level was less than 30 mg. per 100 ml.; in five it was greater than 30 mg. per 100 ml. The survival time in both groups was short. The second group of 16 patients, studied initially in 1956 to 1958, was treated with a minimum dose of 40 mg. of prednisone daily for six months. Seven of these patients have died with an average survival time of 13·4 months from the beginning of treatment. In five the initial serum urea nitrogen level exceeded 30 mg. per 100 ml. The other nine patients are alive an average of 34·2 months later. All had serum urea nitrogen levels below 30 mg. per 100 ml. when treatment was started.

The following case history illustrates the course of lupus glomerulonephritis in a patient with typical clinical and laboratory signs of SLE treated with large doses of prednisone.

A 20-year-old man was first seen in September 1957. At that time he had been ill with SLE for approximately 16 months. Evidence of renal involvement had been present for nine months. For four months before admission he had been treated with 7·5 to 20 mg. of prednisone daily. On admission his blood pressure was 160/110 mm. Hg and there was 1+ ankle oedema. The urine specific gravity ranged between 1·007 and 1·012; his urine contained red cells, white cells and many casts, and he excreted 13·7 g. of protein per day. Serum urea nitrogen was 28 and serum cholesterol 387 mg. per 100 ml. The albumin-globulin ratio was 2·6/2·0 g. per 100 ml. Creatinine clearance

was 78 ml. per minute and PSP excretion 12 per cent in 15 minutes. In the renal biopsy taken at this time there were widespread severe and active glomerular lesions graded as 4+ activity, 3+ severity (Fig. 14A). The patient was then treated with 60 mg. prednisone daily.

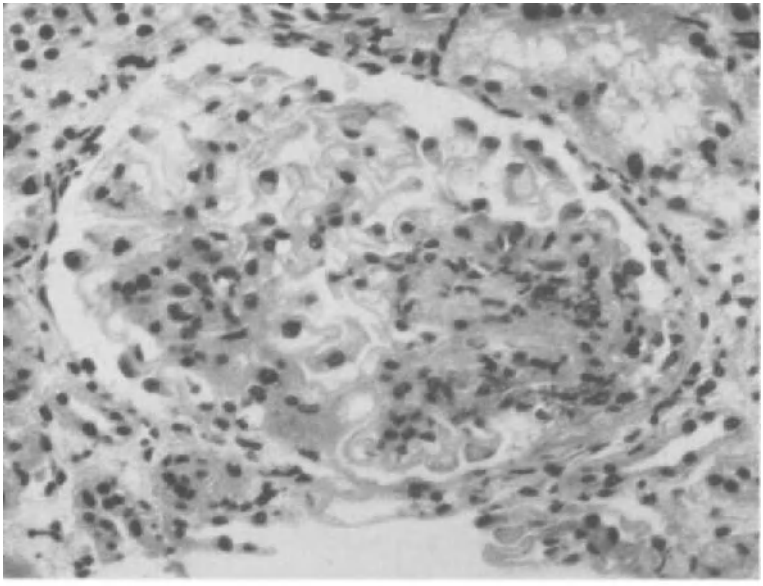
Nine months later, in May 1958, his blood pressure was 155/120 mm. Hg and there was a 3+ ankle oedema. The urine specific gravity was 1·023 with an unchanged urinary sediment, and he excreted 7·4–11·5 g. of protein per day. Serum urea nitrogen was 28 and serum cholesterol 465 mg. per 100 ml. The creatinine clearance was unchanged and there was a slight improvement in the urea clearance and PSP excretion rate. In a second biopsy taken at this time there were less pronounced active glomerular lesions (2+) but persistent and indeed more obvious irreversible changes (Fig. 14B). The patient was given antihypertensive drugs and placed on a low-salt, high-protein diet; the large doses of prednisone were continued because of the persistent activity of the renal lesions, but later the prednisone dosage was reduced gradually, first to 40 and then to 20 mg. per day.

Thirty-three months after the second biopsy, in February 1961, the patient's blood pressure was 230/130 mm. Hg and he was no longer oedematous. The urine specific gravity was 1·010 with an unchanged urinary sediment and he excreted 8·1 g. of protein per day. The albumin–globulin ratio was 2·9/2·5 g. per 100 ml. Serum urea nitrogen was 71, serum creatinine 4·2 and cholesterol 256 mg. per 100 ml. Creatinine clearance was 18 ml. per min. and the urea clearance was also markedly reduced. A third renal biopsy disclosed practically no active glomerular lesions but advanced glomerular and interstitial fibrosis (Fig. 14C).

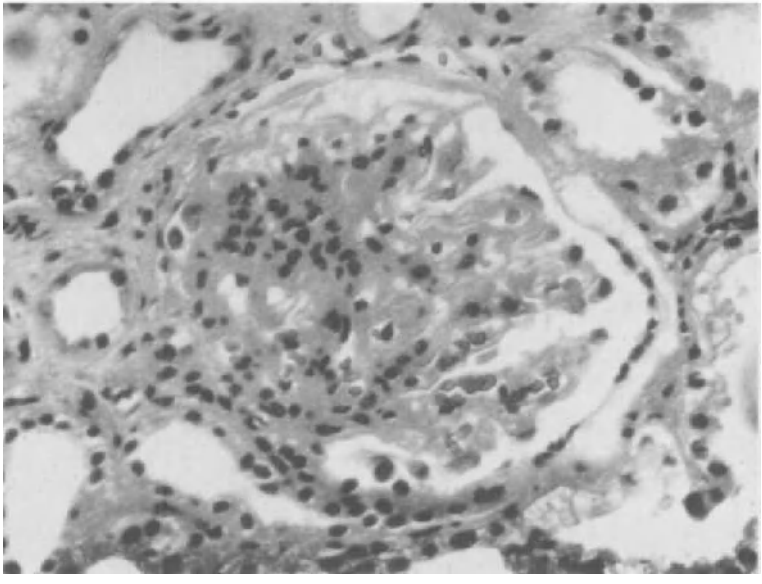
In summary, this patient had been ill with SLE for 58 months and with severe lupus glomerulonephritis for 51 months. The severity and activity of the renal lesions at the time of the first biopsy leave little doubt that renal failure would have developed much earlier without adequate steroid therapy.

The histories of this patient and several others with a similar clinical course indicate that treatment with large doses of prednisone can slow down the progression of the renal lesions and, by preventing the occurrence of exacerbations of SLE and lupus

A



B



C

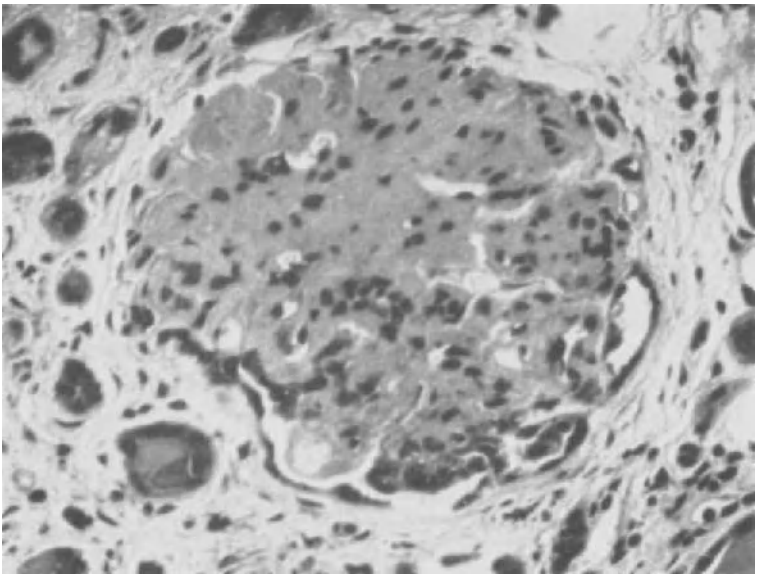


FIG. 14. A: First biopsy. Representative glomerular lesion in a twenty-year-old white man ill with SLE for 16 months and treated with 7·5–20 mg./day of prednisone for four months. Local necrotic and proliferative changes were present in practically every glomerulus, together with mild to moderate thickening of the capillary basement membrane. Lesions graded as 3+ severity and 4+ activity. Haematoxylin-eosin. × 385.)

B: Second biopsy. Representative glomerular lesions of the same patient nine months later following continuous treatment with 60 mg./day of prednisone. Local necrotic lesions have almost completely disappeared. Proliferative changes are still present and the thickening of the capillary basement membrane is now more pronounced. Lesions graded as 3+ severity and 2+ activity. (Haematoxylin-eosin. × 385.)

C: Third biopsy. Representative glomerular lesions of the same patient 33 months later following continuous treatment with 20–7·5 mg./day of prednisone. There is advanced glomerular fibrosis with residual local areas of hypercellularity but no local necrosis. Interstitial fibrosis and tubular atrophy are now prominent. Lesions graded as 4+ severity and 1+ activity. (Haematoxylin-eosin. × 385.)

nephritis, can delay significantly the development of more advanced and diffuse irreversible renal damage. In this connexion it should be noted that twelve patients with lupus glomerulonephritis survived for six months after starting treatment with large doses of prednisone. In ten there was *no* evidence of active lesions in the second renal biopsy done three to twelve months after treatment was started. Only in the patient whose history is given above and in one other was there any evidence of active lesions in the second renal biopsy. Nevertheless, it is apparent that this form of treatment is *not* a cure of lupus nephritis and that, except in an occasional case, it cannot prevent the ultimate development of irreversible renal lesions. Unfortunately at present no adequate clinical or histological data are available to establish a therapeutic regimen tailored to the requirements of the individual patient. In some cases larger doses of steroids might be of greater benefit and in others smaller doses might be adequate.

Summary and conclusions

Single or serial renal biopsies were studied in 105 patients ill with systemic lupus erythematosus (SLE).

Light- and electron-microscopic observations indicate that renal involvement in SLE begins with a local and focal proliferation of endothelial cells in the glomeruli (lupus glomerulitis). Slight thickening of the capillary basement membrane and fusion of epithelial foot processes are associated with and may follow endothelial cell proliferation. In more severe and active renal involvement (lupus glomerulonephritis) the glomerular lesions are characterized by necrosis with fibrinoid, hyaline thrombi, karyorrhexis and haematoxyphil bodies. "Wire-loop" lesions and distinct capillary basement membrane thickening are commonly seen in the more advanced stages, but a local and focal distribution of the glomerular lesions is characteristic until a late stage of the disease. Eventually epithelial cell proliferation becomes a prominent feature with formation of fibroepithelial

crescents and of adhesions. As the glomerular lesions gradually become more diffuse and generalized their specific character becomes less apparent.

Symptomatic treatment with small doses of cortisone or prednisone, sufficient to control the general clinical symptoms of SLE, does not modify significantly the clinical and histological signs of lupus nephritis.

Prolonged treatment with large doses of prednisone appears to control the "active" lesions of lupus nephritis and to slow down the progression of the renal lesions. As a result the average life expectancy of these patients is significantly prolonged.

REFERENCES

ARNOLD, J. D., and SPARGO, B. (1959). *Circulation*, **19,** 609.

FARQUHAR, M. B. (1959). *In* Proc. Xth Ann. Conf. Nephrotic Syndrome, p. 2, ed. Metcoff, J. New York: National Kidney Disease Foundation.

FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A. (1957). *J. exp. Med.*, **106,** 649.

HARVEY, A. M., SHULMAN, L. E., TUMULTY, P. A., CONLEY, C. L., and SCHOENRICH, E. H. (1954). *Medicine (Baltimore)*, **33,** 291.

KARK, R. M., and MUEHRCKE, R. C. (1954). *Lancet*, **1,** 1047.

KLEMPERER, P., POLLACK, A. D., and BAEHR, G. (1941). *Arch. Path. (Chicago)*, **32,** 569.

LILLIE, R. D. (1954). Histopathologic Technic and Practical Histochemistry, 2nd edn. Philadelphia: Blakiston.

MUEHRCKE, R. C., KARK, R. M., PIRANI, C. L., and POLLAK, V. E. (1957). *Medicine (Baltimore)*, **36,** 1.

MUEHRCKE, R. C., KARK, R. M., PIRANI, C. L., POLLAK, V. E., and STECK, I. E. (1955). *Ann. rheum. Dis.*, **14,** 371.

PALADE, G. E. (1952). *J. exp. Med.*, **95,** 285.

PIRANI, C. L., MUEHRCKE, R. C., and KARK, R. M. (1954). *Proc. Inst. Med. Chicago*, **20,** 170.

POLLACK, A. D. (1959). *J. Mt Sinai Hosp.*, **26,** 224.

POLLAK, V. E., PIRANI, C. L., and KARK, R. M. (1961). *J. Lab. clin. Med.*, **57,** 495.

POLLAK, V. E., PIRANI, C. L., MUEHRCKE, R. C., and KARK, R. M. (1958). *Guy's Hosp. Rep.*, **107,** 353.

SOFFER, L. J. (1959). *J. Mt Sinai Hosp.*, **26,** 297.

SPARGO, B., and ARNOLD, J. D. (1960). *Ann. N.Y. Acad. Sci.*, **86,** 1043.

VERNIER, R. L., WORTHEN, H. G., PETERSON, R. D., COLLE, E., and GOOD, R. A. (1961). *Pediatrics*, **27,** 181.

DISCUSSION

Movat: Have you any data, on the basis of serial biopsies, on the fate of the osmiophilic material in the wire-loop lesion? Have you evidence that this material becomes hyaline or "basement membrane-like" by a process such as ageing or polymerization?

Pirani: No. We have not had the opportunity to study serial biopsies as yet by electron microscopy. This material is PAS-positive, as fibrinoid is of course, and has a somewhat greater density than the basement membrane proper. I think that some of the thickening of the basement membrane that one sees is due at least in part to the presence of this material, especially on the inside of the capillary walls.

Movat: In the light microscope you have shown an acidophilic material. After a number of years of doing biopsies do you have the impression that this acidophilic red material is the source of the "basement membrane-like" material?

Pirani: Yes, I think so, although I have no absolute proof.

Rich: How much proteinuria can you have in lupus with normal foot processes?

Pirani: I would say up to 3–5 g./day. A similar situation applies to diabetic glomerulosclerosis.

Hardwicke: Some of your sections look very much like what we have been diagnosing as proliferative glomerulonephritis, and the response to treatment is very similar. I wonder whether perhaps we are diagnosing as proliferative some cases that are really lupus. Could you give us some idea how you diagnose lupus when you have only the proliferative changes without the haematoxyphil bodies, without pyroninophilia?

Pirani: I don't think there are specific lesions in lupus except haematoxyphil bodies. Local glomerular necroses are not specific; they appear in other conditions, especially in the other collagen diseases. The wire-loop lesion is certainly not specific, nor is the presence of "fibrinoid". However, the combination of these changes and their local and focal distribution are strongly suggestive for lupus nephritis.

Hardwicke: Would you regard pyroninophilic staining of your interstitial infiltration as specific?

Pirani: That indicates only that these are plasma cells or cells actively producing protein.

Movat: Perhaps antibody?

Pirani: Yes.

Kark: Certain diagnosis of any lesion is very difficult. In our unit the biopsies of each patient are seen separately by the clinician and by Dr. Pirani. Each makes his decision about the histology. The clinicians have made a diagnosis, before seeing the biopsy, based on the clinical data. Then we all come together once a week or even more frequently to discuss the patient and the laboratory findings together, and argue about it. On the basis of all the evidence we make a decision on a diagnosis. Many times Dr. Pirani will suggest that our clinical diagnosis may be incorrect, and then we study the patient again, and may find a disease we had missed in our original clinical assessment.

As far as the diagnosis of lupus is concerned, we have described our criteria for diagnosis (Muehrcke, R. C., Kark, R. M., Pirani, C. L., and Pollak, V. E. (1957). *Medicine* (*Baltimore*), **36,** 1) so I am not going to waste time by discussing them now. In recent years we have been helped very much by serological tests such as assay of antinuclear factors. The long history of repetitive attacks of illnesses, the positive serological tests, and, more recently, the findings of antinuclear factors in healthy members of the patient's family, all give us a picture which bears on the patient and helps us make a final diagnosis of lupus.

Hardwicke: Would you diagnose lupus without antinuclear factors or without serological tests?

Kark: Yes. Either clinically or histologically.

Earle: Would you tell us how many you have diagnosed without positive serological tests in this group of 105 patients?

Kark: I couldn't do that. We have only been doing antinuclear factors now for 18 months, but even before that time we never relied on the LE cell phenomena.

Earle: How many patients did have positive cell preparations?

Kark: Not all. About 80 per cent.

Pirani: Clinicians often feel that pathology is a more accurate science than clinical medicine. This is true, but only to a limited extent. We have to interpret the changes we see very much as a clinician has to interpret clinical symptoms, and pathology is still somewhat

subjective in spite of all our grading and attempts to put this on a semi-quantitative basis.

Movat: As far as the morphology of glomerular injury is concerned it seems to me that we are always looking for differences, and tend to forget that the glomerulus has only a limited way to react to injury whether it is lupus or some other condition.

Black: It might be relevant to consider the effects of high steroid dosages in another collagen disorder. We have obtained renal biopsies from a patient with acute polyarteritis nodosa who presented with massive oedema, established nitrogen retention, and very moderate hypertension. The biopsy showed changes that were largely non-glomerular, in the interlobular arteries. He was put on 100 mg. prednisone daily, but this made no difference to his clinical course and he died in renal failure. However, on autopsy it was very hard to find any vascular lesions; all we could find was a renal vessel with some obliteration.

Hamburger: We have had 20 cases of lupus—a very small series compared with that of Dr. Kark and Dr. Pirani. All these had positive serological and LE cell reactions, and their pathological features were quite similar to what has been described. We were not as fortunate with high steroid dosages as Dr. Kark's group has been. In some cases the clinical condition deteriorated. On the other hand, I should emphasize that we have had some cases with demonstrated lupus nephritis without any steroid therapy living for more than four years.

Pirani: We have not had patients surviving without steroid therapy when the first biopsy showed necrotizing lesions or severe proliferative changes. If they showed only very mild glomerulitis, minimal changes, they may survive even without treatment as long or longer than the patients treated with high steroid therapy. One wonders if this mild proliferation of cells may not be something separate from the other process, which obviously is a much more active one.

Hamburger: I find it extremely difficult to predict from biopsy how long a patient will live.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

PERCUTANEOUS RENAL BIOPSY IN PYELONEPHRITIS

CLAUS BRUN and FLEMMING RAASCHOU

Central Clinical Laboratory and Medical Department 3, Kommunehospitalet, Copenhagen

IT was with many misgivings that almost three years ago we began a study of renal biopsies from cases of pyelonephritis, and as the study has progressed our misgivings have certainly not regressed. The major problem concerns the difficulties in defining this disease clinically and histologically, difficulties which may be so severe as to make interpretation of the observations almost impossible at the present time.

Pyelonephritis in man has been little studied by means of renal biopsy, and we do not feel it necessary here to review in detail the existing literature on pyelonephritis and renal biopsy.

However, brief mention may be made of a few papers by Jackson and his group (Kipnis *et al.*, 1955; Jackson, Poirier and Grieble, 1957), who in thorough clinical, bacteriological and histological studies of patients with pyelonephritis found that the symptomatology of the disease is not a reliable index of the severity or even the presence of the disease. In some cases they found normal microscopic appearance of the renal tissue in biopsies and normal kidney function in patients with histories of protracted urinary tract infection.

At the Henry Ford Hospital International Symposium on the biology of pyelonephritis (in Detroit, 1959) a wealth of information on all aspects of pyelonephritis was presented, but only two papers dealt with the histological changes in pyelonephritis studied by means of renal biopsy (Brun and Raaschou, 1960; Pirani, 1960).

From the first International Congress of Nephrology at Evian in 1960 the following paper on this topic may be mentioned: Almeida, Taunnay, Cruz, Pena, Brito and Cintra (1961) studied the incidence of pyelonephritis in bilateral diffuse renal disease by means of percutaneous renal biopsy; and at this congress a number of reports from Yugoslavia and Bulgaria described an endemic renal disease which histologically apparently is related to what is generally described as pyelonephritis (Radošević and Radonić, 1961).

In addition, studies have been made by Parrish and Howe (1953), Schreiner and Berman (1957), Muehrcke (1961) and Hutt *et al.* (1961).

Kark and his colleagues (1955) have published observations on kidney biopsies from what was termed "subacute bacterial nephritis". They found marked periglomerular interstitial inflammation plus hypercellularity in the glomeruli and epithelial crescents. At the same time they found positive cultures from the biopsies with repeatedly negative urine cultures. This disease may be a special type of pyelonephritis.

The problems which we shall deal with in the present paper are the following:

(1) The occurrence and nature of histological changes found in renal biopsies from patients with pyelonephritis and the clinical signs and symptoms of these patients.

(2) The incidence at different levels of renal function of the different types of pyelonephritis (acute, recurrent and chronic).

(3) The incidence in relation to functional level of the following characteristics: (a) pyelonephritis and phenacetin consumption in past history; (b) diagnosed renal papillary necrosis; (c) typical histological changes.

(4) The comparison of bacteriological cultures from renal biopsy material with those of urine.

(5) The incidence of renal papillary necrosis in cases of chronic pyelonephritis and in our renal biopsy material.

(6) The prognosis of dialysed cases of renal failure verified by kidney biopsy as severe pyelonephritis.

Material

Our material consists of 80 biopsies from 78 patients. In the majority of these cases the diagnosis was based on clinical criteria, but in six cases it was suggested by the histological changes found in the biopsy.

Patients with diabetes mellitus were excluded from our material and will be dealt with later in this session by Thomsen (p. 281). Patients with acute renal failure were also excluded, simply because we did not have enough strength to disentangle the clinical and histological findings in acute renal failure from acute pyelonephritis. However, we shall conclude by returning to this question. Finally, a number of cases of pyelonephritis associated with obstruction were excluded.

Technique

Our biopsy technique, which has been described elsewhere (Iversen and Brun, 1951; Brun and Raaschou, 1958*b*), allows us to obtain a cylinder of renal tissue with a diameter of 1·7 mm. and a length of anything up to 40 mm. but typically 15–25 mm. After the cylinder has been cut loose by the needle, it is kept in the needle during withdrawal by a vacuum created by a special syringe. When the needle is out of the patient the tissue moves into the syringe, and can be secured from there.

Renal biopsy in acute and chronic pyelonephritis has not been found to be accompanied by any risk of complication over and above that involved in other kidney diseases (Brun and Raaschou, 1958*a*).

The bacteriological examinations were carried out by culturing from the inside of the syringe, which contains the tissue and a mixture of renal urine and blood. A catheter specimen, or in recent years a clean voided midstream specimen, has been

studied bacteriologically. Quantitative bacteriological studies are not included in this paper.

Criteria

The clinical criteria we have used in the selection and evaluation of our patients are the following: acute pyelonephritis in history; cystitis; costovertebral angle pain and/or tenderness; fever; pyuria; bacteriuria; reduced kidney function; positive X-ray.

The generally accepted histological criteria which we have used are: cell casts; invasive glomerulitis; interstitial infiltration; periglomerular fibrosis; tubular atrophy; peritubular fibrosis; dilated tubules with acellular casts ("thyroid-like").

As is well known, neither the clinical nor the histological criteria are specific, let alone pathognomonic. It would certainly be unreasonable to require any one patient to have all the enumerated criteria before a diagnosis of pyelonephritis is made.

Results

It has repeatedly been stated that renal biopsy is of little value in this typically focal disease, and we too have joined the chorus.

If we look at our 78 patients to see in how many cases there are histological changes compatible with the diagnosis of pyelonephritis (acute, recurrent or chronic), we find (Table I) such

Table I

PRESENCE OR ABSENCE OF HISTOLOGICAL CHANGES IN RENAL BIOPSIES FROM PATIENTS WITH CHRONIC AND RECURRENT PYELONEPHRITIS

	Total	*Selected*
Patients	78 cases	44 cases
Histological pyelonephritis	73	39
Normal biopsy	5 (6%)	5 (11%)

changes in 73 cases, while in 5 cases (or about 6 per cent) no histological changes are found.

This can be interpreted in one or both of the following ways: (1) The tissue obtained by renal biopsy is not representative of the kidney tissue at large. (2) The renal tissue in the biopsied kidney is not involved in the pathological process.

In an attempt to differentiate between these two possibilities we have tried to select a group of patients who were considered on the basis of clinical symptoms and signs to have the greatest probability of having pyelonephritis. So we selected 44 cases with pyuria or bacteriuria, who in addition had a history of either attacks of pyelonephritis or costovertebral angle pain and tenderness, indicating that the kidney was involved. Amongst these 44 cases, where the clinical diagnosis of pyelonephritis has the highest degree of accuracy, we still found 5 cases with a normal biopsy, but they now amount to 11 per cent of the selected cases. This rise in the percentage of normal biopsies when the clinical criteria are made more stringent suggests that renal biopsy in some cases of pyelonephritis is not representative.

Pyelonephritis and kidney function

Table II shows the distribution of the total material of 78 patients in clinically acute, recurrent and chronic pyelonephritis. Only 4 cases were acute.

Table II

TYPES OF PYELONEPHRITIS

24-hr. Endogenous creatinine clearance ml./min.	>70	20–70	<20	*Total*
Acute pyelonephritis	1	2	1	4
Recurrent pyelonephritis	7	5	7	19
Chronic pyelonephritis	7	9	39	55

Our total material was divided into three groups according to the level of renal function (expressed as 24-hr. endogenous creatinine clearance (Table III). The first group had a creatinine clearance of 70 ml./min. or above and consisted of 15 patients,

Table III

INCIDENCE, AT DIFFERENT FUNCTIONAL LEVELS, OF PYELONEPHRITIS AND PHENACETIN CONSUMPTION IN HISTORY. INCIDENCE OF RENAL PAPILLARY NECROSIS

24-hr. Endogenous creatinine clearance ml./min.	>70	20–70	<20
Acute or recurrent pyelonephritis in history	10 (67%)	8 (47%)	10 (21%)
Excessive phenacetin consumption	8 (53%)	7 (44%)	27 (56%)
Renal papillary necrosis	2 (13%)	4 (24%)	15 (31%)

10 of whom (67 per cent) had a history of acute or recurrent pyelonephritis. Eight patients admitted to an excessive phenacetin consumption, and two were shown to have renal papillary necrosis.

In the second group, creatinine clearance ranged from 70 to 20 ml./min. Out of a total of 17 patients, 8 (47 per cent) had a past history of acute or recurrent pyelonephritis, 7 had excessive phenacetin consumption, and 4 renal papillary necrosis.

In the third group, with a severely reduced kidney function (creatinine clearance below 20 ml./min.), we found only 10 (21 per cent) out of 48 patients with a past history of pyelonephritis; excessive phenacetin consumption was found in 27 cases and papillitis necroticans in 15 cases.

To summarize, the figures show that the frequency with which acute or recurrent pyelonephritis appears in the past history is low in the group with severely reduced renal function, while it is high in the group with unimpaired renal function. This is probably well known to most clinicians. The incidence of papillary necrosis in the three groups seems to increase with decreasing levels of kidney function, while the frequency of patients with admitted excessive phenacetin consumption seems to be relatively independent of the kidney function.

Returning to the five cases where normal renal tissue was found in patients who fulfilled even the most strict criteria of pyelonephritis, we can state that four had normal renal function and one only moderately reduced function.

However, all of the 48 patients with a renal function below 20 per cent showed pathological renal tissue in the biopsy.

The conclusion concerning the representativeness of renal biopsy in pyelonephritis thus seems to be that definite histological changes are present in nearly all the cases of clinical pyelonephritis with impaired renal function.

We have attempted to examine the frequency with which the different histological changes occur in biopsies from our 44 patients with recurrent or chronic pyelonephritis who fulfilled the strict clinical criteria mentioned earlier.

As shown in Table IV, it appears that the most frequent change

Table IV

OCCURRENCE OF HISTOLOGICAL CHANGES IN RENAL BIOPSIES FROM 44 CASES OF PYELONEPHRITIS FULFILLING STRICT CLINICAL CRITERIA

24-hr. Endogenous creatinine clearance ml./min.	>70	20–70	<20
Number of biopsies	10	13	21
No histological changes found	40%	8%	—
Interstitial cell infiltration	60%	93%	100%
Cell casts	10%	31%	33%
Periglomerular fibrosis	40%	54%	76%
Vascular changes	40%	54%	72%
Tubular atrophy and interstitial fibrosis	70%	93%	95%
Tubular dilatation, "thyroid-like" tissue	10%	31%	57%

found is interstitial infiltration with inflammatory cells, a change which increases in frequency with decreasing level of renal function.

Cell casts occur with a much lower frequency—maximally in about one-third of the cases when the kidney function is impaired.

According to Weiss and Parker (1939) periglomerular fibrosis is pathognomonic of the pyelonephritic contracted kidney. This statement is probably not tenable today. In our cases periglomerular fibrosis is found with increasing frequency with decreasing renal function, but even in the most advanced cases this change

is absent in about one-fourth of the biopsies. Vascular changes occur with the same increasing frequency as periglomerular fibrosis in the three functional groups. Periglomerular fibrosis and vascular changes do not necessarily occur in the same biopsy, especially not in the two groups with moderate or no functional impairment.

Invasive glomerulitis may occur in acute or chronic recurrent pyelonephritis, although infrequently. Isolated crescents are seen only very infrequently.

Tubular dilatation, eventually appearing as "thyroid-like" tissue, so often described as a characteristic feature of chronic pyelonephritis, was found in from 10 to 57 per cent of our three categories of biopsies.

The histological changes discussed here are to a large extent non-specific and no single change is present in all biopsies. Probably cell casts are the most valuable diagnostic criterion when present, but they occur with a low frequency.

It is our experience that it is difficult to estimate the renal function from the histological changes found in the biopsies from cases of pyelonephritis. This is thought to be due partly to the presence of preserved glomeruli amongst atrophic tubules, and partly to the focal nature of the disease.

Cultures from renal biopsies

In 60 patients with pyelonephritis bacteriological studies were performed from renal biopsies and from urine. The results are shown in Table V. The culture from the biopsy was positive in 21 cases, while the urine specimen was positive in 35 cases. In 18 cases both biopsy and urine gave positive cultures, but in only 13 of these was bacteriological agreement ascertained. In 3 cases out of 60 a positive culture from the renal tissue was combined with a negative urine culture. It is noteworthy that the incidence of positive cultures from the kidney is low in the group of patients

Table V

CULTURES OF RENAL BIOPSY MATERIAL CORRELATED WITH QUALITATIVE URINE CULTURES

	Positive culture	*Negative culture*
Biopsy and urine	18	22
Biopsy	21	39
Urine	35	25
Bacteriological agreement	13	22

with normal renal function (1 out of 12) in spite of positive histological findings (8 out of 12).

The idea of studying the bacterial flora directly from the kidney is naturally appealing. Yow and colleagues (1960) were generally unsuccessful in cultivating bacteria from renal biopsies even when large numbers of bacteria could be cultured from the urine at the same time; in only three of over 100 biopsies were the cultures of the biopsy specimens positive. So far in our cases we have had only limited therapeutic benefit from this technique. Further experience may very well change this point of view.

Renal papillary necrosis in pyelonephritis

In our 78 cases of pyelonephritis we found 21 cases of papillary necrosis. The condition was diagnosed mainly by means of pyelography, though in some cases a necrotic papilla was found in the urine. Necrotic tissue appeared in the renal biopsy in only 5 cases (Table VI).

Table VI

RENAL PAPILLARY NECROSIS IN 23 BIOPSIED PATIENTS

Diagnosed by:	*In number of cases*
Pyelography	9
Papilla in urine	6
Post-mortem	7
Renal biopsy	5

All cases but one had pyuria and 17 out of 21 of them had bacteriuria. Excessive phenacetin consumption was admitted in 13 cases, while this could not be ascertained in 8 cases.

