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Environmental Health Criteria 119

Principles and Methods for the Assessment of Nephrotoxicity Associated with Exposure to Chemicals

d under the joint sponsorship of the United Nations Environment Programme,
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Environmental Health Criteria 119

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PRINCIPLES AND METHODS FOR THE ASSESSMENT OF NEPHROTOXICITY ASSOCIATED WITH EXPOSURE TO CHEMICALS

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The **International Programme on Chemical Safety (IPCS)** is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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NOTE TO READERS OF THE CRITERIA DOCUMENTS

Every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors that may have occurred to the Manager of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda, which will appear in subsequent volumes.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Palais des Nations, 1211 Geneva 10, Switzerland (Telephone No. 7988400 or 7985850).

PREFACE

The preparation of this monograph was undertaken jointly by the International Programme on Chemical Safety (UNEP/ILO/WHO) and the Commission of the European Communities.

A joint WHO/CEC Task Group on Principles and Methods for the Assessment of Nephrotoxicity Associated with Exposure to Chemicals met at the National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands, from 4 to 8 December 1989. The meeting was opened by Dr K.A. van der Heijden on behalf of the Netherlands and the host institute. The Secretariat responded and welcomed the participants on behalf of the three cooperating organizations of the IPCS (UNEP/ILO/WHO) and the Commission of the European Communities. The Task Group reviewed and revised the draft criteria document and prepared a final text.

The drafts of this Monograph were prepared by DR P. BACH, Guildford, United Kingdom, PROFESSOR W.O. BERNDT, Omaha, USA and PROFESSOR R. GOYER, London, Ontario, Canada. During the preparation of the monograph, many scientists made constructive suggestions and their contributions are gratefully acknowledged. The photographs in Figure 2 were supplied by Dr N.J. Gregg, in Figure 4 by Professor W. Guder, and in Figure 14 by the Department of Toxicology, Institute for Medical Research and Occupational Health, University of Zagreb. Following the Task Group, Dr P. Bach and Dr M. Robbins collated the text for IPCS. Dr E. Smith and Dr P.G. Jenkins, both of the Central Unit, IPCS, were responsible for the overall scientific content and technical editing, respectively.

ABBREVIATIONS

ADH	anti-diuretic hormone
AIN	acute interstitial nephritis
ARF	acute renal failure
BEA	2-bromoethalamine
BEN	Balkan endemic nephropathy
BUN	blood urea nitrogen
CIN	chronic interstitial nephritis
DBCP	1,2-dibromo-3-chloropropane
DCVC	*S*-(1,2-dichlorovinyl)-L-cysteine
DTPA	diethylenetriamine pentaacetic acid
EDTA	ethylenediaminetetraacetic acid
ESRD	end-stage renal disease
GAG	glycosaminoglycan
GBM	glomerular basement membrane
GFR	glomerular filtration rate
GSH	glutathione-SH
H & E	haematoxolin and eosin
HCBD	hexachloro-1,3-butadiene
HPLC	high-performance liquid chromatography
LDH	lactate dehydrogenase
3MC	3-methylcholanthrene
NAC	*N*-acetyl cysteine
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NNM	*N*-nitrosomorpholine
NSAID	non-steroidal anti-inflammatory drugs
PAH	*p*-aminohippurate
PAS	periodic acid Schiff stain
PCBD	*S*-(1,2,3,4,4-pentachloro-1,3-butadienyl
PG	prostaglandin
PoG	proteoglycan
RPN	renal papillary necrosis
TEA	tetraethyl ammonium
UCM	urographic contrast medium
UDP	uridine diphosphate

1. SCOPE OF THE HEALTH SIGNIFICANCE OF NEPHROTOXICITY

Over the last 20 years it has become increasingly obvious that the kidney is adversely affected by an array of chemicals. Man is exposed to these as medicines, industrial and environmental chemicals, and a variety of naturally occurring substances. The level of exposure varies from minute quantities to very high doses. Exposure may be over a long period of time or limited to a single event, and it may be due to a single substance or to multiple chemicals. The circumstances of exposure may be inadvertent, accidental, or intentional overdose or therapeutic necessity. Some chemicals cause an acute injury and others produce chronic renal changes that may lead to end-stage renal failure and renal malignancies. The extent and cost of clinically relevant nephrotoxicity has only started to become apparent during the last decade. However, the full extent of the economic impact of chemically induced or associated nephropathy is difficult to define because the diagnosis of early injury and the documentation of the cascade of secondary degenerative changes have not been adequately identified. Instead most chemically associated renal disease is only identified as an acute renal failure or as chronic renal failure at a very late stage when therapeutic intervention is impossible. More importantly at this stage, the etiology may be obscured by lack of reliable information on the likely causative agents, the levels and duration of exposure, and other possible contributing and exacerbating factors. At present, epidemiological evidence indicates that nephrotoxicity leading to acute and/or chronic renal failure represents a substantial financial burden to society (Nuyts et al., 1989). Indeed, there is some indication that chemical exposure could play a much greater influence in the very high incidence of end-stage renal disease encountered in nephrology and dialysis clinics than is currently considered to be the case.

There are already several examples of this type of chemically associated disease that went unrecognized for some time. These include those nephropathies caused by cadmium, other environmental heavy metals, and, more

recently, the organo-metallic compounds used as therapeutic agents, anti-cancer drugs, cyclosporin, analgesic abuse, and antibiotics.

Owing to its diverse functions and small mass in relation to the resting cardiac output that it handles, the kidney is a target both for chemicals that are pharmacologically active and for toxic material. The nephron and its related cells perform a diversity of physiological functions. It is the major organ of excretion and homeostasis for water-soluble molecules; because it is a metabolically active organ, it can concentrate certain substances actively. In addition, its cells have the potential to bioconvert chemicals and metabolically activate a variety of compounds. There are a number of other processes described below that establish the potential for cellular injury. Specific physiological characteristics are localized to specific cell types. This makes them susceptible to, and the target for, chemicals. The effect of any chemical on a cell may be pharmacological, in which case the effect is dose related and occurs only as long as the concentration of the effector is high enough to be active. Alternatively, the chemical may cause damage to the cell. The cell responds to injury by repair and the kidney responds to cellular lesion by renal and extrarenal adaptation to compensate for loss of that cell function. Although there is a substantial capacity within the kidney for repair, there are also several circumstances where damage may be irreversible. In general, the proximal and distal tubules and urothelia can be repaired, but the glomeruli and medulla may have a significantly lower repair facility. It is, therefore, possible to initiate a series of degenerative changes as a result of interfering with one or more of the normal physiological processes.

The Environmental Health Criteria monographs normally focus on industrial chemicals, but at present most of the experimental and human information on nephrotoxicity is based on therapeutic substances. These data are most useful because there are animal and human comparisons for specific chemicals where the levels of exposure and the nephrotoxicological consequences are well documented. From these data it has been possible to glean some understanding of mechanisms of primary injury and the long-term consequences and health significance. Thus, these com-

pounds are generally well studied, and the more rational understanding of the mechanism of their nephrotoxicity in animals and man provides the basis for validating extrapolation between species and making rational risk assessment.

Most risk assessment decisions are currently based on information concerning the aminoglycosides, halogenated anaesthetics, several heavy metals, and lithium, where there is an excellent concordance between animal data and findings in humans exposed to these agents (Kluwe et al., 1984; Porter & Bennett, 1989). This has provided some predictive indication of what will take place in humans exposed to analogues of these compounds. On the other hand, the demonstration that the occurrence of light hydrocarbon-related adenocarcinomas is specific to male rats shows that there are examples where the molecular understanding of a renal lesion in animals is irrelevant to humans.

There are also therapeutic agents where attempts to extrapolate from animals to man have not been as successful. These include compounds such as cyclosporin, analgesics and non-steroidal anti-inflammatory agents. It has, however, been possible to develop some model lesions that parallel those in humans using these compounds. Generally, different protocols have had to be used, such as water deprivation and renal injury, but these have in turn provided the basis for developing improved screening methods for such chemicals and also for probing the molecular nature of the lesion. There are, however, a number of chemicals, such as renal carcinogens, mycotoxins, other natural toxins, and anti-cancer drugs, and some types of lesion, such as the immunonephropathies, where it has been difficult to establish good models in animals. A host of chemicals alter glomerular filtration rate (GFR) or some other aspect of renal function, but the long-term health significance is still not known and it is uncertain how to extrapolate such data to man.

2. NEPHROTOXICITY

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines, and industrial or environmental chemicals. It is well established that toxic nephropathies are not restricted to a single type of renal injury. Some chemicals target one discrete anatomical region of the kidney and may affect only one cell type. Chemical insult to the kidney may result in a spectrum of nephropathies that are indistinguishable from those that do not have a chemical etiology.

2.1 Target selectivity

It has become increasingly apparent that there are a number of chemicals that may adversely affect one or more of the anatomical elements of the kidney, such as the glomerulus, proximal, intermediate, and distal tubules, and medullary, endothelial, and urothelial cells. Although some of these cell types (such as the proximal tubular cells) have a marked ability to repair damaged regions, others, such as the glomerular epithelium and the "type 1" medullary interstitial cells, do not. It is for this reason that the dynamic process that follows any renal injury can affect the outcome of the chemical insult.

2.2 The dynamics of renal injury

The renal response to injury is dynamic, and the kidney adapts to maintain homeostasis during the cascade of repair and recovery that follows the primary insult (Bach, 1989). Depending on the type and frequency of the damage, and the region of the kidney that is damaged, the organ can respond by a recovery, a reduced functional reserve, or by a progressive degenerative change. A reduced functional reserve may play a very important role in sensitizing the kidney to subsequent renal injury, and an initiated degenerative cascade may either stabilize or progress to acute or chronic renal failure. It is not possible to differentiate between a kidney that has totally recovered, one with a reduced functional reserve, and an organ with early progressive degenerative change,

except in animals where function and morphology can be assessed under well controlled conditions.

2.3 Classification of renal disease

Classification of renal disease can be based on clinical manifestations, pathological changes, or etiological agents. WHO has prepared a number of detailed and illustrated publications in recent years on the classification of renal disease (WHO 1982, 1985, 1987, 1988). The general approach is to subdivide the kidney into major anatomical components (i.e. glomeruli, tubules and interstitium, and blood vessels) and to relate these to the major clinical syndromes characteristic of renal diseases. Table 1 contains a modified classification of renal disease that focuses on major disorders of the kidney that may be associated with nephrotoxins. This classification is consistent with previous WHO publications and textbooks of nephrology and pathology and provides a framework for discussing the mechanisms and pathology of nephrotoxicity. It must be appreciated that nephrotoxic agents may have multiple anatomical targets and that toxicity manifests itself in more than one clinical syndrome. Further discussion of renal effects due to specific agents are discussed below.

2.4 The epidemiology of nephrotoxicity

Putting the health significance of nephrotoxicity into perspective is difficult because of the diverse array of chemicals that target different parts of the kidney, the spectrum of disease consequences, and the many interacting factors. There is also uncertainty in assessing changes in renal function before they reach the point where preventive medicine can no longer be practised and therapeutic intervention may be appropriate.

Many industrial and environmental chemicals and therapeutic agents have been shown in experimental studies and from acute toxic exposures to be nephrotoxins, but the extent of their contributions to the overall incidence of chronic renal failure is not known. Data extracted from the European Dialysis and Transplant Association Registry identified only about 4% of patients starting renal

Table 1. Classification of renal disease due to nephrotoxins in humans

1. Immunologically mediated

Antibody mediated

Membranous glomerulonephritis or immune complex type disease

metals (gold, mercury)
D-penicillamine
drugs responsible for a lupus-like syndrome
(hydralazine, procainamide, diphenylhydantoin)

Anti-glomerular basement membrane antibody mediated

organic solvents
hydrocarbons

2. T cell mediated (?)

Nephrotic syndrome with minimal glomerular changes

lithium salts
non-steroidal anti-inflammatory agents

replacement therapy in 1984 as having drug or chemical associated renal disease. However, nearly 50% of these patients were considered possible (but not diagnosed) cases of toxic nephropathy (Dieperink, 1989). Of those patients identified as having chemical-related renal disease, analgesic nephropathy is the most important recognized outcome. In an analysis of the European Dialysis and Transplant Association Registry (1986), the prevalence of analgesic nephropathy was found to vary greatly between countries. It is highest in Switzerland (18.1%) and Belgium (11.8%) and accounts for over 4% of patients in Denmark, Germany, Czechoslovakia, and Austria. In 20 countries the prevalence is lower than one patient in every hundred (European Dialysis and Transport Association Registry, 1986; Wing et al., 1989). Other specific drug nephropathies recorded less frequently include those due to cisplatin (*cis*-platinum) and cyclosporin A. A small number of patients had other specific drug or chemical-related nephropathies.

The role of the toxic agents that may contribute to the 50% of cases of chronic renal failure of undiagnosed etiology is less certain. There is opportunity for expo-

sure to a number of chemicals in the workplace or ambient environment (drugs included) that are possible nephrotoxins. It has been estimated that there were nearly four million workers in the USA with potential occupational exposure to known or suspected nephrotoxins in the 1970s (Landrigan et al., 1984). The major occupational exposure is to workplace solvents, but toxic metals and organic compounds, including pesticides, are of great concern. The well documented occurrence of subclinical nephropathies in subjects occupationally exposed to nephrotoxins such as lead or cadmium (see section 5.5), the excess of mortality for renal diseases in cohorts of workers with previous exposure to these two heavy metals (Bennett, 1985; Bernard & Lauwerys, 1986), and, more recently, the suggestion that subclinical renal effects caused by cadmium are early signs of an accelerated and irreversible decline of renal function (Roels et al., 1989) should all be noted. The linkage of these risks to the actual occurrence of chronic renal failure has not, however, been possible. A review of the end-stage renal disease (ESRD) population in the USA has shown that at least 19% have a renal disease of unknown or non-specific etiology (Burton & Hirschman, 1979). If other diagnostic groups that have uncertain etiologies, such as the 30% with glomerulo-nephritis, 5% with interstitial nephritis of suspected etiology (lead, analgesics, etc.) and possibly the 8% diagnosed as having pyelonephritis, are added, it becomes apparent that the etiology of a large portion of patients with chronic renal failure is unrecognized or undefined. Environmental factors may have a previously unrecognized role in the etiology of these lesions.

There are many reasons for the failure to recognize toxic etiologies (Sandler, 1987). A major reason is that chronic renal failure develops slowly over a number of years, so that retrospective identification of a toxic agent requires knowledge of lifestyles, therapies, or workplace environments that might provide risk factors. However, such data are generally not available. Unless the drug or toxic chemical is persistent in tissues, it is usually not possible to confirm or quantify exposure. The inability to recognize multiple etiologies or confounding factors adds further complexity to the problem. There is also lack of uniformity in clinical and pathological

diagnoses. A further complexity is that there is a tendency to categorize chronic renal failure by a mixture of pathological and etiological classifications. For example, in one instance a patient may be classified as having chronic interstitial nephropathy on the basis of a renal biopsy, whereas another patient with the same pathology might be classified as having toxic nephropathy due to exposure to a nephrotoxic chemical because of knowledge of exposure. In a survey of patients requiring dialysis in Israel, Modan et al. (1975) found diagnostic inconsistencies between hospital diagnosis, autopsy reports, and diagnosis made by the study reviewers. Disagreement was most often seen for chronic glomerulo-nephritis, chronic pyelonephritis and nephrosclerosis. The stage of the pathological process or severity influences classification. Interstitial nephritis tends to be diagnosed more frequently in the early stages of chronic renal failure, whereas glomerulonephritis is a more common diagnosis for patients undergoing dialysis. There may be a rational basis for this in that the scarring in persistent interstitial nephritis does impede blood supply to the glomerulus. This could lead to glomerular disease and interstitial nephritis despite the fact that there are different etiologies or risk factors for the two conditions.

The identification of chronic pyelonephritis is made more precise by following established criteria. These include the presence of gross irregular scarring, inflammation, fibrosis and deformity of calyces underlying parenchymal scars, predominant tubulointerstitial histological damage, and relative lack of glomeruli. There is evidence that some of the chronic interstitial nephritis that is labelled chronic pyelonephritis is due to something other than bacterial infection.

Environmental Health Criteria 27: Guidelines on Studies in Environmental Epidemiology (WHO, 1983) provides guidelines for obtaining human data concerning the health effects of exposure to chemical agents. For agents that produce acute renal failure, long-term follow-up may identify those instances where chronic renal disease has persisted. For agents that give rise to accidental poisoning, clinical case reports can provide important information. In the case of agents where exposure to larger population segments occurs, information may be

obtained by using statistical and epidemiological methods to investigate possible nephrotoxicity from such exposures (as compared to a non-exposed control group). Specific segments of the population that might be at higher risk to a potential nephrotoxic drug or workplace chemical should be particularly closely monitored for renal effects.

There are marked differences between the incidence of analgesic-associated ESRD in different countries and within the same country (Table 2). This varies from up to

Table 2. National prevalence of analgesic nephropathy in patients with end-stage renal failure

	%		%
South Africa	22	Scandinavia (1979)	3
Switzerland (1980)	20	France (1979)	2
Belgium (1984)	18	USA	2
Australia (1985)	15	United Kingdom (1979)	1
Federal Republic of Germany (1983)	13	Italy (1979)	1
Canada (1976)	3	Spain (1979)	0.4

22% in Australia (in 1982) and in parts of some of the European countries to as low as 0.2% in the USA. It is generally considered that the withdrawal of phenacetin has lead to the disappearance of the high incidence of renal papillary necrosis (RPN) in Scandinavia, Canada, and Australia, but a high incidence remains in Switzerland, Belgium, and the Federal Republic of Germany (Gregg et al., 1989). Specific geographical locations may have analgesic abuse problems such as the Winston-Salem area (USA). Worldwide variability in the prevalence of analgesic nephropathy has long been recognized. The correlation of the incidence of this disease with local analgesic consumption has been demonstrated. However, the relation between both phenomena is not well established since comparable consumption data, focussed on the sales of analgesic mixtures, are not available in most countries. The high frequency abuse area in Belgium is situated in the north (Fig. 1a), where up to 51% of dialysis patients are analgesic abusers, but this is markedly

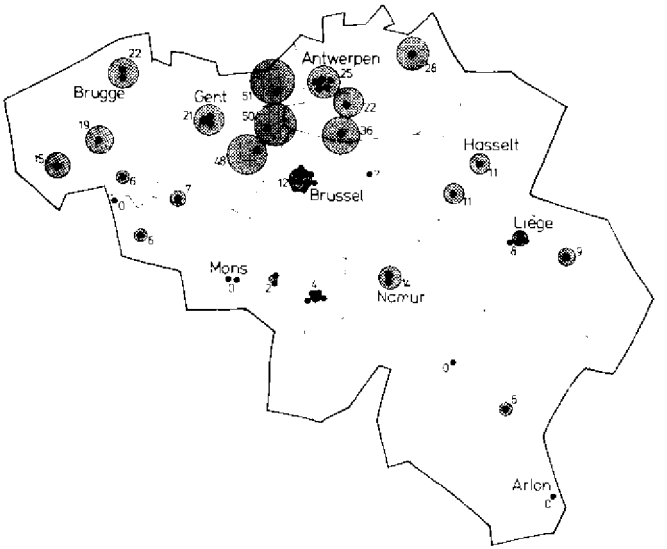


Fig. 1a. Analgesic nephropathy as a cause of end-stage renal failure in Belgium (1984). Population, 10 million; area, 30 515 km^2; chronic dialysis units (•), 54; total numbers of patients under chronic dialysis, 2334. (O) and number, patients with end-stage renal failure caused by analgesic nephropathy expressed as % of patients treated in the dialysis unit(s). From: Elseviers & De Broe (1988).

