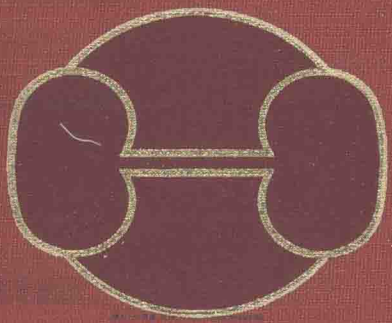
Toxicology of the Kidney

Editor
Jerry B. Hook



TARGET ORGAN TOXICOLOGY SERIES

Editor-in-Chief
Robert L. Dixon

Raven Press

Target Organ Toxicology Series

Toxicology of the Kidney

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Foreword

The *Target Organ Toxicology* monographs have evolved from the need for periodic review of the methods used to assess chemically induced toxicity. In each monograph, experts focus upon the following areas of a particular organ system: (1) a review of the morphology, physiology, biochemistry, cellular biology, and developmental aspects of the system; (2) a description of the means routinely used to assess toxicity; (3) an evaluation of the feasibility of tests used in the assessment of hazards; (4) proposals for applying recent advances in the basic sciences to the development and validation of new test procedures; (5) a description of the incidence of chemically induced human disease; and (6) an assessment of the reliability of laboratory test data extrapolation to humans and of the methods currently used to estimate human risk.

Thus, these monographs should be useful to both students and professionals of toxicology. Each provides a concise description of organ toxicity, including an up-to-date review of the biological processes represented by the target organ, a summary of how chemicals perturb these processes and alter function, and a description of methods by which such toxicity is detected in laboratory animals and humans. Attention is also directed to the identification of probably toxic chemicals and the establishment of exposure standards which are both economically and scientifically feasible, while adequately protecting human health and the environment.

Robert L. Dixon
Editor-in-Chief

Acknowledgments

We are indebted to the National Institute of Environmental Health Sciences, the Society of Toxicology, and the community of academic and federal scientists for the symposia upon which this set of monographs is based. The successful efforts of Joseph R. Borzelleca and Perry J. Gehring in initiating and coordinating the symposia are greatly appreciated.

Preface

The kidney is a frequent target of toxic chemicals. This toxicity may be manifest as immediate organ failure or as a more insidious alteration of function that may not become manifest for many years. In either case, the results may be catastrophic as normal kidney function is necessary to maintain life. How does this happen? How do chemicals produce their toxic effects on the kidney? Why is this organ so susceptible to chemically induced damage?

The kidney is a highly dynamic, complex organ whose normal function is to regulate the composition and volume of our body fluids. The two kidneys together comprise less than 1% of total body mass, and yet these two bean-shaped organs receive nearly 25% of the cardiac output. Consequently, the cells of the kidney are constantly exposed to more chemicals than are the cells of most other organs. Subsequent to its normal functions of filtration, reabsorption, and secretion, the kidney may concentrate these foreign chemicals to toxic levels.

Yet most chemicals are not toxic. Why is this? Which chemicals are toxic? How can we tell? Can we develop methods for identifying toxic compounds? Is there anything we can do once toxicity has occurred?

This book focuses on these and related questions. For the first time in a single volume, in-depth reviews from experts in many fields relating to renal toxicology have been brought together. Each chapter is based on the function of the organ, building on the underlying anatomy and biochemistry. The initial chapters in this volume deal with basic renal physiology using the effects of toxic chemicals to illustrate points. Newer methods of assessing renal physiology including urinalyses, quantitation of enzymes in the urine, and the use of nonmammalian species as tools to evaluate the effects of chemicals are described in depth. Currently approved methods and their shortcomings are evaluated in depth so that the new investigator in this field can use this book as a textbook of physiology and as an introduction to the methods for assessing renal function.

The second group of chapters is concerned with animal testing as a means of evaluating the potential human hazards of a variety of chemicals. The initial chapter describes sophisticated physiological studies performed to evaluate specific mechanisms of acute toxicity. This is followed by the extraordinary detective work of a group of anesthesiologists who observed signs of renal failure in patients after administration of methoxyflurane, an agent that had not been known to produce renal toxicity in animals. This study clearly reiterates the need to evaluate the toxic effects of chemicals in a variety of species before extrapolation of the data to man.