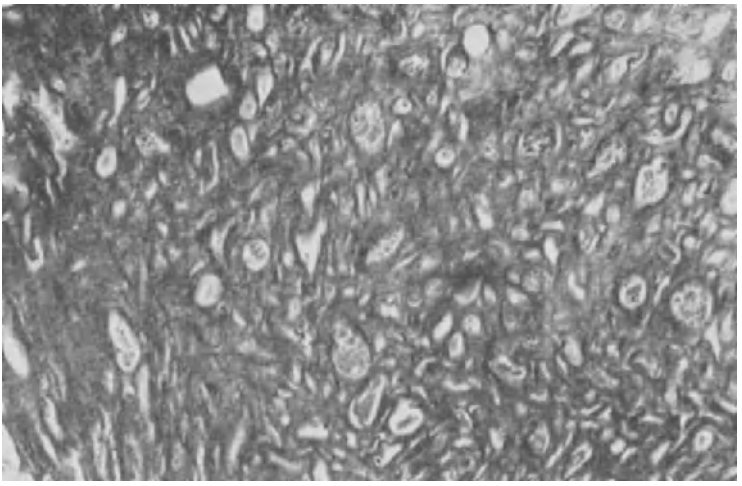


FIG. 1. Biopsy no. 543: 56-year-old woman with bacteriuria, pyuria, uraemia and hypertension. Biopsy showed chronic pyelonephritis and a long piece of necrotic papillary tissue (shown above). (v. GH-stain. Reduced from × 105.)

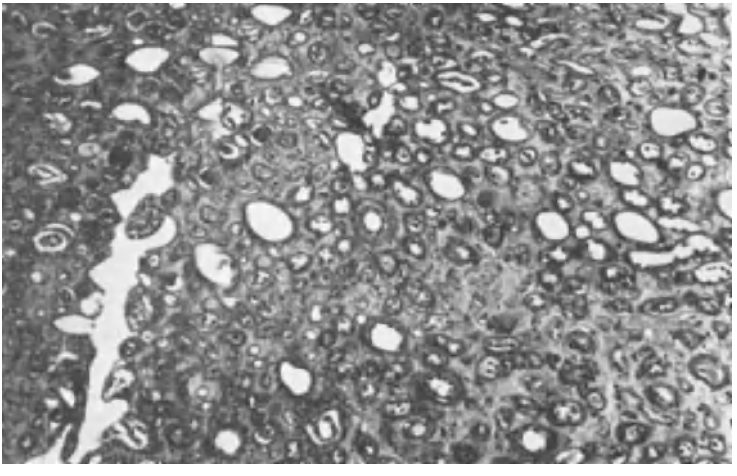


FIG. 2. Biopsy no. 674: 63-year-old male with an admitted excessive phenacetin consumption due to neurotic headache. No pyelonephritis in history. Several urines and biopsy sterile. Creatinine clearance 30 ml./min. Biopsy showed chronic pyelonephritis and necrotic papillary tissue. PAS and H-stain. Reduced from × 105.)

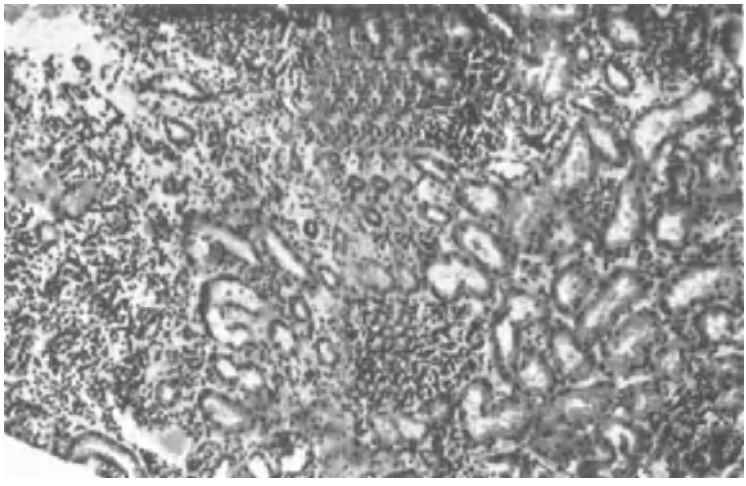
In one case of obvious renal papillary necrosis we found no growth from the renal tissue, and the urine was found sterile on several occasions. This 63-year-old man had had an excessive phenacetin consumption for years. This single patient does of course not prove a causal relationship between excessive phenacetin consumption and renal papillary necrosis. We might add that in our opinion it still has to be proved that a causal relationship exists between excessive phenacetin consumption and chronic interstitial nephritis.

Biopsies from cases of renal papillary necrosis are shown in Figs. 1 and 2.

Before concluding we may briefly mention a field where we feel that renal biopsy is of special value. Among cases admitted to a unit dealing with the diagnosis and treatment of acute renal failure, a considerable number turn out to be not acute renal failure, but aggravation of some chronic renal disease. In this situation a renal biopsy may clarify the situation and prevent dialyses which with our present technical possibilities might be called unnecessary. We are not at the present time prepared to dialyse over prolonged periods of time patients with no renal function and no anatomical possibility of at least some functional recovery. This means that while we will go on dialysing cases of acute renal failure following shock etc. over very protracted periods of time until they eventually recover or die, this is not so in cases with generalized destructive glomerular lesions.

If the biopsy reveals chronic pyelonephritis in which some nephrons have escaped destruction we feel a more active attitude is justified. It is obvious that a great amount of experience will be needed before a sufficient degree of accuracy can be reached in this field.

We have dialysed a number of patients where the biopsy showed severe pyelonephritis, and the results are shown in Table VII. Of the 19 cases at least 11 survived for more than 3 months after dialysis in spite of an initial renal function which for practical



3 A

Table VII

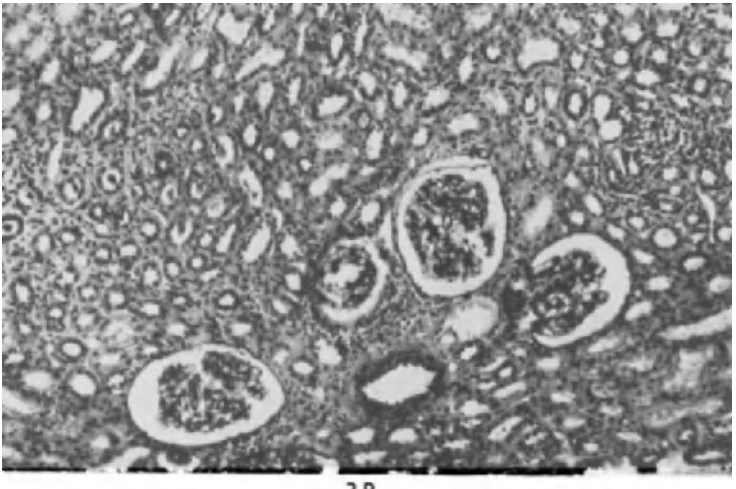
SURVIVAL OF PATIENTS WITH PYELONEPHRITIS AFTER HAEMODIALYSIS

	<1 mo.	*1–3 mo.*	*3 mo.–2 yr.*	*>2 yr.*
Number of cases	5	3	8	3
Minimal creatinine clearance (mean value) ml./min.	1·0	0·6	0·8	0·1
Maximum creatinine clearance (mean value) ml./min.	2·1	14·4	17·2	56·1

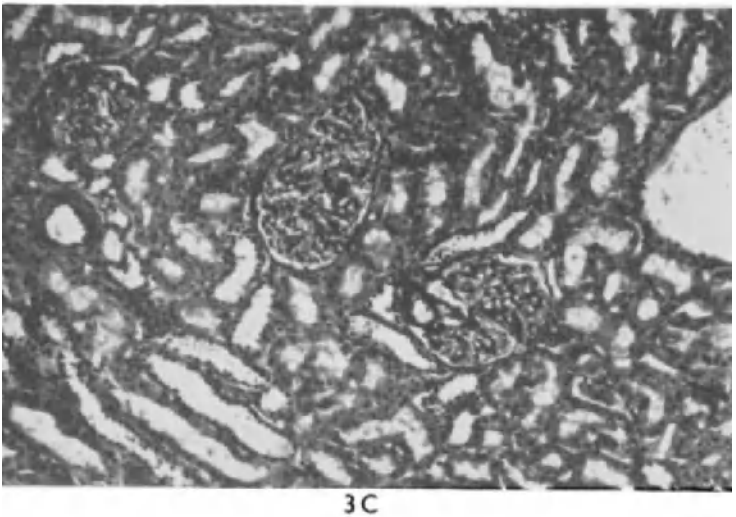
purposes was nil. The degree of functional recovery was very satisfactory. At least for a few of the patients the final results seem surprisingly good, as 2 patients lived for at least four years.

Serial biopsies have been carried out in some patients of this type.

A 32-year-old woman was admitted as a case of acute renal failure *post partum* with a creatinine clearance of 0 and serum urea of 358 mg./100 ml. The first biopsy (Fig. 3A) showed diffuse acute pyelonephritis with severe interstitial infiltration, mainly leucocytes. After one dialysis she gradually recovered and subsequent biopsies (Figs. 3B and 3C) taken three weeks and four

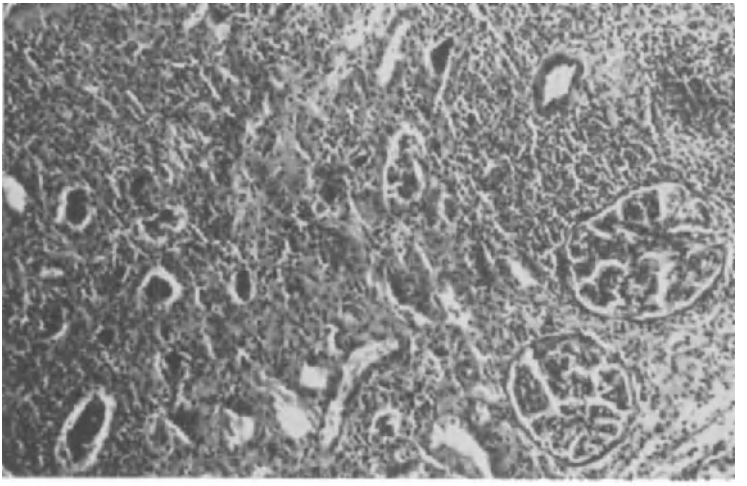


3B

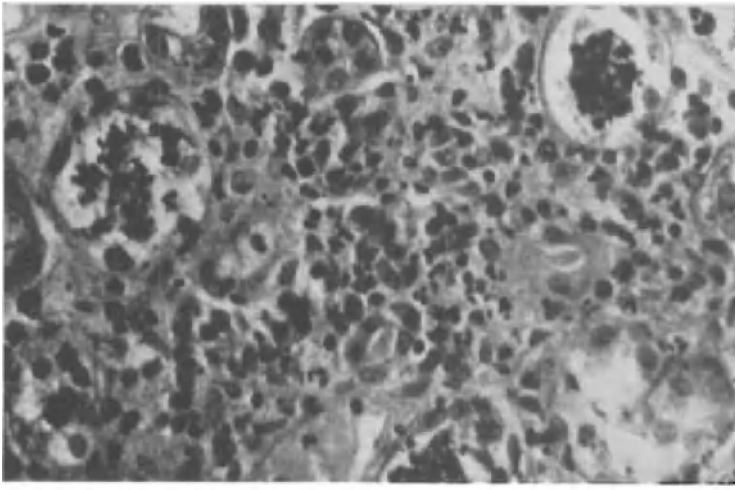


3C

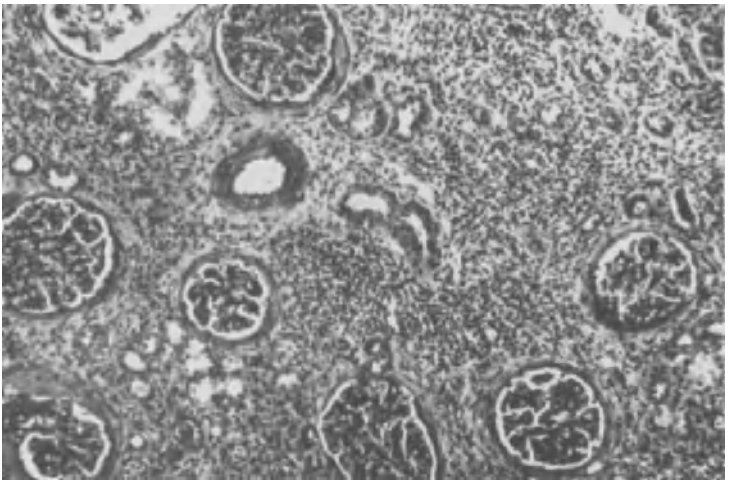
FIG. 3. Biopsy no. 336a, b, c: 32-year-old woman with severe acute anuric pyelonephritis following parturition. Biopsy on fifth day (A) shows massive interstitial infiltration with polymorphs. Two subsequent biopsies, after 3 weeks (B) and 4½ years (C), show gradual return to normal. (H and E-stain. Reduced from × 105.)



4A



4B



4C

FIG. 4. Biopsy no. 584a and c: 57-year-old woman with clinical acute anuric pyelonephritis. Renal function remained below 5 per cent for three weeks, and two dialyses were necessary. Renal biposy on sixth day (A) shows normal glomeruli, heavy interstitial infiltration, mainly with leucocytes, and many cell casts (B). Repeated biopsies after 10 weeks and 1½ years (C) show development of chronic pyelonephritis with periglomerular fibrosis, tubular atrophy and vascular changes. (PAS- and H-stain. A and C reduced from × 105; B from × 333.)

and a half years after onset show return to normal (serum urea 35 mg./100 ml., creatinine clearance 80 ml./min.).

Another case of this type with initial complete abolition of the renal function showed a rather similar picture in her first biopsy (Figs. 4A and 4B). In this case two later biopsies, after ten weeks and one and a half years, show development of a typical chronic pyelonephritis (Fig. 4C). Functionally her creatinine clearance only rose from 0 to 15 ml./min.

These two cases seem to demonstrate that acute pyelonephritis can be a cause of acute renal failure.

Summary

In conclusion we may say that as for the representativeness of renal biopsy in pyelonephritis—definite histological changes are present in nearly all the cases of clinical pyelonephritis with impaired renal function; all of 48 patients with a kidney function below 20 per cent showed pathologically changed renal tissue in the biopsy.

In 60 patients with pyelonephritis a combined bacteriological study was performed on renal biopsy and urine and in 21 cases bacteria were cultured from the biopsy; so far in our cases we have had only limited therapeutic benefit from this technique.

In 78 patients with pyelonephritis 21 patients had renal papillary necrosis; some of them were diagnosed by renal biopsy. Forty-two of the 78 patients admitted to an excessive phenacetin consumption.

In a group of patients with anuria and renal failure biopsies have shown severe pyelonephritis. The value of the renal biopsy method as an indication for haemodialysis is discussed and the surprisingly good prognosis for some of these patients is mentioned.

REFERENCES

ALMEIDA, S. S., TAUNNAY, A. E., CRUZ, H. M. M., PENA, D. O., BRITO, T., and CINTRA, A. B. U. (1961). *In* Ist int. Congr. Nephrology, Evian, France. In press.

BRUN, C., and RAASCHOU, F. (1958*a*). *Arch. intern. Med.*, **102**, 716.

BRUN, C., and RAASCHOU, F. (1958*b*). *Amer. J. Med.*, **24**, 676.

BRUN, C., and RAASCHOU, F. (1960). *In* Biology of Pyelonephritis, p. 225. Boston: Little, Brown.

HUTT, M. S. R., CHAMBERS, J. A., MACDONALD, J. S., and DE WARDENER, H. E. (1961). *Lancet*, **I**, 351.

IVERSEN, P., and BRUN, C. (1951). *Amer. J. Med.*, **11**, 324.

JACKSON, G. G., POIRIER, K. P., and GRIEBLE, H. G. (1957). *Ann. intern. Med.*, **47**, 1165.

KARK, R. M., MUEHRCKE, R. C., PIRANI, C. L., and POLLAK, V. E. (1955). *Ann. intern. Med.*, **43**, 807.

KIPNIS, G. P., JACKSON, G. G., DALLENBACH, F. D., and SCHOENBERGER, J. A. (1955). *Arch. intern. Med.*, **95**, 445.

MUEHRCKE, R. C. (1961). *In* Ist int. Congr. Nephrology, Evian, France. In press.
PARRISH, A. E., and HOWE, J. S. (1953). *J. Lab. clin. Med.*, **42,** 152.
PIRANI, C. L. (1960). *In* Biology of Pyelonephritis, p. 241. Boston: Little, Brown.
RADOŠEVIĆ, Z., and RADONIĆ, M. (1961). *In* Ist int. Congr. Nephrology, Evian, France. In press.
SCHREINER, G. E., and BERMAN, L. B. (1957). *Sth. med. J. (Bgham, Ala.)*, **50,** 733.
WEISS, S., and PARKER, F., JR. (1939). *Medicine (Baltimore)*, **18,** 221.
YOW, E. M., MONZON, O. T., ORY, E. M., and BRENNAN, J. C. (1960). *In* Biology of Pyelonephritis, p. 391. Boston: Little, Brown.

[Discussion after this paper was postponed until after the paper by Dr. Hutt and Prof. de Wardener, and may be found on p. 271.—EDS.]

CORRELATION BETWEEN RENAL BIOPSY AND OTHER DIAGNOSTIC PROCEDURES IN PYELONEPHRITIS

M. S. R. HUTT

Louis Jenner Laboratory, St. Thomas' Hospital, London

and

H. E. DE WARDENER

Department of Medicine, Charing Cross Hospital Medical School, London

IN acute infections of the upper urinary tract it is difficult on clinical grounds to assess the extent of the infection although in recent years it has been increasingly recognized that the renal substance may be involved in attacks of so-called "pyelitis". It is also recognized that chronic upper urinary infections may be clinically silent or may present as cases of hypertension or chronic renal failure. Kleeman, Hewitt and Guze (1960) found that a clinical diagnosis of pyelonephritis had been made in only 17 per cent of 629 cases diagnosed at autopsy. The object of our investigation has been to try and find the relationship of the clinical history, urine culture, the rate of urinary white cell excretion before and after the administration of a pyrogen (Pears and Houghton, 1959), radiological abnormalities and renal biopsy findings, in assessing the presence and extent of acute and chronic upper urinary infections. The results, together with other investigations into the pyrogen test, have been previously published (Hutt *et al.*, 1961).

Acute pyelonephritis

Renal biopsy

Ten patients were biopsied within 90 days of an unequivocal attack of acute upper urinary infection with loin pain and tender-

ness, fever, positive bacterial culture and increased white cells in the urine. None of these patients had a previous history of infection or evidence of an underlying renal abnormality. At the time of the biopsy all the patients' acute symptoms had been relieved with antibiotics. In four of these ten cases renal biopsy showed the appearances of acute pyelonephritis; in these four the biopsies were performed from 5 to 70 days after the onset of symptoms. In two of these patients two renal biopsies were obtained from the same kidney at the same time and in both instances one biopsy showed gross acute pyelonephritis while the other showed normal renal cortex (Fig. 1). In the four positive biopsies there was focal or diffuse inflammatory cell infiltration with an increase of interstitial tissue and areas of tubular degeneration. A closer examination of the tubules showed that polymorphonuclear leucocytes were frequently present within the cytoplasm of the tubular cells as well as in "pus" casts. Although polymorphonuclear leucocytes were identified in all four biopsies they were relatively infrequent in the two cases who were biopsied after an interval of 56 and 70 days. Some of the tubules were dilated, with degeneration and destruction of the epithelial cells, while in others the tubular cells appeared to be regenerating. In some tubules the basement membrane appeared to have split or to have been disrupted by the inflammatory process. In the affected areas the glomeruli were relatively well preserved, in contrast to the tubules, which were often difficult to identify among the inflammatory cells. Some glomeruli, however, showed slight thickening of the basement membrane of Bowman's capsule and this was occasionally associated with swelling and proliferation of the capsular cells and some shrinkage of the tuft. The over-all appearances suggested that restoration of the affected renal cortex to normal was most unlikely.

The most striking feature of this group was that more than one-third of the cases showed foci of acute cortical inflammation following an attack of uncomplicated upper renal tract infection

A

B

which had been diagnosed as pyelitis and treated with antibiotics. It is also evident that renal biopsy must give a falsely low estimate of the frequency of renal inflammation following "pyelitis" because of the focal nature of the inflammation, and the fact that usually only cortical tissue is obtained. In a recent case of severe acute pyelonephritis in which the kidney had to be removed surgically we found small bands of acute inflammation stretching from cortex to medulla, similar to those described in our biopsy cases. It was estimated that there would be only a 1 in 5 chance of hitting one of these areas by renal biopsy. We feel it is reasonable to suggest therefore that foci of renal inflammation probably occur in the majority of cases of so-called pyelitis.

Other investigations

In order to assess the value of various diagnostic procedures in acute pyelonephritis we have studied fifteen patients with exactly similar clinical histories. These include the ten cases already described who had renal biopsies performed, one case in whom a nephrectomy was performed seven days after the clinical onset and four in whom no histology was available. The results of investigations in this group are shown in Table I.

Table I

RESULTS OF INVESTIGATIONS IN 15 PATIENTS WITH ACUTE PYELONEPHRITIS

	All cases		*Biopsy positive cases*	
	Positive	*Total*	*Positive*	*Total*
IVP	2	15	0	4
Proteinuria	3	15	1	4
Urine culture	5	15	4	4
Abnormal white cell excretion rate or positive pyrogen test	8	14	2	3

FIG. 1. Renal biopsy showing normal renal cortex (A) together with another renal biopsy of the same kidney showing acute pyelonephritis (B).

All fifteen patients had an intravenous pyelogram (IVP) performed but in only two was there an unequivocal abnormality and in the four patients whose renal biopsies showed acute pyelonephritis the IVP was within normal limits. Twelve patients, including four of the five cases with positive histology, surprisingly had no protein in the urine. The resting white cell excretion rate was greater than normal in six of the fourteen patients in whom the excretion rate was measured. In two patients, although the resting white cell excretion rate was normal, it rose significantly after the administration of a pyrogen; in four of these patients with abnormal white cell excretion rates the urine was sterile. Conversely the excretion rate was normal in one patient with an infected urine.

Chronic pyelonephritis

Renal biopsy

The group of patients from whom biopsy evidence of chronic pyelonephritis was sometimes found is shown in Table II.

Table II

CLINICAL PRESENTATION OF PATIENTS WHOSE BIOPSIES WERE EXAMINED FOR CHRONIC PYELONEPHRITIS

		Biopsy Positive	*Total*
1. Suspicious history	IVP Negative	3	26
2. Suspicious history	IVP Positive	3	7
3. Symptomless proteinuria or hypertension	IVP Negative	6	27

Twenty-six patients were biopsied because of a suspicious history and clinical findings but in whom the intravenous pyelogram was normal. Only three of these patients showed the biopsy appearances of chronic pyelonephritis. Seven patients were biopsied who in addition to suspicious clinical evidence had an abnormal intravenous pyelogram. Three of these patients showed chronic pyelonephritis on biopsy. In addition to these

thirty-three patients, who were suspected as cases of chronic pyelonephritis on clinical grounds, six out of twenty-seven patients with symptomless proteinuria or hypertension, in whom the IVP was normal, showed histological features of chronic pyelonephritis on renal biopsy.

Examination of these sixty renal biopsies emphasized the difficulties of diagnosing chronic pyelonephritis in renal biopsy material. Firstly, owing to the focal nature of the disease the diseased area will frequently be missed and only normal renal tissue obtained. Secondly, even when histological abnormalities are present, their interpretation is often difficult in a small piece of tissue, and indeed pathologists vary in their criteria for diagnosing chronic pyelonephritis even when the whole kidney is available. In considering the histological diagnosis we have taken into consideration the criteria laid down by Weiss and Parker (1939). These include infiltration of intertubular tissues with lymphocytes and plasma cells, glomerular hyalinization with periglomerular fibrosis, dilatation of tubules containing colloid casts—the so-called "thyroid" areas—and the focal nature of the disease. If all these features are present in a renal biopsy the diagnosis of chronic pyelonephritis can obviously be sustained with reasonable certainty. These criteria are, however, those of a longstanding fully developed disease and it is evident that in an early stage one of these features such as "thyroid" areas may not be present. The diagnosis is made easier if in a renal biopsy there are areas of obviously normal renal tissue as this excludes that the appearances are due to generalized renal disorders, such as glomerulonephritis, diabetes or amyloid disease. However, in biopsies where focal lesions are present the problem is to differentiate early chronic pyelonephritis with inflammation and tubular destruction from foci of lymphocytic infiltration occurring as a reaction to degenerate glomeruli and tubules, in nephrosclerosis. In assessing these difficult problems the extent, type and distribution of chronic inflammatory cell infiltration, the degree of tubular and

glomerular degeneration and the presence or absence of arteriolar-sclerosis must be considered. Using these criteria, we considered that evidence of chronic pyelonephritis was present in 12 of the 60 renal biopsies examined.

Other investigations

In order to assess the relationship of various diagnostic procedures in chronic pyelonephritis we have investigated 26 cases in whom we considered the diagnosis to be proven. These comprised the 12 cases in whom there was a positive biopsy and 14 other patients in whom there was both a history of recurrent upper urinary infection and an abnormal IVP. The results are shown in Table III.

Table III

RESULTS OF INVESTIGATIONS IN 26 PATIENTS WITH CHRONIC PYELONEPHRITIS

	All cases		*Biopsy positive cases*	
	Positive	*Total*	*Positive*	*Total*
IVP			3 (2)	12
Proteinuria	17	26	10	12
Urine culture				
(a) No recent ℞	7	15		
(*b*) Recent ℞	2	11	4	12
Abnormal white cell excretion rate or positive pyrogen test				
(a) No recent ℞	11	15	7	10
(b) Recent ℞	7	11	1	2

℞ = Antibiotic treatment.

In the twelve patients with biopsy evidence of chronic pyelonephritis there were two in whom the IVP showed no opacification while three others showed some definite abnormality consistent with chronic pyelonephritis. The IVP in the other seven was normal. Nevertheless six of these seven cases had some additional evidence of pyelonephritis such as positive urine culture, increased white cell excretion rate or a history of recur-

rent urinary infection. It is evident that a normal IVP does not exclude chronic pyelonephritis as a cause of hypertension or symptomless proteinuria.

In contrast to the cases of acute pyelonephritis, proteinuria was present in 17 of the 26 patients, and in those with positive biopsies, only two had no proteinuria.

Fifteen of the 26 patients had had no antibiotic treatment within the previous month and the urine was definitely infected in seven of these patients. Eleven patients were studied within one month of a course of antibiotics although treatment had ceased at least three days before. In this group the urine was infected in only two patients. Four of the patients with positive renal biopsies had infected urine.

The white cell excretion rate before and after administration of a pyrogen was measured according to the technique of Pears and Houghton (1959). The test was considered to be positive if the white cell excretion rate rose by more than 100 per cent and reached a rate greater than 400,000 per hour after the administration of the pyrogen. In the 12 patients who had had renal biopsies, the tests were positive in eight. Four patients who gave negative results with this test had biopsy evidence of pyelonephritis. However, two of these four cases had chronic renal failure, which is compatible with the finding of Y. Katz (personal communication) that in advanced cases of chronic pyelonephritis in dogs the pyrogen test becomes negative. In the whole series of cases, 11 of the 15 patients who had not had any recent treatment and seven of the 11 patients who had had recent treatment gave positive results with this test.

Only 14 of the 26 patients with chronic pyelonephritis had a diastolic pressure over 95 mm. Hg and in the two patients with the most gross histological changes the blood pressure was normal. Furthermore, a rise in diastolic arterial pressure was not related to a fall in creatinine clearance, which suggests that the rise in blood pressure is not simply related to a reduction in nephrons. These

observations are in keeping with the findings at necropsy, when it has been shown that only 30–60 per cent of patients with chronic pyelonephritis have hypertension (Platt and Davson, 1950; Kincaid-Smith *et al.*, 1958) and that the histological lesions are not well correlated with the finding of hypertension (Merriam, Sommers and Smithwick, 1958).

In summary, therefore, we have shown that renal biopsies from patients with what is usually termed "acute pyelitis" frequently show gross pyelonephritis. The evidence leads us to suppose that these lesions probably occur in all cases of acute upper urinary infection. We have also shown that these lesions may be present in patients with a normal IVP and no proteinuria. If it is accepted that all our cases of acute upper urinary infection had cortical inflammation then it appears that the use of the white cell excretion rate and the pyrogen test is the best method for assessing the continued presence of renal parenchymatous inflammation.

It is evident that in the diagnosis and assessment of cases of chronic pyelonephritis or of cases such as hypertension or proteinuria which might be due to occult chronic pyelonephritis many parameters may be needed to establish a diagnosis. It has been shown that evidence of chronic pyelonephritis on renal biopsy may be obtained from patients with a normal IVP and no previous history of urinary infections and that the white cell excretion rate before and after the administration of a pyrogen is useful both in diagnosis and in assessing the effectiveness of treatment.

REFERENCES

HUTT, M. S. R., CHAMBERS, J. A., MACDONALD, J. S., and DE WARDENER, H. E. (1961). *Lancet*, **1**, 351.

HOUGHTON, B. J., and PEARS, M. A. (1957). *Brit. med. J.*, **1**, 622.

KINCAID-SMITH, P., MCMICHAEL, J. and MURPHY, E. A. (1958). *Quart. J. Med.*, **27**, 117.

KLEEMAN, C. R., HEWITT, W. L., and GUZE, L. B. (1960). *Medicine (Baltimore)*, **39**, 3.

MERRIAM, J. C., SOMMERS, S. C., and SMITHWICK, R. H. (1958). *Circulation*, **17**, 243.
PEARS, M. A., and HOUGHTON, B. J. (1959). *Lancet*, **2**, 1167.
PLATT, R., and DAVSON, J. (1950). *Quart. J. Med.*, **19**, 33.
WEISS, S., and PARKER, F. (1939). *Medicine (Baltimore)*, **18**, 221.

DISCUSSION

Hamburger: The material that we have in Paris seems to be a little different from what has been described. We have a very high proportion of cases in which the intravenous pyelogram or other X-ray examination methods show urological reasons for the pyelonephritis. Methods such as studying the dynamics of the ureter and bladder by the electron dynamometer, measurement of pressures inside the urinary tract, cystography during micturition and also radiocinematography have led us to the conclusion that in practically all our cases of chronic pyelonephritis, when carefully studied, there was an explanation in the urinary tract. When no excretory functional disorder was found, it usually was in patients with too severe renal insufficiency to allow a satisfactory urological examination, so that it is difficult not to wonder whether the excretory aetiology could not have been found if the patient had been seen earlier. But of course only a small proportion of these urological abnormalities are gross obstruction or evident malformation.

Raaschou: We have excluded these cases with obstruction in the urinary tract from our biopsy material.

Blainey: We have had six patients who have shown a histological pattern of pyelonephritis, with a clinical story which has been completely different. These have been mostly young people who have presented with severe haematuria without casts, with a normal serum albumin, usually with a rather high γ-globulin and with a small amount of protein in the urine. One patient, a girl of 18, had a severe impairment of creatinine clearance which showed a steady improvement after two courses of tetracycline. However, she has recently had a relapse, and on two out of three occasions we have had positive urine cultures for coliform organisms.

Kark: Did the red cells come back again in the urine before the second treatment with tetracycline, then disappear once more on treatment,

and start up again all of a sudden with gross haematuria, when you stopped treatment?

Blainey: Yes, with massive haematuria.

Raaschou: In pyelonephritis massive haematuria may occur on the basis of haemorrhagic pyelitis or cystitis.

Blainey: The biopsies, however, showed well-marked "thyroid-like" lesions of dilated tubules, periglomerular fibrosis and lymphocytic infiltration which I think most people would be quite happy to accept as pyelonephritis.

Pirani: Were there a lot of red blood cells in the tubules?

