Environmental Health Perspectives
Vol. 15, pp. 55-71, 1976

Renal Function Tests: What Do They Mean? A Review of Renal Anatomy, Biochemistry, and Physiology

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Renal physiology, biochemistry, and anatomy are reviewed. For the most part, those aspects of these disciplines will be discussed which relate directly to the question of the evaluation of nephrotoxicity. In addition, emphasis is placed on those procedures and techniques which are useful in the evaluation of nephrotoxicity. A detailed discussion of histological and anatomical considerations is not given, since this is probably the least useful criterion for evaluation of renal damage. This information is intended as background for the remainder of the symposium which will be directed toward an understanding of specific nephrotoxicity phenomena.

There are several references or textbooks in which suitable review material is available (*1-4*), all relatively up-to-date, and which 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. Pitts' text is not only complete, but is also much more detailed than the previous two texts. The *Handbook of Physiology* presents the most detail of these four. Furthermore, this reference work contains many citations to the original literature, and can be used therefore as a guide to much of the original literature which pertains to renal physiology, biochemistry, and anatomy.

The intrarenal localization of the nephron is depicted in Figure 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, dip into the medullary regions.

An analysis of the relationship of the renal blood supply to the nephrons is very important and, in part, is also depicted in Figure 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 a substance producing a nephrotoxic insult once it is in the vascular system. The renal artery supplies the interlobar arteries (neither of which is shown in Fig. 1) and the latter divides into the arcuate arteries. These course between the medulla and cortex and send interlobular arteries 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 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

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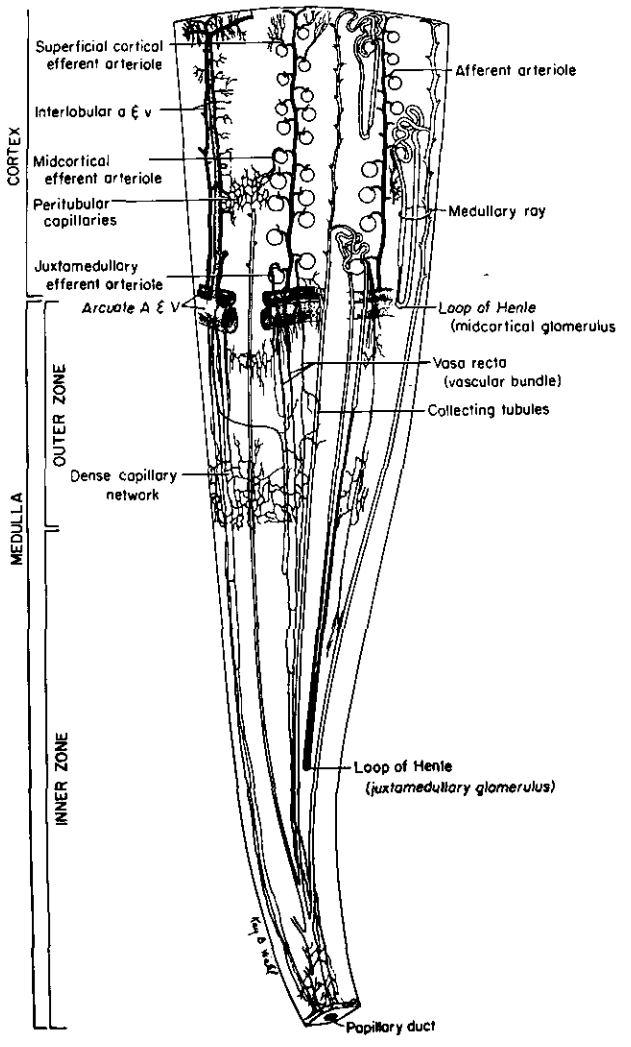


FIGURE 1. A cross section of the renal architecture. From Sullivan (*1*) with permission of the author and Lea and Febiger.

branch into capillaries of approximately equal size, the *vesa recta*. Some branches immediately break into capillary networks around loops of Henle in the outer medullary regions. Other branches form bundles of vessels which course deep into the medulla before developing into capillary networks.

The intrarenal blood flow 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 outer medullary areas, still less to the inner most medullary regions, for example, the renal papillae, and the remainder being distributed to 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. Essentially comparable data are obtained, and an example of krypton washout data is seen in Figure 2. For this procedure a bolus of saline containing radioactive krypton is injected into a renal artery. The disappearance of the radioactivity from the kidney is monitored by an external probe placed over the region of the kidney. The experimental data are depicted by the dark, continuous curved line. The straight lines indicate that this curve can be resolved into multiple components, indicating three or four blood flow compartments. Autoradiographic analysis by Barger and others (*5,6*) permitted assignment of these kinetic compartments to the anatomical entities mentioned above. As much as 80–85% of total renal blood flow can be associated with cortical regions; 10–12% is thought to perfuse the inner medulla, and the remainder of the total blood flow distributes to other aspects 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 quite consistent with what is known about in-

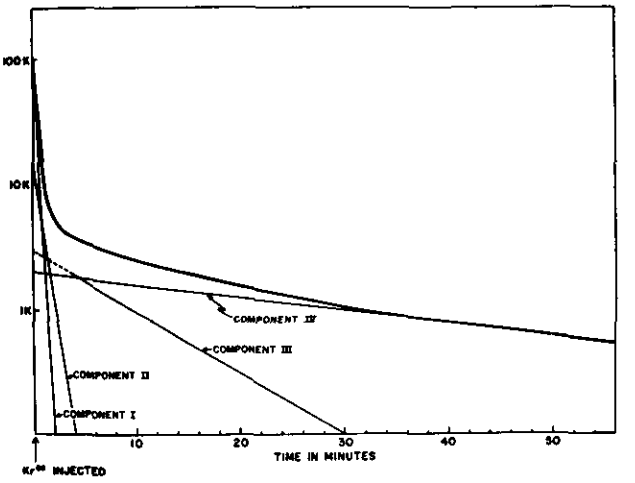


FIGURE 2. An idealized Kr^{85} disappearance curve for the analysis of the intrarenal distribution of blood. The continuous curved line represents experimental data and the straight lines the results of the graphic analysis. From Pitts (*3*) with permission of the author and Yearbook Medical Publishers.

trarenal metabolism at least as a first approximation. Simply stated, the cortical regions are much better oxygenated than the medullary regions, and the types of metabolism, that is oxidative vs. glycolytic, are appropriately distributed (*7,8*). 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 which might be inhibited by, for example, diuretics (*9*). It is likely that similar sites of action could be associated with the effects of nephrotoxins. However, specific experimental data relating to this possibility are lacking, or are incomplete.

A variety of substrates are metabolized in the renal cortical tissue. There seems to be little doubt that free fatty acids are metabolized to some extent, but Pitts (*3*) would deny that this substrate type is a predominant fuel of respiration in the kidney. 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 suggests 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. Despite what is known, it is difficult to pinpoint a specific, single site of action within the energetic mechanism on which nephrotoxins might act. However, as biochemical techniques and approaches become more commonly used, attention will have to be given to these energetic mechanisms as potential sites of action.

In Figure 3 is presented an idealized view of the gross anatomy of the nephron. In the superficial cortical area the nephrons have short loops of Henle, and correspondingly relatively longer proximal convoluted tubules and straight parts of proximal tubule, that is, the 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

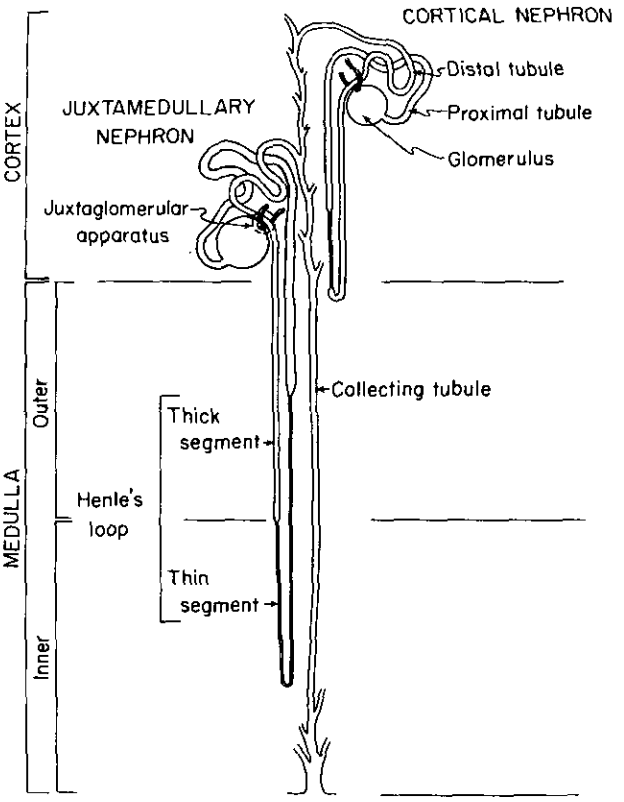


FIGURE 3. The anatomy of the nephron. From Sullivan (*1*) with permission.

