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Washington 1966**

Vol. 2

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Preface

A congress is a coming together. Like any 'happening', there is no accurate quantitative measurement for either its ingredients or its impact. If a congress is successful, one can choose to underline the depth and breadth of its planning, the diversity of its scope, the admixture of talented guests, the quality of presentation, the status of the science behind the presentation, the level of excellence in the particular scientific field, the interplay of personalities, the geographical setting, and even the weather. Judging from comments received, the III International Congress of Nephrology was a success. We hope that success is not measured merely by the 2134 scientists registered, the 624 abstracts submitted, or the 75 invited papers and 224 free communications presented. We hope that these tangible items are outweighed by the intangibles,—the new ideas appreciated, the constructive criticisms received, the new directions indicated, the new friendships created, and the old ones confirmed. The Congress served as a much needed worldwide inventory of the 'state of the art' of nephrology with its related basic and clinical components. It demonstrated the rapidity with which progress has been made in this remarkable new field of medicine.

In the beginning neither President BERLINER nor myself was in favor of publishing a Proceedings. The lead time for preparing presentations for international congresses is usually so long that publications are often dated or repetitious of already published work. In the end our minds were changed, as they should be, by the evidence at hand: the quality and breadth of the symposium presentations which represented a remarkable cross-section of the entire range of nephrology and the only currently available inventory of the field of nephrology as of 1966. The dramatic advances in dialysis and transplantation were matched by equally important additions in the basic fund of knowledge in related physiology, morphology, bacteriology, pharmacology, and immunology. The challenge of the kidney had obviously been a stimulating force in clinical investigation in the three years since Prague. So we have proceeded with these Proceedings which contain all but one of the invited presentations which comprised the symposia

at the Congress. We have divided them into three volumes, roughly designated as Physiology; Morphology, Immunology, Urology; and Clinical Nephrology. Those who were not able to attend the Congress and who have special interests may obtain the material of their choice from the publisher. For those who registered for the Congress, we hope that these three volumes will recall the happy and fruitful days of September, 1966.

For their work and cooperation we wish to express our sincere gratitude to the individual volume editors, Drs. JOSEPH S. HANDLER, ROBERT H. HEPTINSTALL, and E. LOVELL BECKER, to our Congress Manager, Mrs. HELENA B. LEMP, and to our publisher. Most of all, we wish to thank the authors, who deserve the real credit for writing these Proceedings of the III International Congress of Nephrology.

GEORGE E. SCHREINER, M. D.
Secretary-General
Coordinating Editor

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Washington, D. C. 1966

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I. Electron Microscopy

Proc. 3rd int. Congr. Nephrol., Washington 1966, Vol. 2;
pp. 1-16 (Karger, Basel/New York 1967)

Electron Microscopy of the Normal Tubule

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Stockholm

The present paper summarizes results of studies on the fine structure of renal tubules carried out in our laboratory at the Department of Pathology, Karolinska Institutet Medical School, Stockholm, Sweden, and at the Armed Forces Institute of Pathology, Washington, D.C., U.S.A. The aim has been to establish a foundation for studies of the correlation between structure and function of normal and diseased renal tubules at the electron microscopic level.

One of the goals in these studies has been to determine the effects of different methods used in the preparation of the tissues on the fine structure. Comparisons of the appearance of freeze-etched, frozen-dried, and conventionally 'chemically' fixed tissues described by various workers in the field have suggested that the general structure of membrane-delimited organelles within the cells is essentially similar with the different methods. This means that the appearance of chemically fixed tissues may be considered to reflect the general organization of the cytoplasm.

The studies have accordingly been mainly devoted to the effects of the method of fixative application on fine structure and cellular organization in renal tubules. There seems to exist a delicate balance between hydrostatic, osmotic, and mechanical forces in the tubules and adjacent tissues *in vivo*. This balance is profoundly disturbed in immersion-fixed tissues, as revealed by the collapse and cellular distortion seen in sections of such tissues (Fig. 1). The interruption of blood supply along with mechanical damage would seem to be the two main causes of such distortion.

Fixation by dripping the fixative onto the surface of the kidney of anesthetized animals only partially solves the problem. Although one or two layers of tubules situated immediately below the renal capsule become well fixed with this technique (Fig. 2), the deeper structures are not so satisfactory (Fig. 3). In order to obtain uniformly good fixation, we have perfused the kidneys with glutaraldehyde in lightly nembutal-anesthetized rats and mice. A syringe connected with a flask containing the fixative (1–3% purified glutaraldehyde in 0.1 M phosphate or cacodylate buffer, pH 7.4) was inserted into the abdominal aorta. Immediately after clamping the aorta above the renal artery, perfusion was started at a pressure of 100–110 mm Hg. In order to allow free flow of the fixative solution, the renal vein was opened. Fixation times ranged from 1 to 15 min. With this technique, tubules throughout the nephron showed patent lumens (Fig. 4) and fixation of the cytoplasm was uniformly good, as demonstrated with the electron microscope. Indeed, preservation of fine structure in glutaraldehyde-fixed and 'postosmicated' tissues was superior to purely osmium-fixed tissues in that the cytoplasmic matrix with its abundance of microtubules and fibrils was better revealed.