Blainey: Yes.

Hutt: Was there any family history?

Blainey: None whatsoever.

Slater: What was the sex incidence?

Blainey: I have seen six cases, of whom three have been in women and three in men. They have all been young, with the exception of one 40-year-old woman.

Kark: Our oldest case of this type was 45, and we have studied a man who was, I think, 37; all the rest were between 18 and 30.

Rich: Did you see any glomerular changes?

Blainey: Only periglomerular fibrosis.

Earle: Were there any vascular changes?

Blainey: There were some in the second biopsy, none in the particular patient I mentioned. There were areas of sclerosis around the scars.

Hutt: Was your first biopsy done shortly after the onset of haematuria?

Blainey: It was done three months after the onset of haematuria. The condition was regarded as a glomerulonephritis until the biopsies were done.

Rich: What was the blood pressure?

Blainey: Normal.

Earle: Did you get streptococcal antibodies?

Blainey: No: they were not determined.

Hardwicke: In one instance we cultured *Streptococcus viridans* from the urine.

Ross: Did you culture from the biopsy needle in these cases?

Blainey: I tried to, but in only one case were we successful, in a 16-year-old boy who had been regarded as having glomerulonephritis for five years. He had recurrent episodes of haematuria and similar biopsy findings to the others. There was a positive culture for a coliform.

Darmady: In all cases was there a cyst-like appearance of the tubules?

Blainey: It was most marked in the biopsy from the 18-year-old girl, and present to lesser extent in the others.

Hutt: Was there an abnormal white cell excretion rate at any time?

Blainey: Yes. It was high.

Rosenheim: Was the intravenous pyelogram normal?

Blainey: It was reported as normal in all of them, but I am not sure that we are very good at deciding what is the variation in normals.

Hutt: This raises again the question of where the red cells come from in haematuria of renal origin. I still don't think that we have an answer to this. In this case there might be good reason for suspecting that they get straight into the tubule Perhaps that is the answer also in glomerulonephritis.

Kark: In some of our cases it is quite clear that the red cells are coming through the tubule: you can see the break, and you can see the red cells going into the lumen (Kark, R. M., Muehrcke, R. C., Pirani, C. L., and Pollak, V. E. (1955). *Ann. intern. Med.*, **43**, 807). However, we also have some cases where there are red cells in Bowman's space as Dr. Earle reported.

Rich: It is not at all uncommon to find red cells in Bowman's space, is it?

Kark: At autopsy, yes. But not at biopsy.

Rich: But haemorrhage in Bowman's capsule is ordinarily so focal that you may have to search for it even in autopsy sections, in which there are far more glomeruli than in biopsy sections.

de Wardener: Does this acute haematuria, which one might call chronic or recurrent pyelonephritis, respond to antibiotics?

Blainey: Yes. I told you about the one case that relapsed. The other five have shown a satisfactory response. Although we had to treat one man three times, he is now quite well and symptom-free, five years later.

Hardwicke: The woman who had *Strep. viridans* had been on

continuous oral penicillin. She used to have six attacks of haematuria a year, continuing for about three years when we first saw her. She has now been free of haematuria for nearly two years. We haven't repeated the biopsy.

Heptinstall: These attacks cleared up without any treatment, just spontaneously?

Hardwicke: They cleared up previously without any treatment, but she had six of them a year.

Kark: I am not at all sure that these finally clear up, because we have seen recurrences.

Raaschou: Prof. de Wardener, how was the kidney function of your cases of chronic pyelonephritis with positive renal biopsy findings and negative intravenous pyelography—the three cases out of 26 in your Table II?

de Wardener: We had only one that had a creatinine clearance below 20 ml./min. The other two and the majority of the patients were in your second group.

Raaschou: Then there are some differences between our patients.

Blainey: I should like to confirm Dr. Brun's and Prof. Raaschou's findings of pyelonephritis as a cause of acute renal failure. We have had three patients referred to us for dialysis in whom the diagnosis histologically and clinically has been of an acute pyelonephritis, in one case superimposed upon pre-existing chronic nephritis.

Rich: Were bacteria recovered from all of them?

Blainey: Yes.

Bucht: I think that one reason that there is a difference between the results of the Danish and the British group is that the Danish technique gives much larger biopsies; they may be as much as ten times as big as the British ones. If you want to obtain the papilla with a small needle, you can do that by giving an extra push to the needle after you have inserted it; then you can get 3–4 cm. of biopsy, and thus a more representative piece of kidney.

Earle: Dr. Hutt's remarks about the incidence of hypertension not being related to impairment of renal function have dashed some of my hopes. From the literature and from our own cases of renal hypertension, I gained the impression that there had to be rather marked damage in at least one-quarter of one kidney before hypertension

developed in pyelonephritic patients. In Parker and Weiss's, in Longcope's and in other published series, all those with hypertension had considerable impairment of renal function. In our biopsy series we had relatively few that we could call pyelonephritis, three of some 250 patients. Isn't it conceivable in some of the chronics with hypertension, that the histological lesions might be secondary to vascular disease? Did you find any vascular changes in your patients?

Hutt: I think there are two points to be considered here: first of all, are the lesions we are seeing those of chronic pyelonephritis, or are they just a reaction to ischaemia associated with vascular lesions? We thought on histological grounds that the appearances were not simply those of ischaemia. The other point is that assuming that these patients did have pyelonephritis it does not necessarily mean that the pyelonephritis is the cause of the hypertension. The disease may have been coincidental or indeed the hypertension may have predisposed to the development of pyelonephritis.

Earle: Did you observe any vascular changes in the biopsies?

Hutt: We didn't have much opportunity for observing the larger vessels. In a number of the patients who had hypertension their small arterioles showed some degree of arteriolar sclerosis.

Kark: You said that from the group with asymptomatic proteinuria and high blood pressure you took 27 biopsies, of which six were positive. Do you mean that the other 21 were normal biopsies?

Hutt: I meant that they did not show pyelonephritis. In the cases with asymptomatic proteinuria many of the biopsies were normal and some showed minimal or equivocal glomerular changes such as proliferation. In the patients with hypertension, many showed arteriolar sclerosis with occasional ischaemic glomeruli and areas of lymphocytic infiltration which we considered were just the result of the vascular lesion.

Rich: Prof. Raaschou, I was interested in your putting what you call the phenacetin group of interstitial nephritis along with pyelonephritis. Did you find bacteria in any of those?

Raaschou: Yes, most of them have urinary infection. We believe that the order of events is the following: the patients take phenacetin preparations because of headache, lumbar pains and other pains coming from the urinary infection. We do not believe that there is any proof

that phenacetin is the cause of the chronic interstitial nephritis. However, we may add that in Denmark many doctors are convinced that there is a causal relationship between excessive phenacetin consumption and chronic interstitial nephritis. Have you seen this type of patient in the United States and in England? Don't people in these countries take phenacetin preparations?

Rich: Apparently not as much as in Switzerland. But we do see very marked interstitial nephritis caused by sensitizing drugs, even to the extent of great swelling of the kidney, with acute renal failure. I am just surprised that you did find bacteria in most of your cases.

Darmady: In the cases in which you were unable to find any bacteria could the "pyelonephritis" be due to a change in the kidney, or some congenital abnormality, which might then have produced a chemical interstitial nephritis? One of the things that has interested me in seeing healing lesions is this infiltration into the interstitial tissues. Recently we have found certain children with cystic changes in the tubules, whether primary or secondary I don't know, which might tie up with what you have been saying. These are large cysts in the proximal tubules, some of which show thyroid-like lesions in histological and micro-dissection preparations. We have found these in two families (Darmady, E. M. and Stranack, F. (1960). *In* Biology of Pyelonephritis, Henry Ford Hospital International Symposium, p. 173, eds. Quinn, E. L. and Kass, E. H. Boston: Little, Brown). We wonder whether such lesions are congenital and might not form a focus for subsequent infection or possibly produce a chemical interstitial change because the proximal convoluted tubules are inadequate.

Pirani: I should like to make a point which was also raised at the Henry Ford Hospital International Symposium on pyelonephritis (1959): We should try to separate among the chronic cases the ones which are active from those which are not. This can be done mainly by evaluating the proportion of plasma cells in the interstitial infiltrates. The recognition of chronic pyelonephritis in the very late and inactive stages is often impossible, at least in my experience. For example, an old pyelonephritic scar at times is indistinguishable from an arteriosclerotic scar.

Renal biopsies have been very useful in revealing unsuspected pyelonephritis. When we were not studying clinical pyelonephritis at all,

we found about 2 per cent of pyelonephritis in a large series of biopsies, and some of these cases actually did respond to treatment.

Raaschou: Have these patients with biopsies showing unsuspected pyelonephritis had pyelonephritis in childhood?

Pirani: There was no past history in these cases, and no positive culture; it was completely unsuspected. The histological evidence indicated that this probably was a pyelonephritic lesion, associated with some other lesions. There seems to be some evidence that renal disease of other type may predispose to pyelonephritis.

Raaschou: Does anybody know the prognosis of acute pyelonephritis in children? Or in adults?

Rich: In children it must have a very good prognosis by and large, because "pyelitis" is so common in little girls.

Rosenheim: Careful follow-up would suggest that the prognosis in children was not so good (Macaulay, D. and Sutton, R. N. P. (1957). *Lancet*, **2**, 1318).

Slater: Is there not considerable question whether pyelitis exists as an entity, or whether these infections are truly a pyelonephritis, usually ascending in girls, involving the urinary collecting system and kidney?

Rich: I think that is true. The term pyelitis is often used clinically with no evidence that only the pelvis, and not the kidney substance, is infected.

Raaschou: Thiemich in 1910 and, later on, Chown in 1927 and Wilson and Schloss in 1929, have shown that in acute pyelitis in children the kidneys are the seat of pronounced interstitial inflammatory cell infiltration (Thiemich, M. (1910). *Jb. Kinderheilk.*, **62**, 243; Chown, B. (1927). *Arch. Dis. Childh.*, **2**, 97; Wilson, J. R., and Schloss, O. M. (1929). *Amer. J. Dis. Child.*, **38**, 227); thus acute febrile pyelitis in the great majority of cases is actually an acute pyelonephritis.

Rosenheim: Does everyone believe that "chronic pyelonephritis" is always infective? Could not reflex back pressure up the ureter over a lifetime produce fibrotic changes in the kidneys, without there necessarily being any coliform infection? Very often there is no clear history of acute pyelonephritis.

de Wardener: How do you explain the presence of the white cells? I don't see how an increase in back pressure would increase the white cells in the urine.

Rosenheim: If there are white cells it is probably infective, secondary perhaps to obstruction, but I think the fibrosis may possibly be consequent on back pressure.

Raaschou: As for the frequency of bacteriuria I may repeat that in our biopsy material we have found bacteriuria in only one-third of our cases.

Rich: At autopsy it is very uncommon to find pyelonephritis (that is, if we really mean *pyelo*nephritis, the pelvis being involved, too, as well as the kidney) without finding cystitis and ureteritis also.

Raaschou: At the Symposium on "Biology of Pyelonephritis" in Detroit (October, 1959) Paul Kimmelstiel, on the basis of relatively rigid histological criteria, found only 2·8 per cent of chronic pyelonephritis in his large autopsy material.

Kark: One must not overlook potassium nephropathy, the result of potassium depletion, where the histological picture looks very much like chronic pyelonephritis. Although Dr. Muehrcke and Dr. Milne have suggested very strongly that this change is related to superimposed infection, there still is a question in our minds as to whether this is a metabolic disturbance producing a picture that looks like chronic pyelonephritis.

Milne: The sex incidence of infected urines from pyelonephritis in life is very different, as has been pointed out in a recent paper by S. E. Kleeman and L. R. Freedman (1960. *New Engl. J. Med.*, **263**, 988): in a large series, the incidence of urinary infection was ten times as common in women as in men, but at autopsy histopathological changes interpreted as chronic pyelonephritis were equal in the two sexes.

Rich: In our experience, frank pyelonephritis in males at autopsy, if you exclude calculi, enlarged prostates, and other gross obstructions, is very uncommon. It is much more common in females. This has made us wary of thinking of pyelonephritis as being simply a blood-borne infection of the normal urinary tract. The short female urethra predisposes to bladder infection from without, and cystitis is far more common in females than in males. I think that this is important in determining the higher incidence of pyelonephritis in females.

Heptinstall: I don't think that there is any doubt that chronic pyelonephritis is overdiagnosed by a lot of pathologists; it is often just a rubbish heap into which things that you can't diagnose are thrown.

Rich: The accumulations of mononuclear cells in the kidney, which can arise from very many causes, are very commonly called pyelonephritis.

Heptinstall: Dr. O. Saphir in Chicago has taken a series of cases of clinical malignant hypertension at autopsy and tried to assess how many of these were due to chronic pyelonephritis (using criteria similar to those of Weiss and Parker). He came out with a figure of 86 per cent. This is quite contrary to other people's experience. In the original paper of Weiss and Parker, the incidence was 15–20 per cent, and that is what most other people have found. There is a very big difference between 15–20 per cent and 86 per cent. Now is chronic pyelonephritis very common in Chicago?

Jennings: I assume you are speaking of "pyelonephritis lenta". This diagnosis has been very uncommon at the hospitals associated with Northwestern University in Chicago. The consensus is that the name is a misnomer until objective evidence is presented to show that the lesion arises on an infectious basis.

Pirani: With Dr. B. Chomet I have just reviewed 50 autopsy cases of malignant hypertension, in two hospitals, and the incidence of pyelonephritis was extremely low, much lower than that found by Dr. Saphir. I would like to point out again that some of the histological criteria which are supposed to be characteristic of pyelonephritis I don't think are characteristic at all. We have seen thyroid-like areas, for example, in malignant hypertension; I could project these slides here and a lot of people would call them pyelonephritis. Unless all or most of the morphological criteria are present at the same time no definitive diagnosis of chronic pyelonephritis can be made.

Joekes: Prof. Kark, in 1955 you published four or five cases of what you called bacterial nephritis, which presented rather like acute poststreptococcal nephritis. You grew organisms from the kidney and treated the patients successfully with antimicrobial agents. Have you seen such cases since that time?

Kark: Yes, we have. Some of them were exactly like the cases which Dr. Blainey described. However, I must say that the histological changes that one sees in these cases vary widely. In one case there was a tremendous periglomerular infiltration of polymorphs which cleared up completely on antibiotic therapy (Kark, R. M., Muehrcke, R. C.,

Pirani, C. L., and Pollak, V. E. (1955). *Ann. intern. Med.*, **43**, 807). We haven't seen another case as severe as that. We have seen some cases which have had haematuria and positive cultures from the kidney on biopsy, with a positive culture from the urine the next day, —*all urine cultures being negative before the biopsy*—in which there were actually minimal changes in the glomerulus. The vascular changes were of a mild degree. We haven't felt that we had enough material yet to write these cases up.

Raaschou: Did you see crescents in these cases?

Kark: Yes, rarely.

Raaschou: We have never seen crescents in our biopsied cases of pyelonephritis.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

THE SIGNIFICANCE OF RENAL BIOPSY FOR THE DIAGNOSIS OF PYELONEPHRITIS IN DIABETIC PATIENTS

ÅGE CHR. THOMSEN

Medical Department 3, Kommunehospitalet, Copenhagen

PYELONEPHRITIS is generally considered to occur more frequently among diabetic patients than among other groups. Acute pyelonephritis in diabetics has received special attention, as it often produces severe symptoms in the form of papillary necrosis.

Some figures will first be quoted for the frequency of acute and chronic pyelonephritis in various post-mortem series, either consecutive, or omitting diabetic subjects. The lowest frequency of chronic pyelonephritis, 2·8 per cent, was found by Kimmelstiel (1960), who based his diagnosis on well-defined, microscopic criteria only. Raaschou (1948) found chronic pyelonephritis in 5·6 per cent, Zollinger (1958) in 7 per cent, Jackson, Dallenbach and Kipnis (1955) in 9 per cent, whereas Rhoads, Billings and O'Conor (1952) considered signs of active or healed pyelonephritis to be present in at least 20 per cent of all autopsy cases. Gibson (1928) found purulent infection of the kidney in 5·8 per cent. Acute pyelonephritis was the cause of death in 1·6 per cent of Robbins and Tucker's cases (1944), and in 3·3 per cent of those of Edmondson, Martin and Evans (1947), whereas Aye (1954) estimated the frequency at only 0·7 per cent. Thus, chronic pyelonephritis is found in 5 to 10 per cent of all post-mortems, while acute pyelonephritis as the cause of death occurs in about 2 per cent.

The considerable variations in the figures quoted may arise from different criteria for the anatomical diagnosis, from varying

use of microscopic examination, and, in addition, may depend on the interest with which the diagnosis was sought. Geography may exert an influence, and changes in treatment in the course of time may also leave their mark on the figures. As to the sex ratio, Raaschou (1948) found that chronic pyelonephritis attacks both sexes equally, but this, naturally, will depend on the age composition of the material.

Compared to the series mentioned, the frequency in diabetic subjects is considerably higher. In Aarseth's series (1953) the frequency of chronic pyelonephritis among diabetic autopsy cases was about 40 per cent, whereas it was about 20 per cent in that of Young and Clancy (1955). Acute pyelonephritis was found to be the cause of death in 6·8 per cent of the cases examined by Robbins and Tucker (1944), and in 12·4 per cent of those of Edmondson, Martin and Evans (1947). In Aye's material (1954) the frequency was 14·4 per cent. Both acute and chronic pyelonephritis occur about five times more frequently in these patients than in other groups. Chronic pyelonephritis also shows an equal sex distribution in diabetic subjects.

Clinical studies, however, give another picture of the occurrence of infections of the urinary tract. Table I shows the frequency of bacteriuria in some clinical series, in which diabetic subjects were compared with a control material. The figures vary considerably, but in general much higher frequencies were found than in the post-mortem series, both for diabetics and controls. Even though bacteriuria is possible without infection of the kidney, these figures arouse the suspicion that the usual technique of culture from urine involves too great sources of error. When Kass introduced quantitative evaluation of bacteriuria, therefore, this was an advance which made it possible to decide with greater certainty whether a case of bacteriuria was significant. Kass (1956) found that bacteriuria was three times as frequent among female diabetic patients as among the controls, but it was not more frequent among male diabetic patients. Huvos and Rocha (1959),

Table I

FREQUENCY OF BACTERIURIA IN SERIES OF DIABETIC SUBJECTS COMPARED WITH CONTROLS

Author	*Number of patients*	*Sex*	*Incidence of bacteriuria*	*Incidence in control material*	*Comments*
Bowen and Kutzman (1942)	83	F	43%		Ureter urine
	—	—	65%	75%	Bladder urine
Harrison and Bailey (1942)	50	M+F	54%	8%	Microscopy of urinary sediment
Joron *et al.* (1955)	259	F	61%	43%	Catheter specimen
	150	M	61%	59%	Midstream specimen
Young and Clancy (1955)	62	F	42%	10%	Catheter specimen
Kass (1956)	54	F	18%	6%	$> 10^5$ bacteria/ml.
	37	M	5%	4%	Bladder urine
Huvos and Rocha (1959)	50	F, M	26%	22%	$> 5 \times 10^4$ bacteria/ml. Bladder urine
Kalliomäki and Kasanen (1960)	63	F	32%	12·2%	Bacteria + pyuria or recurrent symptoms
	37	M	3%	8·1%	

however, were unable to confirm this. Thus, a good deal of disagreement exists between the post-mortem findings and the bacteriological findings. In addition, disagreements are often found between the clinical and the bacteriological findings.

Renal biopsy would appear to be an ideal way out of this dilemma, as it provides an opportunity of making concurrent clinical and histological studies.

Only very few studies have been published in which the diagnosis of pyelonephritis has been made by means of renal biopsy material from diabetic subjects. Brun *et al.* (1953) diagnosed the condition from the histology in only one case out of 12, of whom eight had a clinical diagnosis of chronic pyelonephritis. They decided, therefore, that the method of renal biopsy was not of particular diagnostic value in diabetic subjects.

Taft, Finckh and Joske (1954) (20 biopsies), Darnaud *et al.* (1956) (20 biopsies) and Miatello, Machada and Medel (1958) (20 biopsies) also found a poor correlation between the clinical and histological diagnosis of pyelonephritis.

The largest material published so far has been that of Gellman *et al.* (1959), who attached much weight to the diagnosis of pyelonephritis. This material consisted of 63 renal biopsy specimens and nine post-mortems, in a total of 53 subjects with diabetes mellitus. No clear histological diagnosis could be established on the basis of Weiss and Parker's criteria (1939). In six biopsy specimens (10 per cent), however, chronic pyelonephritis was strongly suspected in view of the degree of interstitial fibrosis and cellular infiltration. There were five post-mortems (55 per cent) with this diagnosis. The discrepancy between the biopsy specimen and post-mortem frequency could have been caused by incomplete representativeness in the biopsy specimen, in view of the focal nature of the disease. Another explanation was that pyelonephritis might be a terminal event in chronic diabetic renal disease. The authors found a correlation between increasing changes in the urinary sediment (leucocytes and casts) and the

degree of tubular atrophy. However, no mention was made of the relation between the renal histology and the bacteriuria or the diagnosis of clinical pyelonephritis.

To throw some light on the problems mentioned above, an analysis was made of the author's material of renal biopsy specimens from diabetic subjects.

Method

Histological material. Renal tissue was obtained by percutaneous aspiration biopsy of the right kidney with the patient in the prone position, according to the technique of Iversen and Brun (1951), modified by Kark and Muehrcke (1954). The biopsies were always carried out during a quiescent phase of the disease and with no particular aim of revealing a case of pyelonephritis.

The tissue was fixed in 4 per cent formaldehyde or buffered formaldehyde, embedded in paraffin and cut into sections 5–10μ thick. These were stained with haematoxylin-eosin, van Gieson-Hansen's method and periodic acid-Schiff.

All preparations were evaluated by the author.

Preparations which contained less than five glomeruli were rejected.

The following were the histological changes of significance assessed in the present study: *glomeruli*, diffuse and nodular glomerulosclerosis, hyalinized glomeruli, pericapsular fibrosis; *tubules*, atrophy, dilatation, leucocyte casts, acellular casts, thyroid-like appearance; *interstitium*, increase in amount, infiltration by lymphocytes, plasma cells, polymorphonuclear leucocytes and eosinophilic cells; *vessels*, changes in the walls of the juxtaglomerular arterioles. With respect to tubular, interstitial and vascular changes, a classification according to grade was made wherever possible (from 0 to 3).

Clinical material. In addition to the usual clinical examination, microscopy and culturing of the urine were done. Clean voided specimens were used for most of the men, and catheter specimens

for women. A standardized method was used in the microscopy, so that a semi-quantitative evaluation of the pyuria was possible. The urine samples were cultured on blood agar and Conradi-Drigalski agar. Bacterial counts of the urine were not made.

Material

There were 102 biopsy specimens in the total material of diabetic patients. After excluding those patients in whom the

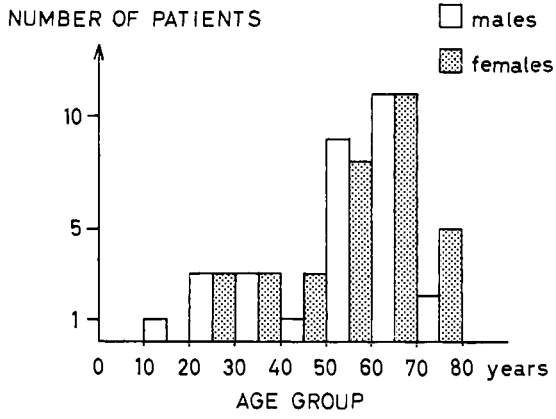


FIG. 1. Age distribution of 63 diabetic subjects.

urine had not been cultured, the material consisted of 58 patients (29 men, 29 women). All suffered from a well-defined diabetes mellitus. The biopsy was repeated in one man and four women after a lapse of some months, and as these are regarded as individual investigations, the material may be regarded as consisting of 63 biopsy specimens, 30 from men and 33 from women.

Fig. 1 shows the age distribution of the patients. The range was from 14 to 77 years. The distribution of men and women was more or less the same for each age class. Thirteen of the patients were juvenile cases, in which the diabetes started before the age of 20.

Fig. 2 shows the distribution according to duration of diabetes. There were many long-term cases, 23 having had the disease for at least 15 years.

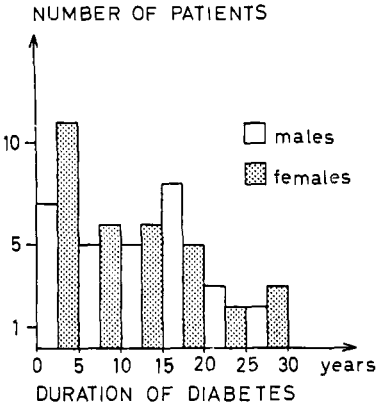


FIG. 2. Distribution of 63 diabetic subjects according to duration of diabetes.

Histological classification

The histological changes suggesting pyelonephritis are shown in Table II. About one half of the biopsy specimens showed a greater or lesser degree of interstitial infiltration. Lymphocytes always constituted the dominant type of cell, while plasma cells and histiocytes constituted only a slight proportion of the cells. Infiltration by polymorphonuclear leucocytes alone was not seen, just as true abscess formation did not occur. Only one specimen showed cellular casts of polymorphonuclear leucocytes in the lumen of the tubules, together with invasive glomerulitis. Round cell infiltration was often found near the hyalinized glomeruli.

Atrophy of the tubules, and often an increase in the interstitial tissue, were observed in more than half the biopsy specimens. Dilated tubules were less common, and only one specimen

showed a typical, thyroid-like appearance, with dilated tubules and colloid casts. A common finding, of doubtful significance pathologically and so not discussed further here, was slightly dilated thin limbs of the loop of Henle, with small colloidal precipitates.

Signs of papillary necrosis were not observed.

Table II

HISTOLOGICAL SIGNS OF PYELONEPHRITIS IN RENAL BIOPSY SPECIMENS FROM 63 DIABETIC PATIENTS

	Males	*Females*
Number of biopsies	30	33
Interstitial infiltration		
Round cells	12 (40%)	20 (61%)
Polymorphonuclear leucocytes	2 (7%)	5 (15%)
Cell casts	1 (3%)	0
Invasive glomerulitis	1 (3%)	0
Increased interstitial tissue	14 (47%)	11 (33%)
Tubular atrophy	16 (58%)	22 (67%)
Dilated tubules	3 (10%)	6 (18%)
Thyroid-like appearance	0	1 (3%)
Pericapsular fibrosis	8 (27%)	15 (45%)
Hyalinized glomeruli	25 (83%)	21 (64%)

The presence of periglomerular fibrosis around the glomeruli, with a capillary tuft of normal appearance, is considered to be a valuable sign of pyelonephritis (Allen, 1951). However, diabetes itself often gives rise to changes in the capillary tuft, so this symptom is of limited significance and is not recorded here. Likewise, hyalinized glomeruli occurred in the majority of the specimens.

On the basis of the changes mentioned, an attempt was made to group the biopsy specimens according to histological diagnosis. Table III indicates the criteria for this grouping. The main criterion for diagnosing pyelonephritis was the presence of interstitial infiltration. The diagnosis of chronic pyelonephritis was made only when the infiltration by lymphocytes and plasma cells was moderate or severe, and accompanied by pericapsular

Table III

HISTOLOGICAL CRITERIA FOR THE GROUPING OF BIOPSY SPECIMENS WITH RESPECT TO THE DIAGNOSIS OF PYELONEPHRITIS

Histological type of pyelonephritis	*Criteria*	*Number of biopsies*	
		Males	*Females*
Chronic	Moderate or severe infiltration by lymphocytes and plasma cells + presence of pericapsular fibrosis	2	3
Chronic ?	Slight infiltration by round cells, or presence of pericapsular fibrosis alone	11	18
Acute	Infiltration by polymorphonuclear leucocytes alone	0	0
Acute in chronic	Like chronic, + infiltration by polymorphonuclear cells	0	1
Acute (in chronic ?)	Like chronic ?, + infiltration by polymorphonuclear cells	1	0
Healed	Thyroid-like appearance without interstitial infiltration	0	0
No pyelonephritis	Neither interstitial infiltration, pericapsular fibrosis nor thyroid-like appearance	16	11

fibrosis. In all other cases of interstitial round cell infiltration, and in cases with pericapsular fibrosis without interstitial infiltration, the diagnosis was considered to be doubtful. The presence of polymorphonuclear leucocytes was taken as expressing an acute condition. Pyelonephritis would appear to be excluded when there is no interstitial leucocyte infiltration, periglomerular fibrosis nor thyroid-like structure.

A sure histological diagnosis of pyelonephritis was made in only seven cases (five cases of chronic pyelonephritis, two cases of acute in chronic). In 27 cases there were no changes which aroused suspicion of pyelonephritis.

It might be mentioned that in the five patients who underwent two biopsies each, there were only two who showed small variations in the diagnosis, depending on differences in the degree of interstitial infiltration.

Clinical classification

Table IV indicates the occurrence of pyuria, bacteriuria, and clinical signs and symptoms of infection of the urinary tract. The clinical symptoms include dysuria, frequent micturition, lumbar pain and fever. The symptoms were usually not particularly pronounced, presenting most often as periodic attacks of dysuria and subfebrile temperature. There were a few cases of isolated pyuria and isolated bacteriuria. It will be seen that the

Table IV

SIGNS AND SYMPTOMS OF URINARY TRACT INFECTION IN 63 DIABETIC SUBJECTS

	Males	*Females*
Number of biopsies	30	33
Pyuria	6 (20%)	18 (55%)
Bacteriuria	8 (27%)	14 (42%)
Clinical signs and symptoms	3 (10%)	11 (33%)
Pyuria + bacteriuria	3 (10%)	12 (36%)
Pyuria + clinical signs	2 (7%)	8 (24%)
Bacteriuria + clinical signs	2 (7%)	9 (27%)
Pyuria + bacteriuria + clinical signs	2 (7%)	7 (21%)
No signs or symptoms of urinary tract infection	18 (60%)	12 (36%)

various symptoms occur two to three times more frequently among women than among men.

The clinical diagnosis was made on the basis of the case history and the presence or absence of clinical signs and symptoms as well as pyuria and bacteriuria. Infection of the urinary tract was considered to be present when two or more of the symptoms of pyuria, bacteriuria or dysuria were found. It was regarded as chronic when the symptoms had persisted for more than one month, or were recurrent. Pyelonephritis was diagnosed when, in addition, there was lumbar pain and fever. Groups were also established corresponding to isolated pyuria or bacteriuria (always coliuria).

Clinico-pathological correlation

Table V shows a comparison between the clinical and the histological diagnoses.

In the group with no histological signs of pyelonephritis, agreement with clinical diagnosis was found in 13 cases. Several other cases in this group disagreed to a greater or lesser extent. Two patients had a sure clinical diagnosis of chronic pyelonephritis, but no histological signs whatever were present in these cases. In some of the other 12 cases with signs of infection of the

Table V

CORRELATION OF HISTOLOGICAL AND CLINICAL DIAGNOSES IN 63 RENAL BIOPSY SPECIMENS FROM DIABETIC PATIENTS

	Clinical diagnosis											
		Pyelonephritis				*Urinary tract infection*						
Histological type of pyelonephritis	*No signs or symptoms*	*Chronic*	*Acute in chronic*	*Acute (in chronic ?)*	*Acute*	*Chronic*	*Acute*	*Previous*	*Pyuria + Bacteriuria*	*Pyuria*	*Coliuria*	*Total*
No pyelonephritis	13	2				1	2	2	2	1	4	27
Chronic	4			1								5
Chronic ?	10	4	1			4		1	2	7		29
Acute												0
Acute in chronic					1							1
Acute (in chronic ?)	1											1
Healed												0
Total	28	6	1	1	1	5	2	3	4	8	4	63

urinary tract, but without lumbar pain or fever, pyelonephritis might nevertheless be strongly suspected: five had hypertrophy of the prostate, three of them with signs of urinary stasis; the patient with chronic urinary tract infection had a moderate hydronephrosis. In the other cases with no histological changes from pyelonephritis, infection of the urinary tract may have existed without inflammation of the kidneys. This might contribute to explaining the discrepancy between the reports of frequency of pyelonephritis based on post-mortem data and clinical

data, respectively. All the patients in this group had normal renal function, except two patients, in whom serum creatinine concentration was slightly elevated.