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 many of the common laboratory rats, etc. fall between these extremes. Very detailed gross anatomical considerations such as these were described by Sperber in the 1940's (*10*), and were the anatomical basis for the development of the countercurrent multiplication theory of urinary concentration first proposed by Wirz, Hargitay, and Kuhn in the 1950's (*11*).

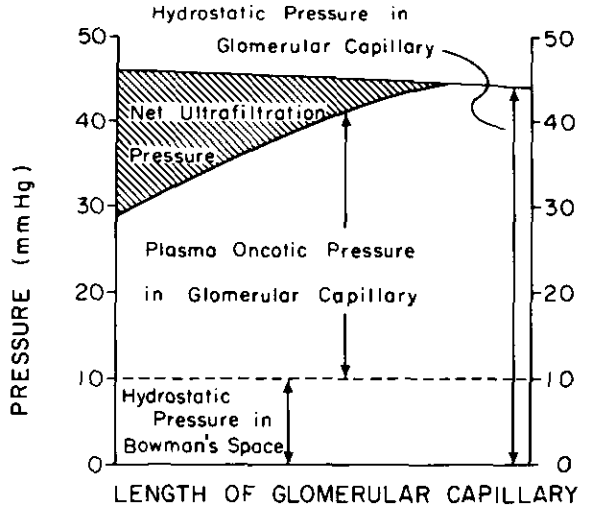
In an effort to examine the important nephron functions related to nephrotoxicity, it is important to examine glomerular and tubular events that occur throughout the nephron. As much as possible emphasis will be placed on those functional characteristics that are particularly important in the evaluation 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 we presented in Figure 4. The arterial blood pressure is responsible for generating the glomerular capillary pressure that serves as the main driving force 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. In any event, a mean net filtration pressure of the order of 6-10 mm Hg results and drives a virtually protein-free filtrate into the proximal convoluted tubule. The proximal tubular fluid is not completely free of protein, but the quantity is small with normal, healthy glomeruli. For the most part the protein that passes the glomerular filter is reabsorbed later on in the proximal tubule. Ordinarily a concentration of protein of the order of 30 mg/100 ml of glomerular filtrate is acted upon by proximal tubular reabsorptive mechanisms so that no protein appears in the final urine of man.

There are so-called functional proteninurias, which occur in absolutely 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 laboratory animals with normal renal function, the production of a protein-free urine is generally anticipated. As indicated above, not only is this because the glomerulus is an effective filter, but also partly because the modest amount of filtered protein is reabsorbed in the proximal tubule.

All of the other constituents of plasma that are not bound to plasma proteins are filtered freely at the glomerulus and exist in the proximal tubular fluid at concentrations the same as those in plasma. Obviously anything bound to the plasma proteins (whether normally occurring such as calcium, or foreign, such as drugs) is retained by the glomerular sieve just as effectively as the protein itself. Hence, a total plasma concentration of 10 mg/100 ml of drug X which is 50% bound to plasma albumin and cleared from the blood only by filtration, would result in a proximal tubular fluid concentration of 5 mg/100 ml.

It is possible to quantitate the magnitude of this filtration process. This is done (Fig. 5) by adding to the plasma a substance that is freely filterable at the glomerulus, is not reabsorbed by the nephron, has minimal pharmacologic effects,



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

FIGURE 4. Forces involved in glomerular ultrafiltration in the rat. From Valtin (2) with permission of the author and Little, Brown & Co.

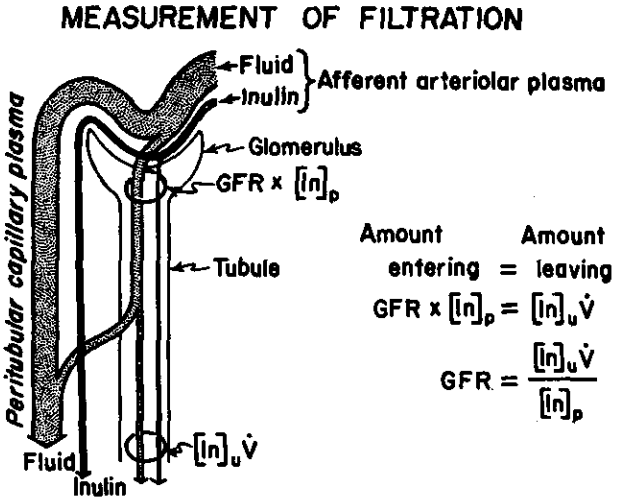


FIGURE 5. Measurement of filtration. From Sullivan (1) with permission.

etc. Then precisely timed urine samples are collected quantitatively, along with plasma samples. Quantitative measurements of the marker substance are made both in urine and plasma, and glomerular filtration rate (GFR) is calculated by the well known clearance formula (see Fig. 5). Usually inulin is used as the marker, although creatinine is used under some circumstances.

In the proximal tubule several events of importance to the pharmacologist-toxicologist occur. These activities can be conveniently divided into those that deal with organic compounds and those that deal with inorganic substances. Glucose, which is filtered at the glomerulus, is reabsorbed virtually completely by the proximal tubule as the fluid passes through it. In the human subject with diabetes mellitus the appearance of large amounts of glucose in the urine is indicative of filtration of larger than normal amounts of glucose. In a human subject or an animal treated with a nephrotoxin, the appearance of glucose in the urine is also a common event. Under these circumstances, the abnormal excretion of glucose is not a reflection of enhanced filtration, but probably of damage to the proximal tubular reabsorptive mechanism.

The reabsorptive process can also be quantitated, and this is depicted in Figure 6. By knowing the amount of material filtered per unit time and the amount excreted in the urine per unit time, it is possible to determine whether or not reabsorption occurs, and the magnitude of this event. In this example (Fig. 6), the amount of reabsorption is calculated as the difference between the filtered load (i.e., GFR multiplied by the plasma concentration) and urinary excretion

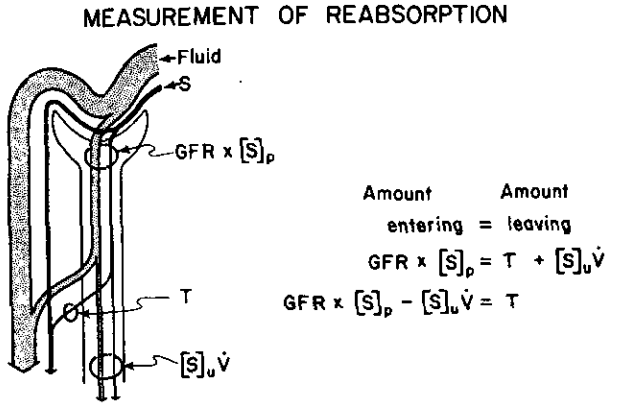


FIGURE 6. Measurement of reabsorption. From Sullivan (*1*) with permission.

for the substance in question, and this difference is expressed as *T*.

A variety of organic compounds are secreted actively in the proximal tubular region of the nephron. These secretory activities are typified by two rather general types of secretory systems, the so-called organic anion secretory process and a similar one for organic cations. These activities can be quantitated exactly as with reabsorption except that the filtered load is now less than the quantity excreted (Fig. 7). *T*, the quantity transported, is still calculated as the difference between filtered load and quantity excreted. That is, the amount of material appearing in the urine is greater than that filtered by an amount equal to *T*.