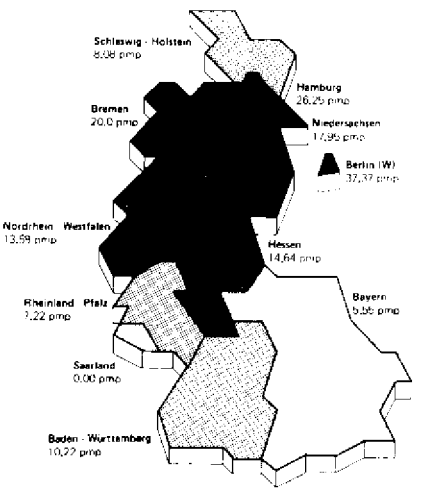


Fig. 1b. Prevalence of terminal analgesic nephropathy in the Federal Republic of Germany (1986). From: Pommer et al. (1986); pmp, per million population.

lower in the south (Elseviers & De Broe, 1988). In Germany (Fig. 1b) the highest prevalence is in West Berlin (up to 50%), Hamburg, and Bremen (Pommer et al., 1986). These data indicate that the prevalence of this disease has been underestimated on a national basis. There are indications that the overall prevalence of this disease has also been underestimated in several other countries. Local well-conducted studies of the prevalence of analgesic nephropathy showed higher prevalences than the European Dialysis and Transport Association Registry. In the Federal Republic of Germany, a prevalence of 13% of analgesic nephropathy in dialysis patients was found, while the appropriate European Dialysis and Transport Association data was never more than 6% (Pommer et al., 1986). In Belgium, the prevalence of analgesic nephropathy was 18%, whereas the European Dialysis and Transport Association registered a prevalence of 12% (Elseviers & De Broe, 1988). The percentage of nephropathies of unknown etiology and of pyelointerstitial nephritis may also indicate an underestimation of analgesic nephropathy. These percentages are low in countries with a high prevalence of analgesic nephropathy (Switzerland and Belgium) and are high in countries such as Italy and Spain (Wing et al., 1989) where analgesic nephropathy is considered to be rare. Analgesic nephropathy progresses silently over a long period, and so the diagnosis is difficult. In addition, most patients deny being analgesic abusers, which further confounds diagnosis. Symptoms are nonspecific until the degenerative cascade affects the cortex, when renal failure occurs. Moreover, even when the renal failure is recognized, the diagnosis of analgesic nephropathy remains difficult unless diagnostic criteria are established.

2.5 Risk factors for toxic nephropathies

The risk of developing a clinically significant nephrotoxicity depends on pre-existing clinical conditions and may be identified in specific patient populations. Hypertension, diabetes, cardiovascular disease, etc. are all thought to have the potential to exacerbate nephrotoxicity, but many of these conditions have not been systematically investigated for all types of nephrotoxicity. There are examples where both chemicals and other disease

factors cause a lesion. For example, sickle cell disease and diabetes can cause renal papillary necrosis, a condition that is also common in individuals who abuse analgesics. The risk factors that predispose individuals to renal papillary necrosis are not clear, and it is also unclear whether diabetics are at greater risk of developing the lesion if they take high doses of analgesics. This question cannot be resolved until better diagnostic criteria are developed to identify the lesion before it involves the cortex. Risk may also vary for different nephrotoxins. While Bence-Jones protein excretion considerably increases the risk of radiocontrast-induced renal injury, the effects of other types of chemicals in patients with multiple myeloma are not clear. Therefore recognition of the risk factors is necessary for the understanding and prevention of renal damage.

There are also many examples of animal data that have not yet been translated into risk terms in humans. For example, the immature kidney may be resistant to aminoglycosides (Marre et al., 1980) and cephalosporins (Tune, 1975), but the reverse is true for other chemicals such as hexachlorobutadiene (Hook et al., 1983). Other factors, such as electrolyte and volume changes or an alteration in the renin-angiotensin system, may affect some types of drug-induced acute renal failure (Bennett et al., 1983).

Risk factors as a measure of vulnerability to potential nephrotoxicity that could be caused by drugs and chemicals are not well defined at present. Clearly, wide variation exists among individuals and even groups of people. Multiple exposure to toxic agents and multiple drug usage are certainly factors, but the ability to estimate risks to multiple exposure is limited. Factors intrinsic to the nature of renal function and risk factors presented by clinical disease have been reviewed (Porter, 1989) and are discussed below.

2.5.1 *Factors related to renal function*

The vulnerability of the kidney to toxicity from exposure to a particular drug or chemical is the product of several groups of risk factors. In any one person, multiple factors may be operative. The nature of normal renal function in itself contributes to the vulnerability

to toxins. The intimate association of the capillary endothelial surface during the process of ultrafiltration provides opportunity for direct toxicity. A further contribution to the glomerular capillary vulnerability is the positive hydrostatic pressure required for producing the plasma ultrafiltrate. Adding to this vulnerability is the "hyperfiltration injury" hypothesis proposed by Brenner (1983) and Brenner et al. (1978, 1982), who showed that when a nephron ceases to function, the remaining nephrons hypertrophy and the flow rate per functioning nephron is raised, thus increasing the exposure to the drug or chemical. A further aspect is the concept of renal reserve, i.e. fewer functioning nephrons further increase the vulnerability of the kidney to toxicity.

Another aspect of the structure of the glomerular endothelial cells that can lead to injury to the kidney is the negative charge of the filtration membranes. Positively charged ligands can become electrostatically attached and alter the permeability coefficient of the glomerulus. In addition, cationic proteins can be sequestered in the glomerulus and act as "planted antigens", and a circulating antibody can attach to such antigens resulting in an *in situ* immune complex formation; hydrocarbons from petroleum products can act in a similar way (Ravnskov, 1985). This is but one of a wide variety of immunologically mediated glomerular injury patterns that have been identified. This variety is not surprising when one considers the heterogeneity of the biochemical composition of the glomerulus and the wide spectrum of antigenic compounds to which the body is exposed (Glassock, 1986).

Tubular vulnerability to nephrotoxins is related to the nature of normal tubular function. The medullary countercurrent multiplier system provides a mechanism for eliminating body waste products while minimizing body water loss. A consequence is the reabsorption and recycling of compounds of low relative molecular mass, in particular urea and neutral toxicants and/or their metabolites, which can accumulate in the medullary interstitium. Depending on their chemical properties, they may initiate an inflammatory response through activation of mediators, a factor that may be relevant in the pathogenesis of analgesic nephropathy (Mudge, 1982).

The organic acid and base transport systems of the proximal tubule serve to excrete certain molecular species. Several commonly used drugs, including the organic acid penicillin, utilize this mechanism of transport. Toxicants that are involved in these systems might induce renal injury directly because of high cellular concentration or by acting as competitive inhibitors to block the elimination of endogenously produced toxic metabolites. Tubular mechanisms for acidification may play a part in tubular injury by drugs or chemicals that induce an acidification defect, e.g., lithium (Batelle et al., 1982). Drugs or chemicals that are absorbed by pinocytosis become concentrated in lysosomes where they are subjected to digestion by hydrolytic enzymes. Some toxicants, however, may inhibit the hydrolytic process, resulting in drug accumulation and tubular cell toxicity that may resemble lysosomal storage disease (as occurs in aminoglycoside nephrotoxicity).

2.5.2 *Clinical risk factors*

The application of multivariate analysis for investigating clinical risk factors in the onset of acute renal failure (Rasmussen & Ibels, 1982) may provide some insight into factors that may increase vulnerability to nephrotoxicity from drugs and chemicals. The risk factors summarized in Table 3 show that multiple risk factors coexist in the majority of patients with acute renal failure. Although age has been recognized as a factor in a number of studies (Porter, 1989; Porter & Bennett, 1989), it may simply be a convenient marker for the change in renal vulnerability that relates to the decline in glomerular filtration rate (GFR) occurring beyond the age of 50 (Davies & Shock, 1950). The pathological basis for this decline is not certain but may be related to vascular changes that accompany aging (Avendano & Lopez-Novoa, 1987). Another possible explanation is that the kidneys of people over 50 years of age no longer respond to hypertrophic growth factors. Renal donors aged 50 or more show little or no functional increase after the loss of one kidney (Boner et al., 1972). Indeed, renal function reserve declines linearly with time after 30 years of age (Anderson & Brenner, 1986). Age may also reflect a loss of the ability of the renal tissue to repair. In young indi-

Table 3. Frequency of combined risk factors in 143 patients with acute renal failure (ARF)[a]

	Age	Hyper-tension	Gout/ hyper-uricaemia	Diabetes	Renal disease	Diuretics
Age (> 59 years)	30					
Hypertension	29	4				
Gout/hyperuricaemia	21	18	4			
Diabetes	11	6	4	1		
Renal disease	18	12	12	6	4	
Diuretics	29	27	21	8	13	0
Multiple risks[b]	108	63	37	14	13	0

[a] Modified from: Rasmussen & Ibels (1982).
[b] Significant risk contribution to ARF based on discriminant multiple linear regression analysis.

viduals nephrotoxicity in terms of tubular necrosis may be compensated for by constant repair, while in older patients this repair capacity may be diminished, resulting in the clinical expression of renal injury (Laurent et al., 1988).

Pre-existing renal disease is an obvious risk factor predisposing to abnormal accumulation and excess blood levels of many nephrotoxic drugs and chemicals. It is not clear whether sex is a predisposing risk factor in humans, but male rodents are considerably more susceptible than females to nephrotoxicity and carcinogenicity from many environmental toxins (NTP, 1983, 1986, 1987).

Factors such as short-term and high-dose exposure versus chronic and/or low-dose exposure influence vulnerability via the mode of metabolism, rate of excretion, etc. Long-term, low-dose exposure to substances that have a long biological half-life, such as lead or cadmium, increases risk from these nephrotoxins, but their role as co-risk factors is not known.

2.5.3 *Extrapolation of animal data to man*

The use of animals has been essential to help define the molecular basis and the progression of model nephro-

pathies, but it may be inappropriate to extrapolate animal toxicology data directly to man because of marked species, strain, dietary, and sex differences. In addition, there may be differences in dosing levels and regimen and in the absorption, distribution, metabolism, and excretion of potential nephrotoxins. There are also very significant differences in renal structural and functional characteristics in the common laboratory species used for risk assessment.

Chemical safety assessment has generally been undertaken in relatively few strains of animals, such as the Sprague-Dawley, Wistar and Fisher-344 rats. There is limited information on inter-species comparisons. In addition to assessing the renal differences in each of the species or strains used, it is necessary to examine extra-renal differences. Thus, for example, there are marked species differences in the hepatic handling of chemicals (Smith 1974; Testa & Jenner, 1976) and the metabolic capacity of each of the major organ systems (Litterst et al., 1975a; Kluwe, 1983). This will have profound consequences on the amount of a parent chemical and the pattern of metabolites that reach the kidney. Dietary factors such as carbohydrate, lipid, and protein intake alter renal function, and the presence of contaminants and natural toxicants may add to the toxic burden of the kidney (Bridges et al., 1982).

If the mechanistic basis of a renal injury is clearly established, it is easier to assess the risk of chemical injury in man, but such data are at present only available for a few chemicals. There are relatively few examples of nephrotoxic chemicals where there is a full profile of information from experimental animals and man, and in the vast majority of cases data are available only in rats.

In order to extrapolate animal data for risk assessment, each screening procedure should cover a sensible level of exposure and a comparable condition to that found in man. The experience gained should provide a foundation from which a rational basis can be developed to identify potentially exacerbating risk factors and from which nephrotoxicity can be reduced. Some lesions can only be induced in rats with difficulty, and there may be a need

to use sensitive species or strains and/or to adapt certain experimental manoeuvres to produce a lesion similar to that which occurs in man.

2.5.4 *Risk assessment from nephrotoxicity studies in animals*

Risk assessment from nephrotoxicity studies in animals has been best defined for therapeutic agents. Many have been widely tested in animals as a pre-clinical safety evaluation or used to study the mechanism of renal injury where there are adverse reactions caused by these compounds in clinical usage. The risk assessment for a number of workplace or environmental chemicals has been developed from animal models that have been used to study the mechanisms of these effects, especially those of the heavy metals and some of the industrial organic chemicals.

2.5.5 *Special risk groups in humans*

The marked variability in the response of any study population to potentially nephrotoxic compounds establishes clearly that there are groups at risk. There are a number of factors that could be responsible for increasing the risk of nephrotoxicity. These include existing renal disease, loss of renal parenchyma, high protein diet, chemical exposure, predisposing factors, multiple myeloma, and other conditions where there is an added level of protein excretion when the kidney is under an additional work-load.

So far there has been relatively little interest in the individuals that do not appear to be at risk from exposure to potential nephrotoxins. While this is generally assumed to be the result of an absence of predisposing factors, there may well be other chemical, dietary, or disease considerations that provide a protective effect. There is experimental evidence to suggest that a pre-existing streptozotocin-induced diabetes and also poly-aspartic acid protect against aminoglycoside-induced renal injury, and that fish oil diets (high in ω-3 polyunsaturated fatty acids) reduce cyclosporin-A nephrotoxicity. These factors could well be used to reduce the health impact of nephrotoxicity.

2.5.6 Multichemical exposure

At present, there is virtually no information on the effects of multichemical exposure in man and very little data on the effects of more than one chemical administered simultaneously in animals. Simultaneous exposure to several chemicals represents a major toxicological problem, as man is generally exposed to more than one substance in medicines, in food, and from environmental factors. However, most experimental studies have investigated only single chemicals. Interactions have been studied between mercuric chloride, potassium dichromate, citrinin, and hexachloro-1,3-butadiene (HCBD) *in vivo* and *in vitro* using a rat model (Baggett & Berndt 1984a,b; 1985). Dichromate potentiates the mercuric chloride effect, i.e. the effects produced by the combination of metals are always greater than the sum of the individual effects. There appears to be no simple kinetic explanation, i.e. no enhanced renal accumulation of the mercuric ion. The plasma membrane may be a site for the interaction of these metal ions and could be the preliminary step that leads to overall renal dysfunction and an ultimately enhanced acute renal failure. Dichromate-citrinin and dichromate-HCBD interactions have been demonstrated by alterations in urine flow, glucose excretion, and transport processes. Some experimental data suggest that a synergistic interaction may occur in analgesic nephropathy. The mechanisms that underlie these interactions are not understood, and at present there is no rational basis to predict them. Experimental studies have shown that tubular cell injury, induced by trichloroethylene and carbon tetrachloride, is potentiated by exposure to polyhalogenated biphenyls, e.g., polychlorinated biphenyls (Kluwe et al., 1979).

2.5.7 Renal functional reserve

The concept of renal functional reserve is a simple one in which not all of the nephrons nor all of the cellular functions in a single nephron are available or used at any one time (Friedlander et al., 1989). Thus there is a buffering capacity in the kidney that can cope with short-lived or protracted demands on function that exceed the normal level. Part of this functional reserve is used to

meet the response to perturbation of the homeostatic system by water or electrolyte loading. Most of the studies on and understanding of renal functional reserve relate to changes in glomerular filtration rate and renal blood flow. It is likely that additional approaches are needed to test for other types of functional reserve.

2.5.8 *The effects of chemicals on kidneys with pre-existing renal lesions*

Although it is generally acknowledged that there are several types of renal lesions that exist as a nephropathy in the general population at a low but significant level (e.g., nephrotic syndrome), little is known about how these pre-existing lesions affect the response of the kidney to subsequent nephrotoxic insults.

2.5.8.1 *Nephrotoxicity in the presence of renal and extrarenal disease*

Safety screening is conducted on young, disease-free animals, housed under optimal conditions, fed contamination-free, high-protein food. By contrast, man is exposed to a variety of dietary and environmental chemicals and to a poly-pharmacy of both prescribed and self-administered medications over many years. In addition, screening is generally undertaken using normal experimental animals. This may be inappropriate because, with the exception of occupational and environmental exposure, man is exposed to potentially nephrotoxic therapeutic substances to treat disease. Pre-existing diseases can have a profound effect on the direct or indirect response of the kidney to handling chemicals (Bennett, 1986). There is an increasing wealth of animal data to demonstrate that common clinical conditions in man, such as hypertension, renal compromise, and renal ischaemic injury, exacerbate cyclosporin nephrotoxicity and bacterial endotoxins in animals and that systemic infection increases the sensitivity of the kidney to aminoglycoside toxicity (Bergeron et al., 1982).

The role of pre-existing renal lesions on nephrotoxicity is important, but there are few clear indications of what can be predicted from existing clinical data and from animal studies. Diabetes is generally associated with reduced renal function, diabetic nephropathy, and renal

papillary necrosis, and might be expected to exacerbate chemical-associated nephrotoxicity. Untreated streptozotocin-induced diabetes, however, protects rats against gentamicin, low-dose cisplatin, and uranyl nitrate nephrotoxicity (Teixeira et al., 1982; Vaamonde et al., 1984). Recent studies on the acute effects of intravenous radiocontrast media on anaesthetized diabetic rats were inconclusive (Reed et al., 1983, Golman & Almen, 1985, Leeming et al., 1985).

3. KIDNEY STRUCTURE AND FUNCTION

An in-depth review of kidney structure and function is beyond the scope of this monograph. Only sufficient information will be given to provide a general background against which nephrotoxicity can be framed. A fuller insight into the complexities of the kidney in health, disease, and nephrotoxicity has been described by Valtin (1973), Orloff & Berliner (1973), Hook (1981), Porter (1982), Bach et al. (1982, 1989), Bach & Lock (1982, 1985, 1987, 1989), Seldin & Giebisch (1985), Brenner & Rector (1986).

3.1 Renal anatomy

The two kidneys are situated retro-peritoneally, on either side of the vertebral column, and process 25% of the resting cardiac output via an arterial blood supply. Much of the fluid and most of the solutes in blood are filtered through the glomeruli into the proximal part of the nephron (the functional unit of the kidney) from which essential small molecules are reabsorbed. Numerous macromolecules are reabsorbed into the tubular cells by an endocytotic process and are digested in tubular lysosomes. Many organic acids and bases (including many drugs) are secreted (and reabsorbed) by carrier-mediated processes located principally in the proximal tubule. There is some secretion, mainly of waste solutes, from the blood into the distal part of the nephron, and much of the water in which they are dissolved is subsequently reabsorbed.