Five patients had a histological diagnosis of chronic pyelonephritis, but in four of them this could not be confirmed clinically or by intravenous urography. The four cases all had a moderately decreased renal function, and in two of them the duration of diabetes was very long (29 and 32 years).

In the group in which chronic pyelonephritis was doubtful, 10 patients had no signs or symptoms of urinary tract infection. Five of them had a long duration of diabetes, at least 10 years. Another patient had previously had four attacks of renal colic on the right side. Of the four patients with a clinical diagnosis of chronic pyelonephritis, three had normal renal function; the same holds good for the four with chronic urinary tract infection. Five of the seven patients with isolated pyuria had decreased renal function. All of them had had their diabetes for several years, with an average duration of 13 years, and all biopsy specimens showed pronounced glomerular and vascular diabetic changes.

Finally, two patients showed histological signs of acute inflammation. In one of them this was consistent with the clinical diagnosis. The other patient had suffered from acute renal failure some weeks before, but renal function was re-established at the time of examination, and there were no signs of urinary tract infection.

The material contained no cases of simple acute pyelonephritis or healed pyelonephritis.

One patient was admitted with clear-cut signs and symptoms of renal papillary necrosis. The biopsy specimen could not confirm this diagnosis as it showed only a severe chronic pyelonephritis.

The clinico-pathological correlation thus showed a discrepancy between the clinical and the histological picture in a considerable number of cases. A histological diagnosis of pyelonephritis was

made in some cases without clinical evidence of the condition. This false-positive diagnosis suggests that the histological criteria used (moderate to severe infiltration by lymphocytes and plasma cells in connexion with pericapsular fibrosis) were not specific, but that the changes had other causes than infection of the urinary tract. (It may be added here that for the whole material of diabetic patients there was no association between the intensity of pyuria and interstitial infiltration.) In some other cases, where a clinical diagnosis of pyelonephritis was established or suspected, no histological signs of the disease could be detected. The failure of the renal biopsy to reveal the condition in these cases may result either from the presence of a unilateral pyelonephritis, or from the pyelonephritis not having been diffusely spread throughout the kidney, so that the biopsy specimen was not representative. In most of these cases in which histological diagnosis was missed, renal function turned out to be nearly normal. Thus, the morphological changes were probably focal. Investigations by Kellow *et al.* (1959) have also shown that focal pyelonephritis was the condition in which renal biopsy specimens were least representative.

The conclusion to be drawn from the study must be that the value of renal biopsy for the diagnosis of chronic pyelonephritis in diabetic patients is limited: first, on the grounds of the inadequate diagnostic criteria; second, on account of the focal nature of the disease.

Diabetic arteriolosclerosis as a cause of interstitial changes

It was shown above that pyelonephritis cannot in all cases explain the interstitial cell infiltration in diabetics. It would therefore seem reasonable to seek the cause of the condition in the diabetes itself.

One of the factors decisive for the development of late diabetic complications is the duration of the diabetic condition. Fig. 3

shows the relation between degree of interstitial infiltration and the duration of diabetes, in a material of 102 renal biopsy specimens from diabetic subjects. Even though the values show a very great scatter, suggesting that several factors play a part, it seems clear that interstitial infiltration is correlated with the duration of the diabetes. Average values of duration of diabetes for grade 0, 1 and 2 were 8·0, 11·1, and 16·4 years, respectively.

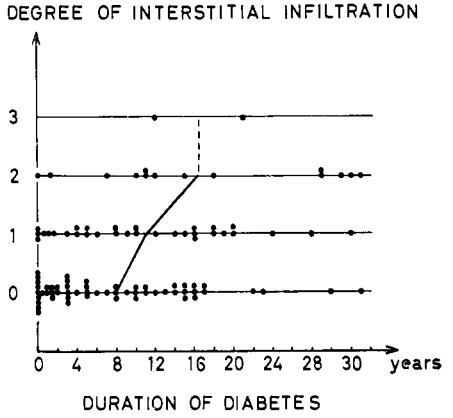


FIG. 3. The relation between degree of interstitial infiltration and the duration of diabetes in a material of 102 renal biopsy specimens from diabetic subjects. The heavy line connects average values.

The difference between averages of grade 0 and 1 was not statistically significant ($P = 0{\cdot}1$), whereas duration of diabetes in grade 2 was significantly longer than both grade 0 and grade 1 ($P < 0{\cdot}01$).

In the author's opinion, based on a study of these 102 renal biopsy specimens, the central element in diabetic nephropathy is arteriolosclerosis. As shown in Table VI, an association undoubtedly exists between the degree of arteriolosclerosis and the degree of interstitial infiltration by lymphocytes and plasma cells.

Table VI

RELATION BETWEEN THE DEGREE OF ARTERIOLOSCLEROSIS AND THE DEGREE OF INTERSTITIAL INFILTRATION IN 102 RENAL BIOPSY SPECIMENS FROM DIABETIC SUBJECTS

Degree of arteriolosclerosis	*Degree of interstitial infiltration*				*Infiltration with polymorphonuclear leucocytes*
	0	1	2	3	
0	20	4	2	0	2
1	26	7	2	0	2
2	9	12	4	0	3
3*	0	8	6	2	6

* In 5 biopsies in this group interstitial infiltration with eosinophilic cells.

While polymorphonuclear leucocytes occur in all degrees of arteriolosclerosis, eosinophilic cells occur to a noteworthy degree

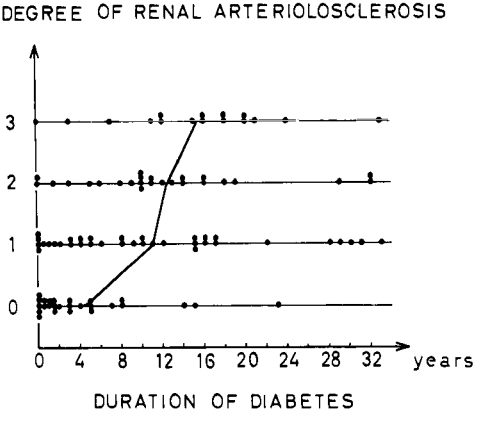


FIG. 4. The relation between the duration of diabetes and the degree of arteriolosclerosis in 102 renal biopsy specimens. The heavy line connects average values.

only in the severe cases of arteriolosclerosis, independent of the degree of infiltration by lymphocytes and plasma cells.

A close association between arteriolosclerosis and tubular atrophy could be demonstrated in this material. Further, a

pronounced association was evident between tubular atrophy and interstitial infiltration. Finally, Fig. 4 shows the connexion between the duration of diabetes and arteriolosclerosis. Although these values also show a considerable scatter, average values of duration of diabetes increase with the degree of arteriolosclerosis (4·3, 11·0, 12·4 and 15·4 years for grade 0 to 3, respectively). The difference between grade 0 and grade 1 was statistically significant ($P < 0{\cdot}01$). Between grades 1, 2 and 3 the differences were not significant.

As an explanation of the relationships discussed, the hypothesis may be postulated that with increasing duration of diabetes, progressive changes develop in the arterioles (and glomeruli). These vascular changes lead to tubular atrophy and a reactive infiltration of the tissue by round cells, mainly lymphocytes.

The relation between arteriolosclerosis and other conditions in diabetes

Although the method of renal biopsy has had only limited value with regard to the diagnosis of pyelonephritis, it has revealed

Table VII

RELATION BETWEEN THE DEGREE OF ARTERIOLOSCLEROSIS AND THE FREQUENCY OF KIMMELSTIEL-WILSON (K.-W.) NODULES IN 102 RENAL BIOPSY SPECIMENS

Degree of renal arteriolosclerosis	*Number of renal biopsies*	*Number of biopsies with nodular glomerulosclerosis (K.-W.)*
0	26	0
1	35	4 (11%)
2	25	11 (44%)
3	16	12 (75%)

other significant aspects of diabetic nephropathy, some of which will be mentioned briefly. Renal arteriolosclerosis is closely correlated with the occurrence of Kimmelstiel-Wilson nodules in the glomeruli (Table VII) and to the grade of diabetic retinopathy (Table VIII), as well as to the blood pressure and renal

function (Table IX). However, these topics lie outside the scope of the present discussion.

Table VIII

DISTRIBUTION OF 95 DIABETIC PATIENTS ACCORDING TO THE DEGREE OF RENAL ARTERIOLOSCLEROSIS AND DIABETIC RETINOPATHY

Degree of arteriolosclerosis	*Degree of retinopathy*			
	0	1	2	3
0	21	3	1	0
1	21	9	3	0
2	2	3	13	1
3	1	0	7	6

Retinopathy grade 1: microaneurysms; grade 2: haemorrhages and exudates; grade 3: proliferative changes.

Table IX

RELATION BETWEEN THE DEGREE OF RENAL ARTERIOLOSCLEROSIS AND VARIOUS CLINICAL FACTORS IN 102 DIABETIC SUBJECTS

Degree of arteriolosclerosis	0	1	2	3
Number of biopsies	26	35	25	16
Average values:				
Duration of diabetes (yr.)	4·3	11·0	12·4	15·4
Age (yr.)	43·6	56·7	54·3	42·6
Age at onset (yr.)	39·3	45·7	41·9	27·2
Blood Pressure mm. Hg				
Systolic	138	143	161	177
Diastolic	82	82	88	105
Creatinine clearance ml./min.	81·5	78·7	61·7	28·9
Insulin dose I.U./24 hr.	35·6	30·8	27·6	29·2

Summary

In a material consisting of 63 renal biopsy specimens from diabetic subjects, a clinico-pathological correlation was sought with respect to the diagnosis of pyelonephritis. Moderate to severe interstitial infiltration with round cells, together with pericapsular fibrosis, were found to be non-specific changes which could also be ascribed to the presence of a diabetic angiopathy. In some cases, the renal biopsy specimen was itself not representative.

REFERENCES

AARSETH, S. (1953). *Acta med. scand.*, suppl. 281, 191.

ALLEN, A. C. (1951). *In* The Kidney, p. 336. New York: Grune and Stratton.

AYE, R. C. (1954). *Diabetes*, **3**, 124.

BOWEN, B. D., and KUTZMAN, N. (1942). *Ann. intern. Med.*, **17**, 427.

BRUN, C., GORMSEN, H., HILDEN, T., IVERSEN, P., and RAASCHOU, F. (1953). *Amer. J. Med.*, **15**, 187.

DARNAUD, C., DENARD, Y., MOREAU, G., and SUC, J. M. (1956). *Sem. Hôp. Paris*, **32**, 8.

EDMONDSON, H. A., MARTIN, H. E., and EVANS, N. (1947). *Arch. intern. Med.*, **79**, 148.

GELLMAN, D. D., PIRANI, C. L., SOOTHILL, J. P., MUEHRCKE, R. C., and KARK, R. M. (1959). *Medicine*, **38**, 321.

GIBSON, A. G. (1928). *Lancet*, **2**, 903.

HARRISON, J. H., and BAILEY, O. T. (1942). *J. Amer. med. Ass.*, **118**, 15.

HUVOS, A., and ROCHA, H. (1959). *New Engl. J. Med.*, **261**, 1213.

IVERSEN, P., and BRUN, C. (1951). *Amer. J. Med.*, **11**, 324.

JACKSON, G. G., DALLENBACH, F. D., and KIPNIS, G. P. (1955). *Med. Clin. N. Amer.*, **39**, 297.

JORON, G. E., DEVRIES, J., REID, G., MATHEWS, W. H., and MCKAY, J. W. (1955). *Diabetes*, **4**, 99.

KALLIOMÄKI, J. L., and KASANEN, A. (1960). *Ann. Med. intern. Fenn.*, **49**, 47.

KARK, R. M., and MUEHRCKE, R. C. (1954). *Lancet*, **1**, 1047.

KASS, E. H. (1956). *Trans. Ass. Amer. Phycns.*, **69**, 56.

KELLOW, W. F., COTSONAS, N. J., CHOMET, B., and ZIMMERMAN, N. J. (1959). *Arch. int. Med.*, **104**, 353.

KIMMELSTIEL, P. (1960). *In* Biology of Pyelonephritis, p. 215, ed. Quinn, E. L., and Kass, E. H. Boston: Little, Brown and Company.

MIATELLO, V. R., MACHADO, E., and MEDEL, R. P. (1958). *Prensa med. argent.*, **45**, 1027.

RAASCHOU, F. (1948). *In* Studies of Chronic Pyelonephritis, p. 35. Copenhagen: Ejnar Munksgaard.

RHOADS, P. S., BILLINGS, C. E., and O'CONOR, V. J. (1952). *J. Amer. med. Ass.*, **148**, 165.

ROBBINS, S. L., and TUCKER, A. W. (1944). *New Engl. J. Med.*, **231**, 865.

TAFT, H. P., FINCKH, E. S., and JOSKE, R. A. (1954). *Aust. Ann. Med.*, **3**, 189.

WEISS, S., and PARKER, F. (1939). *Medicine*, **18**, 221.

YOUNG, K. R., and CLANCY, C. F. (1955). *Med. Clin. N. Amer.*, **39**, 1665.

ZOLLINGER, H. U. (1958). *In* Verhandlungsbericht der deutschen Gesellschaft für Urologie, Wien 1957, p. 165, ed. Boeminghaus, H., and Stoltze, M. Leipzig: Georg Thieme.

DISCUSSION

Blainey: Dr. Thomsen has now shown convincingly that pyelonephritis is not a common concomitant of the living diabetic, as was suggested by renal biopsies in diabetics studied by Gellman and his colleagues (1959, *loc. cit.*). I should like to present the results of two years' investigation by my colleagues—Dr. O'Sullivan, Dr. Fitzgerald, Dr. Malins and others—in a large diabetic clinic in Birmingham (O'Sullivan, D. J., Fitzgerald, M. G., Maynell, M. J., and Malins, J. M. (1960). *J. clin. Path.*, **13,** 527; O'Sullivan, D. J., Fitzgerald, M. G., Maynell, M. J. and Malins, J. M. (1961). *Brit. med. J.*, **1,** 786; O'Sullivan, D. J., Blainey, J. D., Brewer, D. B., Fitzgerald, M. G., and Malins, J. M. (1961). *Quart. J. Med.*, in press). Of 3,841 diabetics attending this clinic, 14 per cent had proteinuria; the distribution by age of the proteinuria appeared to be that of the diabetes itself, except for a slight excess of proteinuria in young males. A random sample of one hundred of these patients was chosen for more detailed study, and careful assessment of clinical state, urine protein examination, renal function tests, including differential clearances, Addis counts, bacterial viable colony counts, and measurements of 24-hour urines, were all done on these 100 patients. From the data it was possible to build up a fairly comprehensive picture of the cause of proteinuria in these patients. In only 12 of the 100 patients was there evidence to suggest the presence of pyelonephritis, as judged by bacterial colony counts of over 100,000/ml., or of more than six white cells per high-power field. In all the other patients the proteinuria was regarded as being due to diabetic renal disease. Of these 12 infected patients, 11 were females over 60.

In order to compare diabetics with the normal population, a sample of 150 diabetics randomly sampled, with or without proteinuria, was taken, and 150 controls (matched for age and sex) from the ordinary population attending the Casualty Dept. for minor trauma. Using viable colony counts as the criterion for pyelonephritis it was found that the incidence of infection in the diabetic population was identical with that in the controls.

We then tried to establish the cause of the proteinuria by renal biopsy. Forty-four biopsies were done in 30 patients, and once again

we found no evidence, as I think has been published in previous series, to suggest that pyelonephritis was a cause of proteinuria in any of these patients. We too saw occasionally the changes of periglomerular fibrosis that Dr. Thomsen has reported, but the main lesions were those of diabetic glomerulosclerosis of varying severity; only three of the biopsies were regarded as completely normal. In two cases with proteinuria the biopsies showed normal glomeruli to light microscopy, while others without proteinuria showed the scattered diffuse lesions of diabetic nephropathy. With periodic acid-Schiff and silver stains one can see clearly early lesions, which may be present in diabetes even of very short duration; in one case these lesions were present three weeks after the acute onset. In more advanced lesions one sees the vascular changes that Dr. Thomsen has mentioned, sometimes associated with periglomerular fibrosis and round cell infiltration. I am sure he is right when he says that these are predominantly vascular disturbances.

As Dr. Thomsen has done, we tried to correlate the grade of histological change, using a simple classification, with various abnormalities of renal function. Again this confirms that advanced diffuse diabetic changes are associated with progressive deterioration of function and that the nodular lesion is relatively unimportant. This series includes more patients of the younger age group who have not previously been reported.

We also tried to correlate the changes with the lipoproteins, which have been used as evidence of biochemical derangement in the diabetic state. There was merely evidence of increasing histological abnormality in every instance where the lipoproteins were grossly abnormal, with little evidence to suggest that these lipid disturbances were in fact the cause of the renal lesions.

Black: I am getting a little worried because while it is possible to exaggerate the importance of a disease by having criteria which are too lax, it is equally possible to minimize its importance by having criteria which are too strict. This applies particularly when part of the evidence is based on biopsy, which we all realize will underestimate the frequency of pyelonephritis. In one particular instance, malignant hypertension in young people, I have only seen two patients under the age of 15 with malignant hypertension; both of those were young girls,

non-diabetic. They showed improvement with sympathectomy, and renal biopsy done at operation showed gross pyelonephritic scarring. I think Prof. Rosenheim has had similar cases.

Rosenheim: Yes, I think we would say that renal ischaemia is quite common as a cause of malignant hypertension—ischaemia rather than pyelonephritis.

Thomsen: Weiss and Parker have also mentioned this picture of interstitial changes in nephrosclerosis.

Black: Yes, but one would be unlikely to have nephrosclerosis under the age of 15 without major renal vessel anomaly.

Slater: Dr. Blainey, in your biopsies of patients with diabetes which showed early changes in the lobular stalks, did those patients have renal dysfunction, for example, proteinuria? Alternatively, did you find similar structural changes in patients with early diabetes who had no signs of renal dysfunction?

Blainey: Of the 31 patients who had biopsies, 12 of them had no proteinuria. We were surprised to find very advanced histological lesions even in people who had no proteinuria and little demonstrable abnormality of renal function.

Thomsen: I would not be sure that the patients you showed with the silver stain had an early stage of diabetic nephropathy. I wonder if you could not find a similar picture in normal persons.

Blainey: We feel that this is definitely an abnormal picture; the electron microscope shows thickening of the basement membrane, with the lamina densa three or four times the normal width.

Pirani: When you say that the duration of diabetes was three weeks, I presume you meant with clinical evidence of diabetes. However, they might have had a prediabetic state.

Blainey: Yes.

Thomsen: One always underestimates the duration of diabetes. We had one patient who was admitted for nephrolithiasis. We discovered he had glucosuria, did a biopsy on him and found nodular lesions typical of late diabetic complications. This patient must have had diabetes for several years.

I could find no correlation between nodular and diabetic glomerulosclerosis and proteinuria. Proteinuria in diabetes may have other causes, for instance, cardiac failure.

Blainey: None of these 100 patients of ours was in cardiac failure. We believe that it is not the nodular lesion, but the diffuse thickening of the glomerular basement membrane that is the change associated with the development of proteinuria. The same or even more advanced lesions of the diffuse change may occur *without* proteinuria, and it is difficult to explain, as in so many other types of renal disease, the precise relationship of the structural changes in the glomerulus and the degree of proteinuria. In our series there was rather poor correlation of structural change and functional abnormality except that all the very advanced lesions with nodules had proteinuria, as they did in your series.

Hardwicke: I assume, Dr. Blainey, that although you showed that early lesion in association with diabetes, you wouldn't regard it as in any way specific?

Blainey: No, I wouldn't.

Milne: Could someone enlighten me on the histological difference between the ischaemic kidney and the cases described as chronic pyelonephritis? It seems to me that in clinical medicine the importance of ischaemia as a cause of malignant hypertension has only become really apparent since the radiologists have been showing ischaemic lesions in large vessels. I think one slide Prof. Raaschou showed (Fig. 1) is rather similar to what I thought an ischaemic lesion should look like, with great crowding of the glomeruli. Perhaps we could just see that slide again.

Pirani: I think that the pathologist should always indicate, especially in renal biopsy, if he suspects pyelonephritis, even if the suspicion is very slight, because pyelonephritis is one of the few renal diseases which can be treated.

Milne: Could you, Dr. Pirani, looking at that slide, certify that the changes were not due to ischaemia?

Pirani: I cannot certify anything, but I think that this slide is very convincing for pyelonephritis because the pericapsular fibrosis, interstitial infiltration and tubular atrophy are completely out of proportion to the vascular damage.

Hutt: This is one of those cases where you could argue either way. One frequently sees in renal biopsies ischaemic glomeruli with tubular degeneration and evidence of arteriolar sclerosis without any chronic

inflammatory cells at all. One would like to know what it is that determines why some ischaemic lesions induce the chronic inflammatory cell reaction, and others do not.

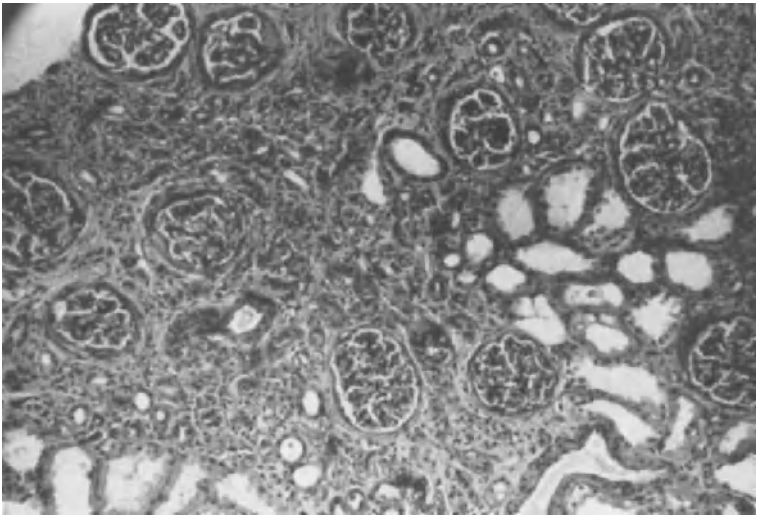


FIG. 1 (Raaschou). A 36-year-old man was admitted with symptoms of chronic uraemia (thirst, vomiting, epigastric pains) of four months duration. He had never had renal colic, dysuria or haematuria, acute pyelonephritis or known arterial hypertension; no misuse of medicine.

When renal biopsy was performed, the status was as follows: haemoglobin: 44 per cent; serum creatinine: 20·2 mg. per cent; serum urea: 472 mg. per cent; 24-hour endogenous creatinine clearance 3·4 ml./min.; blood pressure: 140/80 mm. Hg; urine: + protein. Urine microscopy: 0 erythrocytes, 0 casts, 0 epithelial cells, 0 leucocytes, 0 bacteria. Culture from clean voided urine specimen: growth of numerous coliform bacilli. Ophthalmoscopy: no hypertensive retinopathy. Electrocardiogram: nothing abnormal. X-ray of the heart: no heart enlargement.

Thirteen months after the renal biopsy the patient died from uraemia. Autopsy revealed uniform small kidneys with papillary necrosis everywhere in both. Microscopy of kidney tissue at autopsy showed: chronic pyelonephritis, renal papillary necrosis; no certain pathological changes in the vessels.

Rich: If that slide were shown to me on an examination, I would call that an ischaemic scar. In the ischaemic scar you do get so-called periglomerular or capsular fibrosis, and great atrophy of the tubules, to

complete disappearance even, leaving the glomeruli visually intact for a time.

Ross: I have always thought of the glomerular changes such as we saw there as being ischaemic. Is it not possible that they are secondary to an obliterative proliferative obstruction of the arterioles by the pyelonephritic process, and that the glomerular changes are absolutely identical in pyelonephritis and ischaemic renal disease?

Heptinstall: This is what put Priscilla Kincaid-Smith on to the thesis that the scarring in chronic pyelonephritis was probably very largely due to vascular sclerosis. I agree with Dr. Rich that that is quite compatible with an ischaemic picture. That is the sort of picture you get in kidneys that have been excised for the relief of hypertension due to renal artery stenosis.

Jennings: If we are going to have a vote I think that I would have to go along with Dr. Pirani, mainly on the basis of the glomerular tufts.

Pirani: The glomerular tufts are pretty well preserved.

Heptinstall: If you look at the two glomeruli at the top of the slide you will see some hyaline material appearing just inside Bowman's capsule. That surely is the change that McManus describes in the so-called obsolescent glomerulus, the ischaemic type.

Pirani: I agree that there are changes in the tufts. But if this lesion were due exclusively to vascular stenosis or occlusion, I would have expected more advanced changes in the glomerular tufts. There is no diffuse glomerular fibrosis. There are obsolescent changes of course, because there is a tremendous amount of interstitial fibrosis and therefore a secondary disturbance of circulation.

Rich: I think that it is the typical result of narrowing of the intra-renal arteries, with ischaemic atrophy of the tubules. The striking, almost pathognomonic, feature is that when this happens the glomeruli can appear normal for a long time, although gradually they become sclerotic. It is quite common to find a wedge of marked tubular atrophy with normal-looking glomeruli, as a result of arteriosclerotic ischaemia.

Pirani: You mean that this lesion would be secondary to an obstruction of the larger renal vessels?

Rich: Yes. Narrowing of the larger intra-renal arteries can produce

precisely such a wedge. Narrowing of the still larger extra-renal hilar arteries can produce larger areas of the same type.

Bergstrand: Another feature of this picture is how homogeneous the changes of the glomeruli appear to be; they are all alike. In kidneys with vascular scars you will find some glomeruli which are entirely destroyed, others which are less or not at all. This I think speaks in favour of the diagnosis of chronic pyelonephritis.

Rich: The glomeruli in many ischaemic wedges are homogeneously alike, all either relatively normal in appearance or all sclerosed; and in other ischaemic wedges there are both normal-appearing and partially or completely sclerosed glomeruli. The latter condition can result from differences in the degree of stenosis of the arteriolar branches of the stenotic artery that supplies the area.

Blainey: You would be quite happy about this if the patient were sixty years old, but would you be happy about this being ischaemic in a patient of twenty-five?

Rich: It would depend on whether the patient had intra-renal arterial narrowing or not.

Milne: I think that I have made my point! The pathologists did not warn us about this ten years ago; it has taken the radiologists to emphasize the importance of this in clinical medicine. I think that Prof. Rosenheim would agree to that.

Rosenheim: I would agree with this entirely. At University College Hospital we had the greatest difficulty with this until we got the radiologists and the pathologists together. Now our pathologist, Dr. J. Smith, has gone so far as to agree with the radiologists that the best criterion of chronic pyelonephritis is dilatation of the calyx underlying the scar.

Earle: Prof. Raaschou, do you know whether this particular kidney was small or not?

Raaschou: The kidney biopsy was done because of uraemia of four months duration. The patient died of the chronic uraemia 13 months after the kidney biopsy. The autopsy showed bilateral chronic nephropathy that macroscopically might resemble pyelonephritis, and also numerous papillary necroses in both kidneys. Unfortunately I am unable to state the weight of the kidneys but they were small.

Bergstrand: We should remember also that pyelonephritis may be

superimposed upon other previous renal disease. If you have a chronic pyelonephritis in a patient 60 years old, there may be extensively vascular damage previous to the pyelonephritis. We have recently examined 15 cases of familial renal cystinuria, all of whom showed very severe chronic pyelonephritis and hydronephrosis. Lack of enzymic activity in the tubular epithelium, which is the primary disease, is entirely obscured by this secondary pyelonephritis.

Milne: Is the pyelonephritis due to the enzymic change? Why is it not due to cystine crystals or cystine stones?

Bergstrand: The cause of the pyelonephritis most probably is stone formation, but the hydronephrosis and pyelonephritis obscure the real (enzymic) cause of the disease.

Rosenheim: But doesn't this hold for hyperparathyroidism, for gouty kidneys, and for cystinuria: if the tubules are blocked, they will dilate and there may be associated periglomerular fibrosis and the picture of chronic pyelonephritis?

Raaschou: Even in the nephrotic syndrome you may sometimes find a superimposed chronic pyelonephritis, as Dr. Kark and his collaborators have described.

Bergstrand: And the reverse. In this cystinuria I found one case of chronic glomerulonephritis.

Darmady: I am sure that this type of change in the kidney should not be regarded as "pyelonephritis". This is not the same change which we were talking about earlier in the discussion. When I used the word "chemical" I meant this to cover chemical or toxic factors which might produce this interstitial change.

Ross: We have followed the development of interstitial changes in rats. We were interested in the interstitial nephritis which is supposed to occur in cadmium workers who develop proteinuria. We had a post-mortem on only one man, and he had obvious interstitial nephritis. We gave rats quite large doses of cadmium for a long period and watched the development of tubular atrophy and dilatation, leading finally to fibrosis, with glomerular changes very late on. This was carefully controlled and there was no evidence of infection. It would appear to have been chemical reaction, with tubular damage leading later to fibrosis.

de Wardener: Did it look like chronic pyelonephritis?

Ross: It looked rather like it: in the early stages there were small areas of tubular damage. On the other hand, there was no inflammatory reaction.

Jennings: Do these cadmium workers have proteinuria in the early stages of their poisoning and, if so, where does the protein come from?

Ross: They do have it in the early stages. Where it comes from, I don't know. It is low-molecular-weight protein, not albumin.

Darmady: This happens also with lead workers, doesn't it?

Ross: Yes, a similar thing was reported a long time ago in lead workers.

Kark: Dr. D. A. Henderson's recent work on lead poisoning—which was done in the Department of Pathology at Brisbane but is unpublished as yet—may bear on this.

de Wardener: Is there agreement that pathologists cannot in fact diagnose chronic pyelonephritis but can only diagnose appearances consistent with chronic pyelonephritis?

Rich: I would say that that is a little exaggerated. When it is well marked, with conspicuous mononuclear infiltration of the pelvis, I don't think there is any difficulty. However, when there are just a few accumulations of round cells here and there, and a little capsular fibrosis, I could not diagnose it with certainty.

de Wardener: It depends on the extent and not on the lesion?

Rich: Yes. If in a biopsy one has just a patch of round cells, I would certainly be very dubious about what anybody called it.

Ross: Is the subintimal hyaline material in the glomerular arteriole that Dr. Blainey showed considered pathognomonic of diabetic nephropathy, or can one see it in other cases of the nephrotic syndrome? I have seen it in a biopsy from a man who had no evidence of diabetes at all.

Blainey: I don't think that this is specific, but I think that it is fairly unusual in people of this age except in diabetes.

Thomsen: Can you see similar changes in hypertension?

Ross: I don't think that it is quite the same.

Thomsen: I think the combination of these hyaline changes in the glomerular arterioles and the diffuse lesions is specific for diabetes.

Pirani: This in our experience has been the big diagnostic difference; in arteriolar sclerosis related to hypertension one sees hyaline in the

arterioles, of course, but as a rule there is a very small amount of hyaline in the glomerular tufts unless the disease is advanced. In diabetes the arteriolar and the glomerular involvement develop *pari passu*, while in arteriolar sclerosis, the glomerular involvement follows that of the arterioles. I believe diabetic nephropathy to be an entirely different process from renal arteriolar sclerosis.