The nonsteroidal antiinflammatory drugs, when used improperly, may produce either acute or chronic damage to the kidney. Questions remain as to the appropriate animal model and by what biochemical mechanism these effects are brought about. The chapter on these agents, with its extensive review of the literature, provides a scholarly assessment of our current understanding of the problem.

Many environmental and industrial chemicals are known to be nephrotoxic, and

this chapter describes the current state of knowledge on the biochemical effects in animals as well as the potential hazards to man based on current exposure standards. A specific chapter details the development of the kidney and focuses on functional assessment of the developing kidney, which may be a more sensitive means of identifying teratogenic effects than the traditional anatomical methods.

The chapter on cephalosporin antibiotics demonstrates how some chemicals can be specific nephrotoxicants as a result of the action of the kidney producing high concentrations within specific cells.

This book is written primarily for those involved in the assessment of the effect of chemicals on the kidney. This includes investigators in industry, academia and government, as well as those who need background in renal toxicology to evaluate the data of others. Graduate students and advanced undergraduates in toxicology and related disciplines as well as physicians and veterinarians dealing with problems of clinical toxicology will find this a valuable textbook of basic information, as well as an authoritative view of the major problem areas in renal toxicology.

Jerry B. Hook

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Use of Renal Function Tests in the Evaluation of Nephrotoxic Effects

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This chapter will review the anatomy, biochemistry, and physiology of the kidney. For the most part, aspects of these disciplines will be discussed that pertain more or less directly to the assessment of nephrotoxicity. Detailed analyses of anatomical or histological material will not be given, since these data probably are least useful for the evaluation of functional nephrotoxicity. Hopefully, the information presented will serve as an appropriate background for the specific topics of nephrotoxicity that follow.

Detailed works on renal function should be consulted as desired (8,44,60,66). These books are all relatively up-to-date, and present renal physiology and biochemistry in various degrees of thoroughness and complexity. The two monographs by Valtin and Sullivan are relatively fundamental, but both are thorough. The two-volume set by Brenner and Rector is the most up-to-date and offers very much more detail than the two previous texts. The *Handbook of Physiology* (44) is also very detailed. Furthermore, this reference work contains many citations to the original literature, and can be used therefore as a guide to much of the earlier works pertaining to renal physiology, biochemistry, and anatomy.

ANATOMICAL AND METABOLIC RELATIONSHIPS

The intrarenal localization of the nephron is depicted in Fig. 1. The anatomical arrangements of the various nephron types are presented as they appear *in situ*. All nephrons have their glomeruli, proximal tubules, and distal tubules in the cortex. Only the loops of Henle of the juxtaglomerular nephrons, along with all the collecting ducts, course deeply into the medulla.

An analysis of the relationship of the renal blood supply to the nephrons is very important and, in part, is also depicted in Fig. 1. This is particularly important as background for studies of nephrotoxicity, because the kidney receives nearly 25% of the cardiac output. This factor alone may increase the possibility of nephrotox-