Each kidney is made up of a large number of nephrons, groups of which unite to continue as collecting ducts or tubules, and these in turn combine to make up the ducts of Bellini, which exit around the papilla tip. The papilla opens into the calix, which is in continuity with the renal pelvis, a funnel-shaped area that narrows to the ureter. The continued production of urine, together with peristalsis of the ureter, carries excreted waste to the bladder. The morphophysiology of the kidney varies markedly between species. Therefore, a generalized description will be provided, and only the important differences between the rat (and other common laboratory animals) and man will be described (Moffat, 1979).

3.1.1 Histology

Renal lesions occur in discrete anatomical regions. This highlights the need to understand changes in terms of the biochemical properties of the specifically affected region and its adjacent cells. While haematoxylin and eosin staining and a number of other routinely used staining procedures identify nephropathies and renal degeneration, these are generally based on a relatively non-specific assessment. The non-specificity of routine histopathology has, in fact, been the strength of these methods in the preliminary assessment of chemically induced nephropathies. It may, however, miss some types of lesions and generally gives little information that can help identify the mechanistic basis of a lesion.

Histochemical techniques can provide insight into primary and secondary cellular mechanisms. One aspect of "histochemistry" is the use of frozen segments of the nephron (Bach et al., 1987) and the application of fluorimetric or radiochemical assays to measure the activities and distribution of specific biochemical characteristics. Microdissection generally fails to give a detailed localization of these properties in relation to specific or individual cells. In addition, the technique is difficult to apply to injured renal cells.

The most widely used histochemical approach is based on obtaining frozen, fixed frozen, or fixed embedded sections of the kidney that are then used with chromo- or fluoro-phores. These react with a selected type of material. The types of materials that can be visualized in section depends on their chemical structure (e.g., carbohydrate), enzymic activity (e.g., lactate dehydrogenase), antigenicity (e.g., specific molecules), or physicochemical properties (e.g., lipophilicity), some of which are shown in Fig. 2. This approach also includes the distribution or incorporation of radiolabelled molecules by their interaction with a photographic film laid over the section (Bach et al., 1987). Immunohistochemical techniques using labelled antibodies permit antigens to be localized at the light microscopical and ultrastructural levels.

These microscopic histochemical techniques provide information on the distribution at, or within, specific

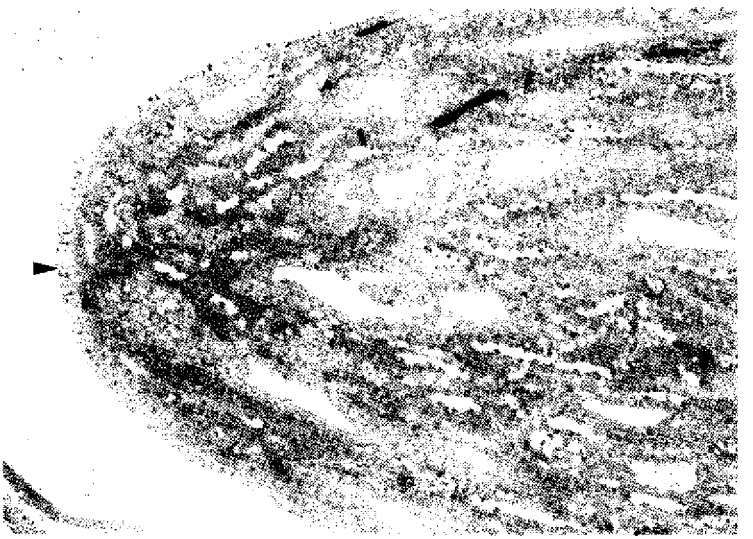


Fig. 2a. Papilla tip from kidney of control animal showing characteristic pink (Giemsa) staining of interstitial matrix and single cuboidal covering epithelium (arrowhead). Giemsa, x 89. All the photomicrographs in Fig. 2 were supplied by Dr N.J. Gregg.

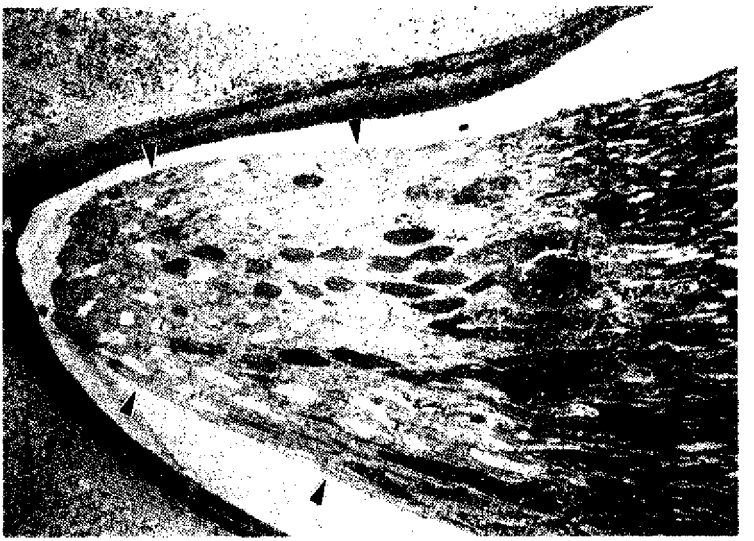


Fig. 2b. Low magnification micrograph showing typical RPN lesion 72 h after BEA. Note denudation of covering epithelium (arrowheads). Giemsa, x 36.

Fig. 2c. Control urothelium from Wistar rat showing three distinct layers: a) epithelium, b) lamina propria, c) lamina muscularis. Giemsa, x 357.

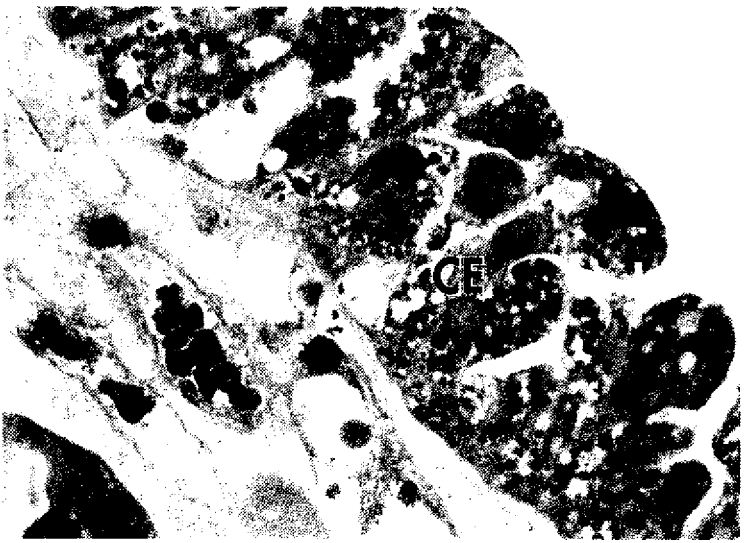


Fig. 2d. Hyperplasia of leading edge of remaining covering epithelium (CE); blebbing, sloughing cells have atypical nuclei and numerous eosinophilic cytoplasmic granule, 72 h after BEA (100 mg/kg), H&E, x 893.

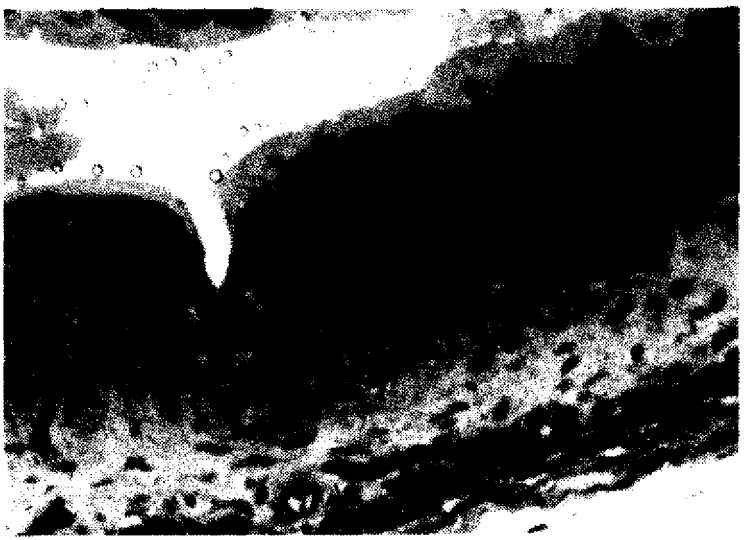


Fig. 2e. Mosaic pattern of alkaline phosphatase staining in hyperplastic urothelium, 18 h after BEA, alkaline phosphatase, x 357.

Fig. 2f. Heterogenous ATPase staining in proteinaceous casts in necrotic tubules in papilla, 72 h after BEA, ATPase, x 36.

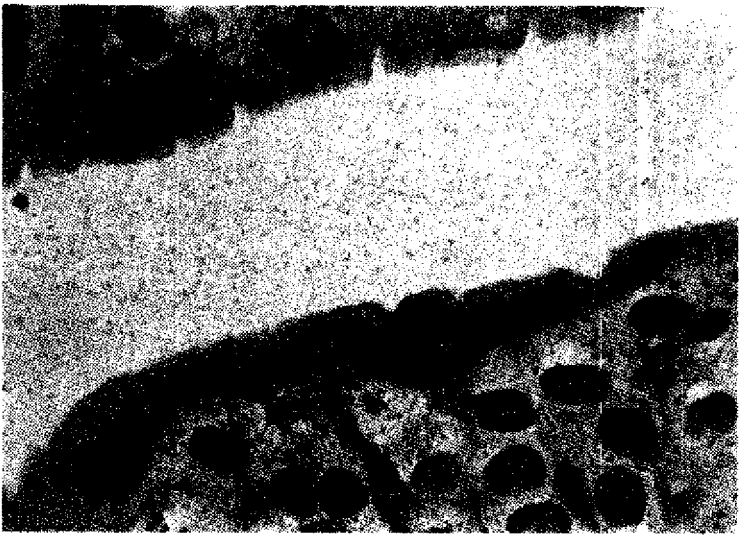


Fig. 2g. Sub-urothelial capillary endothelium stained with ATPase, thickness increasing to occlude lumen (arrowheads), ATPase, x 893.

Fig. 2h. Numerous PAS positive staining granules and inclusion bodies in superficial layer of hyperplastic ureteric urothelium, 21 weeks after BEA (100 mg/kg). PAS, x 893.

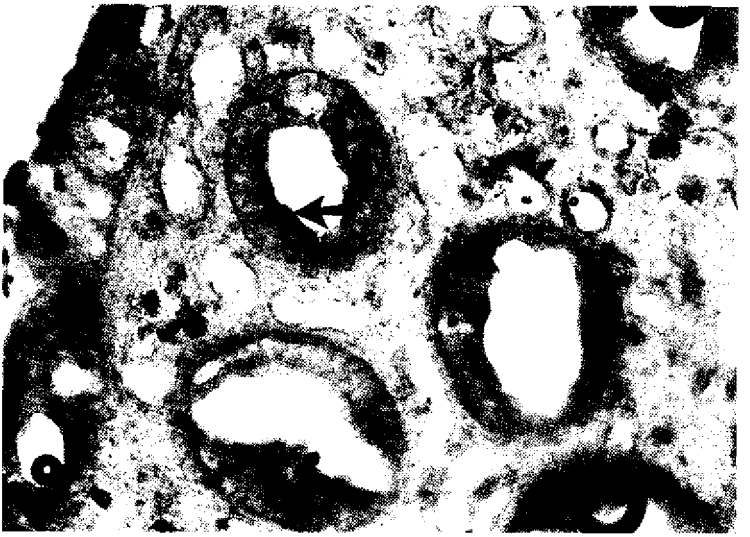


Fig. 2i. Fixed frozen section of papilla 7 days after single iv dose of BEA (100 mg/kg) showing Oil red O stained lipid in collecting duct (arrow) and covering epithelium cells. Oil red O, x 223.

cells and their relative activities, and have been used to define a variety of characteristics of the kidney. It is important to stress that each method has its own inherent strength and weakness, and that it may be difficult to relate data from tissue sections to absolute biochemical measurements. This is a consequence of the complex mixture of materials that are present in tissue sections and the chemical changes that may take place in these sections, particularly once they have been fixed. While the biochemical characteristics of tissue in frozen sections are least likely to be adversely affected, subtle and misleading alterations in chemical properties can still occur. In addition, treatments to conserve morphological features (fixation and embedding) alter these values. It may also be difficult to be certain whether an increased intensity of staining for a certain substance represents *de novo* synthesis, unmasking, or the loss of factors that suppress staining. These two techniques have proved to be very powerful in providing information on the biochemical characteristics of cells and the changes associated with

renal injury. The *in situ* hybridization techniques, using specific labelled nucleotidic probes, permit the detection of protein synthesis at a subcellular level.

3.1.2 Enzyme histochemistry and quantification

Histochemical heterogeneity is evident in each part of the nephron and varies between different species. In addition, the profile of characteristics within any region of the kidney and nephron may be related to sex and age. Antibodies are directed against unique or novel characteristics along the nephron that are present on enzymes, glycoproteins, or other molecules associated with membranes or soluble cytosolic constituents. The biochemical characteristic can be visualized using enzyme, fluorophore, or radioactive labels (Bach et al., 1987). Individual microdissected nephron segments have been studied to determine the quantitative distribution of selected enzymes (Table 4). Table 5 lists biochemical and function parameters for the different nephron segments, showing their individual receptor sensitivity.

3.1.3 Immunohistochemistry

The use of antibodies raised towards enzymes can help in the study of isoenzyme distribution and factors affecting changes in their distribution. For instance, aldolase-B monomers increase in the proximal tubules of rats during renal maturation, but not in the distal tubules. In contrast, aldolase-A monomers increase in the distal tubules but not in the proximal tubules.

Immunohistochemical techniques demonstrate many functional proteins at discrete locations in the kidney, including the glomeruli and the tubular basement membrane, but there are few data on changes in these proteins as a result of nephrotoxicity. A variety of immunodeposits are associated with glomerulopathies including those caused by heavy metals, but these appear to be T-cell mediated. They are assessed by immunofluorescent monitoring in the glomerulus, but this acts as a passive sieve and may be involved as a secondary consequence of immunodeposition.

Histochemistry can also be used to show the distribution of a number of oxidative enzymes. Cytochrome P-450

Table 4. Distribution of enzymes in individual nephron segments in various animal species

Enzyme	Relative activity[a]	Animal
Specific to the glomerulus		
Adenosine deaminase		rat
Specific to the proximal tubule		
Glucose-6-phosphatase	$S_1 > S_2 > S_3$	rat
Fructose-1,6-bisphosphatase	$S_1 < S_2 > S_3$	rat
Phosphoenolpyruvate carboxykinase	$S_1 > S_2 > S_3$	rat, rabbit
Fructokinase	$S_1 = S_2 < S_3$	rat
Fructose-1-phosphate aldolase	$S_1 = S_2 > S_3$	rat
Glycerokinase	$S_1 = S_2 > S_3$	rat, rabbit
Glycerol-3-phosphate dehydrogenase	$S_1 = S_2 \leq S_3$	rat
Glutamine synthetase	S_3	rat
Alanine aminotransferase	$S_1 = S_2 < S_3$	rat
Gamma-glutamyltraspeptidase	$S_1 < S_2 < S_3$	rabbit
Gamma-glutamyl-cysteine synthetase	S_3	rat
Glutathione-*S*-transferase	$S_1 < S_2 \leq S_3$	rabbit
Cytochrome P-450	$S_1 = S_2 \geq S_3$	rat, rabbit
Alanine aminopeptidase	$S_1 < S_2 < S_3$	rat
Alkaline phosphatase	$S_1 = S_2 = S_3$	rat
Leucine aminopeptidase	$S_1 < S_2 < S_3$	rat
D-Amino acid oxidase	$S_1 = S_2 < S_3$	rat
L-Hydroxy acid oxidase	$S_1 = S_2 < S_3$	rat
Fatty-acyl-CoA oxidase	$S_1 = S_2 < S_3$	rat
Choline oxidase	$S_1 = S_2 < S_3$	rat
25(OH)-D_3-1α-hydroxylase	$S_1 < S_2 = S_3$	rat (D_3 deficient) rabbit (fetus)
Relatively specific to the proximal tubule		
Glutamate dehydrogenase	$S_1 = S_2 > S_3$	rat, rabbit
Malic enzyme	$S_1 = S_2 < S_3$	rat
Trypsin-type protease	$S_1 > S_2 > S_3$	rat
β-D-Galactosidase	$S_1 = S_2 < S_3$	rat
N-Acetyl-β-D-glucosaminidase	$S_1 = S_2 > S_3$	rat
Xanthine oxidase	$S_1 > S_2 > S_3$	rat
Superoxide dismutase	$S_1 > S_2 > S_3$	rat
Specific to the lower nephron		
Hexokinase	MTAL = CTAL > DCT > CCD = MCD	rat, rabbit
Phosphofructokinase	MTAL = CTAL = CCD = MCD	rat, rabbit
Pyruvate kinase	MTAL = CTAL < DCT < CCD = MCD	rat, rabbit
Kallikrein	CNT	rabbit
Relatively specific to the lower nephron		
Fructose 1,6-bisphosphate aldolase	MTAL = CTAL > DCT	rat
Glycerol dehydrophosphate dehydrogenase	CCD < MCD	rabbit

Table 4 (contd).

Enzyme	Relative activity[a]	Animal
Relatively specific to the lower nephron (contd.)		
Lactate dehydrogenase	MTAL = CTAL-DCT = CCD	rat, rabbit
Aspartate aminotransferase	MTAL = CTAL > DCT	rat
Citrate synthase	MTAL = CTAL = DCT > CCD	rat
Isocitrate dehydrogenase (NAD^+)	MTAL = CTAL = DCT = CCD	rat
Na,K-ATPase	MTAL < CTAL < DCT > CCD	rat

[a] CCD = cortical collecting duct; CNT = connecting tubule; CTAL = cortical thick ascending limb of Henle's loop; DCT = distal convoluted tubule; MCD = medullary collecting duct; MTAL = medullary thick ascending limb of Henle's loop;
S_1 = early proximal tubule; S_2 = middle proximal tubule; S_3 = late proximal tubule.

mixed-function oxidase activities have been shown immunohistochemically to be localized in the proximal tubule, particularly in the S_2 and S_3 segments in the rat and rabbit. Large numbers of peroxisomes containing D-amino acid oxidase and catalase are localized in the S_3 portion of the proximal tubule, but they are absent from the glomerulus and the distal nephron. There is little immunohistochemical data on the distribution of molecules that are likely to protect renal cells from the effects of reactive intermediates. Ligandin or glutathione-*S*-transferase B is located in the proximal tubule of both animals and man and in the thick limb of the loop of Henle in man. Catalase activity is greatest in the proximal tubule (where it is localized in the peroxisomes), less in the distal tubule, and very low in the glomerulus. Glutathione has been shown by histochemistry to be localized in the proximal convoluted tubule. However, there are some uncertainties as to what is being assessed, since the reaction measures sulfhydryl groups and not only glutathione. The distribution of at least one superoxide dismutase isoenzyme shows a marked species difference between the dog and rat, but is localized in the proximal tubules in both (Bach et al., 1987).