QUANTITATIVE HISTOCHEMISTRY OF THE NEPHRON*

ROBERT M. KARK, HERMANN MATTENHEIMER, SJOERD L. BONTING, VICTOR E. POLLAK,† and ROBERT C. MUEHRCKE

Departments of Medicine, Presbyterian-St. Luke's Hospital and University of Illinois, and the Department of Biological Chemistry, University of Illinois, Chicago

As a result of experience gained over the past few years, one may now consider that percutaneous renal biopsy is a practical method which can be generally applied to the care of the sick. By its use, exact histological diagnoses can be made, to provide a sound background for diagnosis, treatment, and prognosis. Cultures of organisms may be obtained from infected kidneys when they cannot be grown from the urine or blood. Serial biopsies can be taken to study the natural history of disease, and renal tissue can be harvested to investigate the effects of drugs on renal and cardiovascular disease or to study the morphology of the kidney by electron microscopy, cytochemistry, or nephron dissection.

Recently the ultra-microbiochemical methods of Lowry (Lowry *et al.*, 1954*a* and *b*; Lowry, 1957) were adapted to study quantitatively the chemistry of the different functional units of human nephrons taken by renal biopsy. This was done with two broad objectives in view. First, to study quantitively the rôle of enzymes in the formation of urine and, second, to discover enzymes in the kidney which may appear in the urine or blood stream and thus be used as an early index of damage to specific

* Supported in part by a grant from the Surgeon General's Office, U.S. Army (Contract No. DA-49-007-MD-637) and by a grant from the National Institutes of Health, U.S. Public Health Service (H-3912).

† Established Investigator, American Heart Association, supported by the Illinois Heart Association.

structures in the nephron. In essence the techniques used depend on: (1) exact microscopic identification of the structures of the nephron, renal blood vessels and interstitial tissue, using stained, frozen sections; (2) micro-dissection of accurately identified groups of cells, blood vessels and tissue in adjacent unstained, frozen, dried sections of renal biopsies; (3) weighing of the minute pieces of tissue; and, finally, (4) enzyme assay or chemical analysis of the tissue.

The techniques employed are extremely sensitive and assays can be made of five or more renal cells. Quartz fibre balances with a sensitivity and reproducibility of 0·4 mμg. and a useful range of 10 to 100 mμg. are used for weighing. Small quantities of substrate in the range of 2 to 5 μl. are used for incubation of tissues. The product of the reaction produces either a coloured (Figs. 1 and 5) or a fluorescent compound (Figs. 2, 3 and 4) which is measured with electronic equipment. These techniques give higher levels of enzyme activity per unit weight of tissue than the better known standard laboratory procedures used for assay of tissue enzymes (Bonting, 1960).

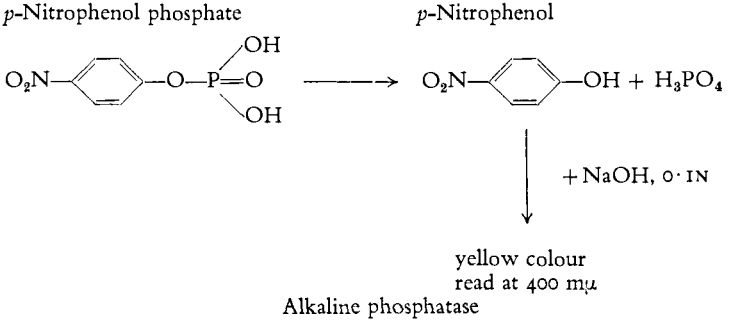
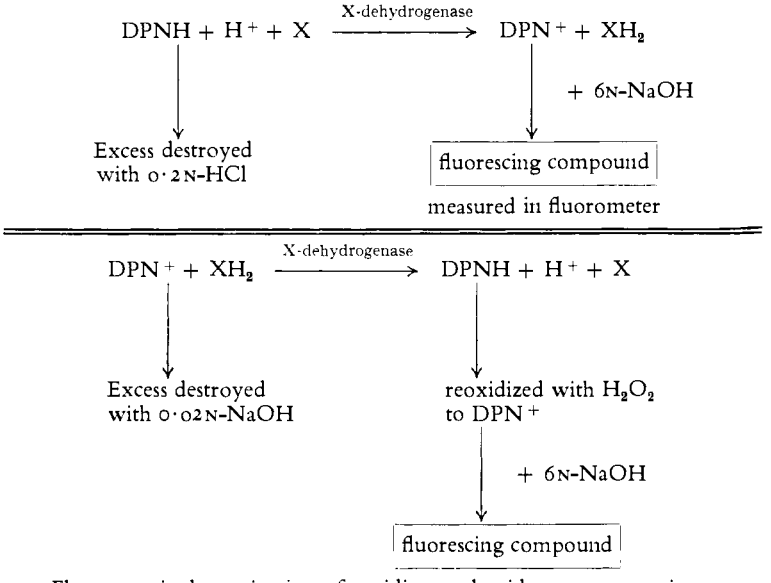


FIG. 1. Measurement of enzyme activity by colorimetric techniques. Example: alkaline phosphatase. A colourless substance, *p*-nitrophenol phosphate, which is included in the incubation medium, is the substrate for monophosphoesterases. This is split by alkaline phosphatase in the tissue to produce *p*-nitrophenol and phosphate. In the presence of sodium hydroxide the *p*-nitrophenol becomes yellow and its concentration can be measured at 400 mμ.



Fluorometric determination of pyridine nucleotide-enzyme reactions

FIG. 2. Measurement of enzyme activity by fluorometry. Advantage is taken of the fact that oxidized diphosphopyridine nucleotides give a fluorescent compound after treatment with 6N-NaOH. If DPNH is the coenzyme (upper reaction) excess DPNH is destroyed by acid at the end of the incubation. On the other hand, if DPN is the coenzyme (lower reaction), excess DPN is destroyed at the end of incubation with 0·02N-NaOH, and the DPNH formed during the enzyme reaction is reoxidized with H_2O_2 to DPN, which is then measured fluorometrically. Two examples are given, one a direct reaction, the other a coupled reaction. (See Figs. 3 and 4.)

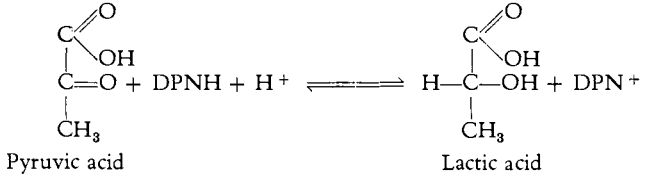
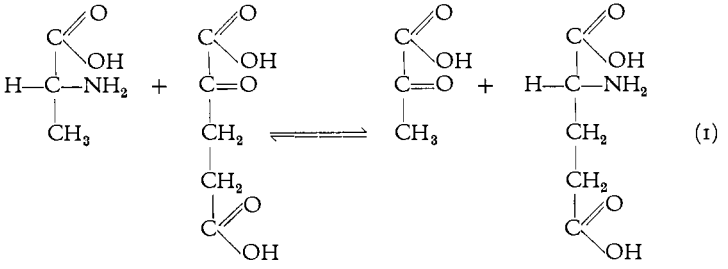


FIG. 3. Measurement of lactic dehydrogenase (LDH) activity. The substrate is pyruvic acid with DPNH as coenzyme. LDH in the tissue reduces pyruvic acid to lactic acid at pH 7·8. DPNH is oxidized in equimolar amounts and is measured fluorometrically.



Glutamic-pyruvic transaminase

(1) transaminase reaction
(2) auxiliary reaction with added LDH and DPNH

FIG. 4. Measurement of glutamic-pyruvic transaminase (GPT) in a coupled reaction. L-Alanine and α-ketoglutaric acid are the substrates. At pH 7·6 GPT in the tissue transfers the amino group from alanine to α-ketoglutaric acid, forming pyruvic and glutamic acids. In a second reaction pyruvic acid is reduced to lactic acid by added LDH and DPNH. The latter is oxidized in equimolar, amounts and is measured fluorometrically.

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

Barbiturate buffer
0·022N, pH 8·1 ⟶ pH 6·5
Bromthymol blue

Blue *Yellow*

Time for colour change measured in seconds at 0°C.
Incubation volume 15 μl.

Carbonic anhydrase

FIG. 5. Measurement of carbonic anhydrase (CA) activity. The enzyme CA in the tissue accelerates the hydration of CO_2 to carbonic acid. This produces a pH-change in the incubation medium, which contains 0·02N veronal-veronate buffer and an indicator to which CO_2-saturated distilled water is added at 0°C. The time difference between the reaction with and without enzyme is measured.

The enzyme topography of the nephron has been studied in a number of species, including man, monkey, dog, rat, rabbit, frog and toadfish. Data for alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, adenosine triphosphatase, and lactic dehydrogenase have been published (Bonting *et al.*, 1958; Bonting *et al.*, 1960*a*, *b* and *c*). Recently we have collected data on glutamic-pyruvic transaminase, glutamic-oxalacetic transaminase, glutamic dehydrogenase and carbonic anhydrase which are given here. We have also reported on the hydroxyproline content of the glomerulus of man in youth and old age (Bonting, de Bruin and Pollak, 1961). Studies have been reported in a variety of diseases in man (Pollak *et al.*, 1960*a* and *b*; Rosenthal *et al.*, 1960) and in experimentally induced renal disease in dogs (Bonting *et al.*, 1960*a*; McCann, 1956) and rats (Dubach and Recant, 1960).

As an example of the kind of observation which can be made, some data are presented on patients with lupus nephritis and on one patient with systemic lupus erythematosus and renal tubular acidosis.

Enzyme assays and glomerulus density in lupus nephritis

The natural history and histological development of lupus nephritis have been studied in detail in our laboratory (Pirani *et al.*, 1961). At all stages of development of lupus nephritis, glomeruli were more severely involved than were the tubules. In the early stages (lupus glomerulitis*) the histological abnor-

* Specimens of renal biopsy assayed for enzyme activity were divided into the following groups:

(1) *Lupus glomerulitis.* This term was used to indicate a proliferative and/or membranous lesion of the glomerular tufts in the absence of significant tubular damage or changes in the interstitial tissue. The glomeruli were involved to a mild degree.

(2) *Lupus glomerulonephritis.* This term was used to indicate a more severe membranous and/or proliferative lesion of the glomeruli, associated with definite

malities were confined to the glomeruli. Only when the glomerular involvement became considerably more severe was there histological evidence of tubular degeneration and interstitial fibrosis and inflammation (lupus glomerulonephritis). Even in lupus glomerulonephritis, histological abnormalities of the tubules and interstitial tissue were considerably less severe than in other types of glomerulonephritis. These histological observations were reflected functionally, in that ability to concentrate the urine and to excrete phenolsulphonphthalein was preserved until comparatively late in the disease.

Glomerular weight and density

During our studies we observed that glomeruli from kidneys with lupus nephritis appeared to be larger and heavier than glomeruli from normal kidneys. The impression was confirmed by a study of weights, diameters and derived densities of 130 glomeruli from 9 healthy kidneys and 234 glomeruli from 17 cases of lupus nephritis. The glomeruli from the cases with lupus nephritis were 40 per cent heavier than the healthy glomeruli. This was particularly true for *active* lupus glomerulonephritis, in which the diameter of the glomeruli was significantly wider. The greatest density was found in *healing* lupus glomerulonephritis (Fig. 6).

These findings are consistent with our histological observations.

tubular and interstitial tissue changes. The kidneys with lupus glomerulonephritis were subdivided into two groups as shown below.

(a) Active lupus glomerulonephritis. Definite morphological evidence of activity was observed in the glomerular lesions, i.e., one or more of the following histological features were prominent: local necrosis, karyorrhexis, haematoxyphil bodies, fibrinoid, "hyaline" thrombi, and "wire loops".

(b) Healed or healing lupus glomerulonephritis. Thickening of the glomerular basement membrane was the predominant lesion found in these biopsies, all or most of the active lesions present in previous biopsies from the same patients having disappeared. The abnormalities found in the tubules and interstitial tissue were similar to or less severe than those found in active lupus glomerulonephritis.

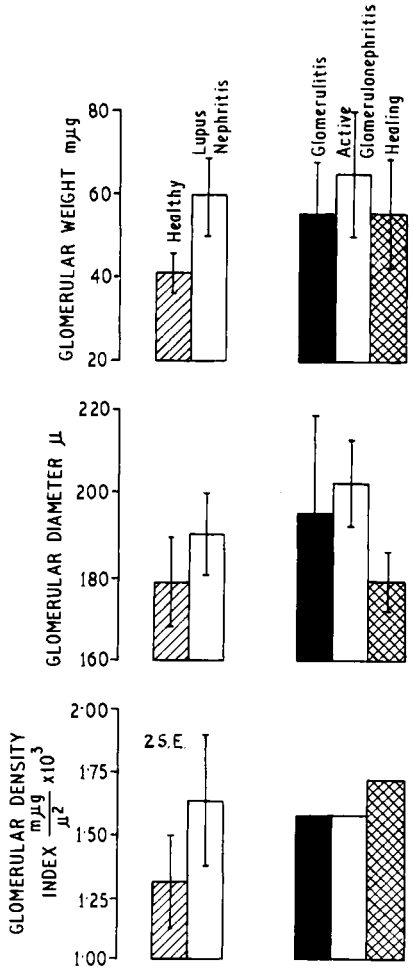


FIG. 6. The weights, diameters and densities of glomeruli from healthy kidneys and from kidneys of patients with lupus nephritis (including lupus glomerulitis and active and healing lupus glomerulonephritis).
The density index of the glomeruli

$$= \frac{\text{glomerular weight}}{\text{glomerular diameter}^2} \times 10^3.$$

The I bars indicate 2 standard errors.

The most severe and widespread histological changes were seen in *active* lupus glomerulonephritis, in which fibrinoid and hyaline materials were deposited on the endothelial surface of the glomerular basement membrane. In addition, endothelial and epithelial cells were often increased in numbers. These changes correlated with the increase in weight and width reported. On the other hand, in *healing* lupus glomerulonephritis the active lesions had disappeared and the major lesion seen was a diffusely thickened basement membrane. This was the initial stage of glomerular scar formation in which one expected to find denser glomeruli than normal. It was gratifying to find that the scarred, healed glomeruli were denser than those in which the disease was active.

Alkaline phosphatase and lactic dehydrogenase activity

Alkaline phosphatase activity (Fig. 7) was found to be decreased in the proximal and distal convolutions of the tubules in patients with lupus nephritis, but the level of enzyme activity was not related to the presence or severity of morphological changes in the tubules. The alkaline phosphatase activity of the glomeruli was within normal limits, and there were no apparent effects of glomerular damage of varying severity on the level of enzyme activity found.

By contrast, there was a slight but statistically significant increase in lactic dehydrogenase (LDH) activity (Fig. 7) in the glomeruli and throughout the tubules in lupus glomerulonephritis, while LDH activity was slightly decreased in lupus glomerulitis. The differences between enzyme activity in lupus glomerulitis and lupus glomerulonephritis were statistically highly significant in the glomeruli and proximal convolutions of the tubules. Thus, there was an inverse relationship between LDH activity and histological evidence of degeneration of the proximal and distal convolutions. These differences were not related to the presence or degree of renal functional impairment, and the differences in

LDH activity did not appear to be related to the dosage of prednisone used to treat these patients.

Glomerular damage in lupus nephritis precedes the appearance

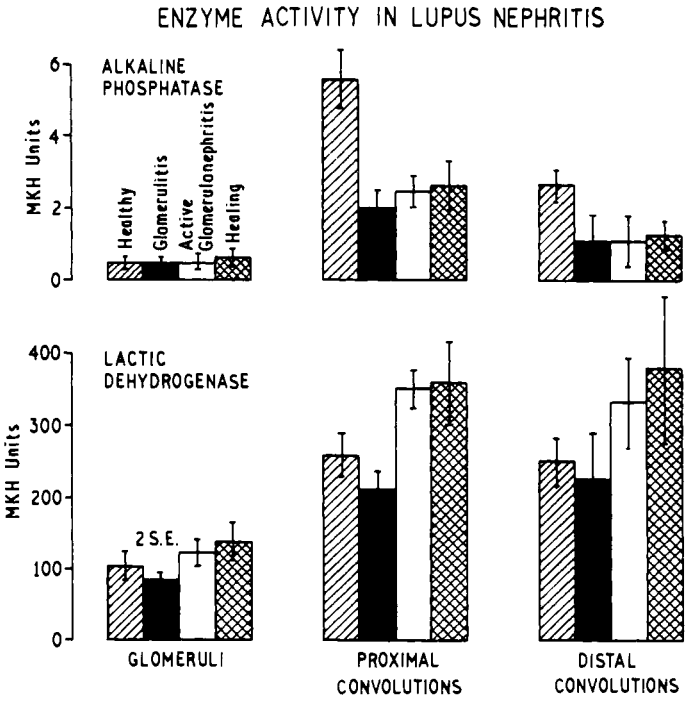


FIG. 7. Alkaline phosphatase and lactic dehydrogenase activity in the anatomical units of nephrons from healthy kidneys and from kidneys of patients with lupus nephritis (including lupus glomerulitis, and active and healing lupus glomerulonephritis). Alkaline phosphatase activity expressed in moles of substrate split per kilogram dry weight of tissue per hour at 37°C.; lactic dehydrogenase activity expressed in moles of DPNH oxidized per kilogram dry weight of tissue per hour at 37°C.

of significant structural changes in the convoluted tubules. The morphological changes in the glomeruli may be very severe before definite tubular damage is evident. In this respect, lupus

nephritis differs from many other renal diseases such as glomerulonephritis and pyelonephritis. This could result in a physiological situation in which the renal tubules, particularly the proximal convolutions, perform increased work in reabsorbing from the tubular lumina substances such as protein filtered in excess by the glomeruli, or in excreting other substances into the tubular lumina. Thus, the relative increase in LDH activity in lupus glomerulonephritis might be an enzymic expression of the preserved function of these tubules and of their ability to respond metabolically to increased metabolic demands. By contrast, in primary renal tubular diseases, the LDH activity of the convoluted tubules was normal in the presence of mild tubular abnormalities and was significantly decreased when the tubules were severely damaged. The view that the tubules in lupus glomerulonephritis are capable of responding to increased metabolic demands would be consistent with the hypothesis of Platt (1952), namely, that in chronic renal disease residual functioning nephrons may be capable of normal activities and even of functional adaptations which may result in an increase of work done per residual functioning nephron. The view of Platt has gained considerable support from the studies of Bricker, Morrin and Kime (1960) on the adaptive changes in residual nephrons of dogs with experimental unilateral renal disease. Their investigations strongly suggest that the surviving nephrons in diseased kidneys function normally, and that specific functional sites are not selectively destroyed in different nephrons, a condition which would result in great functional differences from nephron to nephron.

Renal tubular acidosis

F.P., age 32, had been ill with systemic lupus erythematosus for five years and also had renal tubular acidosis with calcification of the renal papillae. Her inability to respond normally to acidosis produced by ingestion of 48 grams of ammonium chloride

is shown in Fig. 8. Renal tissue was taken three hours *post mortem* for enzyme assay. *Post mortem* there was no evidence of involve-

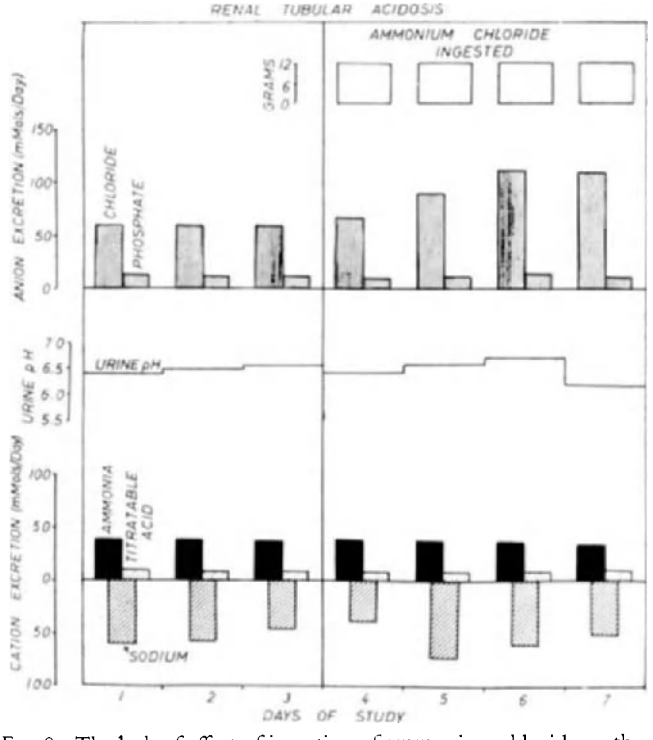


FIG. 8. The lack of effect of ingestion of ammonium chloride on the excretion of urinary constituents in F. P., age 32, ill with renal tubular acidosis and calcification of the renal papillae. Note particularly that ammonia excretion is unchanged and that the excretion of titratable acid is extremely low. When this study was started, the creatinine clearance was 37·0 ml./min. and the serum creatinine was 1·20 mg./100 ml. The serum sodium was 133 m-equiv./l., the serum potassium was 3·1 m-equiv./l., the serum chloride was 1·09 m-equiv./l., and the CO_2 was 19 m-equiv./l.

ment of the kidney by lupus nephritis, but there was extensive fibrosis, particularly of the medullary rays, and much calcification was observed in the papilla.

As is well known, patients with renal tubular acidosis have difficulty in increasing their urinary excretion of ammonia in response to an ammonium chloride load. It would therefore be of great interest to study glutaminase activity in the different parts of the nephron, particularly as it has been shown that the activity of the enzyme is increased in experimentally induced acidosis. Although we have developed an ultramicrochemical method for measurement of glutaminase activity (Mattenheimer, unpublished results), thus far only fresh homogenates have been studied since the enzyme appears to be inactivated by the freeze-drying process.

Glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT) and glutamic dehydrogenase (GDH) activities were measured in renal tissue from the patient and from three healthy subjects. These enzymes are concerned with the transfer of amino groups and in the production of ammonia. Assays were done by Lowry's (1957) technique which we adapted for assay of human renal tissue (Mattenheimer, unpublished results). From Tables I and II it can be seen that no significant differences were found between the diseased and healthy kidneys. These data are preliminary in nature, because we have only studied GPT, GOT and GDH activities in three healthy kidneys and, as can be seen, the variations between normals are considerable.

It has been suggested that carbonic anhydrase activity is suppressed in intracellular and tubular acidosis. It has also been postulated that the enzyme is concerned with proton excretion by the tubule, a defect which is central in renal tubular acidosis. Our observations are shown in Table III. The data, obtained by methods developed in our laboratory (Mattenheimer, unpublished), showed no significant differences between healthy and diseased nephrons. Decreased activity was found only in areas of interstitial fibrosis in which there were no recognizable nephrons.

Table I

GLUTAMIC OXALACETIC AND GLUTAMIC PYRUVIC TRANSAMINASE ACTIVITIES IN THE ANATOMICAL UNITS OF NEPHRONS FROM HEALTHY KIDNEYS AND FROM THE KIDNEY OF A PATIENT WITH SLE AND RENAL TUBULAR ACIDOSIS (expressed in moles of $-NH_2$ transferred per kilogram dry weight of tissue per hour at 37°C)*

	Glutamic oxalacetic transaminase				*Glutamic pyruvic transaminase*		
	Healthy kidneys			*Renal tubular acidosis*	*Healthy kidneys*		*Renal tubular acidosis*
Structure analysed	No. 1	No. 2	No. 3		No. 1	No. 2	
Cortex:							
Glomeruli	1·55±1·61 (7)	6·62±1·17 (4)	8·50±3·16 (9)	3·61±1·84 (8)	0·76±0·46 (6)	0·31±0·24 (5)	1·16±1·49 (7)
Proximal convolutions	19·5±1·97 (7)	20·8±5·80 (5)	14·4±11·5 (7)	15·1±7·27 (7)	2·18±0·49 (6)	3·37±1·21 (5)	4·59±3·55 (6)
Distal convolutions	12·7±5·80 (7)	33·1±15·0 (6)	23·8±11·2 (8)	14·0±13·6 (7)	1·80±1·37 (6)	2·91±1·31 (6)	2·30±2·16 (7)
Degenerated convolutions	—	—	—	18·5±3·40 (10)	—	—	3·18±0·95 (12)
Medullary rays	12·1±5·82 (14)	—	24·6±21·8 (7)	†	2·13±0·93 (11)	—	†
Very fibrotic cortex	—	—	—	3·55±1·74 (10)	—	—	0·23±0·24 (5)
Medulla:							
Outer medullary zone	7·70±4·30 (7)	20·3±3·91 (5)	—	12·2±6·50 (5)	2·80±0·16 (6)	16·1±4·76 (15)	6·08±2·10 (5)
Inner medullary zone	4·05±3·08 (7)	31·7±16·6 (5)	—	8·84±5·70 (5)	1·73±0·67 (6)	4·15±2·26 (5)	1·42±0·55 (5)
Papilla	4·20±3·10 (7)	—	—	2·78±5·70 (5)	0·92±0·36 (9)	3·55±3·90 (4)	0·47±0·30 (5)

* Results are expressed as mean values ± one standard deviation. The number of tissue fragments analysed is given in parentheses.
† Medullary rays extremely fibrotic and not analysed.

Table II

GLUTAMIC DEHYDROGENASE AND LACTIC DEHYDROGENASE ACTIVITIES IN THE ANATOMICAL UNITS OF NEPHRONS FROM HEALTHY KIDNEYS AND FROM THE KIDNEY OF A PATIENT WITH SLE AND RENAL TUBULAR ACIDOSIS (expressed in moles of DPNH oxidized per kilogram dry weight of tissue per hour at 37°C)*

	Glutamic dehydrogenase				*Lactic dehydrogenase*	
	Healthy kidneys			*Renal tubular acidosis*	*Healthy kidneys (7 patients)*	*Renal tubular acidosis*
Structure analysed	No. 1	No. 2	No. 3			
Cortex:						
Glomeruli	0·39±0·24 (5)	0·45±0·14 (4)	0·76±0·37 (5)	0·32±0·10 (7)	31·4±6·4 [6] (46)	31·9±6·7 (7)
Proximal convolutions	2·80±1·06 (5)	3·42±2·99 (4)	4·62±3·72 (5)	3·36±0·94 (6)	74·6±11·5 [7] (55)	78·3±37·4 (7)
Distal convolutions	2·30±0·64 (5)	2·53±1·45 (5)	7·22±4·93 (5)	3·30±1·33 (5)	74·3±12·3 [7] (49)	41·9±17·6 (6)
Degenerated convolutions	—	—	—	2·83±0·57 (11)	—	66·5±15·6 (6)
Medullary rays	2·66±2·23 (10)	—	5·83±3·78 (4)	†	67·1±7·25 [3] (25)	†
Very fibrotic cortex	—	—	—	0·38±0·63 (6)	—	42·3±18·8 (12)
Medulla:						
Outer medullary zone	1·22±0·51 (5)	1·53±1·12 (10)	4·44±1·63 (5)	1·30±0·57 (6)	50·9±23·0 [3] (22)	95·8±18·0 (12)
Inner medullary zone	0·89±0·10 (5)	1·75±1·38 (5)	0·82±0·58 (4)	1·60±0·57 (6)		
Papilla	0·38±0·16 (5)	1·12±0·56 (5)	—	0·22±0·10 (4)	50·9±23·0 [3] (8)	14·1±6·1 (6)

* Results are expressed as mean values ± one standard deviation. The number of tissue fragments analysed is given in parentheses. For LDH activity in healthy kidneys the mean value and standard deviations are given for the averages of each patient. The number of kidney specimens analysed is in brackets. The previously reported values have been divided by factor 3·45 (Bonting *et al.*, 1960*b*).

† Medullary rays extremely fibrotic and not analysed.

Table III

CARBONIC ANHYDRASE ACTIVITY IN THE ANATOMICAL UNITS OF NEPHRONS FROM ONE HEALTHY KIDNEY AND FROM THE KIDNEY OF A PATIENT WITH SLE AND RENAL TUBULAR ACIDOSIS (expressed in enzyme units per microgram dry weight OF tissue at 0°C)*

	Carbonic anhydrase	
Structure analysed	*Healthy kidney No. 2*	*Renal tubular acidosis*
Cortex:		
Glomeruli	1·06±0·20 (4)	0·74±0·45 (8)
Proximal convolutions	4·37±0·64 (5)	2·30±1·50 (12)
Distal convolutions	6·47±2·56 (5)	5·75±3·55 (12)
Degenerated convolutions	—	1·85±0·84 (8)
Medullary rays	1·50±0·84 (5)	†
Very fibrotic cortex	—	0·89±0·49 (9)
Medulla:		
Outer medullary zone	3·65±1·87 (4)	2·25±0·38 (7)
Inner medullary zone	2·22±0·60 (4)	1·91±0·85 (10)
Papilla	—	1·19±0·36 (8)

* Results are expressed as mean values ± one standard deviation. The number of fragments analysed is given in parentheses.
† Medullary rays extremely fibrotic and not analysed.

When lactic dehydrogenase activity was measured (Table II) clear-cut differences were found between healthy and diseased distal convolutions of tubules. These differences were statistically significant, the values in health being 74·3 MKH and in disease 41·9 MKH units. To date this is the only situation we have encountered in analyses of nephrons in which LDH activity was decreased solely in distal convolutions. In this regard the data are strikingly different from what was found in Fanconi's syndrome (Pollak *et al.*, 1960*a*). In passing it can be seen from Table II that

LDH activity was 42·3 MKH units in fibrotic cortex. At present it is impossible to interpret these findings.

Summary

The tissue harvested in health and in disease by renal biopsy can be fruitfully studied by a number of different techniques. We have described above quantitative ultramicrochemical techniques used to study the individual and functional units of the nephron.

REFERENCES

BONTING, S. L. (1960). *Nature (Lond.)*, **185,** 686.
BONTING, S. L., DE BRUIN, H., and POLLAK, V. E. (1961). *J. clin. Invest.*, **40,** 177.
BONTING, S. L., POLLAK, V. E., MUEHRCKE, R. C., and KARK, R. M. (1958). *Science*, **127,** 1342.
BONTING, S. L., POLLAK, V. E., MUEHRCKE, R. C., and KARK, R. M. (1960*a*). *J. clin. Invest.*, **39,** 1372.
BONTING, S. L., POLLAK, V. E., MUEHRCKE, R. C., and KARK, R. M. (1960*b*). *J. clin. Invest.*, **39,** 1381.
BONTING, S. L., TSOODLE, A. D., DE BRUIN, H., and MAYRON, B. R. (1960*c*). *Arch. Biochem.*, **91,** 130.
BRICKER, N. S., MORRIN, P. A. F., and KIME, S. W., JR. (1960). *Amer. J. Med.*, **28,** 77.
DUBACH, U. C., and RECANT, L. (1960). *J. clin. Invest.*, **39,** 1364.
LOWRY, O. H. (1957). *In* Methods in Enzymology, Vol. 4, p. 366, eds. Colowick, S. P., and Kaplan, N. O. New York: Academic Press.
LOWRY, O. H., ROBERTS, N. R., LEINER, K. Y., WU, M. L., and FARR, A. L. (1954*a*). *J. biol. Chem.*, **207,** 1.
LOWRY, O. H., ROBERTS, N. R., WU, M. L., HIXON, W. S., and CRAWFORD, E. J. (1954*b*). *J. biol. Chem.*, **207,** 19.
MCCANN, W. P. (1956). *Amer. J. Physiol.*, **185,** 372.
PIRANI, C. L., POLLAK, V. E., MUEHRCKE, R. C., and KARK, R. M. (1961). This volume, p. 218.
PLATT, R. (1952). *Brit. med. J.*, **1,** 1313, 1372.
POLLAK, V. E., BONTING, S. L., MUEHRCKE, R. C., and KARK, R. M. (1960*a*). *J. clin. Invest.*, **39,** 1386.
POLLAK, V. E., BONTING, S. L., MUEHRCKE, R. C., and KARK, R. M. (1960*b*). *J. clin. Invest.*, **39,** 1394.
ROSENTHAL, I. M., BONTING, S. L., HOGAN, W., and PIRANI, C. L. (1960). *Amer. J. Dis. Child.*, **99,** 185.