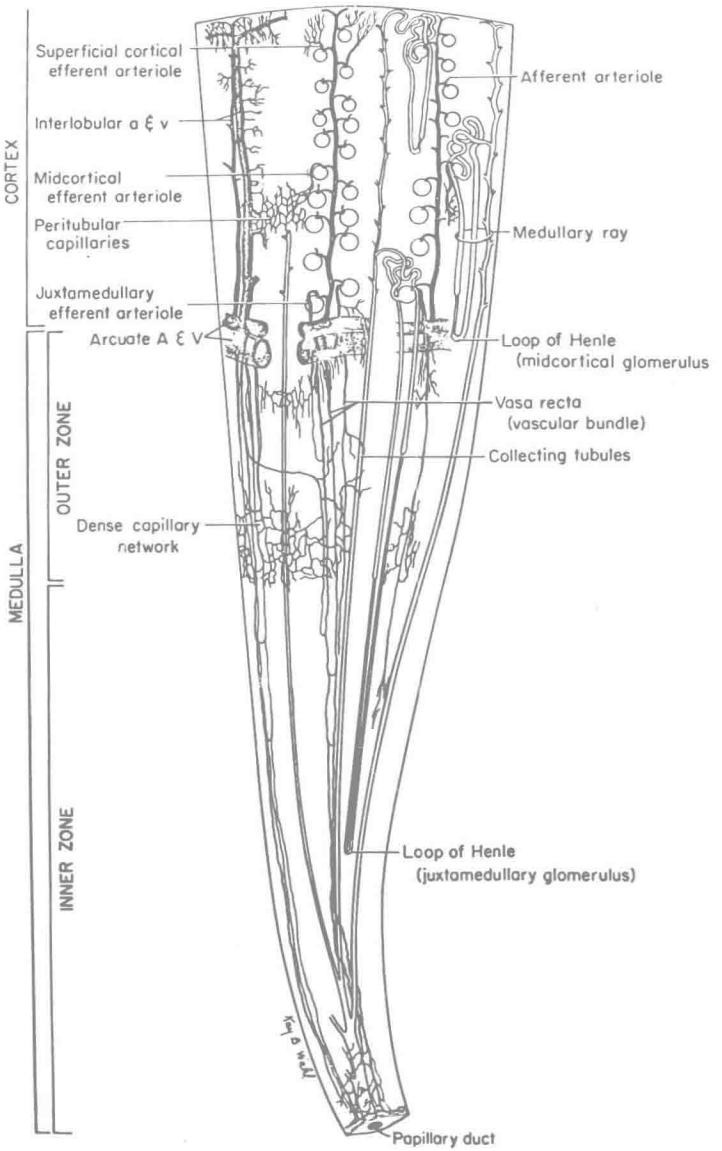


FIG. 1 A cross-section of the renal architecture. (From Sullivan, ref. 60, with permission of the author and Lea & Febiger.)

icity. The renal artery supplies the interlobar arteries (not shown in Fig. 1), and the latter divide into the arcuate arteries. These pass between the medulla and cortex, and from these the interlobular arteries proceed toward the capsule through the cortex. It is from the interlobular arteries that the afferent arterioles leave to form the glomeruli. The renal circulation at this point develops into two capillary

networks that are arranged in series. The glomerulus is the first of these. In the midcortical and superficial cortical areas, the efferent arteriole, which leaves the glomerulus, forms the peritubular capillaries surrounding proximal tubular tissue. The efferent arterioles from the juxtamedullary glomeruli branch into capillaries of approximately equal size, the *vasa recta*. Some branches immediately break into capillary networks around the loops of Henle in the outer medullary regions. Other branches form bundles of vessels that course deep into the medulla before developing into capillary networks.

The intrarenal distribution of blood is not uniform. Under normal circumstances, the cortical regions receive the largest portion of blood supply with lesser amounts being distributed to the inner cortical or medullary regions, the renal papillae, and the perirenal fat and connective tissue. Most of the studies on the renal circulation have been done with either the xenon or krypton washout technique or the localization of radioactive microspheres. With both techniques comparable data are obtained, although the inert gas technique highlights nutrient blood flow and microspheres highlight glomerular blood flow. An example of inert gas washout is seen in Fig. 2. For this procedure, a bolus of saline containing radioactive krypton was injected into a renal artery. The disappearance of the radioactivity from the kidney