3.2 The renal blood supply

Each kidney is supplied by a renal artery (a branch of the abdominal aorta), which divides to form several inter-

Table 5. Summary of nephron heterogeneity[a]

Anatomical region[b]	Biochemical features[c]	Functional features
Glomerulus SF < JM	Renin:(SF > JM)	GFR:(SF < JM)
epithelium	Adenosine-AC	Mesangial contraction (AII, histamine)
endothelium	Histamine-AC (intra-mesangium)	
mesangium	Serotonin-AC (extra-mesangium)	
	ANP-GC	ROM generation (intra- and extra-mesangium)
	ET-PGE2 (intra-mesangium)	
	AVP-PGE2 (intra- and extra-mesangium)	
	AII-PGE2 (intra- and extra-mesangium)	
Proximal tubule	Glucose carrier:	J-glucose:
S_1, S_2, S_3	(brush border)	$(S_1 > S_2, S_3)$
	Gluconeogenesis	
	$(S_1 > S_2 > S_3)$	J-V:$(S_1 > S_2 > S_3)$
	Cytochrome P-450:	$1,25(OH)_2D_3$ synthesis
	$(S_1 < S_2 > S_3)$	
	NADPH-cytochrome c reductase:	J-V decreased by ANP
	$(S_1 < S_2 > S_3)$	
	Ammoniagenesis	P-Cl/P-Na:(SF > JM)
	$(S_1 > S_2 > S_3)$	
	PTH-AC $(S_1 > S_3)$	PAHsecretion:
		$(S_1 < S_2 > S_3)$
	Adenosine-AC	J-aminoacid:
		$(S_1 > S_2, S_3)$
Henle's loop		
Thin: DTL < ATL	AVP-AC(+ATL,-DTL)	P-water:(DTL > ATL)
		P-NaCl:(DTL < ATL)
		P-urea:(DTL < ATL)
Thick: MTAL > CTAL	AVP-AC:(MTAL > CTAL)	AVP stimulation of J-Cl: (MTAL > CTAL)
	PTH-AC:(MTAL < CTAL)	PTH stimulation of J-Ca: (MTAL < CTAL)
	SCT-AC:(MTAL > CTAL)	
	Tamm-Horsfall glycoprotein	
	PGE2 synthesis	J-NaCl:(MTAL > CTAL)
	(MTAL > CTAL)	PG inhibition of J-Na: (+MTAL, -CTAL)
		EGF synthesis
		J-Na decreased by ANP
Distal tubule		
DCT:single cell type	SCT-AC:(+DCT)	SCT suppression of Vt:(DCT)
CNT:multiple cell types	PTH-AC:(+CNT)	PTH stimulation of J-Ca: (CNT)
	AVP-AC:(+CNT)	AVP suppression of Vt:(CNT)
	ISO-AC:(+CNT)	ISO suppression of Vt:(CNT)
	Kallikrein:(+CNT)	
	Aldosterone binding	Vt:(DCT < CNT)
		K-secretion:(DCT < CNT)?

Table 5 (contd).

Anatomical region[b]	Biochemical features[c]	Functional features
Collecting duct system		
two cell types	AVP-AC:(CCD > OMCD)	
CCD (P.cell > I.cell)	ISO-AC:(CCD > OMCD)	Vt:(CCD > OMCD) J-Na and J-V decreased by ANP
OMCD (P.cell > I.cell)	PG-AC:(CCD < OMCD) PGE2 synthesis (CCD < OMCD < IMCD) Aldosterone binding Adenosine-AC (CCD > OMCD) ANP-GC (CCD < OMCD < IMCD)	P-urea:(CCD OMCD < IMCD)
IMCD		J-V decreased by ANP

[a] Based on data obtained from the rabbit and rat kidney.

[b] Parts of the nephron: ATL = ascending thin limb of Henle's loop; CCD = cortical collecting duct; CNT = connecting tubule; CTAL = cortical thick ascending limb of Henle's loop; DCT = distal convoluted tubule; DTL = descending thin limb of Henle's loop; I.cell = intercalated cell; IMCD = inner medullary collecting duct; JM = juxtamedullary nephron; MCT = medullary collecting duct; MTAL = medullary thick ascending limb of Henle's loop; OMCD = outer medullary collecting duct; P.cell = principal cell; S_1 = early proximal tubule; S_2 = middle proximal tubule; S_3 = late proximal tubule; SF = superficial nephrons.

[c] Hormone effects on nephron receptors: AII = angiotensin II; AC = adenylate cyclase; ANP = atrial natriuretic peptide; AVP = arginine vasopressin; GC = guanylate cyclase; EGF = epidermal growth factor; ET = endothelin; ISO = isoproterenol; PG = prostaglandin; PTH = parathyroid hormone; ROM = reactive oxygen metabolites; SCT = salmon calcitonin. Transport substances: PAH = para-aminohippurate; J-x = flux of substance x; J-V = flux of fluid volume; P-x = permeability for substance x; Vt = transcellular voltage.

lobar arteries (Fig. 3). These in turn give rise to the arcuate arteries, which run between the cortex and medulla parallel to the kidney surface. Many cortical radial arteries arise from the arcuate vessel and pass through the cortex. Here a small amount of blood reaches the surface to supply the kidney capsule, but most of the blood flow is directed through branches that form the afferent arterioles to the glomeruli. Each afferent arteriole breaks up to form the capillary plexus of the glomerulus; this is drained into the efferent arteriole. The efferent arterioles form two types of capillary networks

- In the "mid" and "superficial" cortical regions, they form the peritubular capillaries surrounding proximal and distal tubules (in the superficial regions some peritubular capillary networks interlace the nephron from which they were derived, but such an

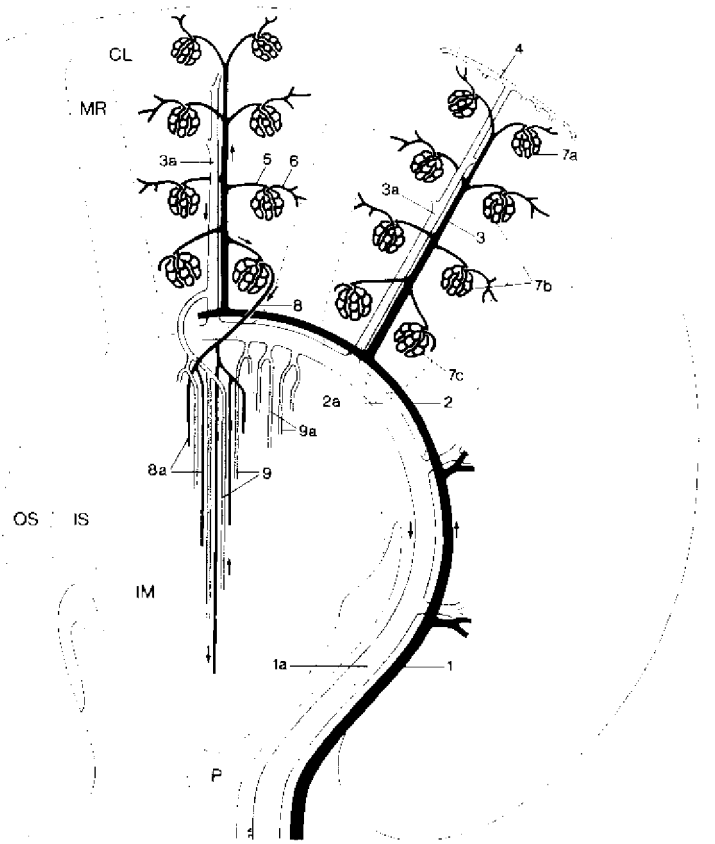


Fig. 3. Scheme of renal vessels. This scheme depicts the course and distribution of the intrarenal blood vessels; peritubular capillaries are not shown. Not drawn to scale. Within the cortex the medullary rays of the cortex (MR) are delineated from the cortical labyrinth (CL) by a dashed line. OS = outer stripe; IS = inner stripe; IM = inner medulla; P = renal pelvis. 1/1a = interlobar artery and vein; 2/2a = arcuate artery and vein; 3/3a = cortical radial artery and vein; 4 = stellate vein; 5 = afferent arteriole; 6 = efferent arteriole; 7a/7b/7c = superficial, midcortical and juxtamedullary glomerulus; 8/8a = juxtamedullary efferent arteriole, descending vasa recta; 9/9a = ascending vasa recta (those ascending within a vascular bundle and those independent from a bundle). From: Kriz & Bankir (1988).

association appears to be the exception rather than the rule).

- In the juxtamedullary region (and some mid-cortical areas in man) each efferent arteriole is directed into the medulla, where it branches into the vasa recta bundles. Each bundle consists of up to 30 descending vessels, the peripheral vessels of which give rise to a highly branched capillary network in the outer medulla. The core of the vasa recta bundle continues to the inner medulla where it terminates in a capillary network (Beeuwkes, 1980).

The walls of all peritubular capillaries in the kidney are made up of a thin fenestrated endothelium resting on a basal lamina. The capillaries in the cortex generally open into the cortical radial vein, from which blood flows via the arcuate vein to the renal vein and finally to the inferior vena cava. The capillary plexuses in the medulla drain into the ascending vasa recta, which join the arcuate veins. The arterial branches are terminal, without anastomoses. In contrast, the veins are richly anastomosed.

There is a well-defined structural relationship between the vasa recta bundles and the nephrons in the outer medulla, at least in the animals that have been studied. A central core (consisting of descending and ascending vasa recta) is surrounded by a peripheral layer consisting of a closely intermingled ascending vasa recta and the descending thin limbs of the loops of Henle. Between these bundles are the thick ascending limbs of the loops of Henle, some descending limbs, and the collecting ducts. Within the bundles both ascending and descending vasa recta are in intimate contact with each other (rather than with the same type of vessel). There are more ascending vessels, all of larger diameter, than descending ones, and this increased volume capacity relates to the removal of excess water from the interstitium and the maintenance of the medullary osmotic gradient shown in Fig. 4.

Many of the "major" and "minor" blood vessels in the kidney have either smooth muscle cells as an integral part of their structure, or other cells that may have a contractile function. Thus most of the intrarenal vascular system has both adrenergic and cholinergic innervation. Intrarenal blood flow, the factors which alter it, and its effects on renal function are poorly understood. Although

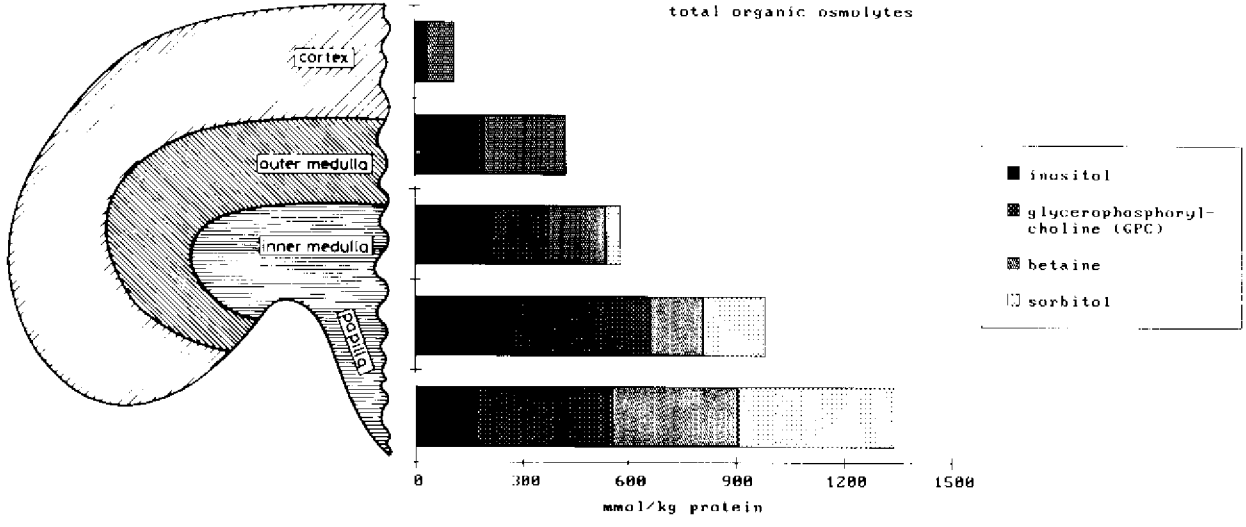


Fig. 4. Cortico-papillary concentration gradient of osmolytes in rat kidney. Results obtained from kidneys of 6 rats are given as means ± SEM. Personal communication by W. Guder, Munich, to IPCS.

there appear to be the facilities for a direct and effective perfusion of the medulla from the arcuate artery, this does not seem to occur (Moffat, 1979; Beeuwkes, 1980).

3.2.1 Renal haemodynamics

The measurement of total blood flow through the kidneys can be measured relatively easily using modern techniques (Grunfeld et al., 1971; Pearson, 1979). Defining the zonal blood flow has given some conflicting results, but assessing regional blood flow within the kidney is fraught with difficulties and is subject to varied interpretations (Grandchamp et al., 1971; Grunfeld et al., 1971; Pearson, 1979; Aukland, 1980; Knox et al., 1984). There are, however, consistent data (derived from a number of fundamentally different techniques) to show that intrarenal blood flow is greatest in the cortex (80-85% of total renal flow) and that it decreases through the juxtamedullary region to less than 10% of the total renal flow in the medulla. It must be stressed that, although the medulla is poorly perfused in comparison to the rest of the kidney, it is, nonetheless (because of the 25% resting cardiac output and therefore abundant renal blood flow), a well-perfused tissue. According to Thurau (1964), the medullary blood flow is about 15 times that of resting muscle and the same as that of the brain. In addition, the capillary volume fraction of the medulla is more than twice that of the renal cortex (Beeuwkes, 1980). Despite this, there is considerable variation in tissue pO_2 in the kidney; a marked decrease in pO_2 levels is seen with increasing tissue depth (Brezis et al., 1984).

3.3 The nephron

The kidney is divided into three main regions, cortex (outer), medulla (inner), and pelvis (Fig. 5). Within the cortex arise the renal corpuscles, defined as superficial, midcortical or juxtamedullary depending on the anatomical location of the renal corpuscle in the cortex. The nephron is the functional unit of the kidney and consists of a continuous tube of highly specialized heterogeneous cells, which show sub-specialization along the length of nephrons and between them. There are marked structural and functional differences between the nephrons arising in the

cortex and those arising in the juxtamedullary regions. The total number of nephrons varies between different species and within any one species as a function of age. The macroscopic differentiation of the kidney into distinct zones arises not only from the regional vascularity but also from the way different functional parts of the nephron are arranged within the kidney. A more detailed account of the ultrastructure of the morphologically definable regions of the nephron and their functional interrelationship has been provided by Moffat (1981, 1982), Bohman (1980), and Maunsbach et al. (1980). Recently the nephron nomenclature has been standardized by the Renal Commission of the International Union of Physical Sciences (Kriz & Bankir, 1988). This is summarized in Figures 3, 5, and 6, and Table 6.

3.3.1 Cellular heterogeneity and cell-cell interaction

There are well over 20 morphologically different cell types (based on light microscopy alone) in the kidney, and when histochemical and immunohistochemical methods are applied to renal tissue sections the diversity of cell types is even more apparent. The spectrum of biochemical (and structural and functional) characteristics in these cells demonstrates the very marked heterogeneity that is the hallmark of the kidney. It is well established that the expression of many of these biochemical characteristics is an integral of the functions of that particular region of the kidney, and there is the potential to change the expression of these characteristics in terms of the demands on the kidney. These include both water and electrolytes, dietary factors, and chemicals with pharmacological and toxic effects, or may be as a result of chemical and other types of injury. More importantly, the characteristics of a cell may make it either resistant or sensitive to the target selective toxicity of a chemical.

3.3.2 The glomerulus

The glomerulus forms the initial part of the nephron and functions as a relatively poorly selective macro-molecular exclusion filter to the hydrostatic pressure of the blood. The number of glomeruli is, in general, related to the mass of the species, and the size of each glomerulus

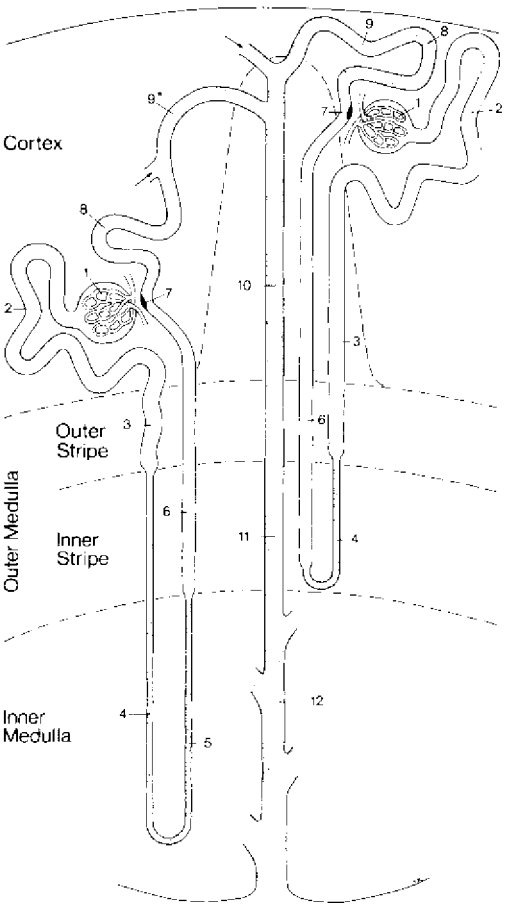


Fig. 5. Scheme of nephron. This scheme depicts a short-looped and a long-looped nephron together with the collecting system. Not drawn to scale. Within the cortex a medullary ray is delineated by a dashed line. 1 = renal corpuscle including Bowman's capsule and the glomerulus (glomerular tuft); 2 = proximal convoluted tubule; 3 = proximal straight tubule; 4 = descending thin limb; 5 = ascending thin limb; 6 = distal straight tubule (thick ascending limb); 7 = macula densa located within the final portion of the thick ascending limb; 8 = distal convoluted tubule; 9 = connecting tubule; 9* = connecting tubule of the juxtamedullary nephron that forms an arcade; 10 = cortical collecting duct; 11 = outer medullary collecting duct; 12 = inner medullary collecting duct. From: Kriz & Bankir (1988).