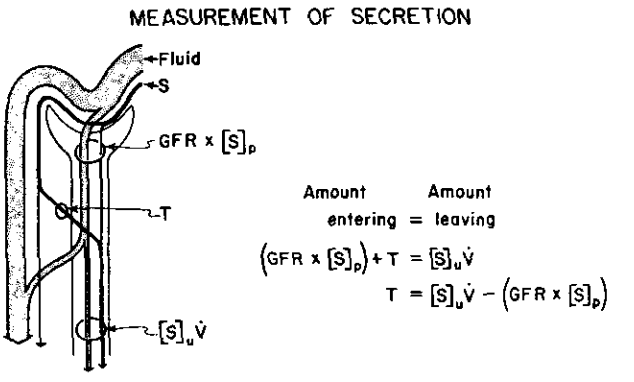


FIGURE 7. Measurement of secretion. From Sullivan (*1*) with permission.

The organic anion process is typified by the active tubular secretion of p-aminohippurate (PAH), which may be used to measure total renal blood flow. This means that by the combined efforts of active tubular secretion in the proximal tubule and glomerular filtration, the PAH entering the kidney by the arterial supply is removed virtually completely from the blood before it leaves the kidney. In man and the dog, approximately 80–90% of the PAH in the arterial blood is removed by the time that blood leaves the kidney as the venous effluent. The proximal convoluted tubule is one of the sites of this secretory activity, but recent studies with the isolated perfused tubule indicate that the region of greatest activity is the pars recta, that is, the straight part of the proximal tubule (*12*) (refer to Fig. 3). Organic cations such as tetraethylammonium (TEA) or *N*-methylnicotinamide (NMN), also are

actively secreted. This event also occurs in the proximal tubule although the details of the anatomical localization of this process are not as well worked out as for the organic anions. In any event, these two processes are tubular functions and might serve as a site or sites of action of nephrotoxins.

There are, however, some relatively poorly understood aspects of these secretory processes. In the early 1950's, Cross, Mudge, and Taggart (*13-15*) were responsible for demonstrating and quantitating the effectiveness of acetate, lactate, and pyruvate in stimulating the transport of PAH. This stimulation was not found to be related to simple metabolism of the acetate for energy, since many other substrates which were metabolized at least as effectively by renal cortical tissue did not stimulate PAH transport, or stimulated it less well. Although some of these considerations have been questioned lately by Hook and his colleagues (personal communication), the issues are still far from clear-cut. Possibly one or another tricarboxylic acid cycle intermediate may serve as a regulator of PAH transport. However, even if true, specific mechanistic explanations are lacking.

The stimulation of PAH transport by several metabolic substrates can be demonstrated both in the intact animal by alterations in the PAH T_m (i.e., the maximal rate of tubular transport), and under *in vitro* conditions by enhancement of the tissue accumulation of PAH. This substrate requirement is even more complicated than it appears. For example, apparently there is a distinct interaction between substrate and potassium in the regulation of this transport process. Clearly there are many transport processes for organic compounds that require inorganic electrolytes (*16*), but the striking involvement of the potassium ion in this renal organic anion transport process is unusual if not unique.

The details of substrate and electrolyte effects with respect to organic cation transport are much less well documented and understood (*17,18*). Some investigators report that acetate will also enhance the transport of TEA or NMN, but not all workers agree. It has also been reported that potassium is required for TEA and NMN transport, but the supporting evidence is less striking than with the organic anions.

Of course, the major activities of the kidney pertain to homeostasis, and to perform this function massive amounts of both fluid and electrolytes are reabsorbed in the proximal tubule.

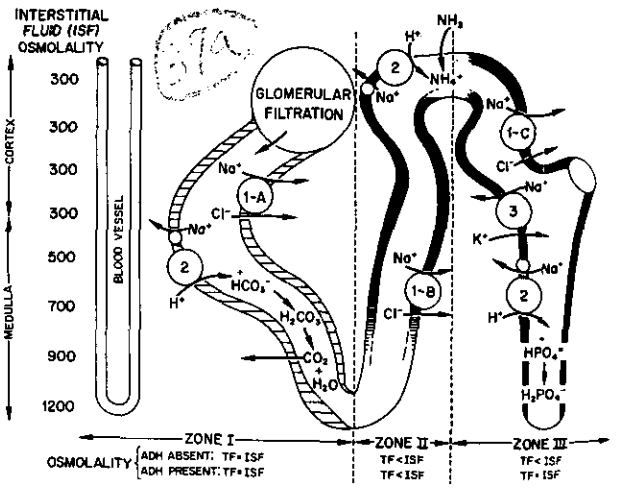


FIGURE 8. A pharmacologist's-toxicologist's view of the nephron. This schematic representation emphasizes the major electrolyte transport systems. From Mudge (*19*) with permission of the author and MacMillan Company.

Figure 8 depicts a pharmacologist's or toxicologist's view of the nephron, emphasizing the various inorganic electrolyte transport systems associated with the maintenance of homeostasis. As much as 50-80% of the volume of fluid filtered at the glomerulus is reabsorbed before the tubular fluid passes into the loop of Henle. The reabsorptive process is an isosmotic one. Hence, the total osmolar concentration of the proximal tubular fluid immediately beyond the glomerulus is the same as the total osmolar concentration of the proximal tubular fluid at the end of the proximal tubule, or at least as far down the proximal tubule as measurements can be made. Said another way, the fluid that is removed from this segment of the nephron is removed with a solute concentration identical to that of the fluid remaining in the tubule. Of course, the major ionic constituents of these fluids are sodium and chloride, although both potassium and bicarbonate also are reabsorbed in this section of the nephron.

As the fluid moves out of the proximal tubule into the loop of Henle, the fluid enters into that segment of the nephron responsible for the development of a concentrated urine. The details of these mechanisms are available from a number of sources and will not be discussed here. Suffice it to say that in the presence of high concentrations of antidiuretic hormone (ADH) in the blood, and due to the differential permeabilities of the loops of Henle, the tubular fluid in Henle's loop, as well

as the interstitial fluid, can become hyperosmotic to plasma. When the tubular fluid flows through the collecting ducts in these regions of high osmolar concentration, passive removal of water along its osmotic gradient occurs, which causes the production of a hyperosmotic urine. This process is assisted by the loops of Henle acting as countercurrent multipliers, the vasa recta acting as countercurrent exchangers, and the kidney being constructed anatomically to allow the final tubular fluid to pass through the regions of high osmolar concentration. Unlike the active transport systems in other segments of the nephron where active transport of sodium seems to predominate, the initiating event for the development of the countercurrent multiplication effect in the loops of Henle is thought to be an active chloride transport located in the thick ascending limb of Henle's loop. These data have been obtained by Burg and others *(20,21)* with the isolated perfused tubule technique. This technique, although very difficult, does allow for a segment by segment analysis of nephron function.

Regardless of the details of the mechanisms involved in the development of a concentrated or dilute urine, it is important to be aware that these processes occur. In the development of nephrotoxicity due to various chemicals an early effect noted is the loss of the ability of the kidney to concentrate urine. Much of the morphological evidence indicates extensive proximal tubular damage occurs after an acute nephrotoxic insult, so it is not entirely clear what the loss of urinary concentrating ability means. However, it is possible this effect is mediated through alterations in intrarenal blood flow or by washout of the corticomedullary concentration gradient.

In the distal tubule (cortical diluting segment) active sodium chloride transport occurs essentially independent of fluid movement. Somewhere late in the distal tubule or perhaps the early collecting duct, potassium ion enters the tubular fluid primarily by passive means. The exact mechanisms responsible for the entry of potassium are still debated, but there is no doubt that the major component of potassium entry into the distal tubule is a passive one *(22,23)*. Depending on the species, more or less active transport may be involved. Associated with the secretory function for potassium may also be a modest active potassium reabsorption and some active sodium reabsorption *(22)*. The classical presentation of an exchange mechanism of potassium for sodium is difficult to reconcile on a stoichiometric basis with all of these recent laboratory data. There seems to be little doubt that at a site where sodium is reabsorbed potassium entry into the tubular fluid also occurs, but the stoichiometry of the situation does not seem to dictate the one-for-one exchange that has been depicted so often.

Finally, the tubular fluid passes through the collecting duct and ultimately out of the kidney. As indicated above, it is in the collecting duct that the final urinary concentration occurs, which is due to the passive removal of water down its concentration gradient into the hyperosmolar interstitium when an abundance of antidiuretic hormone is present.