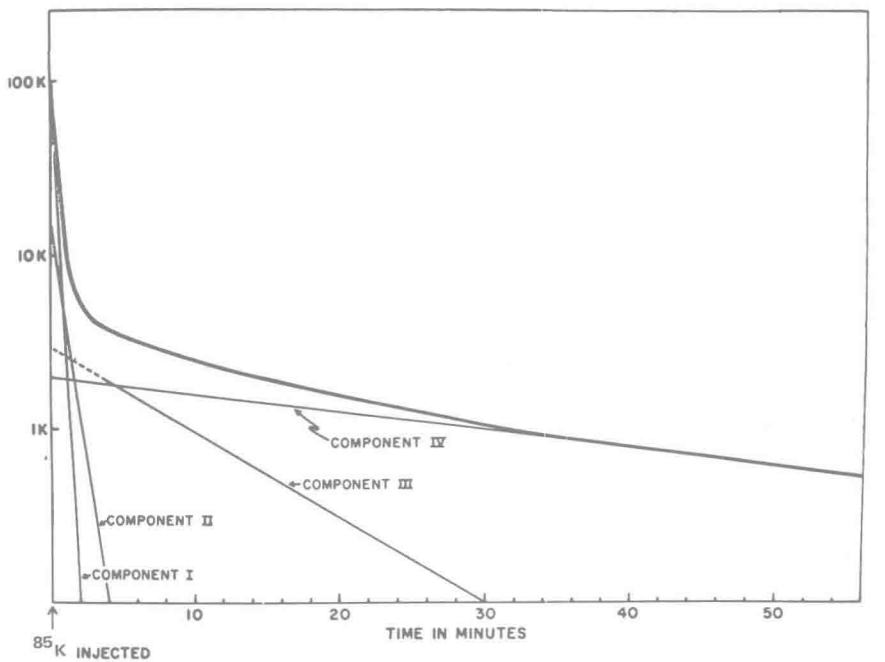


FIG. 2 An idealized ^{85}Kr disappearance curve for the analysis of the intrarenal distribution of blood. The continuous *curved line* represents experimental data, and the *straight lines* the results of the graphic analysis. (From Pitts, 47, with permission from Year Book Publishers, Inc., 1974, as modified from Barger, ref. 2.)

was monitored by an external probe placed over the region of the kidney. The experimental data are depicted by the thick, continuous curved line. The straight lines resulted from graphic analysis and indicate that the experimental data could be resolved into multiple components, each of which is thought to represent a blood flow compartment. Parallel autoradiographic experiments by Barger and others (2,62) permitted assignment of these kinetic compartments to the anatomical entities mentioned above.

Radioactive microspheres have been useful in studies to analyze glomerular blood flow. An analysis of some of the problems encountered have been described by Yarger et al. (70). It is important to know which size microsphere is being used, although both larger (15 μm) and smaller (9 μm) may produce problems. For example, the larger microspheres tended to be trapped in the outer cortex, while the smaller microspheres had a tendency to escape into the medulla. In general, these workers found that radioactive microspheres in the rat overestimated outer cortical blood flow, while an underestimation of deep cortical nephrons was observed. Apparently this was true in both normal and saline expanded rats.

As much as 80 to 85% of total renal blood flow can be associated with cortical regions; 10 to 12% is thought to perfuse the inner medulla, and the remainder of the total blood flow distributes to other areas of the kidney. Just as the total renal blood flow can be altered, so can the intrarenal distribution of blood. Redistribution of the blood supply within these various compartments may be a potential site and/or mechanism of action of various chemical nephrotoxins.

The normal intrarenal distribution of blood is consistent with what is known about intrarenal metabolism, at least as a first approximation. Simply stated, the cortical regions are much better oxygenated than are those of the medulla, and the types of metabolism, that is, oxidative versus glycolytic, are appropriately distributed (32,34). Most of the energy production in the renal cortex comes from oxidative processes, whereas in the medullary regions glycolytic processes appear to be more important. A number of suggestions have been made about renal metabolic processes that might be inhibited by, for example, diuretics such as ethacrynic acid (1). Such effects are thought to be reversible and may underlie diuretic action. It is likely that similar sites of action could be associated with the effects of nephrotoxins. Unlike the proposed diuretic effects, the effects of nephrotoxins would be essentially irreversible. However, specific experimental data relating to this possibility are lacking, or are incomplete.