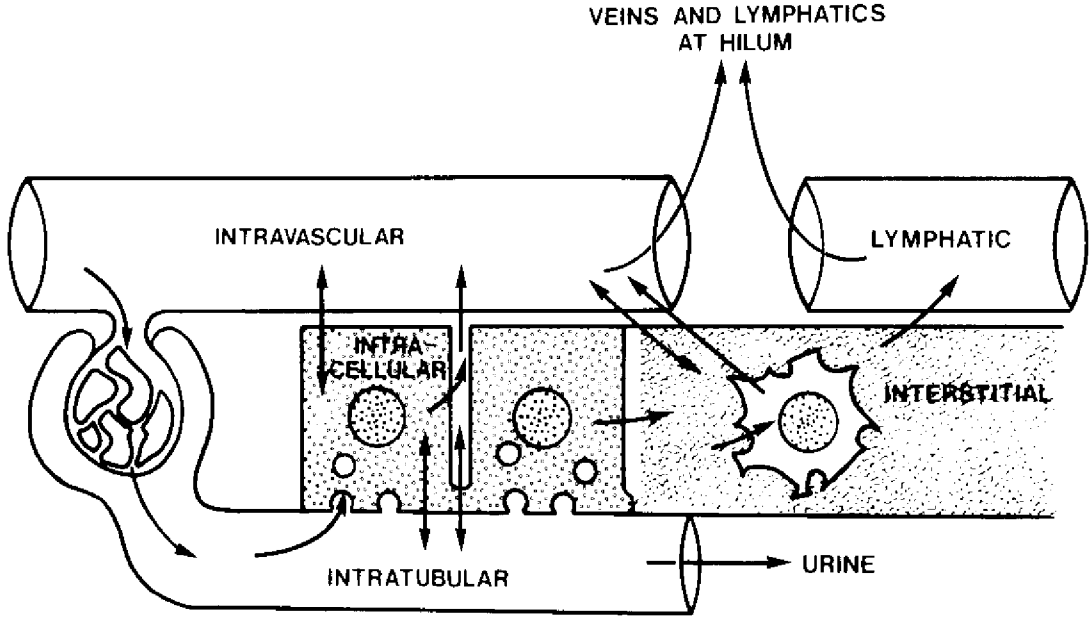
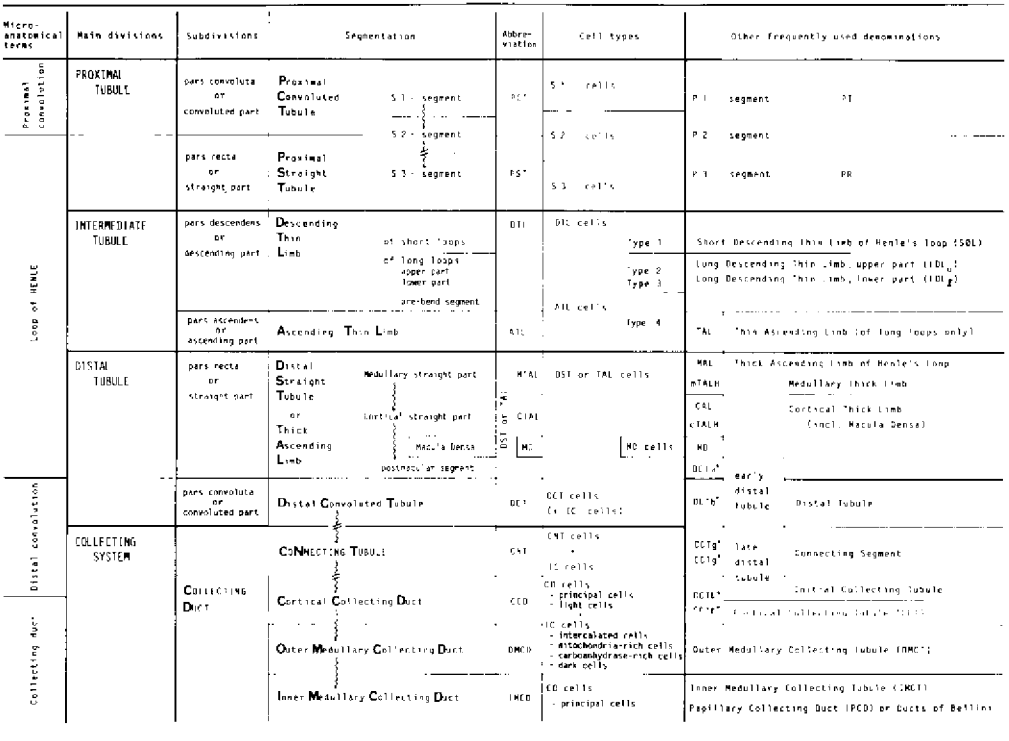


Fig. 6. Schematic representation of the different cellular and extracellular components in the kidney. From: Moffat (1982).

Table 6. Summary of nomenclature of segments and cells of the renal tubule (From: Kriz & Bankir, 1988). A continuous serpentine arrow (→) means that the transition between the two structures is gradual. An interrupted serpentine arrow (⇢) means that the transition is gradual in some species, abrupt in others. Abbreviations marked by a star were introduced by Morel and coworkers (Kidney Int 9: 264, 1976). They mean: DCTa = Distal convoluted tubule, initial portion; DCTb = Distal convoluted tubule, bright portion; DCTg = Distal convoluted tubule, granular portion; DCTl = Distal convoluted tubule, light portion; CCTg = Cortical collecting tubule, granular portion; CCTl = Cortical collecting tubule, light portion



depends, among other factors, on the environmental water balance. Three anatomically distinct types of glomeruli can be identified: those in the superficial cortex, which are part of the superficial nephrons; those arising in the midcortical area; and those of juxtamedullary origin, which continue as nephrons that loop down into the medulla. The structure of the glomerulus is complex (Fig. 7) and has only been defined using scanning and transmission electron microscopy (Maunsbach et al., 1980; Moffat 1981, 1982).

The glomerular "tuft" is made up of a number of capillary branches that arise from the afferent arteriole, anastomose, and drain to the efferent arteriole. There are also communicating vessels between the branch capillaries. The fenestrated endothelium cannot prevent plasma molecules from leaving the lumen, but a negatively charged cell coat imparts some selective permeability. The capillaries are in direct contact with the glomerular basement membrane (or basal lamina), which, when viewed under the electron microscope, can be divided into three layers: the lamina rara interna on the endothelial side; the central lamina densa; and the lamina rara externa, which is in direct contact with the epithelial cells (the podocytes). The basal lamina contains collagen (mostly Type IV) and sialic acid and is rich in glycosaminoglycans, mainly heparan sulfate (Kanwar & Farquhar, 1979), which provides a strongly anionic macromolecular filtration barrier.

The capillary tuft (ensheathed in its basal lamina) is surrounded by a number of podocytes, each of which gives rise to several primary processes (trabeculae). These in turn give rise to secondary processes, and, finally, to numerous tertiary foot processes that are embedded in the lamina rara externa.

The foot processes of one podocyte interdigitate with those of an adjacent epithelial cell for adjacent trabeculae. The surfaces of the podocytes are covered by a strongly anionic cell coat that extends to the spaces between the foot processes. It is through these spaces that the glomerular filtrate reaches the lumen of Bowman's space. Thus, the podocyte provides a structural support for the basal lamina and may also serve to provide additional anionic forces for the process of biological

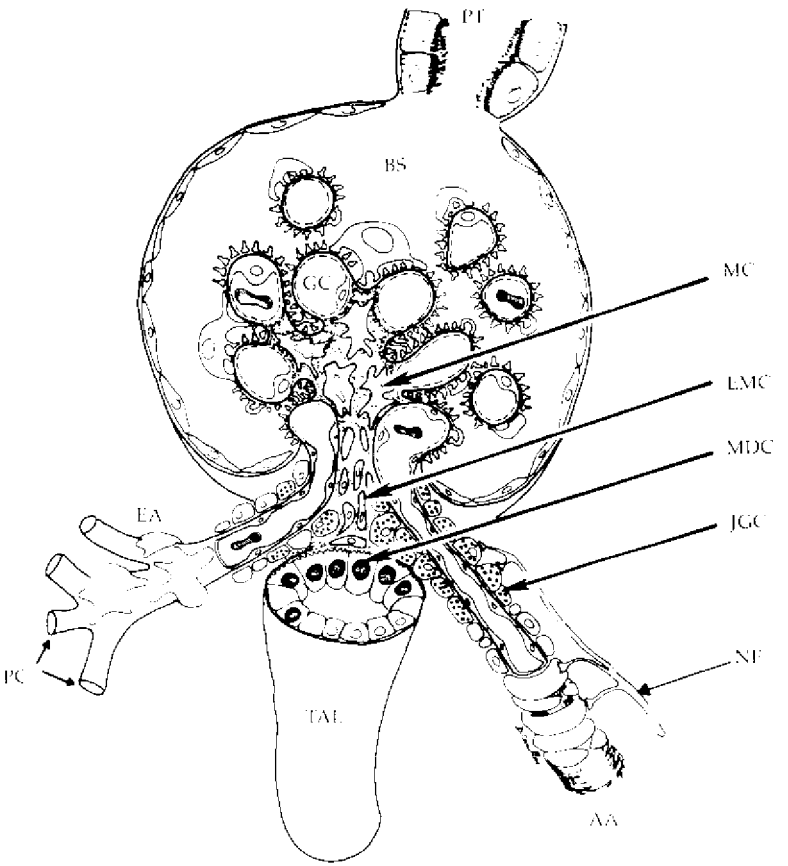


Fig. 7. Glomerulus and juxtaglomerular complex, consisting of afferent arteriole (AA) with the granular cells (JGC) of the juxtaglomerular apparatus, the extraglomerular mesangial cells (EMC), the macula densa (MDC) segment of the ascending loop of Henle, and the efferent arteriole (EA). Also shown are the proximal tubule (PT), Bowman's space (BS), glomerular capillaries (GC), peritubular capillaries (PC), mesangial cells (MC), and nerve fibers (NF). From: Schrier & Gottschalk (1987).

ultrafiltration. It has been suggested that podocytes may have phagocytic properties and undergo contraction (Moffat, 1981).

The axial regions of each glomerulus contain mesangial cells. Information on the structure and possible functions

of mesangial cells has been reviewed by Moffat (1981). In brief, they undergo contraction and may thus control glomerular blood flow via biogenic amine or hormonal control. Of equal importance is the observation that these cells take up large molecules (such as colloids, immune complexes, and protein aggregates), which may eventually be disposed of via the renal lymphatic system.

The driving force for filtration is provided by the glomerular capillary hydrostatic pressure (which is controlled mainly by the vascular tone of the afferent and efferent arterioles), minus both the plasma osmotic pressure and the hydrostatic pressure in the Bowman's space. The resulting "effective filtration pressure" across the basal lamina is about 1.2-2.0 kPa (10-15 mmHg). Selective filtration is achieved primarily on the basis of size restriction by the basement membrane, which impedes the passage of macromolecules with an effective radius greater than 1.8 nm and completely prevents the filtration of macromolecules with an effective radius greater than 4.5 nm. In addition the presence of fixed negative charges on the endothelial, epithelial, and basement membranes hinders the filtration of anionic macromolecules while facilitating the passage of cationic macromolecules. The selectivity of filtration is, in part, a consequence of the anionic nature of the basement membrane, which blocks or slows the passage of negatively charged or neutral macromolecules and leaves those carrying a cationic charge and small molecules (irrespective of charge) to pass unimpeded.

3.3.3 *The proximal tubule*

The proximal tubule is found only in the cortex or subcortical zones of the kidney. Anatomically each proximal tubule can be divided into the convoluted portion (pars convoluta) and the shorter straight descending portion (the pars recta), which then continues to become the descending limb of the loop of Henle. It may be subdivided, by a number of morphological and functional features, into three segments, S_1, S_2, and S_3.

The proximal tubule plays a decisive role in maintaining homeostasis. This is achieved when sodium and chloride ions flux from the tubule lumen to the peritubular capil-

laries under the control of a number of processes such as nonspecific electrophysiological gradients and selective active transport mechanisms. Water follows the ions by osmotic effects. In addition, hydrostatic pressure, attributable to the presence of both proteins and glycosaminoglycans (Wolgast et al., 1973), contributes to water movement from the epithelial cell to the interstitium and thence, by an osmotic gradient, into the capillaries (Valtin, 1973). The flux of ions within the proximal tubule, including the absorption and secretion of HCO_3^- and H^+ and the "lumen trapping" of ammonium ions, controls renal acid-base regulation (Valtin, 1973).

Those proteins that have passed from Bowman's capsule (a significant amount of albumin in the case of normal rats) are reabsorbed in the proximal tubule by pinocytotic removal from the base of the microvillous brush border into the epithelial cells. The vesicles thus formed combine, form protein-filled vacuoles, and fuse with lysosomes, from which the digestion products of the protein diffuse, eventually, to the capillary system or are used in the metabolic processes of the cell.

There are, in addition, other absorptive and secretory mechanisms. These include the co-transport process that reabsorbs glucose and the secretion of both acidic and basic organic compounds (Valtin, 1973; Orloff & Berliner, 1973; Brenner & Rector, 1986; Berndt, 1989).

3.3.4 *The medulla*

The medulla differs from the cortex (Fig. 5 and Fig. 8) both at the macroscopic and at the microscopic levels. This region can be divided into the outer medulla (which is made up of the thin descending and the thick ascending limbs of the loops of Henle, collecting ducts, the vasa recta, and a dense capillary network) and the inner medulla, the free part of which is referred to as the "papilla" (although some researchers apply that name only to the apex of this region). The inner medulla contains the thin limbs of the loops of Henle, collecting ducts, the vasa recta, and a diffuse network of capillaries. Packed into the spaces between these structures are interstitial cells embedded in a matrix rich in glycosaminoglycans.

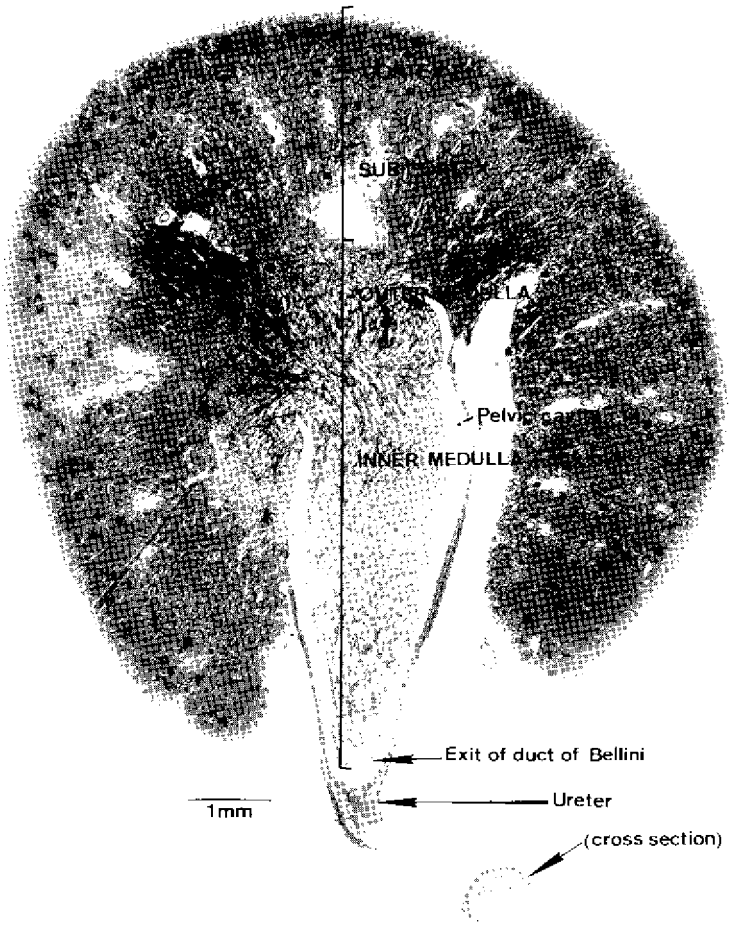


Fig. 8. Coronal section through a normal rat kidney. (Toluidine Blue; bar line = 1 mm). From: Bach & Bridges (1985a).

The collecting ducts terminate as the ducts of Bellini around the tip of the papilla. Whereas the mouse, gerbil, rat, guinea-pig, rabbit, dog, cat, and primate kidneys have only a single papilla, the pig and man have multi-papillate kidneys. There are between 9 and 20 papillae in

each human kidney (Burry et al., 1977), of which there are two anatomically distinguishable types. The conical non-refluxing papillae, where the surface orifices of the ducts of Bellini are slit-like, close when there is an increase in the "back-pressure" of urine from the bladder and so prevent intrarenal reflux when reflux occurs from the bladder. These papillae occur predominantly in the mid zone. The refluxing papillae occur predominantly in the polar regions, and, as they have flattened tips, the collecting duct orifices are wide and prone to retrograde flow of urine into the tubules during vesico-ureteric reflux (Ransley & Risdon, 1979). The microscopic and ultrastructural features of the medulla have been described by several researchers (Moffat, 1979, 1981, 1982; Bohman, 1980; Maunsbach et al., 1980).

3.3.4.1 *The loops of Henle*

The loops of Henle may be divided into two populations on anatomical grounds. Short loops penetrate no further than the outer medulla. The proximal tubule and thick ascending limb are closely associated in the cortex, but in the medulla the descending limb is intimately related to the ascending vasa recta, and the ascending limb to the collecting duct. The association of the ascending and descending limbs of the loop of Henle with the vascular system or with the collecting ducts provides a multi-dimensional network in which solutes or water may undergo countercurrent exchange. These exchanges may either provide a shunt that excludes selected solutes (and water) from the inner medulla or, alternatively, solutes (e.g., sodium chloride and urea) may be trapped in this zone. This exclusion of water and trapping of sodium chloride, urea, and osmolytes helps maintain the osmotic gradient along the inner medulla. In long loops (the length is proportional to the renal concentrating potential), the loop of Henle penetrates the inner medulla. Only about a third of the ascending and descending limbs of long loops lie together; in the other instances the ascending limbs are nearer to collecting ducts than to descending limbs.

3.3.4.2 *Collecting ducts*

Collecting ducts consists of three identifiable segments, which lie, respectively, in the cortex, the outer

medulla, and the inner medulla. These segments demonstrate different permeabilities to water and osmolytes. The difference in permeability may be related to the presence of two cell types, the intercalated and collecting duct (or principal) cells.

3.3.4.3 The distal tubule

The distal tubule connects the thick ascending limb of the loop of Henle to that part of the collecting duct which originates in the cortex. The distal tubules are in-involved in both ion and water reabsorption, but play a much less significant role than the proximal tubules. The underlying mechanisms responsible for reabsorption appear, in essence, to be similar to those already outlined. The major differences include a stronger Na^+ gradient against which to "pump", the ability to reabsorb sodium without reabsorbing water, the controlling effects of anti-diuretic hormone (ADH) and aldosterone (among other mediators), and the very limited (or lack of) protein reabsorption. The secretion of potassium ions appears to be under the control of an active transport mechanism, the regulating factors of which are many and complex (Valtin, 1973; Orloff & Berliner, 1973; Brenner & Rector, 1986).

3.3.4.4 The countercurrent multiplier system and urine concentration

Less than 1% of the glomerular filtrate leaves the kidney as urine (unless there is a state of diuresis), the remainder having been reabsorbed. The process of urine concentration is complex and depends (at least in part) on the countercurrent multiplier system, which establishes a steep osmotic gradient along the inner medulla. The high osmolality is a consequence of the differential permeability of the limbs of the loops of Henle and the collecting ducts to water and ions. The thick ascending limb is thought to have an active mechanism which transports chloride and sodium out of the lumen and into the interstitium, but the limb remains impermeable to water. As a consequence the osmolality decreases in this part of the tubule (the diluting segment). The descending limb, on the other hand, is freely permeable to water, but probably not to sodium ions. The high ion concentration in the interstitium would draw water out of the descending limb, increasing the osmolality towards the turn of the U-loop.