Both in the experimental laboratory and in the clinical situation similar kinds of analyses are done to determine the state of renal function. In the following paragraphs the types of studies that are done routinely in the experimental laboratory are highlighted. In doing this both a discussion of the techniques which utilize whole animals and techniques which involved tissue slices will be presented.

Testing procedures used by the experimentalist are in general less complicated than those employed by the clinician. For example, it is quite easy to inject a nephrotoxin into a rat, put the rat in a metabolism cage, collect urine, and if one wants, fecal samples, quantitatively for any period of time. This straightforward procedure eliminates one of the significant problems associated with any clinical study, namely collecting accurately timed and measured samples of urine. Once the urine has been collected, tests for a number of important substances can be done. These tests can be accomplished with specific quantitative methods, or with the semiquantitative, so-called "dip stick" procedures.

The integrity of the glomerulus is assessed by doing protein determinations. Because the normal, healthy laboratory rat does excrete small amounts of protein in the urine, it is particularly important to have good control measurements on the experimental animals before injection of the nephrotoxin. If quantitative assessments of urinary protein excretion are required, there are a number of methods available. Although some monor difficulties exist, these are easily overcome. For example, with the usual rat metabolism cage there is the possibility of contamination of the urine with both fecal matter and with food. Either or both of these could influence the apparent urinary protein determinations.

Evaluation of the blood urea nitrogen (BUN) is usually considered essential for monitoring renal failure. Again, this measurement pertains primarily to glomerular function, and as filtration slows or ceases, the BUN rises. Paralleling this rise is an increase in the plasma creatinine level. The relationship between the BUN and the plasma creatinine has been well established in the chronic renal failure situation and data from the human are depicted in Figure 9. This graph describes a rectangular hyperbola, and from these relationships accurate predictions can be made about the extent of remaining renal function. Furthermore, the relationship between the BUN and plasma creatinine is a precise one for any given species (except under certain circumstances), and therefore either the BUN or plasma creatinine is often an adequate predictor of remaining renal function. Analyses such as these are most useful, however, when the development of renal failure is relatively slow, and data such as those seen in Figure 9 are most useful for chronic renal failure. Obviously no great advantage is gained by being able to predict the extent of glomerular function remaining in a subject whose urine production goes from normal to zero in 24 hr. However, it is likely that with a chronically developing, chemically induced nephrotoxicity, relationships such as those depicted in Figure 9 would be valid.

From an analytical point of view neither the BUN nor the creatinine determination is particularly difficult. With creatinine, one must always worry about nonspecific chromagens which might react and give the telltale yellow color, but this problem can be avoided (*25*). For the BUN there are quite specific analytical methods, although frequently relatively nonspecific ones are used (*26*). Unusually high values for BUN can be obtained under conditions of tissue damage, bleeding into the gastro-intestinal tract, etc., and such things should be controlled. In man, the ratio of BUN to plasma creatinine is about 10, and significant deviations from this ratio would be indicative of factors such as those just described.

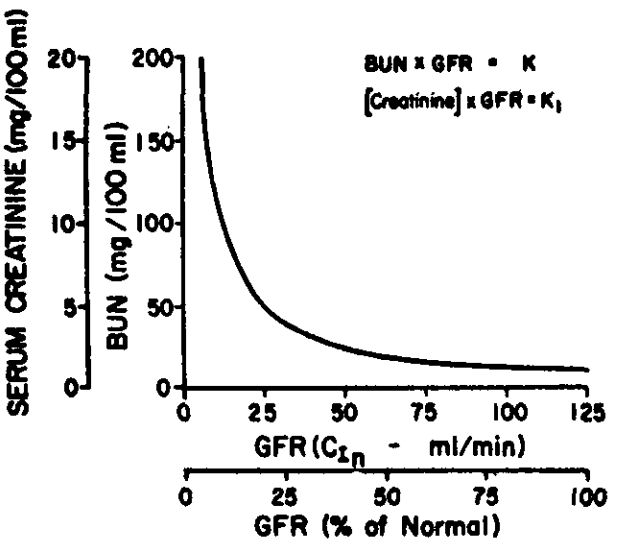


FIGURE 9. Relationship of glomerular filtration rate to serum creatinine concentration and BUN. Modified from Relman and Levinsky (*24*).

Tubular function may also be evaluated by analyses of the urinary excretion of several substances. As indicated above, the reabsorptive capacity of the nephron for glucose is diminished after a nephrotoxic insult. The consequence of this is the excretion in the urine of moderate to extremely large amounts of glucose. This substance can be analyzed semiquantitatively by "dip stick" methods, or very precisely by a number of specific analytical methods (*27*). Other tubular functions, such as the secretion of PAH or TEA are reduced by a variety of nephrotoxins. Hence, after the administration of a nephrotoxin a reduction in glucose reabsorption is the difficulty, while with PAH, etc., a decrease in the net secretory capacity of the nephron is observed.

Incidentally, because PAH secretion or glucose reabsorption fails after ingestion or administration of a nephrotoxin, one is not entitled to conclude that specific effects on these transport processes have been produced. These effects may be indirect. This is not to say that there may not be effects on specific renal transport systems, but caution must be exercised in making such a prediction on the basis of data obtained from *in vivo* experiments. Hence, although methods such as these will allow you to describe the characteristics of the nephrotoxicity, determine the temporal relationships of the renal failure, etc., there are difficulties in the use of these procedures for mechanistic studies.

Nephrotoxins alter the ability of the kidney to concentrate the urine and they also affect the volume of the urine excreted. One type of response noted in the rat is seen in Figure 10 (*28*). In this study potassium dichromate was administered after collecting control urine samples for two days. Urine production, osmolality, etc. were monitored for several days thereafter. The normal ability of the rat to concentrate the urine was lost after the administration of chromium

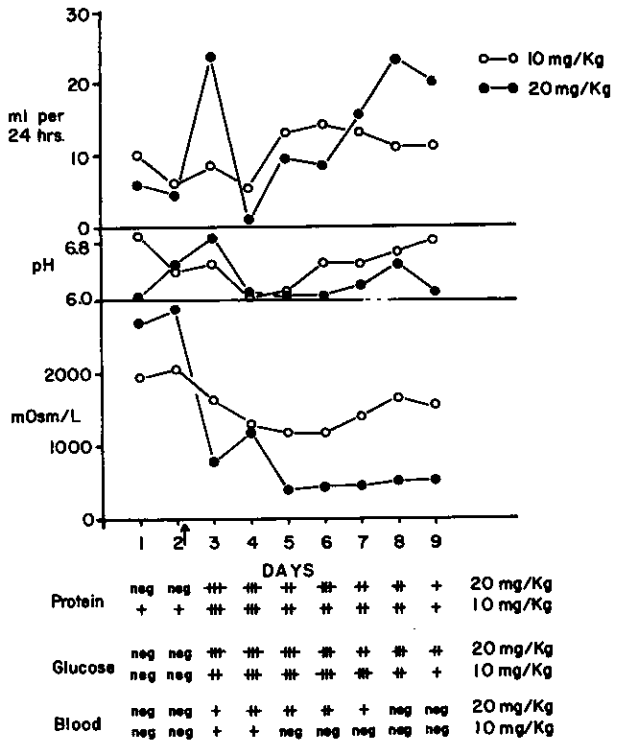


FIGURE 10. Time course of nephrotoxicity after subcutanious administration of two doses of potassium dichromate. From Berndt (*28*) with permission of Toxicology and Applied Pharmacology.

and the rat urinary output was reduced for the first 24 hr, at least with the higher dose of chromium, and a very dilute urine was produced for the remainder of the experiment. In some rats these doses of chromium produce complete anuria, but obviously effects on urinary concentration are lost under these circumstances. Hence, these data which may not be typical for these doses are presented to illustrate the effect of a nephrotoxic insult on urinary osmolality. Measurements of osmolality are very straightforward from a technical point of view, involving simply the measurement of freezing point depression with any number of commercially available instruments. Of course, a somewhat less quantitative apporach would be to measure specific gravity, and when done carefully and precisely this would yield the same kind of information as obtained from the somewhat more sophisticated freezing point depression method.