The studies have been mainly concerned with the structure of mammalian metanephric tubules (the complete nephron of rat and man, proximal tubules of dog, cat, rabbit, and mouse). Since it appeared that observations of the kidneys in lower vertebrates might be helpful in elucidating the functional significance of structural modulations, the mesonephric tubules of two marine teleosts, the glomerular *Cottus scorpius* and the aglomerular *Syngnathus* have been studied. In addition, observations have been made of the actively absorbing mesonephric ureteric duct of *Myxine glutinosa*. Usually, tubules fixed *in vivo* (either in OSO_4 or in glutaraldehyde + OSO_4) were utilized. In the case of human tubules, immersion fixation was the only possible method. This mode of fixation was also used in some instances for tissues from other species. Although conclusions concerning the appearance of extracellular spaces, the configuration of the plasma membrane, and the state of hydration of the cells cannot be drawn from such preparations, reliable information can usually be obtained concerning cytoplasmic organelles, provided peripheral portions of the blocks are investigated.

In order to further study the functional significance of the morphologic observations, techniques for the light microscopic and ultrastructural demonstration of acid phosphatase ('AcPase'), aryl sulphatase, and adenosine triphosphate splitting enzyme activity ('ATPase')

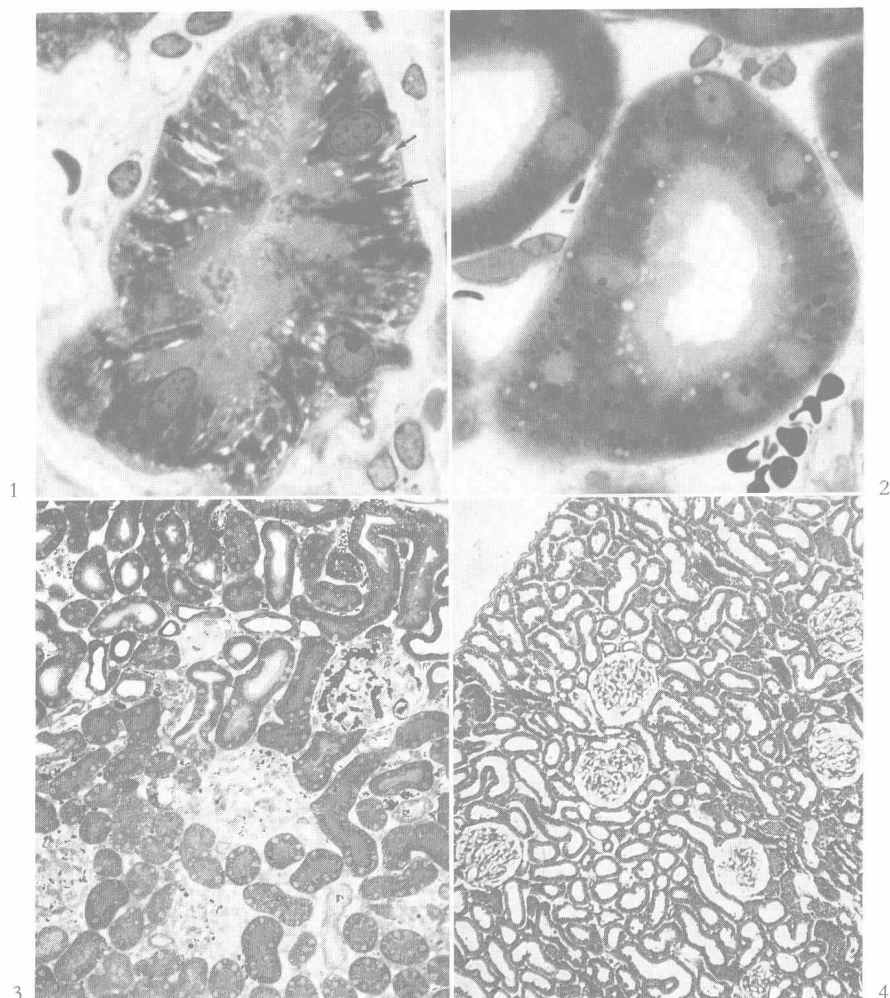


Fig. 1. Proximal convoluted tubule in immersion-fixed normal human biopsy. Note tubular collapse, and widened extracellular spaces at the base (arrows). OsO_4 - γ -collidine; Epon; toluidine blue. $\times 800$.

Fig. 2. Subcapsular, 'drip-fixed' proximal convoluted tubule from rat showing patent lumen. OsO_4 -phosphate; Epon; toluidine blue. $\times 800$.

Fig. 3. Low magnification picture of 'drip-fixed' rat kidney showing collapse of tubules below the well-fixed subcapsular zone (upper portion of the picture). OsO_4 - γ -collidine; Epon; toluidine blue. $\times 110$.

Fig. 4. Glutaraldehyde-perfused kidney from rat. Note patent lumens of tubules throughout the cortex. Glutaraldehyde perfusion 10 min; paraffin; hematoxylin and eosin. $\times 75$.