This is augmented by urea and other osmolytes that leave the collecting ducts and enter the ascending limb via the interstitium, thus being recirculated to the medulla.

The collecting ducts regulate the final urine concentration by controlling the amount of water that is reabsorbed. The passage of water out of the ducts is thought to be mediated largely by cyclic adenosine-monophosphate (cAMP), the synthesis of which is stimulated by ADH, which increases the permeability of the luminal cell membrane to water. Osmotic effects draw the water out of the cell (through the basement membrane) into the hyperosmotic interstitium. In the absence of ADH the collecting duct is thought to be impermeable and relatively little water is reabsorbed from it. The interstitial osmotic gradient is assumed to be maintained by the effective removal of water via the ascending vasa recta, which have both a greater radius than the descending vasa recta and are about twice as numerous. The countercurrent exchange associated with the loops of Henle arising from cortical nephrons offers an important "barrier" zone, which is thought to facilitate solute trapping in and solvent exclusion from the inner medulla, and thus helps to maintain the hyperosmolality in this "compartment".

There are a number of other factors that control, alter, or contribute to urine concentration. Medullary blood flow is complex, as are the factors controlling it. Increased blood flow rates will decrease the efficiency of countercurrent exchange in the outer medulla, as a consequence of which the high osmotic gradient in the inner medullary compartment will be "washed out", and urine will not be concentrated. Diuresis is associated with increased blood flow rates (Earley & Friedler, 1964, 1965; Chuang et al., 1978).

A unique feature of the vasa recta is their permeability to macromolecules, a consequence of which is that the medulla contains a large pool of albumin. The factors controlling the rapid turnover of this milieu are poorly understood. It is generally assumed that (together with the glycosaminoglycans) these proteins provide an interstitial osmotic pressure that facilitates water reabsorption (see Brenner & Rector (1986) for a fuller discussion and list of references).

3.3.4.5 *The interstitial cells*

Interstitial cells occur in most organs. Three types of interstitial cells have been described in the medulla of the rat kidney (Bohman, 1980). Type I cells are the most abundant and represent the typical renal medullary cells. Type 2 medullary interstitial cells are generally round and lack lipid droplets, while Type 3 cells correspond to the pericytes. Types 2 and 3 are sparsely distributed and are often overlooked between the tubules, ducts, and blood vessels. In the inner medulla, however, Type I cells are numerous and especially prominent because they are set in a dense matrix of glycosaminoglycans (previously referred to as mucopolysaccharides or acidic mucopolysaccharides).

The medullary interstitial cells have been described by Moffat (1979, 1981, 1982), Bohman (1980), and Maunsbach et al. (1980). The number of cells and the amount of matrix substance occupies 10-20% of the tissue volume in the outer medulla, and 40% near the apex of the inner medulla (Bohman, 1980). The cells, which are arranged in a regular pattern perpendicular to the tubules and vessels, are irregular in shape and have many long slender processes. These come into close contact with adjacent interstitial cells, capillaries, and the limbs of the loop of Henle, but there is no such relationship with the collecting ducts.

One of the most characteristic features associated with the Type I cells is the presence of lipid inclusion droplets, which occupy at least 2-4% of the total cell volume. The lipid content is largely triglycerides, with variable amounts of cholesterol esters and phospholipids. A number of conditions have been described where there are marked changes in the size and number of lipid droplets. The pathophysiological significance of these changes is difficult to interpret because of varied experimental approaches, species variation, and contradictory reports (Bohman, 1980).

3.4 Species, strain, and sex differences in renal structure and function

There are important differences between the renal structure of animals and man that may have a direct effect

on the interpretation of toxicological data (Stolte & Alt 1980, 1982; Mudge 1985). The kidney varies greatly in structure and function between different species and strains, and there are also more subtle differences between the sexes of several animals. In general, the kidneys can be classified into those that are multipapillate, such as those of man and the pig, those that have more than one papilla (e.g., spider monkey), and those of the vast majority of animal species, which are unipapillate. The papillae may either be present as a well defined pyramidal structure, as in rodents, man, pigs, and dogs, or represent only a ridge as in the non-human primates. Furthermore the kidney may be unilobar (and have a compact structure) or consist of a multilobar structure, as in bovines and elephants.

Table 7 compares some of the structural and functional features of the most species most commonly used in toxicity studies. It illustrates clearly that there are a number of differences between man and the rodents, which are the most commonly used species to assess nephrotoxic potential and study mechanisms of injury. There are also major strain differences between Sprague-Dawley, Wistar, and Fischer 344 rats, which probably account for the vast majority of animals studied. The Brown Norway rat is a most useful model for studying mercuric chloride immunomediated nephropathy (Druet et al., 1987), and the differences between the metabolism of methoxyflurane anaesthetic in Fischer 344, Buffalo, Wistar, Long Evans, and Sprague-Dawley rat was used as the basis for demonstrating the toxicity of the fluoride ion released by hepatic mixed-function oxidase activity (Mazze, 1976, 1981). In addition there is evidence that there are consistent differences between the renal structure and function of male and female mice. Other sex differences have been reported in other species.

Species or strains with unique anatomical and functional attributes can offer an important way of helping to understand toxic mechanisms, but at present there are few published data on non-human primates, marmosets, or the pig.

3.5 Renal biochemistry

There is little doubt that renal metabolism is coupled tightly to specific functions in the kidney. In particu-

Table 7. Comparison between the renal structure and function in man and in commonly investigated species[a]

	Man	Rat	Dog	Pig
Cortical structure				
Nephrons per g body weight	16	128	45	26
Glomerular radius (μm)	100	61	90	83
Proximal tubular length (mm)	16	12	20	30
Tubular radius (μm)	36	29	33	35
Cortical function				
Glomerular filtration rate (ml/min per m^2)	75	35	104	72
Inulin clearance (ml/min per kg body weight)	2.0	6.0	4.3	2.1
p-Aminohippurate transport maxima (mg/min per kg body weight)	1.3	3.0	1.0	-
Drug-metabolizing enzymes[b]				
Mixed-function oxidase	4	5	-	-
NADPH-cytochrome c reductase	15	48	-	-
Medullary structure				
Number of papilla	15-20	1	1	6-10
Percent long loops	14	28	100	3
Relative medullary thickness	3.0	5.8	4.3	1.6
Medullary function				
Maximum urine osmolality (mOsmol/kg)	1400	2610	2610	1080

[a] Data from Mudge, 1985; Stolte & Alt, 1980, 1982; Gyrd-Hansen, 1968
[b] Renal enzyme activity expressed as a percentage of liver activity
- No data published on this parameter

lar, reabsorption of sodium chloride can be correlated directly with oxygen consumption and is probably the most energy-demanding transport function of this organ. The regions of the kidney vary in their ability to metabolize and produce various substrates.

3.5.1 *Biochemistry and metabolism in the cortex*

The movement of the sodium ions from the tubular fluid to the blood is quantitatively one of the the most important functions that the kidney performs. This is accomplished by aerobic metabolism linked to adenosine-

triphosphate (ATP) production and utilization. The exact mechanisms are not fully understood.

Renal cortical nephrons are capable of utilizing a variety of substrates, and the substrate utilization varies from nephron segment to nephron segment. For example, Klein et al. (1981) demonstrated that the convoluted portion of the proximal tubule utilized succinate, glutamate, glutamine and other substrates quite extensively. This same nephron segment, however, utilized glucose, lactate, and palmitate only minimally. The hexose-monophosphate shunt is present at highest activity in the distal segment of the nephron and in the thick ascending limb. Although this pathway may account for relatively little glucose oxidation, it would appear important as a source of reduced nicotinamide adenine dinucleotide phosphate (NADPH).

The kidney cortex is also capable of producing glucose from non-carbohydrate precursors. Although different substrates are utilized for gluconeogenesis in the kidney, compared with the liver, the gluconeogenic pathways are similar. Changes in hydrogen ion activity do not alter hepatic gluconeogenesis, but markedly effect that in the kidney.

Additionally, renal gluconeogenesis is influenced by the concentration of substrate in the renal arterial blood. Guder & Schmidt (1974) and Schmidt & Guder (1976) have demonstrated that the rate-limiting enzymes for gluconeogenesis are not uniformly distributed throughout the nephron. For example, the highest activity is found in the proximal convoluted tubule, there being relatively little activity in the thick ascending limb. The glycolytic enzymes, on the other hand, are present in the thick ascending limb, the distal tubule, and the collecting duct. A specific role for glucose in supporting the various renal transport processes has not been adequately described. Renal phospholipid metabolism, however, may be important in support of transport and may play a direct role in sodium movement.

3.5.2 *Biochemistry and metabolism in the medulla*

Guder & Ross (1984) have highlighted the biochemical aspects of heterogeneity along the nephrons. Much of the

published information has been derived from whole medulla or medullary slices and fails to differentiate between the metabolic contribution from the nephrons (loops of Henle), as opposed to the collecting duct epithelia, versus the interstitial cells.

3.5.2.1 The biochemistry of renal prostaglandins (PG)

The PGs and endoperoxides are a group of ubiquitously distributed hormones with a broad spectrum of potent biological activity that shows marked receptor specificity. They are synthesized from the C20:4 fatty acid arachidonic acid by an enzyme system (which includes cyclo-oxygenases, peroxidases, isomerases, and reductases) collectively called PG synthetase.

The PGs (Fig. 9) are structurally similar and are only present in minute concentrations. Several are labile and undergo spontaneous chemical changes. Thus, most of the methods (both qualitative and quantitative) needed for their biochemical investigations are fraught with subtle pitfalls (Frolich & Walker, 1980). The literature on renal PG biology is large, complex, contradictory, and difficult to interpret. The subject has been reviewed recently by Dunn & Hood (1977), Dunn & Zambraski (1980), Horrobin (1980), Morrison (1980), Zusman (1980), Dunn (1981), Frolich et al. (1981), and Levenson et al. (1982).

PGs are not stored in renal tissue but are synthesized *de novo* from arachidonic acid, which is released from stored phospholipid or triglyceride pools by the action of phospholipase A_2. The factors that regulate the release of arachidonic acid include both receptor-mediated responses (such as vasoactive peptides and biogenic amines) and non-specific stimuli (ischaemia). The prostaglandin precursor may be drawn from different lipid pools. Any arachidonate that is not channelled into prostaglandin synthesis may be re-acylated (as are the *de novo* synthesized molecules) or disposed of via several other metabolic routes. Arachidonic acid (the availability of which is rate limiting) is converted to PGG_2 and thence to other PG-related substances.

The anatomically identifiable areas of the kidney each synthesize a different pattern of PGs *in vitro*. The *in*

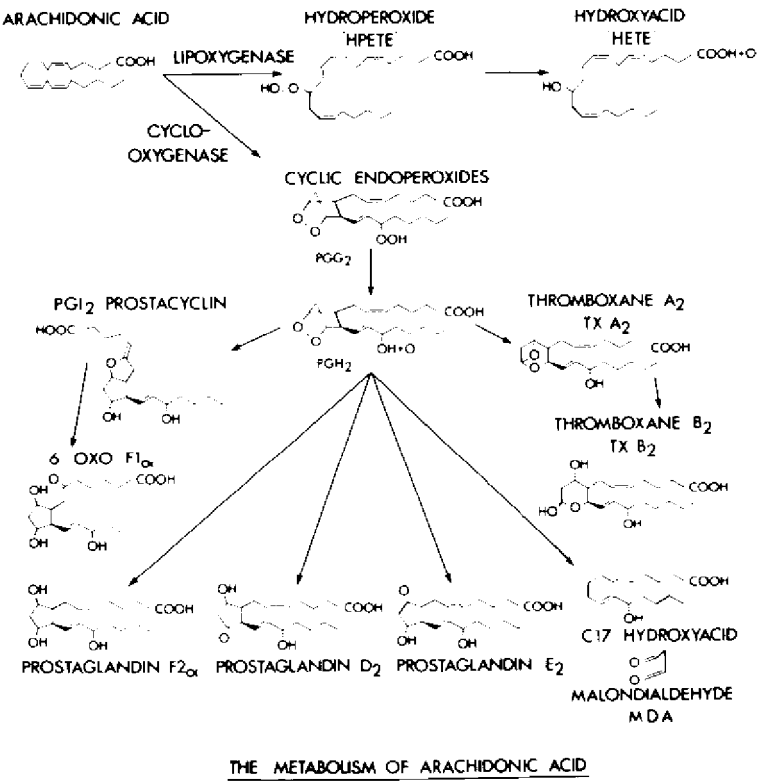


Fig. 9. The bioconversion of arachidonic acid to prostaglandins (PGs) and thromboxanes (TXs). From: Bach & Bridges (1985a).

vivo contributions of each area to PG synthesis and the function of each PG remains largely a matter of speculation at present. Total PG synthesis is several times higher in the medulla (where typically it is greatest in the papilla) than in the cortex (Dunn & Hood, 1977). However, the distribution of, for example, PGE_2 synthesis

reflects a more complex picture, its concentration being lower in the papilla than the rest of the inner medulla (van Dorp, 1971). A recent study using isolated nephrons showed that the highest PGE_2 production is in the medullary collecting tubules, followed by cortical collecting ducts and glomeruli. Furthermore, there are marked sex-related differences in the effects of cofactors on medullary PG synthetase activity (Hirafuji et al., 1980). Some PGs break down spontaneously (e.g., PGI_2 to α-6-keto-PGF), but the majority are metabolically degraded (Morrison, 1980). The enzymic conversions are mediated by a number of enzymes, including dehydrogenases, reductases, and β- and ω-oxidases. The enzymes that degrade PGs are located mainly in the cortex, but there are species differences in the corticomedullary ratio of these enzymic activities (Powell, 1980).

The factors regulating the biosynthesis of each type of PG are only poorly defined (Horrobin, 1980). A large number of endogenous and exogenous substances have been reported to alter renal PG synthesis, and several pathophysiological conditions have been described in which renal PG synthesis is increased. Most attention has been focussed on the inhibitory effects of the anti-inflammatory drugs. The steroidal compounds (e.g., corticosteroids) prevent the release of arachidonic acid from its lipid pools, and the non-steroidal products (e.g., indomethacin) inhibit cyclo-oxygenase. It is, however, essential to be aware that any factor that perturbs PG synthesis may act differently at different sites in the synthetic (or degradative) pathway.

Indomethacin (one of the most extensively studied cyclo-oxygenase inhibitors) produces several alterations in renal PG dynamics. Uncertainties in defining PG-"related" pathophysiological changes are compounded by the observation (Attallah & Stahl, 1980) that PGE_2 synthesis in slices from each zone of the kidney has a different dose response to indomethacin inhibition: the cortex is most sensitive and the papilla least sensitive. The cyclo-oxygenase inhibitors are generally classified as either reversible or irreversible, but the multiplicity of effects and the possibility of enzymic polymorphism in different regions of the kidney suggest that such a classification may be an over-simplification.

The exact physiological roles of the PGs in normal renal function and the way in which these are altered in the development of nephropathies are not clear. Firstly, indomethacin, for example, has been shown to cause biochemical changes that may be classified as either related or unrelated to altering PG dynamics. Secondly, many attempts to define renal PG function have been based on the hypothesis that urinary PG excretion reflects *de novo* renal synthesis (Dunn & Hood, 1977; Dunn & Zambraski, 1980; Dunn 1981), notwithstanding analytical difficulties of measuring very low levels of various PGs and apparently ignoring the fact that *de novo* synthesized PGs may have undergone extensive degradation. The measurement of urinary PGs, as an estimate of their *de novo* renal synthesis, remains equivocal because, firstly, seminal PGE_2 is an unavoidable and variable contaminant in the urine of males (Suzuki et al., 1980) and, secondly, Brown et al. (1980) have demonstrated that both rabbit and rat urinary bladders can synthesize PGE from arachidonic acid. Finally, the physiology of renal function is controlled by several hormonal systems, the detailed functioning of which has not been clearly established. It is known that renal PGs may be altered by (or may alter) the renin/angiotensin II/aldosterone system (Hackenthal et al., 1980; Lee, 1980; Weber, 1980; Baer, 1981), the kallikrein-kinin system (Margolius, 1980; Rockel & Heidland, 1980), and the regulation of fluid balance and water reabsorption via ADH (Blair-West et al., 1980). Furthermore, each of these hormonal systems may interact with the others via direct or indirect mechanisms. It seems likely that a full understanding of the pathophysiology of the renal hormonal systems will take some time to crystallize.

In spite of the rather abstruse biology, there is general consensus (Dunn & Hood 1977; Dunn & Zambraski, 1980; Morrison, 1980) that PGs have a central role in renal function. It seems, however, that renal PGs play little, if any, major regulatory role in basal renal blood flow in normal conscious animals. There is evidence that PGs are released in response to ischaemic and vasoconstrictive stress, where their role seems to be to provide a protective effect by maintaining glomerular dynamics. The role of PGs (especially PGE_2) in preventing experimentally induced acute renal failure is conflicting.

Arachidonic acid does stimulate renin release, a response that is blocked by cyclo-oxygenase inhibitors, but it remains uncertain which of the PGs mediate this effect *in vivo*. It is also unclear from which renal zone such mediators are synthesized and released. Renin release may, in turn, affect PG synthesis and the kallikrein-kinin system (which in turn may modulate PG synthesis and the renin system). ADH is assumed to stimulate PGE_2, but published data on the controlling effects of PGs on salt and water balance are very difficult to interpret. Similarly, the mass of literature on hypertension and PGs favours the concept that the two are related, but fails to propound a unifying hypothesis.

3.5.2.2 *Lipid metabolism*

The contents of the interstitial lipid droplets are too specialized to be used as a metabolic energy store (Bohman, 1980), although this droplet population undergoes marked and rapid change during the short periods that precede various pathophysiological conditions. The interstitial cells produce the ground substance matrix that surrounds them. Early evidence that the interstitial cells of the renal medulla are only a highly specialized PG-producing cell type has become equivocal. The interstitial lipid droplets do not, in fact, provide the sole source of arachidonic acid for PG synthesis. Only 50% of the medullary capacity for synthesizing PG is confined to the interstitial cells; the rest is in the collecting ducts (Bohman, 1980). The significance of PG synthesis in the medullary cells cannot, however, be overlooked, as it may play an important role in regulating blood pressure and other renal functions. It has also been suggested to occupy a central position in the pathogenesis of renal papillary necrosis.

In recent years the importance of the endocrine function of the medullary interstitial cells in regulating blood pressure has been highlighted by several workers (Mandal & Bohman, 1980). Three groups of vaso-active compounds have been isolated from the medulla or cultured interstitial cells. Experimentally induced, spontaneously occurring, and pathologically precipitated hypertensive states have been reversed by subcutaneous transplants of

renal papillary fragments and by cultured interstitial cells. In addition, the systemic administration of both the polar and neutral reno-medullary lipids reduces arterial blood pressure (Muirhead & Pitcock, 1980).