Most nephrotoxins when given in an adequate dose will produce anuria or oliguria. These disturbing events are potentially life threatening for both the human subjects and experimental animals. However, there are also reports of so-called "high output" renal failure. An elegant example of such a syndrome is presented later in this volume in the discussion of methoxyflurane nephrotoxicity, although the general phenomenon has been reported previously (*29*). When "high output" renal failure occurs, copious volumes of dilute urine are produced. In this case as with the more common oliguric or anuric syndrome, the kidney loses its ability to perform regulatory functions. The loss of the balance function of the kidney is noted in each of these syndromes by increases in the BUN, plasma potassium, so forth. With the "high output" type of failure, the situation is even more complicated because of the large fluid loss. Hence, increases in plasma protein and sodium concentrations are also noted.

Another approach to this general problem of measuring renal function in an experimental setting is to evaluate the effects of a nephrotoxin with isolated tissue techniques. One of the simplest and most commonly used procedures is described subsequently in this volume (*30*).

Probably the renal slice technique is more sensitive than most whole animal procedures for the evaluation of nephrotoxic effects on renal transport processes. It has been suggested, however, that the urinary excretion of enzymes of renal origin might be a measure that is still more sensitive for the detection of effects of chemical nephrotoxins. The measurement of renal enzymes is not new, but the application of this to the question of nephrotoxicity is (*31*). For example, lysosomal enzyme(s) (lysozyme, muramidase) have been examined extensively. Chromium has been reported to increase dramatically urinary lysozyme levels. Although mercury also does this, a lesser effect is noted. Probably these effects are representative of tubular damage only, since an antiglomerular antiserum does not increase urinary lysozyme excretion. However, it is not clear how much cellular damage is needed to see the lysozymuria although probably considerable damage is needed.

More recently maltase, a brush border enzyme (*32*), has been proposed as a potentially useful candidate. This enzyme is located only on the renal brush border and in the intestinal tract, and therefore the possibility of a nephrotoxin causing a nonspecific release of this enzyme is unlikely. Also, release may be accomplished with modest cellular damage. The reasoning for this proposal

was that the brush border location might yield relatively specific effects. Although investigations on the relationship of urinary maltase excretion to nephrotoxicity are at an early stage, in some preliminary studies Hook (personal communication) has been able to demonstrate an effect of mercury on the urinary enzyme level before any effect can be detected on the tissue level of maltase.

Several problems must be considered when measuring urinary enzyme levels. First, care must be taken to insure that the enzyme being measured is not inactivated in the urine while the urine sample is sitting in a collection vessel. To avoid this, for example, may require technical arrangements which permit collecting of the urine in an ice bath. Secondly, the presence of enzyme inhibitors in the urine may prove troublesome. Frequently this can be avoided by dialyzing the urine samples for 12 or 24 hr before making the enzyme measurements. Finally, if urinary concentration of the enzyme of interest is low, it may prove necessary to concertrate the urine by evaporation procedures that will not inactivate the enzymes.

Whenever possible attempts should be made to determine the renal handling of the nephrotoxins themselves, as well as the general pharmacological characteristics of the compounds under study. Experiments of these types may yield valuable data concerning mechanisms of action. For example, knowing whether the parent compound or one of its metabolites is the actual nephrotoxin goes a long way toward understanding mechanisms. From an analytical point of view these studies can be very difficult, because such a variety of chemical substances are potentially nephrotoxic. Hence, specific analytical procedures have to be adopted for each experimental study.

Lastly, it is important to examine the effects of nephrotoxins on renal morphology. These comments come last, because in all probability morphological studies will contribute least to our understanding the effects of nephrotoxins on renal function. This is not to say that appropriate morphological studies are not important and worthwhile, but probably studies relating to physiology and biochemistry will yield data more relevant for mechanistic explanations of the nephrotoxic events. For example, studies by Oliver (*33*) and others (*34*) have demonstrated a localization of the chromium ion into the proximal convoluted tubule of the kidney. On the other hand, apparently mercury produces its greatest morphological damage in the pars recta. Nonetheless, in terms of understanding the mechanisms underlying the acute renal failure syndrome, these morphological observations are not very helpful, because they do not help pinpoint nephrotoxic effects on specific transport processes. Furthermore, morphological studies contribute little to the sorting out of the pathogenesis of acute tubular necrosis with resultant acute renal failure. If acute renal failure results from a disruption of vascular events (*35*), tubular damage may be secondary, and it is not at all clear what it means to localize a toxin in one or another nephron segment.

In any event, something can and should be said about the morphological events which attend the functional ones. To begin with, it appears that most chemical substances when introduced into the rat will produce a relatively well defined area of necrosis related to the proximal tubule. These necrotic changes are often uniform in contrast to the frequently reported "patchy necrosis" that is observed under conditions of anoxia. Accordingly, the remainder of these comments will be confined to studies in which proximal tubular cells were examined.

Several workers have attempted to correlate changes in renal function with alterations in the cellular or subcellular structures of the kidney. Because many of these studies have much in common, I will present data from only one laboratory, namely from the work of Trump and his colleagues (*36-38*). These workers classified control and renal ultrastructural changes into five groups. For these experiments renal cortex slices were caused to swell by incubating them in bathing solutions with high potassium concentrations, by the addition of ouabain to the bathing solution, or by prolonged cold storage. In all three of these experimental situations, the intent was to interfere with sodium transport. Overall the experiments were designed to correlate changes in structure with the inhibition of sodium transport. The degree of transport inhibition was assessed, for example, by allowing the tissues to demonstrate their ability to recover from cold storage.

Figure 11 is a control electron micrograph depicting what was termed stage 1 by Trump and his colleagues. This picture is typical of healthy renal proximal tubular tissue. An organized brush border exists along with an abundance of well formed mitochondria, the usual endoplasmic

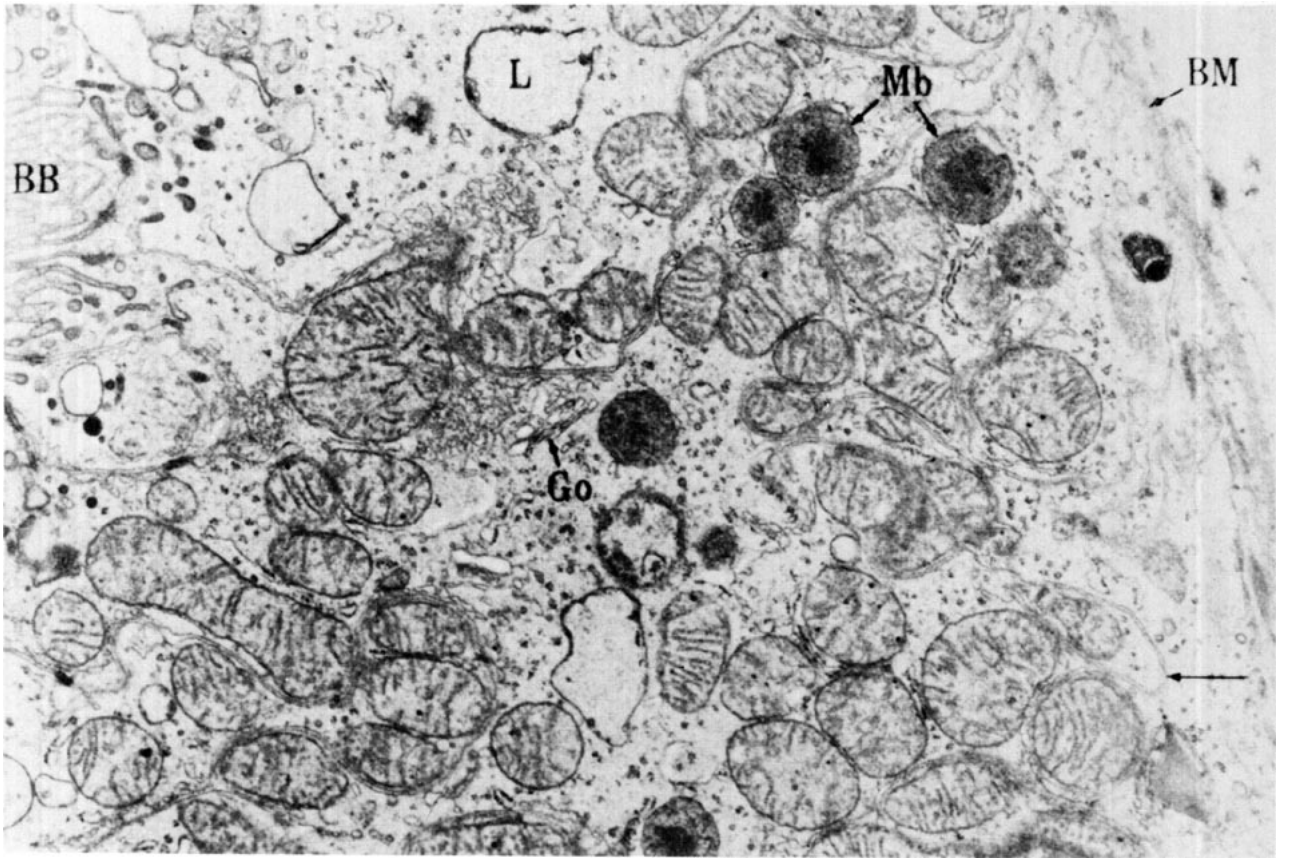


FIGURE 11. Control renal cortex slice. The ultrastructure is representative of normal tissue slices. BB, brush border, Go, Golgi dictyosome; L, secondary lysosome; Mb, microbody; BM, basement membrane. From Trump (*36*) with permission of the author and S. Karger.

reticulum, an intact basement membrane, and some of the so-called "microbodies".