DISCUSSION

Bergstrand: Whether your conclusions are valid or not depends on the exact localization of the tissue as proximal and distal convoluted tubules. Could you give us some more information on how you differentiate the parts of the tubules?

Kark: Details of how we differentiate the tubules are given in a recent paper (Bonting *et al.*, 1960*a*, *loc. cit.*). We started off our study by trying to do in man what W. P. McCann (1956, *loc. cit.*) has done in dogs; that is, injecting various dyes which were known to be excreted by the proximal tubules. We then tried fluorescence microscopy, and finally we came to the conclusion that to obtain the biochemical data we needed we couldn't add any chemical to the kidney, and we couldn't use fluorescence microscopy in dehydrated tissues. We then set off on a long study of the anatomy and histology of the nephron, using animals in which we had dyed the proximal tubules. We have been able to determine the different portions of the nephron quite clearly from the histological staining. We can recognize now not only proximal and distal convolutions, but also the ascending portions of the distal convolutions of the tubules, and descending and ascending loops of Henle, but not as easily. We are not sure about these latter yet, and that is why I haven't included data on them in our paper.

Rich: Do you think that all parts of the proximal convoluted tubule are the same functionally? In view of the differences in the uptake of foreign proteins and in susceptibility to injurious agents in different segments of the proximal tubule, don't you think that they have different functions?

Kark: Groups of cells from the proximal tubules have been dissected out and analysed. Obviously they come from different portions of the proximal tubule. When we analyse these separately for different enzymes, we find a wide variation in values, but statistically no difference in the activity in the different groups of the proximal tubular cells.

Rich: But don't you believe that there must be some differences?

Kark: Yes. It is quite obvious that there are hundreds of enzymes present, and we haven't yet picked out the right ones. This is the difficulty of this kind of study. One studies enzymes which have

important functions in energy transport and in synthetic activity, but this does not mean that they are necessarily the ones which are important in the function of one portion of the tubule.

Bergstrand: Can you separate the pars recta from the upper part of the convoluted tubule?

Kark: I think so.

Bergstrand: Mercurial diuretics evoke changes in enzymic activity only in the pars recta and not in the more proximal part.

Kark: We haven't studied diuretics yet.

Hamburger: When you weigh the glomerulus, do you weigh the whole glomerulus or sections of it?

Kark: We weigh sections 16 μ thick.

Hamburger: How many glomeruli do you take?

Kark: As many as are required to give significant data. This varies from 5 to 46.

Brun: Have you any information on the collecting ducts?

Kark: Very little. The only studies we have made are on potassium depletion. Dr. Muehrcke and Dr. Bonting, in a study in man and in animals, showed a very marked decrease in LDH in the collecting tubules in the papillae about the fourth week after potassium depletion had started. At the same time there was an increase in LDH in the urine. In addition, alkaline phosphatase is depressed in the collecting ducts in potassium depletion (Muehrcke, R. C. (1960). *In* Biology of Pyelonephritis, p. 581, ed., Quinn, E. L. and Kass, E. W. Boston: Little, Brown).

Vernier: In the study of hydroxyproline as a constituent of collagen, have you any figures on the comparative contents of hydroxyproline in scarred glomeruli as compared with healthy glomeruli?

Kark: We do not have many figures, but there is no doubt about the marked increase in hydroxyproline and collagen in scarred glomeruli.

Vernier: I am interested in obtaining chemical confirmation of similarity between the basement membrane and the basement membrane-like material that appears in these glomeruli. They have similar morphological characteristics, but are they similar chemically?

Kark: The problem with collagen is that it exists as a gel and as a fibre, and their chemical composition is probably similar. The same

is true for the neutral salt-soluble collagen of D. S. Jackson (1957. The formation and breakdown of connective tissue. *In* Connective Tissue, ed. R. E. Tunbridge. Oxford: Blackwell). At the moment there is no chemical method of differentiating them. If you find a large amount of hydroxyproline in a scarred glomerulus, then you know there is more collagen there, but you cannot tell whether it is fibrous collagen or soluble collagen except by looking at it with a microscope.

Bergstrand: Do you find hydroxyproline in the glomerulus as a whole, including Bowman's capsule?

Kark: No. This is measured on the glomerular tuft without including the capsule.

Movat: In these pro-collagens (or acid-soluble collagens or neutral salt-soluble collagens) there is a definite proportion between the various amino-acids. It would be interesting to find out what the proportions are in basement membrane; perhaps they are different from those in the pro-collagens.

Kark: I think that it is something we will go into later but we don't have any data at present which indicate any clear-cut differences. The fact that you have some hydroxyproline in the basement membrane doesn't mean that the whole of the basement membrane is made up of soluble collagen; there may be some other ground substance there as well.

Movat: Did you do any carbohydrate studies?

Kark: No. We have not done any mucopolysaccharides yet.

Black: Does the increase in LDH in the lupus tubule correspond to anything that can be demonstrated histochemically?

Kark: I don't think histochemical techniques can be used for quantitation. When we have tried to quantitate, let us say, alkaline phosphatase in tubules by stains, with chemical analyses of enzyme activity, we couldn't demonstrate correspondence.

Heptinstall: Dr. Kark, what was the effect of prednisone on these enzymes? Were these cases of lupus biopsied while they were on prednisone?

Kark: We have done studies on the effects of large doses of prednisone in rats. We have not done any studies on healthy people yet. It did not have any effect on enzyme activity of the tubules of the rat (Bonting, S. L., Pollak, V. E., Muehrcke, R. C., and Kark, R. M.

1960. *J. clin. Invest.*, **39**, 1394). We measured alkaline phosphatase, LDH, GOT, and GPT.

Darmady: Did you measure hydroxyproline?

Kark: No, not in relation to prednisone.

Milne: Have these techniques been applied to patients with a nephrotic syndrome?

Kark: Not really. We've done some alkaline phosphatase and LDH activities in some nephrotics, but there hasn't been very much difference from the other diseases which we have studied.

Brun: Any cases of acute renal failure?

Kark: No, not yet.

Hamburger: Have you studied any patients with congenital tubular abnormality?

Kark: Yes. There were marked depressions of alkaline phosphatase, LDH, and other enzymes. But we have not yet found an enzyme which was specifically depressed in any single type of tubular disease, and the depressions which we have measured we believe are secondary.

Milne: Do you include the specific defect of cystinuria or exclude it from this broad spectrum of tubular diseases?

Kark: We have not studied cystinuria.

Blainey: Do you find evidence of differences in enzyme activity with different levels of physiological activity within the kidney, for example, during diuresis?

Kark: We haven't done this yet. When the kidney is diseased we have found that some tubules which looked pretty normal to us on the stained sections had low levels of alkaline phosphatase.

Pirani: One case in which there was a good correlation between the histochemical staining and the chemical analysis was a case of hypophosphatasia which we studied with Dr. Ira Rosenthal of the Department of Pediatrics, at the University of Illinois. This infant was diagnosed as having congenital hypophosphatasia while he was still *in utero* because of the abnormal bone changes that could be recognized on X-ray. Dr. Kark showed chemically a lack of alkaline phosphatase in the kidneys of this infant and there was practically no alkaline phosphatase that we could demonstrate histochemically. The tubules otherwise appeared entirely normal.

Kark: We have studied the enzyme levels in various renal tubular

diseases—renal glycosuria, hypophosphatasia (in adults and infants) and cystine storage disease and adult Fanconi syndrome. In the distal and proximal convolution, particularly in the hypophosphatasia, alkaline phosphatase is markedly reduced. The lactic dehydrogenase is decreased in cystinosis. In the three adult Fanconis there are different levels—increased, within normal limits, and decreased. There are no uniform changes in these enzymes, except in alkaline phosphatase which is uniformly decreased. In renal tubular glycosuria, the alkaline phosphatase is very markedly depressed. There has been a lot of discussion that alkaline phosphatase controls the reabsorption of glucose, but hypophosphatasic infants have no alkaline phosphatase in the tubules, and no glucosuria; and we don't feel that alkaline phosphatase is concerned with the transport of glucose. We have also done some studies on phlorrhizinized dogs and found no decrease in alkaline phosphatase when they developed glycosuria.

Black: I was interested in the variation of lactic dehydrogenase of the adult Fanconi's. Now if the interpretation is sound that this is linked to overworking of the tubules, then it should be inversely correlated with the number of nephrons. Was there much difference in over-all measures of renal excretory function in those three patients?

Kark: Yes. There were differences in those three patients but I can't recall if they were the right way round.

de Wardener: Have you tried to measure hyaluronidase? Is it possible?

Kark: We haven't tried it, but I feel you can try anything with this technique if you have a good enough biochemist.

Hamburger: Have you studied compensatory hypertrophy, the situation where one kidney is removed and the other hypertrophies?

Kark: We haven't done those yet. I might say that these studies are terribly demanding; you have to be extremely careful about the chemistry and the anatomy, and you have to run controls all the time. Often a whole day's work is lost because you can't rely on it. I think it was four years before we had something we felt we could publish.

RENAL BIOPSY
Clinical and Pathological Significance
G. E. W. WOLSTENHOLME *and* MARGARET P. CAMERON

SOME OBSERVATIONS ON THE FINE STRUCTURE OF HUMAN KIDNEY BIOPSIES IN ACUTE ANURIA AND OSMOTIC DIURESIS

O. Z. DALGAARD

Medical Department 3, and the Central Clinical Laboratory, Kommunehospitalet, Copenhagen

and

K. J. PEDERSEN

Institute of General Zoology and Biophysical Institute, University of Copenhagen

THE methods of electron microscopy and modern histochemistry have been of great importance in offering possibilities for a detailed study of the structure and function of the normal human kidney and as a means of furthering the understanding of the pathogenesis of kidney diseases.

Introduction of biopsy techniques by Iversen and Brun (1951) has made electron microscopic studies of human kidney tissue possible, and a considerable literature already exists concerning the ultrastructural changes underlying the major kidney diseases. However, the majority of these studies have been centred around glomerular changes and only rather few studies have dealt with pathological processes in the tubules.

We have been mainly interested in tubular diseases, and the present report deals with some electron microscopic observations originating from studies on kidney biopsies from five cases of acute anuria due to shock and one biopsy taken from a patient with normal kidney function after intravenous administration

of hypertonic mannitol in order to produce a reversible state of osmotic diuresis.

Biopsy studies of the kidney in acute renal insufficiency have previously been made on routine stained sections with the light microscope by Brun (1954), Gormsen, Iversen and Raaschou (1955) and Brun and Munck (1957). The most striking observation was the contrast between the moderate structural changes and the complete breakdown of function. The most characteristic changes observed were dilatation and flattening of the epithelium in the distal convoluted tubules. These changes were present in almost every case. A further characteristic feature was the presence of pigmented cylinders in the lower part of the nephron. Necrosis of the tubular epithelium was uncommon. The glomeruli were normal.

The various structural lesions of the kidney tubules in shock kidney, as revealed by the electron microscope, have been described in a preliminary communication, based on a single case (Dalgaard and Pedersen, 1959), and at the Ist International Congress of Nephrology in Evian, based on four cases more (Dalgaard and Pedersen, 1961). Regnier (1959) and Putois (1959) have published similar observations.

Three of the patients were in the oliguric phase when the biopsy was taken, while the other two were in the polyuric phase. In one case the diagnosis was verified at autopsy. The biopsies were embedded in methacrylate. The number of glomeruli found in the biopsies were 3, 8, 5, 0 and 5 respectively.

The three components of the glomeruli—the epithelial cells, the basement membrane and the endothelial cells—were seen to be normal.

In one case, pronounced thickenings of the basement membrane were found in three out of five glomeruli, presumably older sclerotic lesions.

Our electron microscopic preparations showed that apparently normal cells in the proximal and distal tubules were present

alongside of damaged ones, so that the morphological changes described can hardly be artifacts from the methacrylate polymerization process (Fig. 1).

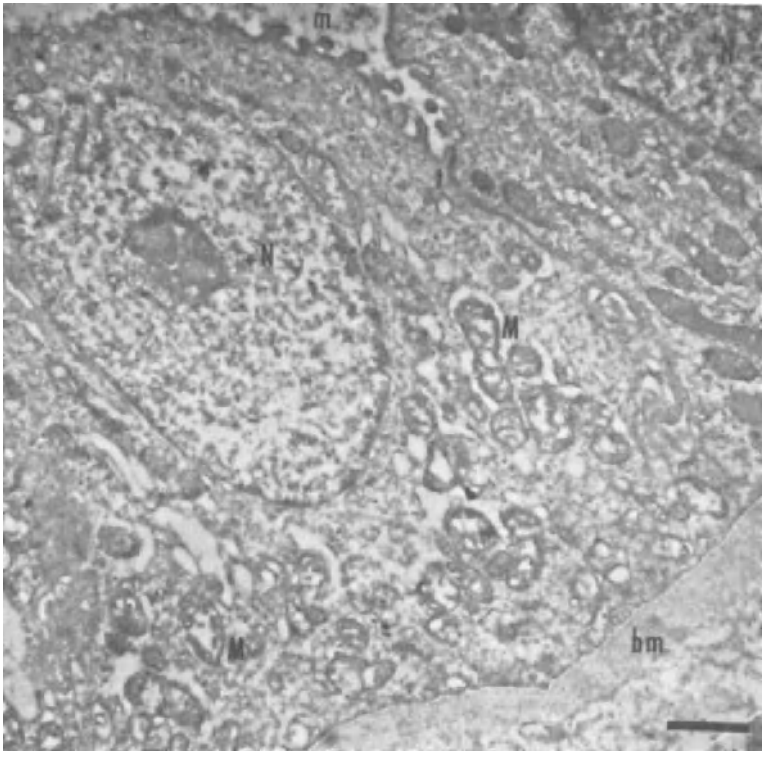


FIG. 1. Kidney biopsy embedded in methacrylate from a case of shock kidney. The central cell of a distal tubule is damaged. The inner structure of the mitochondria is partly destroyed. The cell to the right is apparently normal. (× 10,715.) (For abbreviations see Fig. 8.)

The mitochondria were the most sensitive elements. First they became slightly swollen, then the matrix between the cristae mitochondriales became more coarsely granulated and then more

swollen, then the cristae disintegrated and became converted to a homogeneous finely granulated substance, resembling the matrix; alternatively, the cristae became detached from the surrounding membrane. Examples of more pronounced changes were the poorly defined small vacuoles scattered throughout the matrix, the increasing diffuse hydropia of the mitochondria, the disintegration of the surrounding membrane, and its dissolution.

This type of presumably irreversible mitochondrial change has been described in many pathological states.

In karyopyknosis, shrinkage of the nucleus is usually seen with folding of the nuclear membrane.

In some of the affected tubule cells, the brush border was shed over larger or smaller areas.

In necrobiosis of proximal and distal tubule cells, poorly delimited vacuoles were observed in the mitochondria and the surrounding cytoplasm, distributed in patches. The endoplasmic reticulum of the cytoplasm disappears almost completely. The cytoplasm between the poorly delimited vacuoles may be very coarsely granulated. In autolysis, diffuse hydropia of the cytoplasm develops, and the cytoplasm consists of scattered fine and coarse granules and fragments of organelles.

In shock kidney the basement membrane may be found to be suddenly disrupted (tubulorrhexis) and elsewhere quite thin or split into filaments, and the epithelial coverings may be interrupted and necrotic.

Using classic light microscopy, tubular regeneration appears already after a few days, the degenerative lesions improve, and the epithelial defects become covered after the lapse of a week. The necrotic epithelium is shed and carried to more distant segments (Lucke, 1946).

The newly formed cells are flattened, the cytoplasm is scanty and strongly basophilic.

Flattened tubule cells were present in almost all cases throughout the period of the disease, being observed as early as the second

day of illness and as late as the 40th day after the onset of the disease (Brun and Munck, 1957).

A rim of acid mucopolysaccharides is often found on the inner surface of the lumen in the flattened tubules (Fig. 2). Using

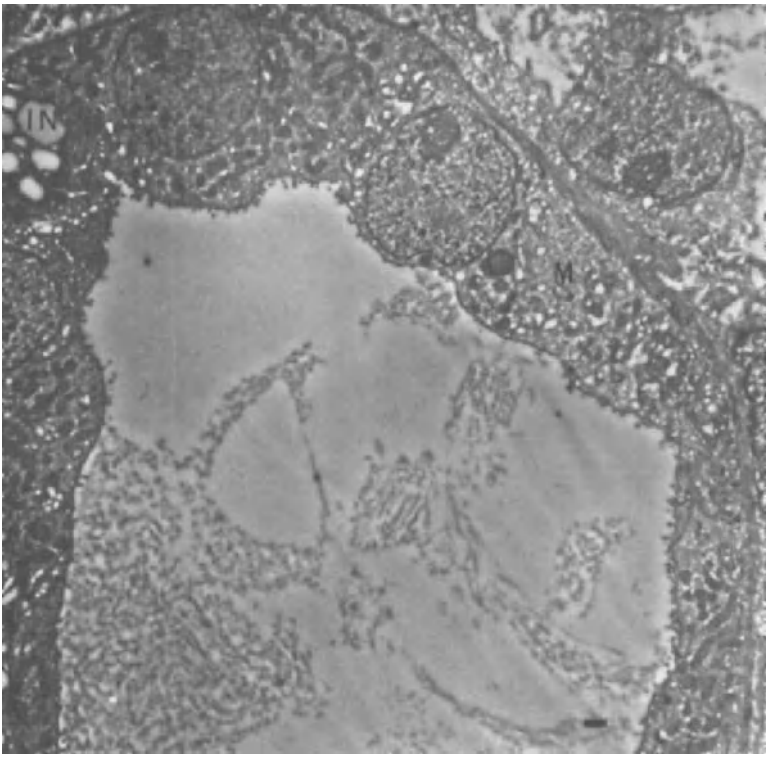


FIG. 2. Same biopsy as Fig. 1. A characteristic flattened distal tubule is seen. (× 2,620.) (For abbreviations see Fig. 8.)

chromotrope staining, the flattened tubule cells are seen to contain varying amounts of mitochondria, the various sections showing both few and many, presumably increasing in step with the maturation process in the individual cells. The flat tubule cells

were also characterized by having a high concentration of sulphydryl groups, as well as a very strong basophilia of the cytoplasm due to ribonucleic acid. The flattened tubule cells are

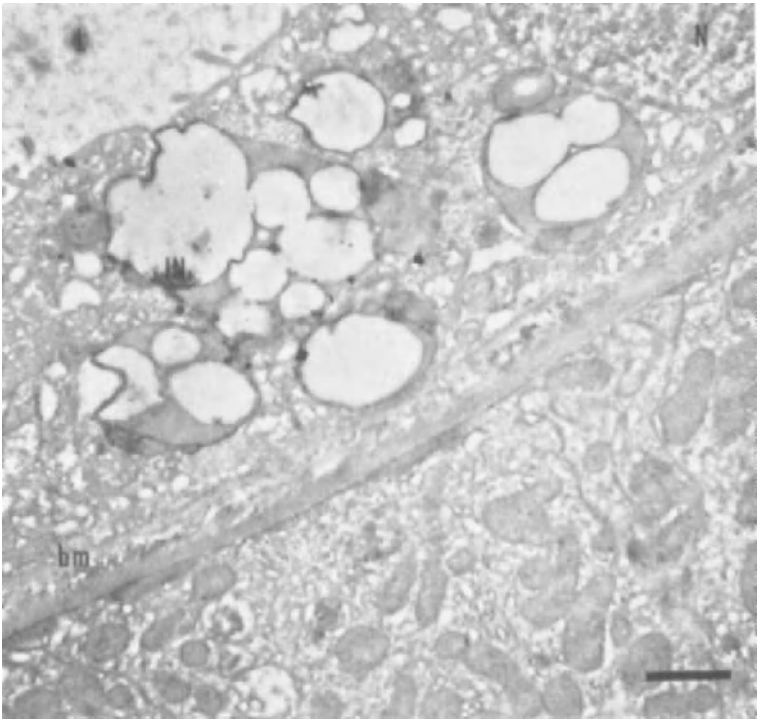


FIG. 3. Same biopsy as the two preceding figures. A multilocular inclusion is seen in a distal tubular cell. (× 10,715.) (For abbreviations see Fig. 8.)

relatively undifferentiated. They contain few mitochondria, and all membranes have a simple contour.

Multilocular vacuolized inclusions are observed, particularly in the undifferentiated flat tubule cells (Fig. 3). They develop from highly dense small granules, which can lie singly or in

clusters, and they increase in size and develop an almost homogeneous central matrix and a dense peripheral membrane-like layer. The vacuoles within one inclusion and between neighbouring inclusions may flow together to form multilocular inclusions almost as large as cell nuclei. These inclusions have also been observed in rapidly regenerating HeLa cells (Harford *et al.*, 1956).

In the flattened tubule cells, the cytoplasmic Palade granules are very numerous, and in most cases not associated with profiles from the endoplasmic reticulum.

All five patients had in common the fact that they had been in shock. The significance of shock for the pathogenesis of damage to the kidney tubules is still an unsolved question. The most important cause of injury is possibly anoxia resulting from reduced peritubular circulation during the state of shock.

Production of osmotic diuresis is sometimes used clinically for therapeutic purposes. In order to try to correlate the ultrastructure of the tubules with kidney function during a very large urine flow, osmotic diuresis was produced by intravenous administration of a hypertonic solution of mannitol to a patient with normal kidney function. The urine flow increased to about 20 ml./min. during three hours. At this time two kidney biopsies were performed (Fig. 4). The biopsy for electron microscopy was embedded in an epoxy resin "Epon" according to the method of Luft (1961). In osmotic diuresis several authors have previously demonstrated by light microscopy a rather striking swelling almost exclusively localized to the epithelium of the proximal portion of the nephron and with complete lack of evidence of renal dysfunction. The changes are produced by regularly distributed vacuoles, reach their maximum in 48 hours after the hypertonic sucrose is administered and are entirely reversible (Helmholz, 1933). Processes from the cytoplasm bulging into the lumen have often been described. Occasionally slight vacuolization of the distal tubules and of the parietal epithelium of Bowman's capsule has been observed.

Electron microscopic studies of osmotic nephrosis have recently been carried out in animal experiments. Rouiller and Modjtabai (1958) provoked the condition by injection of hypertonic glucose intraperitoneally in rabbits. Yolac (1959) used hypertonic sucrose in mice, and Janigan, Santamaria and Trump (1960) induced the changes in rats by intraperitoneal administration of hypertonic sucrose solution.

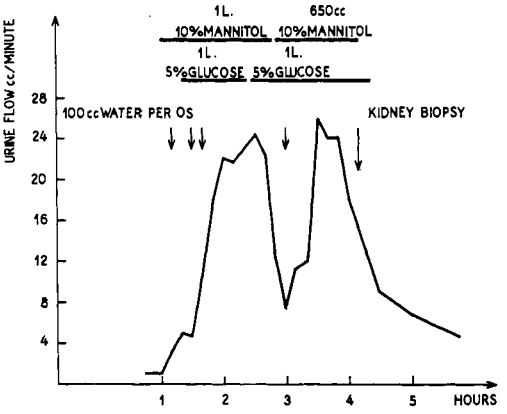


FIG. 4. Osmotic diuresis produced by hypertonic mannitol in a patient with normal kidney function. Kidney biopsy was performed (arrow).

Our electron microscopic observations in a human case have confirmed and extended their results (Fig. 5). The epithelial cells in the proximal portion of the nephron are often swollen as a result of the presence of large vacuoles. However, not all cells are changed in this way. Some are apparently normal. This is also evident in light microscopic preparations stained by Wagner's and Shapiro's (1957) chromotrope 2R method, which probably demonstrates mitochondria: some segments of the proximal

tubules are highly swollen, while adjacent segments display a normal appearance.

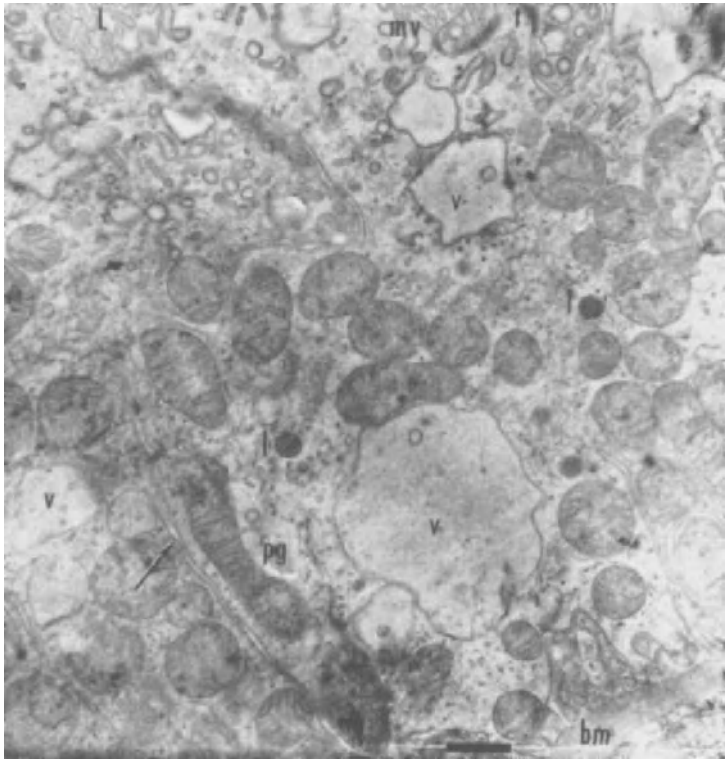


FIG. 5. Proximal tubule cells from a kidney biopsy taken during osmotic diuresis. Note the large vacuoles (v), the dilated intercellular spaces (arrow) and the bulging luminal process at the top of the picture. Many vesicles, presumably pinocytotic, are present. Embedded in Epon. (× 8,570.) (For abbreviations see Fig. 8.)

Often processes from the cytoplasm bulge into the lumen (Fig. 6). Especially here the cytoplasmic ground substance seems to be diluted. The concentration of Palade granules and profiles from the endoplasmic reticulum is highly variable, but always de-

creased. This swelling is probably the expression of water uptake in the cytoplasm.

The processes contain mitochondria, inclusions and large vacuoles. These vacuoles may have a diameter up to 5 μ. They

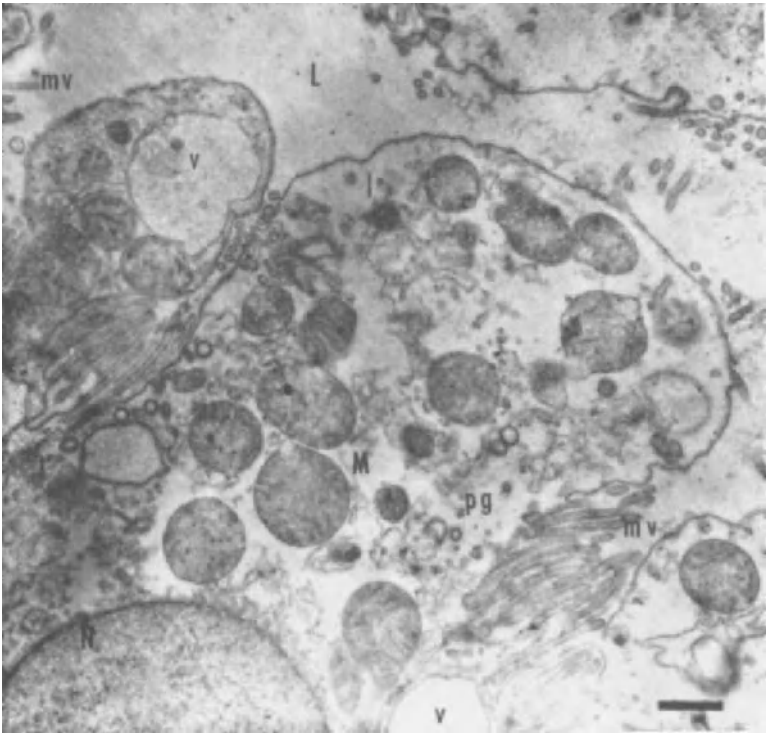


FIG. 6. Same case as Fig. 5. Note the hydropic cytoplasm in the bulging processes from proximal tubular cells and the disappearance of the microvilli from the brush border. Some of the mitochondria are swollen. (× 8,570.)

are bounded by a 70 Å wide membrane. Their contents display low density and are often slightly granular. Similar characteristic vacuoles are also observed in other parts of the cells.

Around the bulging processes the brush border is modified.

As a result of distension the microvilli are separated from each other. They are shorter and wider or have entirely disappeared

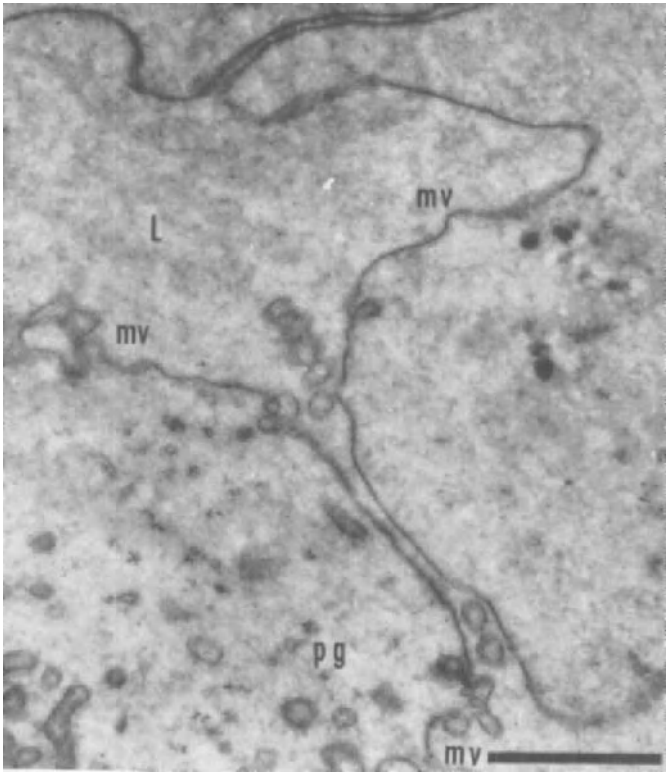


FIG. 7. Large ballooning processes from proximal tubular cells. (Same case as Figs. 5 and 6.) (For abbreviations see Fig. 8.)

by ballooning (Fig. 7). Similar changes in the luminal surfaces have not been observed in the other segments of the nephron.

The mitochondria in the proximal tubular cells often display a variable swelling. This is especially evident in the bulging processes. In clear swelling—probably due to water imbibition—

the mitochondria show a diluted matrix, and the cristae are shorter than normal. Another type of swollen mitochondria has a dense appearance due to accumulation of substance in the matrix. The cristae are obscured or have partly disappeared. In the same cell very swollen mitochondria may be seen among mitochondria of normal size and density.

Mitochondria in other parts of the nephron are apparently normal judged by morphological criteria (Fig. 8).

The opposed plasma membranes between two proximal tubule cells are normally separated by an intercellular space displaying a very constant width, about 200 Å. In osmotic diuresis these spaces are often very conspicuous because of dilatation, and they may be seen in continuity from the lumen to the basement membrane.

The terminal bars ("desmosomes"), which hold together the edges of the cells towards the lumen and seal the intercellular spaces so that material is prevented from passing between two cells, display symmetrical thickenings of the plasma membranes of adjacent cells. Sometimes the spaces between the plasma membranes are also widened at this place. The plasma membranes turn inward at the basal part of the proximal tubular cells to form septa of parallel membranes separated by about 200 Å. In this way the basal parts of the cells are subdivided into narrow compartments packed with mitochondria. Normally the intercompartmental spaces are collapsed, but in osmotic diuresis they are frequently dilated and form an extracellular basal labyrinth, presumably as a consequence of the rapid transport of fluid.

In the cytoplasm of the proximal tubular cells many small vesicles of variable size are found, especially in the luminal part of the cells. These vesicles may be an indication of pinocytotic activity. However, it is not yet known if the number of vesicles is increased during osmotic diuresis.