The numerous lipid droplets in the interstitial cells have been found to contain traces of cholesterol esters, a few percent of phospholipids, mainly phosphatidylcholine, and, rarely, trace amounts of phosphatidyl-ethanolamine. A few percent of free fatty acids and tri-acylglycerols make up the remaining 80-90%, the composition of which varies in different species. The most striking features are the varied types and large amounts of unsaturated fatty acids; most notably those of 20 or more carbon atoms such as arachidonic acid and especially adrenic acid. The large amount of arachidonic acid suggests that the interstitial lipid droplets may be an important pool for PG synthesis in the kidney (Bojesen, 1974).

3.5.2.3 *Carbohydrate metabolism in the medulla*

The metabolism of carbohydrate in the renal medulla has been reviewed by Cohen (1979). Some early observations suggested that the low oxygen tension in the inner medulla (a pO_2 as low as 0.67-2.0 kPa (5-15 mmHg) compared with 10 kPa (75 mmHg) in the cortex) would necessitate anaerobic metabolism. However, aerobic metabolism is only limited at an O_2 availability of less than 0.13 kPa (1 mmHg).

Many metabolic investigations have used medullary slices or homogenates. Thus, the exact contribution of the different cell types in the inner medulla to functional energy dynamics and to the changes that underlie, for example, diuresis or anti-diuresis have yet to be related to the phosphorylation and redox states within these individual cell types.

Carbohydrates are stored in the medulla as either glycogen or as glycosaminoglycan (GAG), the former in collecting ducts and epithelia and the latter as an important constituent of the interstitial ground substance. There is evidence to suggest that either can be mobilized to provide an energy source or the glucose units for the synthesis of the other macromolecular carbohydrates (Darnton, 1967, 1969).

3.5.2.4 *Medullary glycosaminoglycan (GAG)*

The biology of GAGs has been described by Kennedy (1979). GAGs are linear polysaccharides that are made up of repeating disaccharide units, one carbohydrate moiety of which is a hexuronic acid (or a neutral sugar in one case) and the other a hexosamine. The nature of the disaccharide units and the occurrence of *N*-acetyl groups, together with the position of *O*-sulfate groups, define the species of macromolecule. There are seven basic types of GAG. These molecules also show heterogeneity of relative molecular mass (when isolated from the same or different organs; Toledo & Dietrich, 1977), and the molar ratio of sulfate to hexosamine varies by up to 2-fold for the same type of GAG (Suzuki et al., 1976). Most of these substances probably occur *in vivo* as proteoglycans (PoGs). These supramolecular structures are composed of a linear protein backbone that carries GAGs covalently bound at intervals along its length. In theory, any combination and ratio of GAGs may occur. It is only recently that the concept of PoGs has been accepted; before this the presence of protein was assumed to be a contamination and vigorous steps were taken to remove it.

In spite of the ubiquity of PoGs and their composite GAGs, relatively little is known about their physiological functions, with the exception of their anti-coagulant and anti-lipaemic properties, which are best studied in heparin. These molecules are bound to cell surfaces (Kjellen et al., 1977), where they may control the access of endogenous and exogenous molecules to cell membrane receptors. Similarly, the functions of this intercellular polyanionic matrix most probably extend beyond that of "immobilized anti-coagulants" or "space filling", and include controlling the micro-environment of cells (by binding either inorganic or organic cations and by their immense water-holding capacity) and regulating cell-cell communications. GAGs may also control cell recognition and adhesion, and contribute to the control of cell movement, growth, differentiation, and proliferation (Long & Williamson, 1979). The association of GAGs with mitochondria and nuclear membranes suggests that these macromolecules may also play a direct role in controlling some intracellular functions.

The distribution of GAGs has been assessed in tissue either by the autoradiographic distribution of precursor carbohydrates or $^{35}SO_2$ or by histochemical staining. It is generally assumed that sulfate radiolabel distribution is relatively specific for GAGs. However, most of the staining procedures are nonspecific (e.g., toluidine blue interacts with any polyanion to give a metachromatic colour shift) and depend either on *a priori* knowledge of distribution or, for example, the use of control sections that have been exposed to selective enzymic digestion.

The amount and types of GAGs in the kidneys of various species have been reported. The quantity of polyanionic macromolecule has been found to be greater in the medulla than in the cortex for the rat (Jacobsen et al., 1964; Kresse & Grossmann, 1970), pig (Kresse & Grossmann 1970), dog (Castor & Green, 1968; Kresse & Grossmann, 1970), and normal human kidney (Inoue et al., 1973; Constantopoulos et al., 1973). The medulla:cortex ratio in the human kidney was found to be age related, increasing rapidly to a maximum in the fourth decade and then declining slowly (Inone et al., 1973). The heterogeneous distribution of the types of GAG in the kidney is supported by the data of Constantopoulos et al. (1973) for the human kidney and Castor & Green (1968), who reported that hyaluronic acid had a high relative molecular mass in the medulla but a low one in the cortex of dogs. However, other data on the dog, pig, and sheep (Dicker & Franklin, 1966) and the rat (Barry & Bowness, 1975) suggest that the types and quantities of GAG are the same in both the cortex and medulla.

The processes underlying and controlling the biosynthesis of PoGs are complex and incompletely documented (Kennedy, 1979). Muirhead & Pitcock (1980) reported that medullary interstitial cells synthesize PoGs (both *in situ* and in culture) and that these macromolecules are associated with the cellular cisternae (dilated rough endoplasmic reticulum). Darnton (1967, 1969) presented data to show that glycogen associated with the epithelial cells of the collecting duct in the rabbit is mobilized and incorporated into GAGs.

The functions of the medullary GAGs have been the centre of controversy since Ginetzinsky (1958) suggested that the action of ADH was mediated by the release of

hyaluronidase. This would depolymerize medullary GAG and (so it was argued) allow greater water reabsorbtion from the tubules into the interstitium and thence to the blood supply. This hypothesis has been supported by some workers (Jacobson et al., 1964; Farber et al., 1971) but refuted by others (Sun et al., 1972; McAuliffe 1978, 1980; Sun, 1980) in animals with spontaneous diabetes insipidus. These conflicting data are difficult to resolve into a single unifying theory relating the physiological function of GAGs to the urine-concentrating process.

3.6 The metabolism of xenobiotic molecules in the kidney

Chemically induced lesions may depend to varying extents on the metabolic capacity of tissues to deal with "insults". The metabolism of xenobiotic molecules may either prevent lesions (by deactivation), or be directly responsible for damage (by bio-activation). The renal metabolism of chemicals (and its consequences) has been reviewed by Hook et al. (1979), Anders (1980), Connelly & Bridges (1980), Kluwe & Hook (1980), Davis et al. (1981), Rush et al., (1984) and Tarloff et al. (1987).

It is likely that the liver meets the challenge of metabolizing a major proportion of exogenous compounds *in vivo* before they reach the systemic circulation. Most fundamental types of bioconversion have been described for the perfused kidney of several species (Szefler & Acara, 1979; Elbers et al., 1980; Ross et al., 1980; Emslie et al., 1981) and for isolated renal cells and tubular fragments (Fry et al., 1978; Jones et al., 1979; Ormstad, 1982). Similarly, kidney microsomes have been shown to have most of the enzymic and cytochrome-mediated metabolic activities that have been described in other tissues. The xenobiotic-transformating capacity of the kidney is about 3-50% (depending on the system, species, and source of data) of that found in the liver (Litterst et al., 1975a, Navran & Louis-Ferdinand 1975; Fry et al., 1978), but it may be much higher than that of the liver under certain circumstances (Anders, 1980). There are marked qualitative differences between hepatic and renal xenobiotic metabolism. Renal enzymes are stable during the Ca^{2+} method of preparing microsomes (Litterst et al., 1975b). Enzymic kinetic constants vary between microsomes isolated from

the two organs (Navran & Louis-Ferdinand, 1975). Whereas there are marked sex-related differences in hepatic metabolism, there are few in the kidney (Litterst et al., 1977). There is evidence to suggest that liver cytochrome P-450 is similar to that of the kidney, based on electrophoretic and electron paramagnetic resonance studies (Armbrecht et al., 1979), immunological criteria (Guengerich & Mason, 1979), and on immunometabolic studies (Kaminsky et al., 1979). However, these data are most difficult to interpret in "absolute" terms, because the samples of cytochrome P-450 were from organs exposed to different inducing agents. There is now substantial evidence that hepatic and renal tissue respond differently, both quantitatively and qualitatively, to the various inducers of cytochrome P-450 (Litterst et al., 1977; Zenser et al., 1978a; Kaminsky et al., 1979). Ascorbic acid deficiency (Sikic et al., 1977) and carbon tetrachloride pretreatment (Litterst et al., 1977) alter the metabolism of xenobiotics in a different way in the liver and kidney. In addition, the inhibitory effects of 2-diethylaminoethyl-2,2-diphenylvalerate (SKF-525A) on renal and hepatic microsomes studied *in vitro* are similar but not identical (Litterst et al., 1977).

There are several enzymes involved in renal xenobiotic metabolism. It is not possible to comment on all of those that may be relevant to nephrotoxicity nor, indeed, is it clearly established what role each renal enzyme plays in the realization of the potential toxicity of a chemical. Many of the enzymes that metabolize xenobiotics are compartmentalized in specific regions of the kidney. The anatomical localization of these characteristics may play a key role in the toxicological consequence that follows the entry of a xenobiotic into the kidney. The distribution and regulation of these enzymes may predispose to the toxic effects of chemicals. Thus, although intrarenal metabolism may be a prerequisite for the target selective effects of some chemicals, the final outcome of the toxic response relates to the sum of a number of factors. These include the localization of those renal enzymes involved in xenobiotic metabolism (this may be metabolic activation or other processes, perhaps in an adjacent cell) and the processes controlling the intracellular concentration of

toxic chemicals. The intracellular concentration of a chemical can be influenced by xenobiotic metabolism *per se* and by many of the inherent processes in the kidney, such as transport, pH, and solute gradients on either side of a membrane. The outcome of a chemical exposure may also be affected by the numbers and types of organelles in a specific cell type that have critical properties relevant to the functions of that cell and by the presence of a protective mechanism in a particular cell type (such as antioxidants, free radical scavengers). Most of the processes that underlie nephrotoxicity are probably multi-step events that are affected by more than one metabolic pathway and occur via competing and sequential pathways. Little is known about the control of these pathways, so that it is difficult to predict from the structure of a chemical alone what effects it will have on the kidney. In addition, a major role is obviously played by the extra-renal metabolism (in the liver, lung, gut, etc.) of the parent chemical and by a variety of other organ functions. These include the lung (exhalation of volatile metabolites), liver (biliary excretion), and gastrointestinal microflora (enterohepatic circulation, serum protein binding), and they determine the types of chemicals, the concentration that reaches the kidney, and their renal pharmacokinetics.

3.6.1 Oxidases

Oxidases can convert chemicals into active intermediates or generate reactive species by redox cycling. This is potentially important for compounds that contain arylamine, quaternary bipyridyl (paraquat), quinone (adriamycin), or nitro (nitrofurantoin) structures. It is generally accepted that, in the liver, biologically reactive intermediates mediate their toxic effects by binding to cellular macromolecules and blocking normal functional processes (Jollow et al., 1976; Snyder et al., 1981). Similar mechanisms have been proposed to explain various types of chemically induced renal lesions, including papillary necrosis. The metabolically generated reactive intermediates have a relatively short life and are most likely formed in the organ or anatomical area in which they induce damage.

3.6.1.1 *Cytochrome-P-450-dependent mixed-function oxidases (monooxygenases)*

This enzyme system carries out a two-electron flow pathway, and the flavoprotein component can catalyse single electron reductions such as the reduction of quinones to semiquinone radicals (Bachur et al., 1979). Multiple forms of cytochrome P-450 have been identified in the kidney. These include phenobarbital-, 3-methylcholanthrene-, and β-naphthoflavone-inducible cytochrome P-450. Renal cytochrome P-450 induction varies in different species. Polycyclic aromatic hydrocarbons induce cytochrome P-450 in most species, whereas phenobarbital is effective in hamsters and rabbits but not in guinea-pigs, rats, and mice (Smith et al., 1986). Similarly, renal P-450 responds differently to inhibitors of mixed-function oxidases.

The effects of inhibitors such as SKF-525A are further complicated by multiple actions on renal transport, intracellular binding of chemicals at noncatalytic sites, and on cytochrome P-450-dependent metabolism. Other inhibitors do not appear to have been as fully studied, and the paucity of data in this area makes other studies on the effects of inhibitors most difficult to interpret. Some species also have sex-related differences, e.g., male mice have higher concentrations and activities of P-450 than female mice (Krijsheld & Gram, 1984; Smith et al., 1984; Hawke & Welch, 1985), but this is not the case for rats (Litterst et al., 1977; Hook et al., 1982) or rabbits (Litterst et al., 1977). There is no clear data on other species, such as man, nor on how different types of renal disease affect the concentration of renal P-450 or its induction or inhibition.

The specific activity of the renal mixed-function oxidases varies widely between species and is about 10% of the hepatic activity (Zenser et al., 1978a,b; Endou, 1983). This suggests a role for renal P-450 that is quantitatively less important than that of the liver. However, this is not the case for all chemicals, since the renal metabolism of chloroform is about 2-fold higher than the hepatic activity (Smith & Hook 1984). More importantly, mixed-function oxidase activities are intra-renally localized to discrete areas where their significance in

metabolism may be far greater than in the liver. The S_2 proximal segment has a cytochrome P-450 concentration that is 2-3 times higher than the S_1 or S_3 segments. The distal tubules, cortical collecting ducts, and the medulla contain no measurable cytochrome P-450 activity (Endou, 1983). By contrast NADPH-cytochrome-P-450 reductase activity is highest in the S_2 and S_3 segments, but it also extends to the distal tubule and medullary structures.

3.6.1.2 Prostaglandin peroxidase-mediated metabolic activation

Recently, it has been shown that there are marked quantitative and qualitative differences in the regional distribution of microsomal mixed-function oxidase activity within the rabbit kidney (Zenser et al., 1978a,b; Armbrecht et al., 1979). Most mixed-function oxidase activity is located in the cortex and least in the inner medulla in control tissue and in that taken from animals induced with 3-methylcholanthrene. Cytochrome P-450 is not detected in the medulla of controls or even those of induced animals. In addition, laurate hydroxylase activity (the only mixed-function oxidase activity found in the inner medulla) shows marked differences in the pattern of inhibition by carbon monoxide, α-naphthoflavone, and metyrapone in the cortex and the outer and inner medulla. This suggests differences in the genetic expression of the same type of enzymic activity in different zones of the kidney.

Davis et al. (1981) showed that oxidative metabolism in the medulla is mediated in the absence of spectrophotometrically measurable cytochrome P-450. Zenser et al. (1979a) reported that cortex microsomes metabolized 1,3-diphenylisobenzofuran to *O*-dibenzoylbenzene largely via a cytochrome P-450-like system (it was NADPH dependent and inhibited by carbon monoxide and metyrapone). The inner medulla microsomes had the same metabolic capacity in the presence of arachidonic acid (the system was independent of NADPH, and it was inhibited by non-steroidal anti-inflammatory compounds such as indomethacin and not by carbon monoxide or metyrapone). The outer medulla microsomes had both types of activity. The antioxidant ethoxyquin inhibited the arachidonic acid and the NADPH-dependent metabolic processes.

The specific arachidonic-acid-dependent PG cyclo-oxygenase-mediated metabolism of benzidine has been shown to be absent from hepatic and renal cortical microsomes but active in medullary microsomes, especially those from the inner medulla. The metabolism was inhibited by non-steroidal anti-inflammatory drugs, ethoxyquin, and arachidonic acid analogues. Approximately 75% of metabolized benzidine was covalently bound to macromolecules, presumably via a reactive intermediate. Addition of sulfhydryl protectors, such as glutathione, reduced the amount of covalently bound metabolite to 25% (Zenser et al., 1979b,c). Using rabbit renal inner medullary slices, Rapp et al. (1980) have confirmed the arachidonic-acid-dependent co-oxidative activation of low concentrations of benzidine, and its covalent binding to tissue. Mohandas et al. (1981a,b) examined the activation of paracetamol (acetaminophen) and the covalent binding to protein in the inner medulla, outer medulla, and renal cortex, and compared this to the situation in the liver. The arachidonic-acid-dependent pathway showed a ten times greater degree of activity in the inner medulla compared to the renal cortex, intermediate activity being found in the outer medulla. In the liver the arachadonic-acid-dependent pathway activity was approximately 50% of that of the renal cortex. The total activation of paracetamol (acetaminophen) by arachadonic-acid- and NADPH-dependent pathways in the renal cortex and the liver was essentially the same.

3.6.2 Conjugation

Conjugation takes place on existing groups or those produced by oxidation, and greatly increases the polarity of compounds. This facilitates their elimination and generally terminates any pharmacological activity. There are several examples, however, where conjugation may give rise to reactive compounds (e.g., the glucuronides of *N*-hydroxy-2-acetylaminofluorene and *N*-hydroxyphenacetin are potently toxic). Similarly, glutathione conjugates may be toxic (section 6.3.2.1).

3.6.2.1 Glucuronide conjugation

Glucuronide conjugates are formed by the action of uridine diphosphate (UDP) glucuronyl transferase. This

enzyme has at least three isozymes, each of which preferentially conjugates different types of molecules. Only UDP-GT1 occurs in the rat kidney, where the substrates include planar compounds such as 1-naphthol and 4-nitrophenol. UDP-GT1 activity is increased by 3-methylcholanthrene. Human kidneys have UDP-GT1 and high GT2 activities, while rabbit kidneys have all three isoenzymes. UDP-glucuronyl transferase activity is highest in the cortex, and the distal tubule activity is about 50% of that found in the proximal tubule (Cojocel et al., 1983). The enzyme is also measurable in rat kidney medulla, but here its activity may be limited by the availability of the co-substrate, UDP-glucuronic acid. Renal glucuronidation capacity may be comparable to or greater than that of the liver, depending on the substrate, and microsomes from female rats form considerably more glucuronide conjugates than those from male rat kidneys.

3.6.2.2 *Sulfate conjugation*

Sulfotransferases form highly polar and, therefore, rapidly excreted sulfate esters. The concentrations of both sulfotransferase and activated sulfate are higher in the renal cortex than in the medulla, and renal sulfotransferase activity is markedly lower than that of the liver. The capacity to synthesize sulfate conjugates is not increased by standard inducers.

3.6.2.3 *Glutathione conjugation*

Glutathione is the most abundant thiol-containing peptide in the kidney, where it is synthesized in the proximal tubule and provides a scavenger for detoxifying electrophilic radicals formed from alkyl and aryl halides, epoxides, and alkenes (Ormstad, 1987). These compounds are degraded to the cysteine conjugate and are generally excreted as the *N*-acetyl-cysteine conjugate. Glutathione may also have ligand binding and transport properties, and it has been suggested that it is an important carrier in the transfer of amino acids from the extra- to the intracellular space. Glutathione *S*-transferase plays an active role in metabolism, where it catalyses the initial step in glutathione conjugation of halogenated aromatics, epoxides, halogenated alkyls and aralkyls, and α,β-unsaturated

compounds (Reed & Beatty, 1980), drugs such as paracetamol (acetaminophen), and endogenous substrates such as estrogen and PGs (Moldeus et al., 1978; Jones et al., 1979; Kaplowitz, 1980).