A variety of substrates are metabolized in the renal cortical tissue. For example, free fatty acids are metabolized but may not be the predominant fuel of respiration in the kidney (8). This, in addition, means that the free fatty acids are not the primary source of fuel for reabsorption of sodium and water by the nephron. Specifically, Pitts (47) suggested that from about 16 to 22% of renal oxidative metabolism is supported by the combustion of fatty acids. The exact contribution is dependent upon the acid-base state of the animal. In the case of acidosis, glutamine accounts for about 40% of the cortical oxidative metabolism, with lactate assuming about 25% of the load. Under conditions of alkalosis, lactate contributes almost

half of the total oxidative metabolism, with glucose assuming a more important role. Clearly, renal oxidative processes are capable of utilizing a variety of substrates (47). Despite what is known, it is difficult to pinpoint a specific, single site of action within the energy-producing mechanisms on which nephrotoxins might act. However, as biochemical techniques and approaches become more commonly used, attention will have to be given to these energy-producing mechanisms as potential sites of action.

In Fig. 3 is presented an idealized view of the gross anatomy of two major nephron types. In the superficial cortical area, the nephrons have short loops of Henle and relatively longer proximal convoluted tubules and straight parts of proximal tubule, that is, *pars recta*. The juxtamedullary nephrons have longer loops of Henle and, hence, the proximal and distal segments comprise proportionately less of the total nephron length. The frequency with which juxtamedullary nephrons occur, the length of the thin loops, etc., vary considerably with species. For example, in the gerbil or kangaroo rat, the loops are very long, actually reaching deep into the papilla, which in these species protrudes into the ureter. These animals live in arid climates and water conservation is a necessity. In an animal such as the beaver or hippopotamus, there is almost no renal medulla at all. Man, dog, and

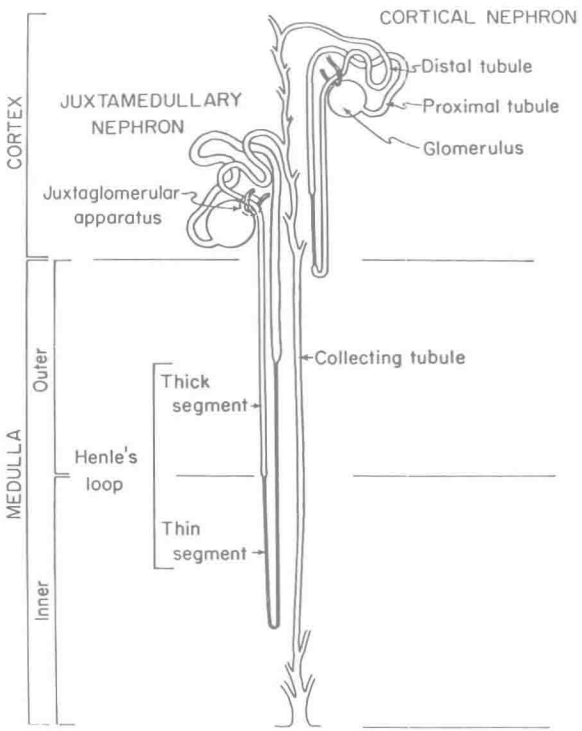


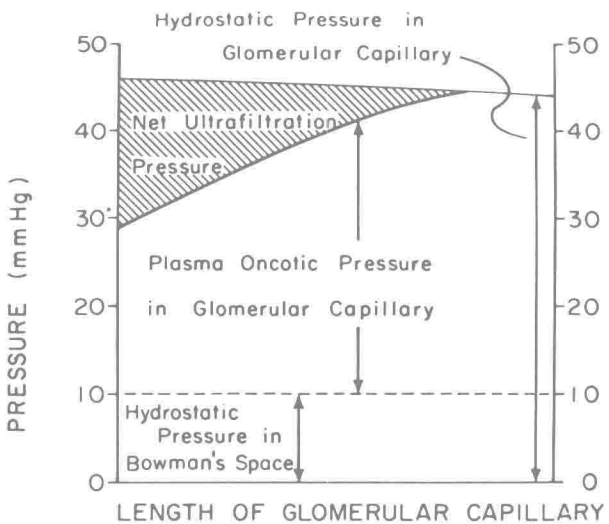
FIG. 3. The anatomy of the nephron. (From Sullivan, ref. 60, with permission of the author and Lea & Febiger.)

many of the common laboratory rodents fall between these extremes. Detailed gross anatomical considerations of nephron architecture were described by Sperber in the 1940s (56), and were the anatomical basis for the development of the countercurrent multiplication theory of urinary concentration first proposed by Wirz et al. in the 1950s (68).