The results of our observations based on a human case are in agreement with previous electron microscopic observations in

animals in a state of osmotic diuresis. Many substances may produce a hydropic vacuolization of the proximal tubular

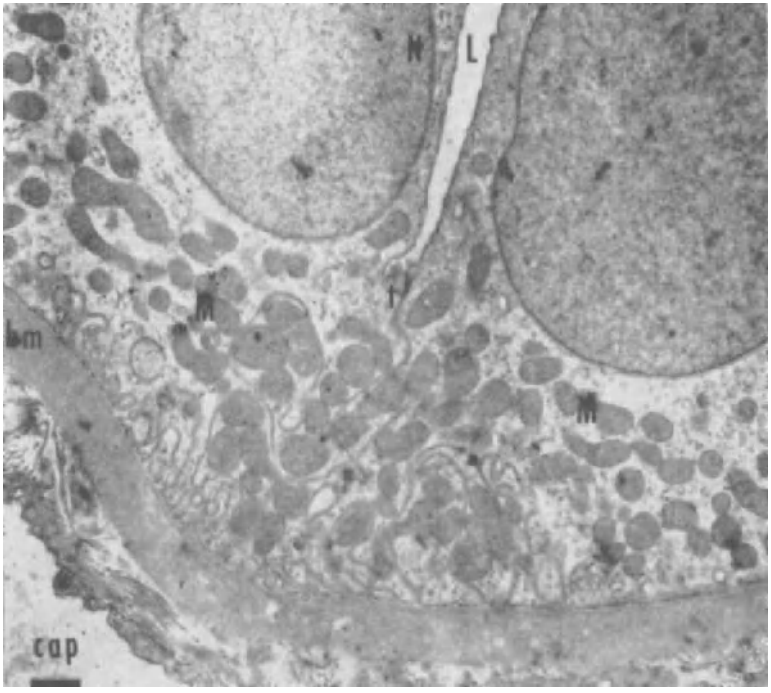


FIG. 8. Same biopsy as the preceding three figures. Two cells from a distal tubule. The cells are normal. (× 6,165.)

Abbreviations for figures:

bm = basement membrane	mv = microvilli of brush border
cap = capillary	L = nucleus
In = inclusion	pg = Palade granules
L = lumen	v = vacuole
M = mitochondria	t = terminal bar

epithelium if they are present in sufficient molar concentration in the lumen.

Wilmer (1944) assumed that sucrose diffuses from the tubular

lumen into the cells and causes cellular uptake of water only by virtue of its osmotic action, a mechanism also considered by Yolac (1959) as a result of his studies of changes in mouse kidney caused by sucrose administration.

The bulging of the luminal cytoplasm of the proximal tubular cells, accompanied by distension of the plasma membrane and ballooning of the microvilli in the brush border, may be the structural expression of uptake of mannitol together with an osmotically equivalent quantity of water into the cell.

Janigan, Santamaria and Trump (1960) have considered the possibility that sucrose is taken up by the cell through pinocytosis and that the pinocytotic vesicles and vacuoles become associated with, or incorporated by "lysosomes".

Morphological studies of isolated mitochondria have demonstrated that the volume of the mitochondria varies according to the tonicity of the medium. The reversibility of the phenomenon suggests that these organelles may function as a kind of osmometer. Electron microscopic studies revealed swelling of the mitochondria in the proximal tubules but not in the thin segment of Henle's loops or the distal tubules. These facts may be interpreted to mean that the luminal plasma membranes of the proximal tubular cells are more permeable than those from the thin segments of Henle's loops and distal tubular cells. This is also in agreement with certain physiological experiments from which it has been concluded that the proximal tubules are relatively permeable to water and that the distal segments—at least during water diuresis—are relatively impermeable to water (Smith, 1956).

These observations have emphasized some completely different fine-structural expressions of irreversible and reversible injury to kidney tubules in man. It is our hope in future work to be able to correlate these changes with studies on the cytochemical organization of tubular cells in similar states and with special emphasis on the enzymic patterns.

REFERENCES

BRUN, C. (1954). Thesis, Copenhagen.

BRUN, C., and MUNCK, O. (1957). *Lancet*, **1**, 603.

DALGAARD, O. Z., and PEDERSEN, K. J. (1959). *Lancet*, **2**, 484

DALGAARD, O. Z., and PEDERSEN, K. J. (1961). *In* 1st int. Congr. Nephrology, in press.

GORMSEN, H., IVERSEN, P., and RAASCHOU, F. (1955). *Amer. J. Med.*, **19**, 209.

HARFORD, C. G., HAMLIN, A., PARKER, E., and VAN RAVENSWAAY, T. (1956). *J. biophys. biochem. Cytol.*, **2**, Suppl. 347.

HELMHOLZ, H. F. (1933). *J. Pediat.*, **3**, 144.

IVERSEN, P., and BRUN, C. (1951). *Amer. J. Med.*, **11**, 324.

JANIGAN, D. T., SANTAMARIA, A., and TRUMP, B. F. (1960). *J. Histochem. Cytochem.*, **8**, 385.

LUCKE, B. (1946). *Milit. Surg.*, **99**, 371.

LUFT, J. (1961). *J. biophys. biochem. Cytol.*, in press.

PUTOIS, J. (1959). Structure et ultrastructure renale. Paris: Arnette.

REGNIER, C. (1959). Etude histologique et electronique du rein normal et pathologique de l'enfant. Toulouse: Soubiron.

ROUILLER, C., and MODJTABAI, A. (1958). *Ann. anat. path.*, **3**, 223.

SMITH, H. W. (1956). Principles of Renal Physiology. New York: Oxford University Press.

WAGNER, B. M., and SHAPIRO, S. H. (1957). *Lab. Invest.*, **6**, 472.

WILMER, M. J. (1944). *Amer. J. Physiol.*, **141**, 431.

YOLAC, A. B. (1959). *Verh. dtsch. path. Ges.*, **43**, 235.

DISCUSSION

Jennings: In your Fig. 1 the proximal tubules appeared to be separated from one another by fibrous tissue or oedema. Is that a uniform finding in your patients with acute tubular necrosis?

Dalgaard: No.

Hamburger: You mean that you have cases of so-called tubular necrosis without oedema?—that is, without any interstitial reaction?

Brun: We do have interstitial reaction. But the amount of oedema depends on the treatment. Our old slides show more oedema. However, nowadays, with our very strict water regimen, we rarely see any oedema in the sections.

Hamburger: Then this type of tubular nephritis might be called tubulo-interstitial—a term I believe you suggested.

Brun: I'm afraid I did.

de Wardener: I am concerned about Dr. Dalgaard's statement that more water goes into the cell in mannitol diuresis. There should be less. One would expect the cell to be shrunken.

Brun: This depends on whether the mannitol goes into the cell and remains there. If mannitol goes into the cell it might be followed by water.

Earle: Very little gets into the cell though.

de Wardener: Assuming a little gets in, the majority doesn't.

Dalgaard: You can find the same swelling with inulin.

de Wardener: Are you sure it is water in the cell?

Black: Water reabsorption should be maximal in antidiuresis. It could be that the water just goes in and passes very rapidly on—that might be the difference.

Darmady: Might it not be that the proximal convoluted tubule is really reabsorbing the mannitol and that the water is a secondary phenomenon.

de Wardener: There is very little evidence of reabsorption, I would have thought.

Brun: If mannitol goes into the cell, it might not be able to get out.

Hamburger: In my group, J. L. Funck-Brentano has studied experimentally the lesion caused by water intoxication, comparing the effect of pure water causing cellular overhydration with the effect of the same quantity of water given with salt and causing extracellular overhydration. His pictures of water intoxication are very similar to what Dr. Dalgaard has shown.

Earle: If there is a high osmotic gradient in the lumen, perhaps it is pulling water across away from the peritubular spaces. I should like to ask Dr. Dalgaard how he thinks water is transported across the epithelial cells. Is it molecule by molecule, or is it in droplets? I would like to report some preliminary studies in another field that may be relevant. Our otolaryngology department has been doing time-lapse photography on tissue cultures. On observing the endolymph sac over 48 hours, it is seen to swell up and shrink periodically. As it swells up you can see droplets, apparently of water, going across the epithelial cells into the lumen, and when it shrinks you see lots of these little droplets going out. I wonder if this is a general phenomenon.

Kark: Surely, if one accepts the whole idea of pinocytosis, then you must have aggregates bigger than molecules; there must be little droplets of water, little vacuoles, going across.

de Wardener: Could somebody explain pinocytosis? Some of us are in the dark.

Vernier: Pinocytosis has been studied most extensively in the amoeba; the experiments are done with living cells floating in a medium, and uptake of various substances placed in the medium can be studied. Prof. H. Holter and his associates at the Carlsberg Laboratory in Copenhagen have done a great deal of this work. In the amoeba, one can show by time-lapse phase microscopy that pinocytosis can occur by at least two mechanisms. In the first mechanism, small projections of the cytoplasm extend, then coalesce and engulf (take a drink of) the contents of the milieu in which the cell is floating. These vacuoles can move down the long channels which extend into the cytoplasm. Prof. Holter thinks that some of the vacuoles may come to the edge of these spaces (perhaps the equivalent in the amoeba of endoplasmic reticulum) and burst there, freeing their contents for transport into the cell, within these spaces. He maintains that while the water (or whatever the cell has taken in) is in a vacuole it is technically outside the cells. The second way in which the cell might take in the water is by formation of invaginations which seal off and form vacuoles within the cell. As far as I can tell, both of these processes are termed pinocytosis, although fancier names such as rhopheocytosis, which Dr. M. Bessis uses, have been suggested. Whether there is any difference between pinocytosis and phagocytosis is highly questionable. We use the term phagocytosis when referring to a reticular endothelial cell and this type of uptake, but I suspect that these are the same phenomenon.

Prof. Holter and his colleagues (1955. *Exp. Cell Res.*, Suppl., **3**, 52) have also shown that if you add to the fluid in which the amoebae are swimming, radioactively labelled protein and radioactively labelled glucose, the amoebae are able differentially to drink more of the protein than of the glucose. He does not believe that the cell takes in both in equal quantities and spits out the glucose; he thinks that somehow it can select, perhaps the larger molecule, in this case the protein. This has, of course, quite a bearing on transport across cells generally and on protein transport in particular.

Movat: We are presently studying inflammation. In most textbooks of pathology exudation is described to occur between endothelial cells which are separated from each other by so-called cementing substance. When silver is injected into the vessel certain areas impregnate with silver, and this was thought to be between two endothelial cells. However, the electron microscope does not show such a cement substance, as Dr. Dalgaard has just pointed out. Two cells are separated just by a very narrow slit, about 160 to 200 Å. Dr. R. C. Buck (personal communication, 1959) in London, Ont., has found that on examining such silver-impregnated sections under the electron microscope, the silver lines are usually not at the border of the cells but are distributed quite haphazardly. In our own studies we have seen what Dr. Vernier has shown, that there are little bulges of the plasma membrane which travel from the lumen towards the connective tissue. This is only a static picture, but we believe that what may happen in inflammation is that little bubbles, presumably containing plasma protein, travel across through the cytoplasm of endothelial cells.

Vernier: However, it is impossible in a static picture to tell in which direction the particle is going. In the labelled transport studies, in particular the studies with ferritin, it is possible to predict the direction in which the injected label is travelling. If we put the ferritin into the capillary lumen and find it in vacuoles within the cytoplasm of the epithelial cell, we have to presume that it has gone across from the capillary lumina into the epithelial cell, as vacuoles. But, as I tried to point out, the process of transport, at least in the human foetus, is also in non-vacuolated form, and one finds ferritin particles free within the epithelial cell, i.e., not in vacuoles. There are at least two mechanisms, and perhaps others not yet studied.

Rich: Both the term "pinocytosis" and the observation of this phenomenon were first put clearly on record by Dr. Warren Lewis many years ago. In tissue cultures he observed just what you described, a constant stream like bubbles of fluid going into the cell. He didn't want to call it phagocytosis, because these cells are really drinking and not eating, so he called it pinocytosis. You can see these droplets streaming into leucocytes, and in the mononuclear phagocytic cells it is quite spectacular. I would think that most cells do not imbibe fluids in that way. The phagocytic cells notably do.

Vernier: I don't think that there is any question that the process of pinocytosis occurs. The question is, how important is it as a method for transport of materials in mammalian cells generally?

Rich: In cells generally, I would think that it was of little importance.

Bergstrand: We are perhaps discussing two different things: one is the intake by the living cell of substances that it needs for its own living processes, and the other is the transport of fluid through the epithelial cell layer. I do not think we can dispose of the intracellular spaces as lightly as we have done. In the normal tubular cells there are basal compartments limited by infoldings of the basement membrane, up to 50 per cent of the height of the epithelial cells. They are very numerous. They may be dilated into large spaces or they may be very narrow. In Dr. Dalgaard's slides they were very low and seemed to be compressed, indicating in my opinion a swelling of the epithelial cells. This may mean that a pathway for the transport of fluid is narrowed and there is less water passing.

Dalgaard: I do not agree with you. Many of our pictures showed the large labyrinth in the basal parts of the epithelial cells.

Darmady: What about the potassium deficiency states? It has always seemed to me very difficult to know what causes the vacuolization—the clear areas in the epithelium of the proximal and distal convoluted tubules. Is this water?

Dalgaard: I don't know. Our patient had normal serum potassium when the biopsy was taken.

Movat: Did you see any micro-bodies in your regenerating tubule cells?

Dalgaard: We saw some very small mitochondria, but perhaps these are only sections that are cut at the end of the mitochondria.

Movat: Recently there has been some controversy as to whether the protein absorption droplets develop from mitochondria or whether they are just absorbed protein. F. Miller (personal communication, 1960) at the Rockefeller Institute has injected haemoglobin as a marker, because it is electron-dense, and showed how haemoglobin apparently entered between the villi of the brush border into the cytoplasm (being surrounded by a plasma membrane) and gradually migrated apparently in the direction of the centre or base of the tubule cells. This finding

suggests that protein or hyaline absorption droplets are not of mitochondrial origin.

Hardwicke: Dr. Brewer in our department has done some similar studies on reabsorption of haemoglobin (Brewer, D. B. and Eguren, L. M. (1961). *J. Path. Bact.*, in press).

I should like to take issue with Dr. Vernier on one point. He described experiments with amoebae, and suggested that they were rather "vitalistic" in their absorption of proteins at the expense of glucose. As a good "mechanist" I would suggest that in the buffer solutions used, if they are at ordinary pH, protein normally probably concentrates on cell membranes and consequently would be automatically preferentially absorbed.

Vernier: This is exactly what Prof. Holter believes, that the cell membrane is perhaps coated with a mucinous material which somehow selects the protein (adsorption), which aggregates along the cell membranes. In the infolding process, the concentration of protein might then be higher in the vacuole than in the medium generally. I believe this is his explanation.

Hardwicke: I think that this is very important for our consideration of how things are reabsorbed by the brush border.

Black: Does this process in the amoeba take minutes or seconds or milliseconds? If it is going to have any quantitative significance for water reabsorption in the kidney it would have to be milliseconds.

Vernier: This is the main objection which is raised to pinocytosis: the process is too slow to explain the bulk transfers that one can demonstrate. In the amoeba the process of pinocytosis is cyclic; it goes on for periods of 10 to 15 minutes, the cell then rests, and then engages in another period of pinocytosis. During these bursts of activity, the process of transfer from the exterior to the vacuoles within the cell goes on in a period of a minute or two I would think from seeing the pictures; it is relatively slow.

Hardwicke: If you put amoebae into hypotonic solution, I believe that they swell extremely rapidly without the accumulation of vacuoles, and then they form excretory vacuoles and get rid of the water. So I think we must assume that water can go in by diffusion, in the absence of vacuoles.

Michielsen: Dr. Dalgaard, you showed that there is some separation

of the cell membranes of adjacent tubular cells. Could this finding not be interpreted as a shrinkage of the epithelial cells? I feel quite unhappy about mannitol causing a dilatation of the epithelial cells because there is no active reabsorption nor secretion of this substance. I also don't understand why mannitol should get passively between the tubular cells, because there is no osmotic gradient, at least not in the proximal tubule, between the capillaries and the tubular lumen.

Dalgaard: From light microscopy we know that the cells are swollen. Why should mannitol not be able to go into a cell?

Michielsen: It is generally accepted as a measure of the glomerular filtration just as inulin is. And even if it goes actively into the cell, this does not explain why the intercellular spaces dilate, unless you accept an intercellular secretion.

Dalgaard: If it passed between the cells with water, it might separate the membranes.

Milne: There is no evidence of swelling of cells other than the proximal tubular cells. Therefore this appears to be a specific reaction of the proximal tubule, and I do not think that we can relate it to simple osmotic effects. There must be something more to it than that.

Hardwicke: I think that it is well known that if you give large doses of dextran of moderately small molecular weight and fix your sections in alcohol, you can show large numbers of PAS-positive granules in the proximal tubular cells. I assume the same thing happens with mannitol: anything that is in the tubule will go back into the cell. Whether it goes back into the circulation is another matter.

Blainey: Dr. Dalgaard, have you studied a large enough population of cells to be able to say that there was a quantitative distribution of the droplets? Do some cells show very many droplets and others no droplets, which might indicate definite differences in physiological activity in various parts of the proximal tubules?

Dalgaard: We have not done quantitative studies.

Michielsen: I would like to ask Dr. Bergstrand if the large infoldings at the base of the tubular cells he spoke about are from human or from animal material?

Bergstrand: Human.

Michielsen: Because in human biopsy material I usually don't see large infoldings and I wondered if this was due to a pathological

change. I have no opportunity to study normal human material in biopsy.

Bergstrand: Our normal material has been embedded only in methacrylate so I don't think that it is quite comparable to Dr. Dalgaard's pictures in Epon. I wondered if he had a normal picture of Epon-embedded material to show us as a comparison.

Dalgaard: We had one patient with acute diarrhoea who developed anuria for one day, and when the biopsy was taken the kidney function was normal again. We did not find any changes in that biopsy.

Bergstrand: Have you compared it to methacrylate-embedded materials and have you seen any difference between the two?

Dalgaard: No, not on the same biopsy.

Bergstrand: Our observations indicate very great variations in this field. I think that the limit between normal and pathological is just a matter of frequency: identical things may appear rarely in the normal but be very frequent in the pathological state.

Michielsen: Do you see a difference in the distance between the cell membranes in Epon and methacrylate-embedded material?

Bergstrand: We find the same distance.

Michielsen: With Epon we usually have better limitation of the plasma membranes, and a greater distance between the cells. This may be due to a lesser degree of dehydration in the embedding technique with Epon.

de Wardener: Can we go back to tubular necrosis? I don't do many biopsies on acute tubular necrosis, but I believe that it is general experience that the amount of damage seen in biopsy is difficult to correlate with the deterioration of function. How much greater is the damage seen by electron microscopy as compared with light microscopy?

Dalgaard: With light microscopy you can't see the damage of the mitochondria, for instance, if you don't do special staining.

de Wardener: What proportion of tubules looking normal by light microscopy are in fact abnormal by electron microscopy?

Dalgaard: I cannot give any quantitative answers. I can only describe the qualitative changes.

Darmady: In our studies we find normal and abnormal nephrons lying side by side. I would have thought that this curious random distribution would make it very difficult to say which you are looking

at. The number of damaged nephrons varies greatly, in different cases: some have a very high proportion of damaged nephrons, and one presumes that whatever one does they won't get better; others are obviously minimally damaged and will probably recover; some are completely intact.

Rich: There are cases in which with the light microscope they seem to be only minimally damaged, and yet the patients die; those are the ones that are most troublesome.

Hamburger: I suppose Dr. Brun has more early biopsies than anyone else in the world. How often do you see optically normal tubular cells in such cases?

Brun: I am not sure they are ever completely normal. There are always some casts or some tubular dilatation. But I am still surprised that anyone is happy about calling this tubular necrosis. If we diagnosed this disease only when we actually find necrotic tubuli, we would see very few cases. This is in biopsy. I think it is much more difficult to diagnose this disease at autopsy because so many autolytic changes take place unless special precautions are taken. The autolytic changes are difficult if not impossible to differentiate from intravital necrotic or necrobiotic changes.

Wilson: What would you like to call this condition?

Brun: I don't think that it should have a new name until we know what this disease is. We can call it "number 17" or whatever you like. It will only add to the confusion if we invent new names now.

Hamburger: The glomeruli in this condition, whatever it is called, have always been supposed to be quite normal in optical views. Has electron microscopy confirmed the fact that the glomeruli are normal?

Dalgaard: Yes, I think so.

Michielsen: Have you ever seen fibrin or similar material in the glomeruli in acute tubular necrosis?

Dalgaard: I have not seen it.

Kark: May I ask the pathologists here what they mean by abnormal tubules? Could not dilated tubules be physiological? I have the greatest difficulty in knowing when a tubule is abnormal on histological examination.

Rich: I think that in practice we regard a tissue as normal when its appearance falls roughly within the limits of what we are accustomed

to see in that type of tissue when there had been no clinical or other evidence of disturbed function. This is obviously unprecise, for a tissue can, of course, look "normal" though it had been functioning abnormally, and *vice versa.* Just where we are to draw a precise line between normal and abnormal, no one can say at the present time, and I doubt whether even electron microscopy will provide the answer. As for the tubules, I think that renal biopsy has supported the long-held view that dilated tubules need not be abnormal unless their cells appear abnormal.

Pirani: In other words, we don't know the limits between physiological hypo- or hyperfunctional and really pathological changes. Resorption of protein occurs in tubules which are perfectly normal. In proteinuria, for example, there is increased resorption and hyperfunction of the tubular cells. The cells can be perfectly viable although morphologically abnormal. I am not sure that in this case we should speak of degenerative changes.

GENERAL DISCUSSION

Rich: This general discussion period affords us an opportunity for the consideration of matters that you may have in mind concerning areas of clinical or experimental renal pathology that may bear upon problems of renal biopsy, whether related or not to the subjects that we have discussed during these three days. It also, and particularly, provides a further opportunity to exchange opinions as to the value of renal biopsy in practice, and in the investigation of the structural alterations and basic mechanisms responsible for the disturbances of function that are encountered in the various forms of renal disease.

If we may begin with the practical aspects: Very important from the purely practical standpoint is the question, "Can renal biopsy really contribute significantly to the welfare of the patient, and if so, under what conditions?" The actual value of renal biopsy in present-day practice—the question whether there are conditions in which it can provide information of distinct value to the patient, and which cannot be obtained by clinical experience aided by the existing standard tests; the question whether there are any conditions which not only justify its use from the patient's standpoint, but make it mandatory for the best practice—these are questions of major importance and concern to many who have hesitated to adopt the procedure. A free exchange of opinions on these questions by the members of this symposium, who have devoted serious thought and study to them, and whose individual experiences may have led to somewhat divergent views, would help to sharpen and to clarify our ideas as to the present clinical value of renal biopsy. That, I take it, is one of the major purposes of this symposium, as presented to us in its title "The Clinico-Pathological *Significance* of Renal Biopsy". The preceding papers and discussions, and the clinical and pathological experience assembled in this room, provide an exceptional background for the discussion of the clinical indications for renal biopsy. May I therefore ask for opinions on this question, which is a question continually asked by physicians who have not yet used renal biopsy: In what circumstances

may renal biopsy be reasonably expected to provide information *of value to the management of the patient*?

Hamburger: I consider that there are three cases in which renal biopsy is of real advantage for the patient. The first is in the choice of treatment for the nephrotic syndrome. The second is acute anuria, when the cause of anuria is not clear and when it is particularly important, not only to know the cause of anuria, but also to know whether the kidney is destroyed so that one can decide whether to go on with the artificial kidney. The third is the large number of cases of apparent chronic nephritis, in which we have not been able to make a diagnosis clinically, and in which biopsy has revealed conditions that we have not spoken of here, such as oxalosis, latent nephrocalcinosis and other surprising diseases.

de Wardener: I can think of three more: persistent proteinuria with no other biochemical abnormalities; recurrent haematuria; and in the diagnosis of chronic infection of the kidney.

Rich: The diagnosis of chronic infection by renal biopsy is full of pitfalls though; as we have seen, the interpretation is very difficult in many cases.

de Wardener: It may be full of pitfalls, but you get some evidence.

Rosenheim: Are you referring to finding organisms or to the histology?

de Wardener: My experience has been with the histology, but I would go with both.

Milne: I agree with Prof. Hamburger about the tremendous importance of renal biopsy as an indication not only for initiating dialysis but also for continuing it. I see no future in continuing dialysis in a patient who is obviously not going to recover, unless you are doing it as Dr. B. H. Scribner in the University of Washington does, as a long planned routine. That is to me a different problem.

Another condition where renal biopsy is of great value is in amyloidosis. We are all agreed that the only method of certain diagnosis of renal amyloid is by percutaneous renal biopsy, the dye tests being tests for only extrarenal, and especially hepatic, amyloid. I think we would also all agree that the early diagnosis of amyloidosis is essential so that one can remove the primary cause, if this is feasible—for example, amputation in osteomyelitis. In addition, the use of

steroid therapy in amyloidosis is open to question, and I would like to get the opinion of the meeting on this. Although many of us have reservations about the value of steroid therapy until controlled trials are done, I think that the balance of evidence indicates that steroids are of use in lupus erythematosus, and in the nephrotic syndrome due to glomerulonephritis, particularly in those cases with no change by light microscopy. In amyloidosis the evidence from the experimental animal is quite clear, that steroids intensify amyloidosis and make it worse. But is this proven in human disease? I think that is a very important question.

Another important point in relation to amyloid is acute oliguric renal failure from renal vein thrombosis. I have tried treating these cases with prolonged anticoagulant therapy, but so far I have not had any marked success because I have not had them early enough. Therefore I think that early diagnosis of amyloid is very important, in that it points to a different method of treatment than our usual for a case of amyloid who develops suddenly an acute oliguric renal failure.

A third issue in which renal biopsy may be of value is in the diagnosis of primary aldosteronism. These cases have presented with a potassium-losing syndrome, and usually with an extracellular alkalosis. The fact that you can find both high excretion and secretion rates of aldosterone is not really helpful, because this does not give you the differential diagnosis between primary and secondary hyperaldosteronism, that is, whether the lesion is adrenal or renal. If percutaneous renal biopsy shows nothing but hypokalaemic changes then I am happier that this is a case of primary hyperaldosteronism. Secondly, it is most important to show by aortography that there are no obstructive lesions in the renal arteries before one advises adrenal exploration or adrenalectomy.

To sum up, three situations in which I feel renal biopsy is of great practical value are: the question of continued dialysis in acute oliguric renal failure which Prof. Hamburger mentioned; the diagnosis of renal amyloid; and in these cases of hyperaldosteronism (or Conn's syndrome).

Earle: With regard to the treatment of amyloidosis with steroids, Dr. Morton Maxwell reported results of treating 28 patients with amyloid disease of the kidney, proved by renal biopsy. In not one was

steroid therapy beneficial. This work was presented at the 1960 meeting of the National Nephrosis Foundation and has not been published yet.

Milne: Was there any deleterious effect?

Earle: I don't know.

Vernier: I would like to elaborate on the use of biopsy in the nephrotic syndrome. In children I have found biopsy to be useful in selecting those patients in whom steroids should not be given, or in whom steroids should be used very cautiously, that is, the group of patients who have by renal biopsy and light microscopy evidence of hyalinization of a significant proportion of the glomeruli. In these patients we have had many difficulties with hypertension and other complications of the drugs. Sometimes the glomerular damage is not detectable by functional tests. I think that renal biopsy is of distinct value in selecting patients for therapy.

Joekes: May I add another indication? With a surgeon, one is occasionally faced with the question of nephrectomy. One can sometimes prevent nephrectomy by showing by renal biopsy that the contralateral kidney is involved in a diffuse renal disease.

Hamburger: Hypertension itself could be an indication for renal biopsy. The results of biopsy seem useful to us in all cases for which we are discussing the decision for surgical treatment.

Rich: Unilateral renal hypertension?

Hamburger: Not only these, but also the few patients whose condition is still regarded as a possible indication for sympathectomy and adrenalectomy.

Joekes: May I amplify the question of hypertension? This may be important for surgery. If you have what you suspect to be unilateral renal disease or renal artery involvement with hypertension, and if there is evidence from renal biopsy that the contralateral kidney is involved in a vascular process, then it makes the importance of not removing the kidney with the artery stenosis even greater than it is normally. This can lead to the decision to go on trying with drugs rather than removing the kidney.

Ross: Can you be certain that the changes are vascular in origin? In our small experience of this we find that we cannot be sure whether we are looking at pyelonephritis, which would contra-indicate

nephrectomy, or whether we are seeing ischaemic changes secondary to the hypertension, which might be reversible.

Joekes: I think that this is irrelevant in this argument. The question is, is the contralateral kidney damaged? If the contralateral kidney is damaged, then one has to think very carefully indeed before accepting a nephrectomy of the supposedly only abnormal kidney.

Rosenheim: I would think that aortography was much more valuable under those circumstances than renal biopsy.

Hardwicke: In Dr. Kark's paper in which he originally described his technique of renal biopsy, hypertension was included as one of the contra-indications. I would like to know whether the opinion of the meeting and his own opinion have changed since then. Certainly we find complications more commonly in patients who are hypertensive than in others.

Kark: We have changed our minds about hypertension as a contra-indication. It always seemed to me more difficult to do a biopsy in hypertensive patients, but maybe we were not giving them enough sedatives beforehand. Now that we are studying unilateral renal artery disease in hypertension, we are extremely interested in knowing what the unaffected kidney looks like, because we want to know whether response to operation might or might not occur. At present I do not know how we can decide how much damage we have to have on the unaffected side as a result of hypertensive vascular disease before it is irreversible. In general, our surgeons nearly always try to use grafts rather than a nephrectomy. As far as we are concerned, renal biopsy is an absolute necessity to us in the study of unilateral renal artery disease.

Raaschou: In 1958 Dr. Brun and I investigated the frequency of gross haematuria in patients with arterial hypertension and found that this frequency did not deviate significantly from the frequency of gross haematuria in patients with normal blood pressure (1958. *Arch. intern. Med.*, **102**, 716).

Rich: Dr. Joekes, if there is a stenosis of the main artery on one side, and a biopsy of the other kidney shows what is interpreted as chronic pyelonephritis (with no bacteria) would you consider that a sufficient contra-indication to nephrectomy?

Joekes: If when you take two separate biopsies you show that both are

involved, then I think that it would be most dangerous to remove the opposite kidney.

Rich: Just how much intra-renal involvement in a biopsy of the opposite kidney would you regard as a contra-indication to nephrectomy?

Joekes: Here the question is vascular changes. As Dr. Kark has just said, we are still trying to decide this. I would think that if marked vascular changes are present then certainly it is of great importance to conserve the part of the kidney with the arterial stenosis.

Rosenheim: Have we evidence about this?

Vernier: We have one case that illustrates just this point: a 14-year-old boy who had had severe hypertension for six years and suffered two strokes in the course of this hypertensive disease. He was found to have a very striking anomaly of the main renal artery on the left side, a stenosis and a post-stenotic dilatation. Two kidney biopsies were taken. The non-stenosed kidney was shown to have severe arterial disease in all the vessels. The biopsy of the stenosed kidney was entirely normal; presumably the shunt had protected this side. It was possible for the surgeon to do a bypass in which the enlarged post-stenotic dilatation was connected directly to the aorta, and neither kidney was removed. The patient continued to have rather severe but somewhat less hypertension for six months. Because of the severe arteriolar changes in the non-stenosed (right) kidney, we elected to take it out. The patient has been well for two years, with normal blood pressure.

Hamburger: This is not always the rule though. In several of our cases the stenosis did not seem to protect the kidney, and the picture was exactly the same on both sides, despite the stenosis of the artery.

Rich: We have seen the protection of one kidney by a marked stenosis, just as in the Goldblatt dog.