Figure 12 shows relatively mild changes in structure denoted stage 2. The major defect noted was a swelling of the endoplasmic reticulum. The mitochondria, although somewhat more rounded than before, are still intact.

Figure 13 presents an example of a stage 3 tissue. The mitochondria appear to be condensed or contracted, perhaps with a more dense matrix. The brush border or microvilli appear to be disrupted or distorted. The swollen endoplasmic reticulum that was seen before still persists.

The morphologic changes represented by stages 2 and 3 apparently are reversible. At least if these changes are permitted to occur over a relatively short period of time (a few hours) one can anticipate that the tissues will recover if introduced into normal bathing solutions. The stage 2 tissue in Figure 13 was prepared from kidney cortex slices incubated for 4 hr in the presence of nitrogen, and the stage 3, disruption (Fig. 14) was produced by short-term incubation in the presence of potassium cyanide.

State 4 is seen in Figure 14. In addition to the changes noted earlier, this stage is characterized by a loss of polysomes from ths swollen endoplasmic reticulum. In addition, there is some mitochondrial swelling noted at this stage. However, the matrix of these mitochondria are still relatively well organized.

The most severe level of damage is depicted in Figure 15. In addition to what was seen at earlier stages, the internal structure of the mitochondria appeared to come apart at the level of Stage 5 damage. The internal material appeared to be a flocculant precipitate.

The value of an analysis of structure such as this will be apparent only if these morphological characteristics can be correlated with alterations

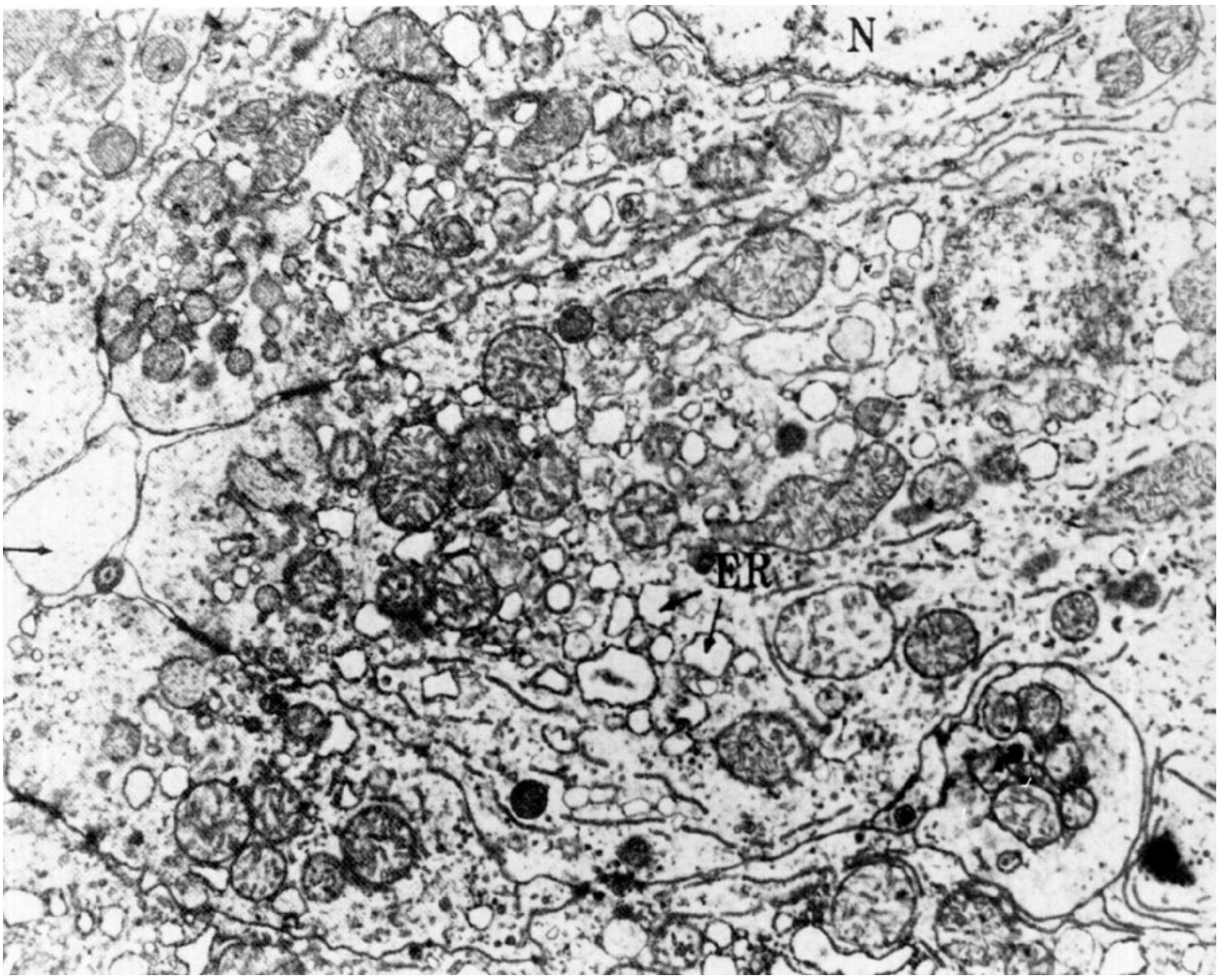


FIGURE 12. A micrograph of a tubule showing stage 2 damage. From Trump (*36*) with permission.

in function. Trump's group have attempted to do this and some of their data are seen in Figure 16. These data are tissue slice sodium values expressed as meq/kg dry weight versus time. These data were obtained form renal slices which were incubated at 4°C for various periods of time, removed, warmed to 37°C, and allowed to extrude the sodium which they had accumulated. Note that tissues incubated in the cold for up to 4 hr returned their tissue sodium values to the control level. Even the samples incubated for as long as 8 hr probably were able to recover virtually normal tissue sodium values. From a morphological point of view all of the tissues samples incubated for up to 24 hr in the cold showed stage 2 or stage 3 morphological disruption. The samples incubated longer showed stage 4 and 5 damage. These data appear to mean that functional changes associated with morphological disruption through stage 3 are reversible. Functional changes associated with morphological damage of Stage 4 or 5 magnitude probably are irreversible.