PHYSIOLOGICAL RELATIONSHIPS

In an effort to examine the nephron functions related to nephrotoxicity, it is important to examine glomerular and tubular events that occur throughout the nephron. Emphasis will be placed on those functional characteristics that are particularly important in the evaluation of nephrotoxicity.

As indicated previously, the blood enters *the glomerulus* through the afferent arteriole and leaves through the efferent arteriole. While the blood is in this capillary net, filtration occurs. The filtration process is regulated by a variety of forces, which are summarized in Fig. 4. The arterial blood pressure is responsible for generating the glomerular capillary pressure that serves as the main driving force



BALANCE OF MEAN VALUES

Hydrostatic pressure in glomerular capillary	45 mmHg
Hydrostatic pressure in Bowman's space	10
Plasma oncotic pressure in glomerular capillary	27
Oncotic pressure of fluid in Bowman's space	0
Net ultrafiltration pressure	8 mmHg

FIG. 4. Forces involved in glomerular ultrafiltration in the rat. (From Valtin, ref. 66, with permission of the author and Little, Brown, and Co., © 1973.)

for filtration. This force is opposed by the colloid osmotic pressure of the capillary and hydrostatic pressure of the capsular fluid, the so-called intracapsular pressure. A mean net filtration pressure of the order of 6 to 10 mm Hg results and drives a virtually protein-free filtrate into the proximal convoluted tubule. The exact value for the net filtration pressure is still debated (44). The proximal tubular fluid is not completely free of protein, but the quantity filtered is small with normal, healthy glomeruli. The protein is reabsorbed from the proximal tubular fluid. In man, as much as 30 mg of protein per 100 ml of glomerular filtrate is reabsorbed, resulting in a urine which is virtually protein free.

There are so-called functional proteinurias, which occur in normal, healthy young adults. For example, proteinuria is noted sometimes with exercise, sometimes with fever, exposure to extremes of heat or cold, and sometimes even with emotional stress. However, in humans and in many laboratory animals with normal renal function, the production of a protein-free urine generally is anticipated.

All of the constituents of plasma that are not bound to plasma proteins are filtered freely at the glomerulus and exist in the immediately postglomerular fluid at concentrations approximately the same as those in plasma. Obviously, anything bound to the plasma proteins (whether normally occurring, such as calcium; or foreign, such as a drug) is retained by the glomerular sieve just as effectively as the protein itself. Hence a total plasma concentration of 10 mg/ml of drug X, which is 50% bound to plasma albumin and cleared from the blood only by filtration, would result in an early proximal tubular fluid concentration of 5 mg/100 ml. Quantitation of the filtration process is done (Fig. 5) by adding to the plasma a substance that is freely filtered at the glomerulus, is not reabsorbed by the nephron, has minimal pharmacologic effects, etc. Precisely timed urine samples are collected, along with plasma samples. Quantitative measurements of the marker substance are made both in urine and in plasma, and glomerular filtration rate (GFR) is calculated by the well-known clearance formula (see Fig. 5). Usually inulin is used as the marker,

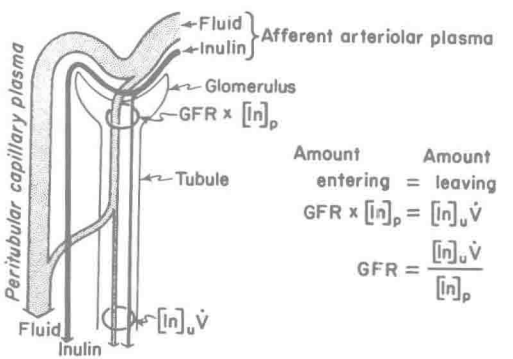


FIG. 5. Measurement of filtration. (From Sullivan, ref. 60, with permission of the author and Lea & Febiger.)