Milne: Is this protection permanent in the experimental animal, or does it only take place for a limited time? Prof. Wilson, you have had tremendous experience of experimental hypertension. Would you say that however long the animal lived the kidney was permanently protected by the stenosis?

Wilson: Yes. In an animal with a clipped kidney I only know of one circumstance when vascular lesions occur beyond the clip and that is

when we take out the opposite kidney; severe vascular lesions may then be found in the clipped kidney. I feel very unhappy about the idea of basing treatment on biopsy findings in the opposite kidney. Some of the most successful results of unilateral nephrectomy have been in young subjects with malignant hypertension, where there is very probably vascular damage in the opposite kidney. In our animal experiments, when we took out the clipped kidney, we never saw the blood pressure rise or renal function deteriorate. If a damaged kidney is removed in a patient, I think it is unlikely that hypertension or renal failure will be aggravated unless there is gross damage in the opposite kidney, which would have been detected by renal function tests. Has it ever been found in a patient with normal renal function tests that removal of a single diseased kidney, whatever the biopsy appearance of the opposite kidney, leads either to rise in blood pressure or deterioration of renal function?

Hamburger: But in the hypertensive patient when we find something wrong with one kidney we are not sure that the other kidney is not primarily involved too. With our gross tests, renal function is rather frequently normal in cases of bilateral stenosis of the arteries with hypertension.

Wilson: I think renal biopsy is of benefit to the individual patient only if it will enable you to decide whether to use some form of therapy which is known with certainty to be effective. In anuria one can decide whether to go on with dialysis or not, purely on the progress of the case. The finding of calcification in the renal biopsy section should not affect one's decision—such cases may be fatal or may recover. In the nephrotic syndrome, we may justifiably use renal biopsy to decide whether steroids are effective or not in a controlled trial, in relation to the type of structural lesions. But I still don't feel that in the *individual patient* renal biopsy is going to make any difference. Has anybody any evidence that it does?

Earle: I think it does help individuals in the nephrotic syndrome. Several groups have shown that the "no lesion by light microscopy" responds quite well to steroid therapy whereas the proliferative or advanced membranous doesn't. Therefore, if the patient has "no lesion by light microscopy" I would treat, and if he has advanced proliferative I wouldn't.

Hamburger: The example of amyloidosis is very clear. When amyloidosis is revealed by renal biopsy in a case of nephrotic syndrome, it is a contra-indication to treatment with steroids.

Wilson: No controlled trials of steroid therapy have been carried out in these conditions, and their value, therefore, is purely a matter of personal opinion.

Milne: I did raise two other questions in amyloid, Prof. Wilson. One was that when there is a clear-cut remediable cause (which I agree is rare) the diagnosis of amyloid by renal biopsy is of real practical value to the patient. The other is the common development of acute renal failure due to renal vein thrombosis. I think that these patients should be treated differently to other cases of acute oliguric renal failure and therefore an accurate diagnosis is essential.

de Wardener: I think we are confining ourselves too much to therapy. You can influence a patient's future and welfare by other means. If someone is found at an insurance examination or on trying to join some firm to have proteinuria and is turned down, one can sometimes enable him to pass through by showing a normal renal biopsy to the medical officer.

Rich: Of course, some of these may turn out to have incipient nephrosis.

Pirani: We have one case where therapy certainly was influenced—a patient with advanced rheumatoid arthritis who was being considered for extensive orthopaedic procedures. On examination it was discovered that this patient had proteinuria; renal biopsy was done and a very mild incipient amyloidosis was observed. On the basis of the mildness of the lesions, it was decided that the patient had a good chance to survive for a number of years so these orthopaedic procedures were done. These would probably not have been done if the damage had been severe.

Wilson: But was the renal function normal?

Pirani: Yes.

Wilson: Wouldn't you allow your orthopaedic surgeons to operate on a patient with albuminuria and normal renal function, even if you didn't know what the structural lesion was, if there was any real indication for the operation?

Pirani: Yes, very probably.

de Wardener: Then there is haematuria. Prof. Rosenheim pointed out earlier that you could save the patient a lot of trauma from the surgeons if you can show them that the lesions are in the parenchyma.

Raaschou: There is another field in which renal biopsy may be of therapeutic value: if it is correct, as Dr. Brun and I postulated yesterday, that acute diffuse pyelonephritis can be the cause of acute anuria, then the cultivation of bacteria from the biopsied kidney tissue may be of significance for antibiotic therapy.

Wilson: Is there any evidence that in fact this is the case? I think that one must put in the proviso that other methods of investigation will not give the information required. We now have many methods of investigation of pyelonephritis or latent pyelonephritis (for example, bacterial counts and provocation by pyrogens). I don't know that renal biopsy in suspected pyelonephritis will give you any more reliable information than the current available methods of diagnosis.

Joekes: We have cultured the urine routinely at intervals from all patients with oliguric renal failure. From at least 50 per cent of such patients at some time or other we obtain a positive urine culture. So that from the urine it is impossible to determine whether or not an infected lesion is causing the acute renal failure. If you are faced with an oliguric patient in renal failure, and the possibility exists that this could be pyelonephritis, then I would accept that as an absolute indication to attempt to culture from the kidney. Certainly on two occasions, when we have grown an organism from the biopsy and treated the patient with the selected antimicrobial agent, the patient recovered, and renal function improved.

Wilson: Do you mean that the relief of anuria has been proved to be the result of antibiotic therapy?

Joekes: No, of course there is no proof of that.

Milne: To return to amyloidosis: is not the question of amputation of a limb with osteomyelitis positive therapy? Or the use of anticoagulants in early renal vein thrombosis? How are you going to get that information otherwise?

Wilson: How does renal biopsy help you to diagnose renal vein thrombosis?

Pirani: One of the criteria is the membranous change of the glomerular capillaries (which of course is not specific). Something that is

much more characteristic is the presence of interstitial oedema and fibrosis out of proportion to the glomerular damage; this is something we have never encountered to the same degree in the nephrotic syndrome due to other conditions.

Rich: Dr. Pirani, wouldn't you say that that doesn't necessarily happen in renal vein thrombosis, and that it may occur in nephrosis without renal vein thrombosis? In our files we have cases of nephrosis without vein thrombosis, in which there is interstitial fibrosis out of proportion to any glomerular damage, and cases of vein thrombosis with nephrosis in which there is no fibrosis.

Pirani: We have been able to diagnose two cases of renal vein thrombosis by renal biopsy in this fashion.

Milne: You have taken a more general view than the cases I was considering. I am merely saying that if I knew without any shadow of doubt that a patient had a renal amyloidosis, and then suddenly, say six months later, that patient developed without any apparent reason an oliguric renal failure, I would be 95 per cent certain that that was due to a secondary renal vein thrombosis. It seems to me that the logical way to treat that patient is to use effective anticoagulant therapy.

Rosenheim: If you believe this, wouldn't you use long-term prophylactic anticoagulants whenever you diagnose amyloid disease?

Milne: One has to weigh the discomforts and dangers of long-term anticoagulant therapy against the possible need for it. It is not a clear-cut problem.

Rosenheim: What percentage of patients with amyloidosis do go on to develop renal vein thrombosis?

Milne: In our necropsy series, on careful examination at least 30 per cent of our cases of secondary amyloid had renal vein thrombosis. Could I just remark that in the renal vein thrombosis of large veins I think venography is infinitely superior to percutaneous renal biopsy as a diagnostic tool.

Rich: Is there any evidence that anticoagulant therapy will do any good after you have a degree of thrombosis that will produce anuria?

Milne: This is a difficult question. One will never know unless one tries.

Pirani: A point that has not been raised is the correlation between renal function tests (and other laboratory tests) and morphological

changes. At times we have found advanced glomerular disease with good functional status of the kidney, and this dissociation may help us considerably in reaching a clinical decision.

Wilson: What kind of cases are these?

Pirani: We have seen this in lupus and in amyloidosis. In amyloidosis I think the involvement of the glomeruli may be quite advanced and still the renal function may be quite good.

Hardwicke: In some of the more chronic proliferative forms you can see very severe damage with practically normal renal function tests—endogenous creatinine clearances of 75 per cent of normal or over.

Kark: I think that the time has come to look upon renal biopsy as one more test which we employ in the study of patients with renal disease. When we feel we have an answer from other tests, then we don't need to do a renal biopsy, but when we don't have an answer, then we do need a biopsy. In the care of my patients, I like to know as much as I can about every part of them.

With regard to conditions in which the biopsy has done something that nothing else can ever do, I first want to point out the example of lupus, where finding a minimal lesion is going to let us use small doses of steroids over a long period of time. I know at present no other way of differentiating between a serious glomerulonephritis in lupus and a glomerulitis. When we get to the point where we will be able to distinguish—by tests on the urine or with antinuclear antibody titres—which patient has glomerulonephritis and which glomerulitis (and therefore the dosage of steroids required for treatment), then I won't feel the need of doing renal biopsies in those patients.

With regard to renal vein thrombosis, Dr. Pirani didn't mention the margination of leucocytes—and perhaps periglomerular lymph space enlargement—which doesn't occur in any type of membranous glomerulonephritis that I know of, except in renal vein thrombosis. We have had three patients now, who were going along with repetitive thrombotic episodes involving their lungs and other parts of their body and in whom a diagnosis of renal vein thrombosis was not certain. From renal biopsy we were able to decide that this was renal vein thrombosis, and have instituted long-term anticoagulant therapy. One of these cases has been reported, and that patient has been very well now for nearly ten years.

I must disagree with Prof. Wilson about the nephrotic syndrome. I know no other way of differentiating patients with so-called adult lipoid nephrosis from those with very severe glomerular damage. I would never dream of using steroids in large amounts in patients with very severe glomerular damage, because I am worried that the patient will deteriorate. I know that I can vigorously use steroids in the ones with the so-called lipoid nephrosis and get them well.

With regard to amyloid, I have a strong feeling (again this is not proven) that in amyloidosis not only does steroid not help, but that the disease increases very rapidly. Again renal biopsy is most helpful in deciding on treatment.

With regard to asymptomatic persistent proteinuria, both patients and their insurance agents want to know what is going on inside the kidney. When lesions are found to be mild, the patient is insurable, since prognosis is good. It is most helpful in this situation.

Another situation in which biopsy is useful is bacterial nephritis, in those cases which we have studied and in the type of case which Dr. Blainey spoke about, with haematuria, with organisms in the kidney and no organisms in the urine. Again we have helped the patient by selecting the right antibiotic and continuing therapy as long as that patient was infected.

On the basis of our experience of over a thousand biopsies, in which we have not had a death and in which we have not had to operate on a single patient, I feel that I would not be able to carry out my function as a physician without using this tool to help me in the management of my patients.

Milne: Could we just be let into the secret of "margination of the leucocytes?"

Pirani: By margination of the leucocytes, we mean adherence of leucocytes to the glomerular capillary wall. I am not as sure as Dr. Kark seems to be that this is absolutely specific, but I have seen it consistently in renal vein thrombosis. There is one other condition in which we have seen it and where I have at times suspected the clinical condition on morphological grounds, and that is in very severe cardiac failure. I suspect the situation might not be too dissimilar in renal vein thrombosis and severe cardiac failure, in terms of proteinuria and renal haemodynamics.

Hardwicke: I would like to support Dr. Pirani in that. I don't think margination of the leucocytes is a constant sign, but we have certainly seen this in two cases of renal vein thrombosis. It looks different from any other form of polymorph infiltration which you can see in a glomerulus.

Pirani: The leucocytes are definitely within the lumen of the capillaries. This process is not associated with exudation between the capillary loops. Of course the other features I mentioned before should be present to support the diagnosis of renal vein thrombosis and to exclude the possibility of an exudative type of process.

Darmady: Could I ask Dr. Milne why he thought that biopsy was going to be helpful in aldosteronism (Conn's syndrome)?

Milne: This is sometimes such a difficult diagnosis that you have to use everything you can to help you make the decision. You are presented with a patient with classical biochemical changes; he is either excreting or secreting a large amount of aldosterone. This can be due to primary adrenal disease or primary renal disease, and the operative decision is obviously different, depending on which condition is primary. To me the knowledge from renal biopsy that there is no ischaemic change from an obstructive lesion of renal arteries and no histological abnormality of the kidney other than that produced by potassium deficiency, helps me to say to the surgeon, "In my opinion, this is primary hyperaldosteronism, and I advise you to explore both adrenal glands. If you find a tumour, excise that gland; if you do not find a tumour, excise seven-eighths of the patient's existing adrenal tissue." I think the physician must give as specific instructions as that. Although we always do air insufflation with tomography, this procedure can give both false positives and false negatives and is only one other possibly helpful addition to the differential diagnosis.

Hardwicke: What would you do if you found evidence of pyelonephritis in a patient in whom you suspect Conn's syndrome? There is a good deal of evidence that the potassium deficiency is associated with a high incidence of pyelonephritis.

Milne: Or changes consistent with pyelonephritis?

Hardwicke: Yes.

Milne: Renal biopsy does not help you here, I quite agree, but how do you know that this will be found until you have done the renal biopsy?

Rich: We agree in general that if a patient has no other recognized cause of potassium loss, the hypokalaemic renal lesion is strong evidence of primary hyperaldosteronism; but potassium deficiency from other causes can produce the same renal lesion, can't it?

Milne: I would say that it is possible to develop kalipenic nephropathy both by gastrointestinal and renal potassium loss.

Hardwicke: I would like to return to Prof. Wilson's points about hypertension. We are constantly asked to do renal biopsies in patients with hypertension and we never know whether we should do them or not. I doubt whether severe histological changes on the contralateral side are compatible with normal renal function. I know that Prof. Rosenheim has a large series of cases with unilateral renal disease in which he has done bilateral renal function tests, and that he has a lot of evidence that they are of value in considering whether it is worth taking out the kidney or not. Have you done biopsies on these?

Rosenheim: We haven't done biopsies on them. We have done bilateral renal function tests. I think that there are two points about differential renal function tests. One is the Howard test to see whether the damaged kidney puts out less water and less sodium. The second is the possibility of finding evidence of hypertrophy of the opposite kidney, with an increased inulin clearance.

Hardwicke: The point here is that if one can get the evidence which is required without doing renal biopsy then one is being meddlesome in doing a renal biopsy.

Rosenheim: I am not at all sure that I wouldn't rather have a renal biopsy myself than have bilateral ureteric catheterization.

Black: In this particular context there is no absolute reason why renal biopsy need be percutaneous. If a renal artery stenosis is demonstrated on one side by aortography, then it is probably worth exploring surgically, provided that renal function is either normal or only very slightly deteriorated. While the surgeon is measuring the threshold gradient across the stenosis, the pathologist can be looking at a frozen section and determine vascular damage. I think that open renal biopsy is probably safer than percutaneous.

Rosenheim: It is not easy to biopsy the contralateral kidney when you are measuring pressures in the renal artery.

Black: The kidney is biopsied first. While the pathologist is looking at it, the surgeon is measuring pressure.

Rosenheim: It is the contralateral kidney we are discussing.

Rich: The decision could be terribly difficult from a frozen section of a biopsy.

Black: Yes, but gross vascular damage should be detectable.

Slater: Despite some opinions to the contrary, we consider that steroid therapy is indicated in the treatment of idiopathic nephrotic syndrome of early childhood. In certain of these patients, initial or later lack of responsiveness to steroids poses the problem of how long to continue therapeutic trials with different types of steroids. We feel that a renal biopsy is indicated in these circumstances but are always a little doubtful about just when this should be done. Thus structural changes in the glomeruli may aid in the decision about the nature of the disease and give some clues to the progress of the pathological process.

Rich: Has there been any clinical change toward the so-called nephritic stage—for example, any nitrogen retention?

Slater: My experience is very limited. We have seen children unresponsive or only partially responsive to massive doses of steroid (who previously were responsive) who show no depression in urea or creatinine clearance. In these, hypercellular proliferative changes in the glomeruli or early appearance of hyaline material are interpreted to reflect progress in the disease toward chronicity.

Rich: Would you conclude that the steroids had done no good at all, and that the disease was going on its natural course to sclerosis of the glomeruli, or would you believe you may have slowed it up? And if you believe that, would you want to stop the steroids? I am just trying to see how it would affect the treatment. What would you do if you found beginning sclerosis of the glomeruli—stop steroids, or go on with them and hope that you could slow it up?

Slater: Each child requires individual management, and I would be interested to hear the experience of others. If continued steroid therapy improved the physical state of a child by at least partial suppression of proteinuria, even if there is present what one might consider as irreversible damage, than I would follow that regimen. If I could achieve the same end by diuretic therapy, then I would be inclined not to give steroids. So we go through a series of clinical trials with each child to

see how much we can achieve. In general, we feel it wise to avoid the use of steroids in chronic renal disease because of the unpredictable tendency to hasten the progress to renal failure.

I am concerned about the lack of acceptance of steroid therapy by some members of this symposium. I wonder if the questions that have been raised here are based upon therapy of the nephrotic syndrome of all types, especially in adults, rather than the more specific situation of idiopathic nephrosis of early childhood.

Rosenheim: My views are related to adults, but I have certainly seen response in children. I am not sure whether the long-term prognosis in children has really been altered, but there is no doubt about the short-term improvement.

Slater: We certainly would agree with that.

Vernier: Until the controlled case studies are done, we will never have the answer to this question. As far as I am concerned, Prof. Wilson is entirely right in being critical of the results that have been published. On the other hand, it seems to me that there is adequate evidence of the increasing frequency of complications from the use of steroids in those patients with significant alterations of the glomeruli in the nephrotic syndrome to make a biopsy useful in monitoring the use of steroids in that group of patients. However, I do not feel that biopsy is essential in selecting patients for treatment; you are either an enthusiast for steroid therapy or you are not, and you are likely to remain in one or the other category until the control studies are done. In general, with steroid therapy the frequency of complications has to be balanced against the benefits expected.

Hardwicke: I would like to have the views of people here on what the major complications of steroid therapy are. We don't think we have very many; in fact the only two that we will really admit to are two patients over the age of 40 who died with acute respiratory tract infections during a winter epidemic of influenza. Our steroid dose does not exceed 200 mg./day cortisone and I think this is lower than is commonly used.

Vernier: Dr. R. A. Good, Dr. R. T. Smith and I have accumulated a "chamber of horrors", which we have published (1957. *Pediatrics*, **19,** 95, 272) relating our experiences in the treatment with steroids of renal diseases and other diseases in children. I know that there are

groups who say they do not have complications: the only factors that I know of which modify the incidence of complications are the dosage, the duration of therapy, and the selection and elimination from treatment of patients who have underlying severe renal disease. We still have complications and I feel it is important to select the patients carefully.

Joekes: Diagnosis is essential if we are to treat something correctly, and surely renal biopsy may serve to look for new treatment, which we would not be able to do if we didn't know the diagnosis.

Jennings: Medicine is a notoriously sloppy business: there is a great deal of art in it and that art is usually good, but it would be better if it were a little more scientific. Whenever we can improve accuracy of diagnosis we have a good chance of improving therapy for patients in general. This is one of the great advantages of renal biopsy, even though it may not be a strict advantage to any given patient.

Wilson: I think we all accept that. But we are trying to distinguish between the benefit to the individual patient, and its scientific value. I should like to know what Prof. Iversen would have said if he had been here. Can anybody tell us his views on this?

★ ★ ★ ★

Raaschou: During proof-reading this question was brought before Poul Iversen, who has answered:

"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 so various that in a dialysis department you have to include the information given by a kidney biopsy before you make up your mind to dialyse (or to repeat a dialysis, if the biopsy is not available till after the first dialysis).

"It is my personal opinion that so long as you are not clinically convinced of the cause of the renal symptoms, you ought to perform a kidney biopsy, 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 prog-

nosis, and that is of course always of value for the advice you give your patients.

"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."

★ ★ ★ ★

Hamburger: Could we speak for a few minutes about accidents of renal biopsy?

de Wardener: Before we start, could we have an idea of how many biopsies we represent in this room? (Editorial note: See Table.)

Hamburger: I should mention that, unlike most of the groups represented here, we do not use the percutaneous method. We make a short incision and insert the needle under visual control.

Kark: Has anyone lost a patient directly as a result of a biopsy—by gross bleeding or soon afterwards by shock? (Editorial note: See Table.)

Table (General Discussion)

RENAL BIOPSIES PERFORMED BY GROUPS REPRESENTED AT THE SYMPOSIUM

(Figures are, for the most part, rough estimates)

Baltimore	130
Birmingham	240
Chicago	
Northwestern University	410
University of Illinois	1000
Copenhagen	840
London	
London Hospital	150
Postgraduate Medical School	100
St. Mary's Hospital and Institute of Urology	400
St. Thomas's Hospital	280
University College Hospital	40
Manchester	40
Minneapolis	300
Paris	550
Stockholm	510
Toronto	130
	5120

In the cases biopsied by these groups there have been no deaths. One kidney has been lost (see text).

Hamburger: Our group has had no deaths, but I know of four unpublished cases: one in France, one in Poland, one in Germany, and one in Switzerland.

Rich: How many kidneys have been lost as a result of biopsy?

Brun: One in our department. Dialysis was necessary 24 hours after the biopsy and, probably due more to the heparinization of the patient than to the biopsy, she started to bleed. The kidney was removed and the patient survived.

Rich: Once I was sent for consultation sections of the biopsy and operative material from a case of nephrosis in which the kidney was removed following protracted severe haematuria resulting from the biopsy.

de Wardener: We had one case which had to be opened up because of continuous bleeding. There was a spurting artery on the surface. We were able to stop it with mattress sutures and the kidney did not have to be removed.

Joekes: In one case of acute renal failure with severe haematuria (not among the 400 biopsies reported for our group), we have had to remove one kidney.

Ross: Has it been a general experience that bleeding is more common with amyloid disease? With our comparatively small figures, it has been more common.

Milne: We have done biopsies on 18 cases of amyloidosis and seen no bleeding.

Hamburger: We have done about 20 renal biopsies in cases of amyloidosis without noting any unusual bleeding.

Kark: How many people have seen severe retroperitoneal or perirenal haemorrhage? By this I mean a diagnosable haematoma of 500 cc. or so, where presence of the haematoma is evident by fall in haematocrit, by X-ray or by palpation, or where the pain from it is not relieved by pethidine or morphia.

Ross: We had three at the London Hospital.

Kark: Seven.

Vernier: Two.

Milne: Four.

Earle: Four.

Brun: One. (Brun, C. and Raaschou, F. (1958). *Arch. intern. Med.* **102,** 716.)

Blainey: Two.

de Wardener: Four.

Rich: One, diagnosed by palpation.

Rosenheim: I cannot recall any.

Black: Our incidence of severe haematoma is one out of 40.

Bucht: In 18 patients I have examined at post-mortem, none of them had any perirenal bleeding

Joekes: I have had five, but they were all in the group of acute fulminating glomerulonephritis that Dr Milne was discussing. These patients have a particular tendency to bleed, and I cannot remember any of them who on post-mortem did not show some perirenal bleeding.

Milne: This has been my experience also.

Slater: We have not recognized any patients with gross retroperitoneal haemorrhage. We have had two with gross haematuria, lasting two to three days and requiring transfusion.

Earle: If you do biopsy under direct vision, you can see that every patient gets a little bleeding.

Hamburger: But with the direct-vision method, you need have no anxiety. You wait as long as you want until it doesn't bleed any more.

Movat: Has anyone who has done biopsies for some time seen a perirenal fibrosis forming in a patient who may have had haematuria?

Several Voices: Yes.

Dalgaard: Can that produce hypertension in a patient later on?

Kark: We have wondered about this. We have never seen that and I wondered if anyone else had.

Rich: Any other complications?

Kark: Yes, transient renal colic in a few patients.

Wilson: May I ask how many people regard renal failure as a contra-indication to biopsy? We feel that it is.

Joekes: Patients with acute renal failure may of course include ones with this acute fulminating type of glomerulonephritis, and such a patient is liable to have quite a severe perirenal bleeding. However, in my experience, this is almost exclusive to that particular group.

Milne: I think that saying that renal failure is a contra-indication to biopsy is in my particular practice frankly ridiculous. As well as the individual patient, one has to consider the community of patients being treated. We have a large number of oliguric patients referred

to us, and it has been absolutely essential to decide which patients are potentially recoverable and should be dialysed until they recover or die. Dr. B. H. Scribner, with his arterio-venous shunting apparatus, can dialyse a patient at least 100 times, and I am certain that we could dialyse a patient at least 50 times. Now if you are receiving one patient with acute renal failure every two days, as we do in a busy period, it is absolutely essential to select the recoverable patients for dialysis. Repeated dialysis in an irrecoverable patient is not a kindness to the patient. It turns a natural death from an illness lasting a week or two into a long, protracted, worrying period of up to six months or a year. And if, on the other hand, you say you will dialyse once and let the patient die if he does not have a diuresis, then you will lose about 50 per cent of patients whom you could otherwise restore to perfect health. They are usually young patients in the most important period of their lives.

Rich: Would anyone like to hazard a guess in what percentage of unselected, run-of-the-mill cases with renal diseases renal biopsy would help the patients by improving the way in which they would be managed?

Rosenheim: This is very difficult, because we all have selected groups. I see a lot of hypertensive patients with renal disease, while Dr. Milne gets cases of anuria sent to him. I think this would not be very valuable.

Hamburger: In our group about ten per cent of the renal patients have a renal biopsy.

Rich: Of those that have a biopsy, in roughly what percentage does it help in treatment or alter the method of treatment? You wouldn't want to hazard a guess?

Hamburger: I can't say

★ ★ ★ ★

Rich: If I may be allowed a personal remark, it has been a very great pleasure to meet old acquaintances again, and to have so many other names that have been familiar in the literature suddenly come so pleasingly to life in person at this symposium. It has been a matter of particular regret to all of us that Prof. Iversen, who is so largely responsible for developing the procedure of aspiration renal biopsy, was prevented by illness from being with us.

The stimulating papers and discussions have held the interest of all of us, and have contained so many points that invite an expression of opinion that it is difficult to select specific problems for mention in these closing remarks. I shall therefore try, only, to summarize one or two of my general impressions regarding the advantages and limitations of renal biopsy. It is not to be expected, of course, that each of these opinions will be shared by all members of the symposium, nor is it really desirable that they should be. Friendly differences of opinion between thoughtful students of a problem usually indicate a need of further investigation. A symposium that ended with complete agreement on all points discussed during three days, would hardly be one from which the participants would depart refreshed and stimulated to further study.

I think that no one would doubt that renal biopsy can contribute significantly to basic research into the finer structure and cellular chemistry of the different parts of the human nephron in its normal state, and when altered by disease. Only biopsy can supply fresh renal tissue at particular desired times during human renal diseases and, as is well known, fresh tissue is required for trustworthy results in electron microscopy and in the study of cellular chemistry, particularly enzyme chemistry. No other organ in the body has so complex a functional unit as the kidney. The advance in knowledge of the finer structure of the nephron, which is being gradually contributed by electron microscopy, has already raised fascinating and basically important problems concerning the correlation between the new structural discoveries and normal and disturbed renal function. Perhaps the most visually spectacular of those discoveries is the extraordinary and hitherto unsuspected complex mesh of foot processes of the surface epithelial glomerular cells, and its alteration in conditions of increased permeability of the glomerulus to protein, such as in lipoid nephrosis. What is the functional significance of that remarkable meshwork? Has it no more subtle function than to bind the epithelial cells to the glomerular surface, to prevent them from being washed away by the flow of fluid?

Though the precise recognition of the different parts of the nephron in biopsy material is difficult at present, the attempt to correlate observed finer structural and chemical alterations throughout the

nephron with the functional state of the kidney as determined by appropriate tests at the time of biopsy in the various forms of renal disease, offers the possibility of improving our information regarding the sites of localization of the normal renal functions in the different parts of the nephron, and their alteration in disease; and biopsy at carefully selected periods holds promise of increasing our knowledge of the pathogenesis and natural history of renal diseases, i.e., the nature of the finer alterations that characterize the lesions and their associated functional disturbances at different stages of each disease, and the manner of their origin, progression and regression. These are matters of great importance to future progress in the treatment and control of diseases of the kidney.

Basic information that helps to clarify pathogenesis extends, of course, beyond the pleasures of the satisfaction of curiosity. Though treatments and cures of diseases have often been discovered empirically while pathogenesis was still obscure, if we are not to be left dependent upon sheer trial-and-error in the search for more effective methods of treatment of the life-threatening renal diseases, it is essential to gain a better understanding of the nature and causes of the cellular and acellular tissue alterations that underlie the functional disturbances; and, also, better means of early recognition of their presence before irreparable damage has occurred. How far renal biopsy will be able to serve these ends cannot be accurately predicted; but when we consider the great gaps in our knowledge and understanding of the structure and function of the different parts of the nephron, and the really woefully inadequate state of the treatment and prevention of most of the serious insults to which the kidney is subject, I think that there can be little doubt that the possibility of increasing importantly our understanding of the structure and function of the kidney through renal biopsy, with its related techniques of electron microscopy and cellular chemistry, holds a sufficiently large measure of reasonable promise to encourage the continuation of a vigorous, enthusiastic, and hopeful pursuit of such studies.

What, now, of the clinical value of the procedure? The degree to which biopsy will contribute to more accurate diagnosis in any environment will depend, obviously, upon the diagnostic skill of the physician in charge of the patient, and the skill of the pathologist who studies

the biopsy. A diagnosis based upon the very limited amount of tissue provided by a needle biopsy is often, of course, much more difficult than that based upon an ordinary microscopic section—and the diagnosis of even a large section of kidney is, at times, not without its pitfalls. But granting excellence in the diagnostic ability of the physician and the pathologist, what does biopsy offer to the welfare of the patient?

I would say that in the great majority of cases, the diagnosis reached by an experienced, competent physician from the history, his examination, and appropriate laboratory tests, will not be improved by renal biopsy in a manner that will affect significantly the management of the patient. Indeed, unless the limitations of renal biopsy are given proper weight, a biopsy diagnosis can be quite misleading in certain conditions, as was clear in some of the discussions in this symposium. I share this evaluation with Dr. W. Gordon Walker, who has been in charge of renal biopsy on the medical service at Johns Hopkins.* I do not expect general agreement.

But one cannot stop on this apparently discouraging note, for even though they constitute only a minority of instances of renal disease, there definitely are cases in which the differential diagnosis may reasonably be in question, even in the hands of the most experienced physician, and in which a correct or an incorrect diagnosis may mean the difference between life and death. The number of such instances encountered in any unselected series of cases will, of course, depend upon chance; but in view of the present lack of specific treatments or cures for so many of the major renal diseases, the proportion of cases in which renal biopsy will make a significant difference in the treatment of the patient will, I believe, be rather small at present. But however small statistically the number may be, an addition to the armamentarium of differential diagnosis which saves the life of even a rare patient, or prolongs it significantly in a state of reasonable well-being, is of course highly important. Furthermore, a specific treatment for one or more renal diseases may be discovered at any time, and if that occurs, the

* Analysis of the first 90 consecutive cases of renal disease in which biopsies were studied in the Johns Hopkins Hospital, shows that the biopsy diagnosis altered the clinical diagnosis in seven instances, but the management of the patient was influenced in only three cases.

value of renal biopsy to the individual patient will, of course, increase proportionately.

The occurrence of serious injurious results of renal biopsy has been rare, but up to the present biopsy has not been in widespread, indiscriminate use.

In the individual decision whether to use renal biopsy, and when to use it, I would think it wise to avoid, on the one hand, a too rigid statistical undervaluation of the worth of biopsy, and on the other hand, to avoid overenthusiastic expectations and interpretations of its present value to the patient. Either of those attitudes could be detrimental to the long-run best interests of a potentially very valuable diagnostic procedure.

INDEX OF AUTHORS

Numbers in bold type indicate a contribution in the form of a paper; numbers in plain type refer to contributions to the discussions, and those in italics to references to an author's work.

INDEX OF SUBJECTS

Printed by Spottiswoode, Ballantyne & Co. Ltd., London and Colchester