This type of study demonstrates that it is possible to see some correlation of morphological changes with functional changes. However, this is a particular type of study in which recovery of a functional characteristic is correlated with morphological changes rather that attempting to correlate specific changes in morphology with specific changes in function. For example, there is no

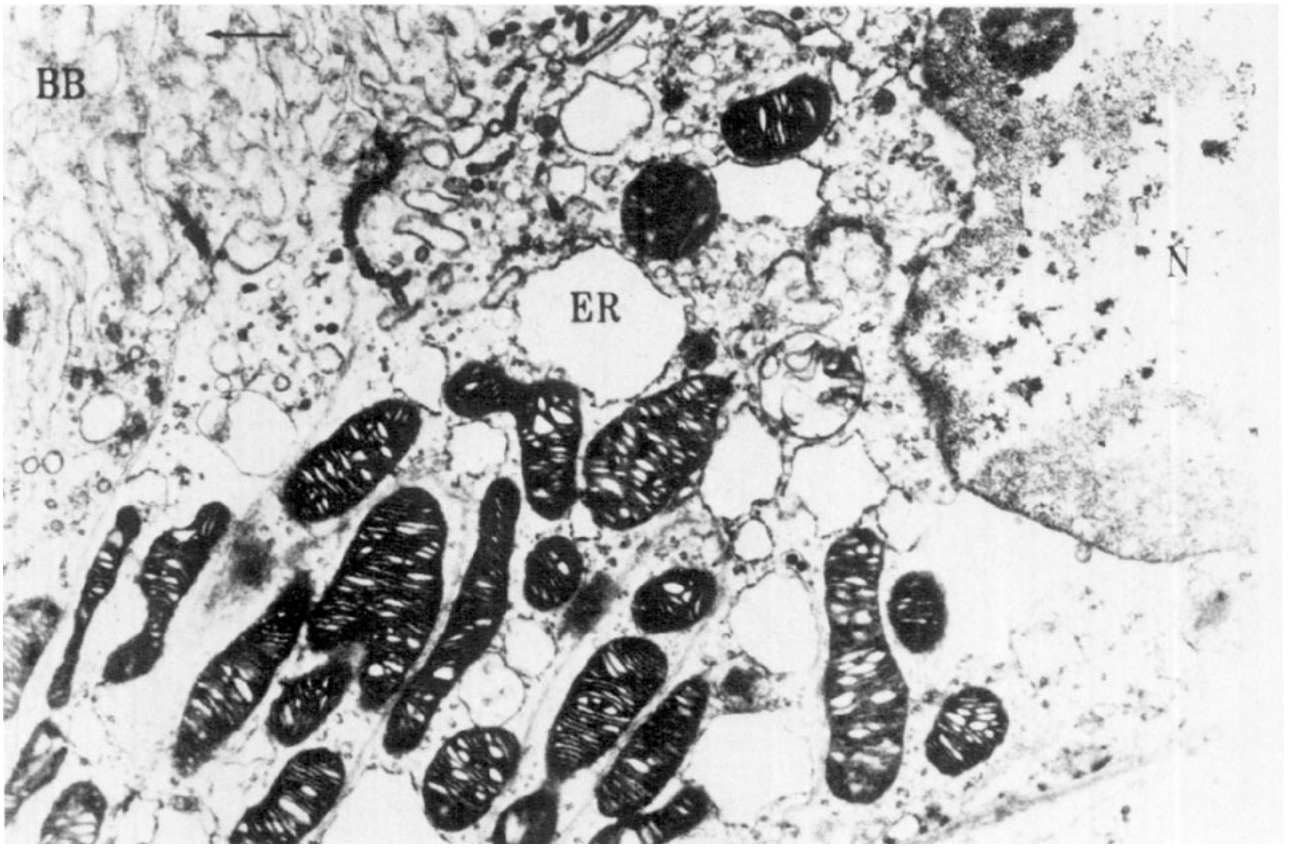


FIGURE 13. A micrograph prepared from a kidney slice demonstrating stage 3 damage. From Trump (*36*) with permission.

doubt that if one stores tissues in the refrigerator for 4 hr or even less and then measures tissue electrolyte levels, these will be abnormal. These abnormalities presumably are associated with structural abnormalities which should, at least in part, be related to tissue swelling. However, tissue swelling is a gross structural alteration and any number of functional alterations might be correlated with it. The significance of studies such as these is not clear. Changes in morphology would be most useful to the experimentalist if these could be used to predict changes in function. Unfortunately, this does not appear to be true.

In a collaborative study with Koschier (*39*), it has been possible to demonstrate an alteration in the transport of a number of organic ions by renal cortex slices *in vitro*, subsequent to pretreatment of intact animals with the organic acid herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). That is, rats are injected on with 90 mg/kg of 2,4,5-T and 24 hr are sacrificed and renal cortex slices are prepared for an *in vitro* experiment. In this experiment measurements were made of the ability of the slices to accumulate 2,4,5-T itself, as well as 2,4-dichlorophenoxyacetic acid (2,4-D), PAH, TEA, and AIB. Data from a number of these experiments are summarized in Table 1. The renal cortex slices obtained from the pretreated animal showed a significant deficit in their ability to accumulate 2,4,5-T,2,4-D, and TEA. That is, the uptake of two organic anions and an organic cation were depressed by the pretreatment procedure. On the other hand, a universal disruption of transport by renal cortex slices did not occur, since neither the transport of PAH nor AIB was affected.

At this point it would be appropriate to show a series of slides depicting changes in morphology of the tissue to correlate with these functional changes. However, no structural alterations were

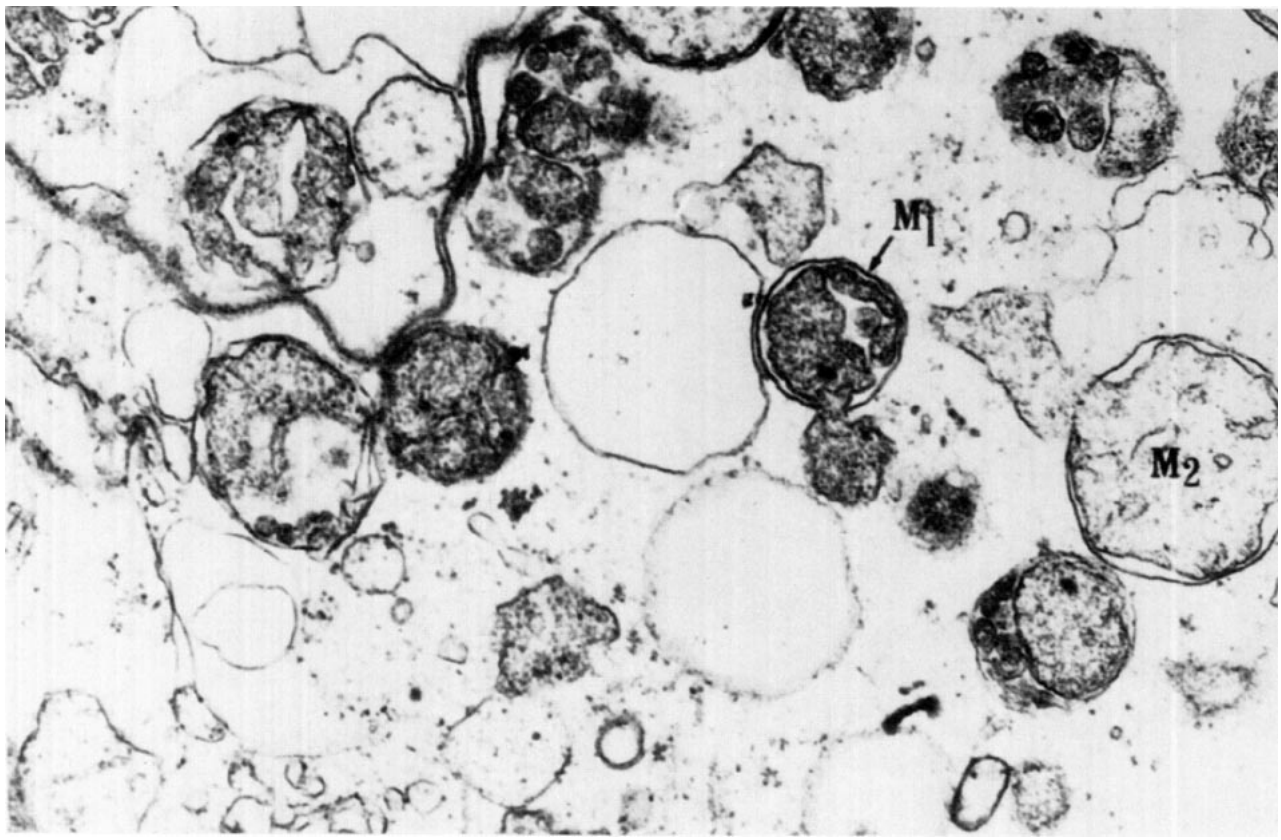


FIGURE 14. A micrograph depicting a tubule with stage 4 damage. From Trump (*36*) with permission.

obtained in renal tissue of rats pretreated with 2,4,5-T. Hence, although relatively dramatic alterations in functional characteristics of the isolated renal cortex slice were noted, structural changes with which to correlate these functional changes were not obtained.

This is not an isolated incident. For example, rat kidney tissue has been submitted to a "blind", light microscope examination (unpublished observations). Kidneys taken from animals within 24 hr of having received nephrotoxic doses of chromium were judged to be normal or, at most, mildly affected when light microscopy was used. Functionally, these tissues had abnormal water and electrolyte distributions, as well as a reduced ability to transport PAH and TEA (*28*). Of course, to some extent the appearance of microscopic damage is dose-related. With very large doses, microscopic damage can be detected early. However, there is no doubt that it is possible to use smaller doses which impair renal function at a time when morphological disruption cannot be detected.

An attempt has been made in the report to demonstrate that it is possible to evaluate experimentally the onset of nephrotoxicity by relatively conventional means. It is possible to examine urinary excretion of protein, glucose, etc. and from these measurements detect damage to renal function. Also determinations of plasma constituents such as the BUN are helpful. Studies of this sort can be used to give the investigator insight into the magnitude of nephrotoxicity and its temporal relationships. The use of renal cortex slices will also allow one to examine the effects of nephrotoxins on specific renal transport processes. Studies of this sort may be useful in the determination of mechanisms of action as well.

Although it is possible to correlate some structural changes with alterations in function, in general it appears that morphological studies are not nearly as sensitive as functional studies. Said

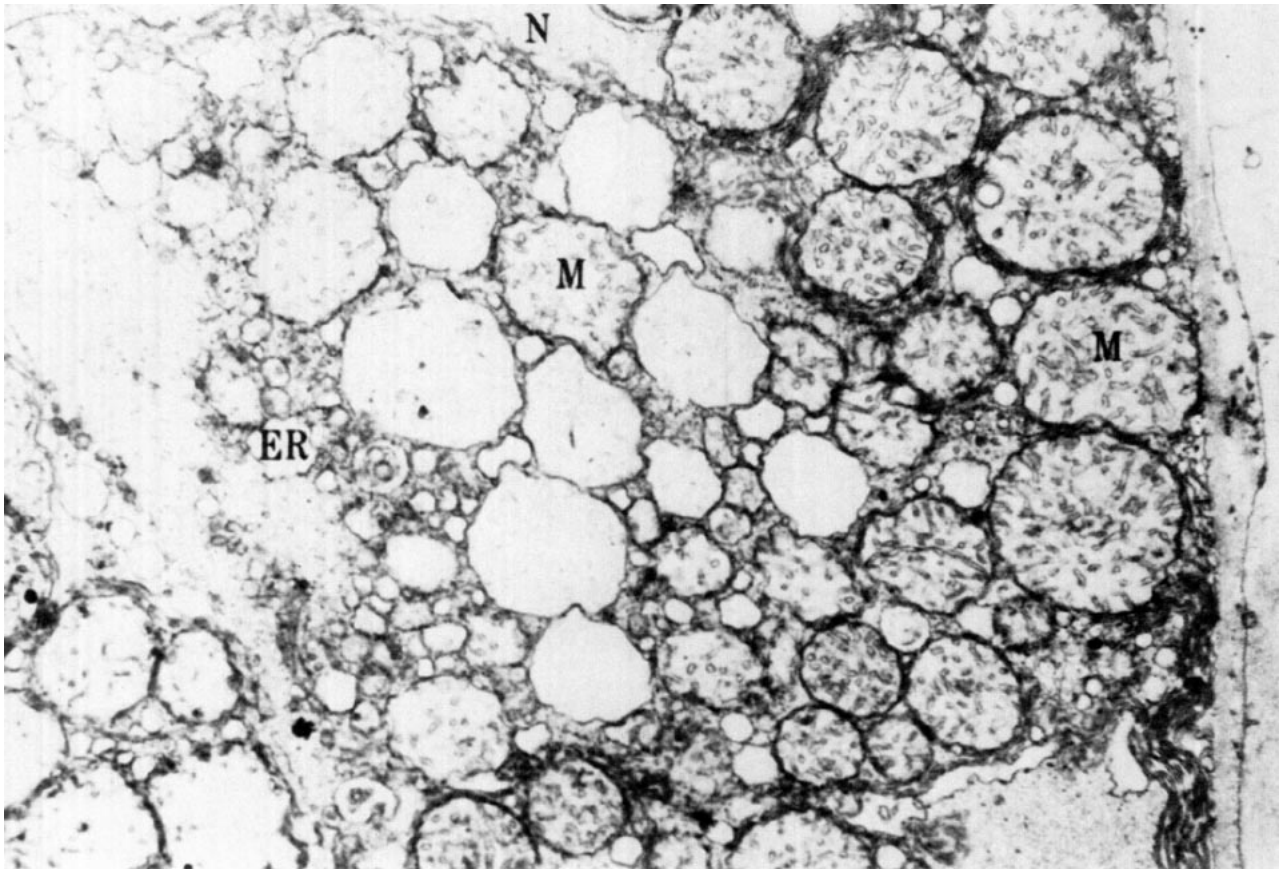


FIGURE 15. A micrograph of a tubule demonstrating stage 5 damage. From Trump (*36*) with permission.

another way, if one is to use morphology as a mechanism for detecting and/or understanding nephrotoxicity, the degree of insult must be sizable before this will be possible. Hence, by the time one sees relatively easily detectable morphological changes in the renal tissue it would be extremely easy to detect functional alterations.

We are left with several enigmas, however, which relate to the production of acute renal failure that are difficult to explain in terms of our present understanding of renal physiology and pathophysiology. For example, there is one school of thought that believes the acute renal failure syndrome is primarily attributable to severe cortical ischemia resulting form the interruption of blood flow to the kidney cortex (*35*). These workers doubt the importance of the chemical characteristics of a nephrotoxin, since they argue that all of these substances act through the same basic mechanism.

If this is true, the meaning of the effects of various nephrotoxins on specific renal tubular transport systems is obscure. Not only are there

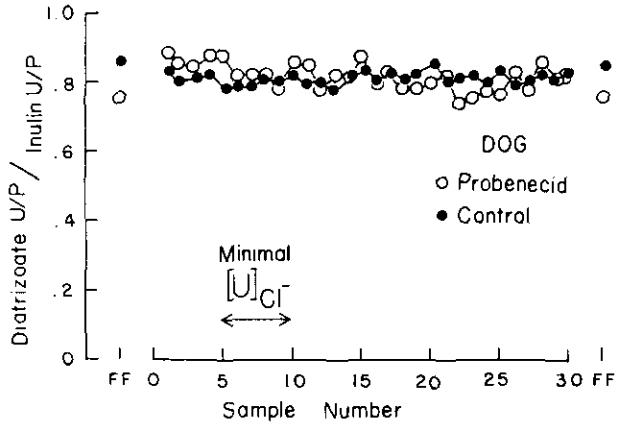


FIGURE 16. Data which demonstrate the reversibility of tissue function. Modified from Trump (*36*).

Table 1. Influence of 2, 4, 5-T pretreatment on organic ion transport by renal tissue.[a]

	Uptake, % of control
2,4,5-T	62[b]
2,4-D	39[b]
TEA	74[b]
PAH	80
AIB	112

[a]All uptakes were measured 24 hr after a single, subcutaneous dose of 90 mg of 2,4,5-T/kg given to 300 g, male, Sprague-Dawley rats. Uptakes were for 2 hr at 25°C in the presence of 100% O_2, 40 mM K^+, and 10 mM sodium lactate.

[b]Significantly reduced from control, $p<0.05$.

effects on transport systems, but different nephrotoxins can produce different effects (*28,40*). Furthermore, one might even argue that all the effects on specific transport systems are interesting but irrelevant to the question of acute renal failure. That is, because the renal ischemia is the primary event, all of the effects on transport systems must assume a position of lesser importance.

Finally, the question of the "high output" renal failure syndrome must be mentioned. If nephrotoxicity indicates either a disruption of tubular function or an interference with glomerular events, it is difficult to understand the "high output" phenomenon. Nonetheless, many workers would believe that virtually every nephrotoxin can produce the high output events under the right experimental circumstances. If so, this may mean that all chemical nephrotoxins have an ability to abolish the urinary concentrating and diluting mechanisms and accordingly may be exerting their initial effects on the thick ascending limb of Henle's loop, as do the more efficacious, reversible diuretics.

The original experimental work from the author's laboratory was supported by NSF grant P4B3159 and PHS grant 18124.

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