The total renal GSH *S*-transferase activity per g wet tissue is considerably less than the corresponding hepatic activity (Hales et al., 1978). There are sex differences in the renal GSH *S*-transferase activities in rats, aralkyl, epoxide, and alkyl transferase activities being lower in males than in females. Rat kidney glutathione *S*-transferases consist of three distinct proteins. One is identical to hepatic transferase B (ligandin), a second conjugates α,β-unsaturated substrates similarly to the hepatic enzyme, and the third, renal transferase, is unique to the kidney and active with *p*-nitrobenzyl chloride (Hales et al., 1978).

Renal GSH *S*-transferases are under complex hormonal control. Hypophysectomy in male rats significantly increases GSH *S*-aryl, aralkyl, and epoxide transferase activities without altering GSH *S*-alkyl and alkene activities. GSH *S*-transferase can be induced by a number of chemicals, but the profile of activities affected is complex. Phenobarbital fails to induce cytochrome P-450 activity in rat kidney, but increases GSH *S*-aralkyl transferase activity without affecting GSH *S*-alkyl, aryl, and epoxide transferases. 3-Methylcholanthrene (3MC) induces renal GSH *S*-aryl and aralkyl transferase activities but not GSH *S*-alkyl or epoxide transferases (Clifton et al., 1975; Chasseaud, 1980).

3.6.2.4 *Mercapturic acid synthesis*

The formation of glutathione conjugates is the first in the pathway of renal metabolism to mercapturic acid. The enzyme γ-glutamyl transpeptidase is localized on the brush border of the proximal tubule, where it cleaves the γ-glutamyl linkage of glutathione to produce the cysteinyl-glycine conjugate in the tubule lumen. This metabolite is a substrate for a number of peptidases that produce the cysteinyl conjugate, which in turn is converted to the mercapturic acid by microsomal *N*-acetyltransferase (Green & Elce, 1975). The cells of the proximal tubule in the outer medulla produce the *N*-acetyl-cysteine conjugate of paracetamol (Jones et al., 1979).

Glutathione conjugation is generally a detoxification pathway, but some compounds may undergo bioactivation by β-lyase (localized in the outer mitochondrial membrane and the cytoplasm of the proximal tubule). This enzyme is now known to be capable of cleaving the C-S bond, leaving a reactive intermediate.

Extrarenal biotransformations are now known to produce substrates for renal enzymes, which convert these metabolites into reactive intermediates that cause target selective toxicity. Hexachloro-1,3-butadiene (HCBD) is metabolized in the liver probably by GSH *S*-transferase-catalysed halogen substitution rather than a cytochrome P-450-mediated reaction to the GSH conjugate. In the process, hepatic but not renal GSH is depleted in the male rat, whereas GSH decreases in the female rat kidney. The HCBD-GSH conjugate may be transported to the bile, returned to the bloodstream via intestinal reabsorption, and excreted via the kidneys (Nash et al., 1984). The cysteine conjugate of HCBD, *S*-pentachlorobuta-1,3-dienyl cysteine, causes the same lesion. The enzyme β-lyase is present in both the liver and kidney. The unique renal susceptibility to the HCBD-GSH metabolite appears to be related to accumulation via the organic anion transport, as this is inhibited by probenecid both *in vivo* and *in vitro* (Fig. 10).

Some alkylhalides, such as 1,2-dibromoethane (Lock, 1987) and 1,2-dibromo-3-chloropropane (DBCP) (Dybing et al., 1989), may form reactive, nephrotoxic intermediates (presumably episulfonium ions) following conjugation with glutathione without further metabolism by β-lyase. 1,2-Dibromoethane and DBCP are metabolized in the liver by both cytochrome P-450 oxidative dehalogenation and by glutathione-*S*-transferase-mediated substitution. The renal cortex of the rat contains substantial quantities of glutathione *S*-transferase with a high activity towards 1,2-dibromoethane (Lock, 1987). In the male rat kidney, studies with perdeutero-DBCP indicate that DBCP is not metabolized by cytochrome P-450, but presumably by glutathione *S*-transferase. Furthermore, inhibitors of γ-glutamyl transpeptidase and β-lyase do not affect DBCP-induced renal tubular necrosis (Omichinski et al., 1987; Dybing et al., 1989).

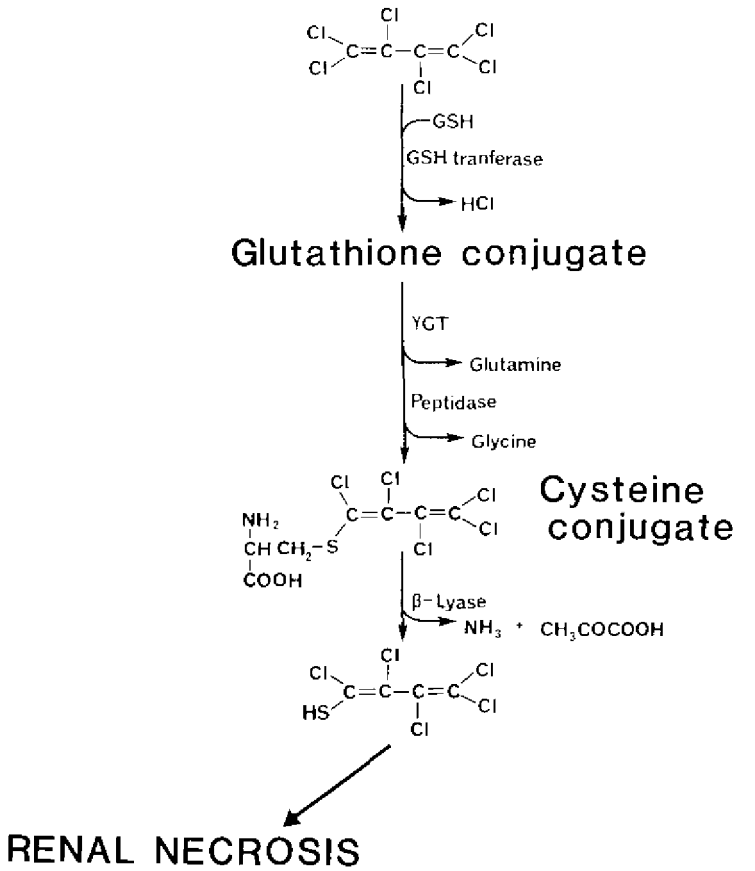


Fig. 10. Schematic representation of the bioconversion of hexachlorobutadiene to its proximate toxic metabolite (Courtesy of Dr E.A. Lock).

3.6.2.5 *Amino acid conjugation*

The amino acid glycine forms a glyco-conjugate via the activation of a carboxylic acid (e.g., salicylic acid) by

coenzyme A. Salicyl-CoA and glycine are co-substrates for acyl-CoA-glycine-*N*-acetyltransferase, which catalyses the condensation to salicyluric acid. Glycine *N*-acetyltransferase activity is present in the kidneys of rabbits, monkeys, and humans (Bekersky et al., 1980). The kidney may be a major site for the metabolism of benzoic acid to hippuric acid, *p*-aminobenzoic acid to *p*-aminohippuric acid, and salicylate to salicyluric acid (Wan & Riegelman, 1972a,b; Wan et al., 1972). The isolated rat kidney perfused with glycine and salicylic acid excretes 3-4% of the salicylic acid as the glycine conjugate (Wan & Riegelman, 1972a,b; Wan et al., 1972; Bekersky et al., 1980). These reactions are reversible in the kidney. About 20% of salicyluric acid is converted to salicylic acid by the isolated perfused rat kidney, whereas the liver does not deconjugate salicyluric acid to salicylate. The freshly deconjugated salicylate is more rapidly excreted than the parent salicylate. Possibly the diffusion of salicylate into the renal cell is the rate-limiting step of elimination. This is not the case for salicyluric acid, which is converted to salicylate within tubular cells (Bekersky et al., 1980).

Other aromatic acids (such as benzoic acid or *p*-aminobenzoic acid) competitively inhibited glycine conjugation. Cellular glycine is limited, and so its use in conjugation would be saturated with increasing concentrations of substrate.

3.6.3 *Other enzymes involved in xenobiotic metabolism*

Highly electrophilic epoxides play a key role in tissue alkylation of macromolecules, especially nucleic acids, leading to mutagenic and carcinogenic effects. Epoxide hydrase (hydrolase) converts aliphatic and aromatic epoxides to their trans-hydrodiols, a process that may generate both toxic and non-toxic products (Anders, 1980).

The oxidation of aldehydes to ketones or carboxylic acids in the kidney is mediated by aldehyde oxidase and aldehyde dehydrogenase (Goldberg & Anderson, 1985). Renal aldehyde oxidase activity is localized in the proximal tubule but represents only 40% of the liver activity (Anders, 1980), but aldehyde oxidase represents only 10%

of renal aldehyde dehydrogenase activity (Anders, 1980). At least two aldehyde dehydrogenase isozymes occur in proximal tubule mitochondria and cytosol. Substrates include formaldehyde, acetaldehyde, acrolein, and malondialdehyde (Hjelle et al., 1983).

DT-diaphorase (NADPH-quinone oxidoreductase) is a cytosolic enzyme that is present at highest concentration in the medulla and catalyses a two-electron reduction of quinones to less reactive hydroquinones. It therefore blocks redox cycling and the generation of superoxide anion radicals (Lind et al., 1978).

4. THE MECHANISTIC BASIS OF CHEMICALLY INDUCED RENAL INJURY

Over the past 20-30 years there has been a growing understanding of the molecular basis of disease and the biochemical mechanisms that are associated with chemically induced cellular degeneration and lesions in target organ systems. The application of this understanding provides a foundation upon which to study chemically induced renal injury and, in particular, a rational basis for the extrapolation of animal toxicity data to man and risk assessment.

4.1 Immunologically induced glomerular disease

Immunologically mediated glomerulonephritis can result from the deposition of free circulating antibodies interacting with a structural glomerular antigen or with a "planted", non-glomerular antigen (such as cationic proteins that fix on anionic sites of the glomerular basement membrane). Alternatively, glomerular injury may be the consequence of the deposition of circulating immune complexes. The two main forms of antibody-mediated glomerulonephritis are the anti-GBM-mediated disease and membranous glomerulonephritis (or immune complex-type mediated glomerulonephritis). The former disease is characterized by the presence of linear IgG deposits along the glomerular basement membrane. The latter form may result from either of the mechanisms mentioned, i.e. deposition of free circulating antibodies against an irregularly distributed antigen, which may be of glomerular origin, or deposition of circulating immune complexes. A membranous glomerulonephritis is characterized by the presence of granular IgG deposits along the glomerular basement membrane.

Besides antibody-mediated glomerulonephritis, it is more and more apparent that there are cell-mediated glomerulonephritides without Ig deposition. An example of such a disease is probably the nephrotic syndrome with minimal glomerular changes at the light microscope level.

The role of genetic factors, which has been well demonstrated in the mercury model in the rat, is also

clear in the human situation. Membranous glomerulonephritis induced by gold and D-penicillamine is much more frequent in DR3-positive rheumatoid arthritis patients and in poor sulfoxydators. The lupus-like disease observed in hydralazine-treated patients is more frequent in those with the DR4 antigen and "slow-acetylators".

Other drugs such as non-steroidal anti-inflammatory agents or lithium salts may induce the nephrotic syndrome with minimal glomerular changes. Immunofluorescence is negative in these cases, and there is indirect evidence that such disease could be due to T cell-mediated immunity.

There are many potential environmental agents, drugs, and toxic chemicals that have been related to this form of glomerulonephritis. Drugs and toxic chemicals that may induce glomerulonephritis in humans include gold and mercury, d-penicillamine, non-steroidal anti-inflammatory agents, and heroin. Other drugs and chemicals, in which association is suspected but not certain, include silica exposure, toxic-oil syndrome, hydrocarbon exposure, and interferon. Other drugs may induce an immune complex type of glomerulonephritis in the context of a lupus-like syndrome (e.g., hydralazine, procanamide, and diphenylhydantoin. Furthermore, there are many other substances that have been implicated by case reports or unconfirmed experiments. Anti-GBM-mediated glomerulonephritis is not the usual mechanism responsible for toxic glomerular nephropathy. However, exposure to organic solvents is thought to be a factor in some cases of Goodpasture's syndrome, which consists of the simultaneous occurrence of necrotizing haemorrhagic interstitial pneumonitis and proliferative usually rapidly progressive glomerulonephritis. This is an auto-immune disease resulting from anti-GBM antibodies that cross-react with basement membranes in lung alveoli.

The mechanisms by which drugs and chemicals induce immunologically related renal disease are both complex and not entirely understood. It is unlikely that drugs or toxins induce renal damage by modifying self antigens or by acting as haptens. On the other hand, many agents such as gold, d-penicillamine, and mercury may have an immunomodulatory effect. This mechanism of action is also

not understood, but Druet et al. (1987) and Druet (1989) have summarized the evidence that this effect is dependent on genetic factors and may be related in the rat to the appearance of anti-class II T cells.

4.2 Direct glomerular toxicity

Glomerular lesions may be caused by the direct toxicity of a drug or chemical. Direct toxicity to components of the glomerular apparatus, apart from immunologically induced injury, is relatively uncommon. However, it has been described following exposure to puromycin or to materials that may be deposited in the basement membrane (Caulfield et al., 1976). Particulate substances such as gold and silica may become deposited in mesangial cells (Burkholder, 1982). Whether material within mesangial cells is actually phagocytosed is unclear, but the reaction to such deposits may be a proliferation of glomerular cells and inflammatory cell response. Injury to the mesangium may alter glomerular permeability. Solutes and water move across glomerular capillary walls through an extracellular pathway that consists sequentially of endothelial fenestrae, the glomerular basement membrane, the pores of slit diaphragms, and the filtration slits. Water permeability is determined by the total area of epithelial slit pores. Contraction of the glomerular mesangium shortens and narrows glomerular capillaries, which in turn narrows epithelial slit pores and reduces glomerular filtration.

Damage to the glomeruli may also occur as a result of fibrin deposition due to local or systemic activation of the coagulation system. This may be induced by a variety of renal diseases including toxic or immunotoxic disorders. Fibrin deposits *per se* may damage glomeruli in several ways, including occlusion of glomerular capillaries, involvement in an inflammatory reaction, or direct toxicity to glomerular mesangial cells (Kanfer, 1989).

4.3 Tubulointerstitial disease

Tubulointerstitial disease may result from hypersensitivity to specific drugs or chemicals or from direct toxicity to tubular epithelial cells. Most forms of

tubular injury also involve the interstitium, and so these forms of renal injury are considered together. However, they may be subdivided, on the basis of clinico-pathological features, into three groups of disorders.

4.3.1 Acute interstitial nephritis

Acute interstitial nephritis (AIN) occurs as an immunoallergic or cell-mediated immune response to a variety of drugs, particularly penicillin and its derivatives (e.g., methicillin), but is also reported after therapy with thiazdes, non-steroidal anti-inflammatory drugs, gold salts, and occupational exposure to mercury (Kleinknecht et al., 1978; Clive & Stoff, 1984).

Both humoral and cellular immunity are involved. Antitubular basement membrane antibodies are probably involved in some cases of methicillin- or diphenyhydantoin-induced immunological nephritis. In the majority of cases of acute interstitial nephritis no immune reactants are found. The most striking feature is the presence of cells infiltrating the interstitium with mononuclear cells and eosinophiles. Most lymphocytes have been shown to be T cells; most of these are T4 cells (helper/inducer cells) and a lesser fraction composed of T8 cells (suppressor/cytotoxic cells). It has been suggested that the T cells may be activated by drug exposure (Druet et al., 1987; Druet, 1989).

When extrarenal signs and clinical symptoms are present, they may reflect a systemic hypersensitivity reaction that includes fever, skin rash, and eosinophilia. Renal involvement is manifested by mild proteinuria and haematuria. AIN tends to be more severe, with a high incidence of renal failure, in adult patients, but is usually milder in patients under 15 years of age. It has also been pointed out that absence of prior allergic reaction to a drug (e.g., penicillin) does not alter the risk of AIN. The kidneys are usually swollen (as visualized radiographically) because of oedema fluid and cellular infiltration, composed most commonly of lymphocytes and plasma cells as well as eosinophilic and polymorphonuclear neutrophils. In some instances the histological appearances may suggest chronic inflammation, and macrophages and giant cells may be present. Renal tubular cell damage

is always present, but there is no fibrosis in the acute stages. Glomerular and vascular lesions are uncommon, and the lesions are usually reversible. However, persistent loss of renal function indicates progression to fibrosis and chronic interstitial disease in an undetermined number of cases. Anti-tubular basement membrane antibodies are probably involved in some cases of methicillin- or diphenylhydantoin-induced nephritis. Deposits of IgG and C3 may be detectable along tubules in biopsies during the acute phase. IgE may be elevated in the serum, confirming the allergic or hypersensitivity process. The reaction can be further indicated by tests of cell-mediated hypersensitivity (lymphocyte transformation test) or antibody-mediated hypersensitivity (circulating antibodies reacting with the drug).

4.3.2 *Acute tubular toxicity*

Acute tubular effects of drugs and toxins are the result of direct cellular toxicity. They may vary from necrosis of tubular cells, leading to acute renal failure, to subtle subcellular lesions and functional effects. The major groups of agents causing acute tubular toxicity are antibiotics, particularly aminoglycosides, contrast agents, non-steroidal anti-inflammatory drugs, and chemotherapeutic agents including cyclosporin A and *cis*-platinum.

Injury to proximal tubular lining cells is manifest by increased excretion of substances normally resorbed by these cells, such as glucose, amino acids, phosphate, and sodium. Extension of the lesion to distal portions of the tubule is accompanied by loss of the ability to acidify the urine and to maintain water and electrolyte balance. Tubular toxicity may be accompanied by glomerular effects, and, if the process is persistent, may lead to a chronic interstitial nephropathy, as in lead and cadmium toxicity.

4.3.3 *Chronic interstitial nephritis*

Chronic interstitial nephritis (CIN) generally has fewer distinguishing morphological features than most forms of acute renal disease. It is characterized morphologically by infiltration with mononuclear cells, prominent interstitial fibrosis, and tubular atrophy. The best documented cause of CIN is analgesic nephropathy which is

often accompanied by acute papillary necrosis. CIN may also occur as a sequela to severe acute tubular disease or acute interstitial nephritis, or as an expression of chronic low-dose exposure to specific nephrotoxins (lead or cadmium nephropathy). The term "tubulointerstitial disease" may be preferable, because it better identifies the primary sites of the pathological process. Progressive fibrosis of the interstitial tissue results in a decreasing number of functional nephrons with eventual reduction in glomerular filtration rate and azotaemia. There may be few symptoms preceding the onset of renal failure.

4.4 Mechanisms of cellular toxicity

There are several mechanisms that are thought to be central to toxicological injury, including impaired lysosmal function, membrane changes, and oxidative stress. It is now widely accepted that Ca^{2+}-homeostasis in the cell and Ca^{2+}-mediated cell functions are critical targets for numerous pathophysiological processes including toxicant-induced cell death (Recknagel, 1983; Pounds & Rosen, 1988). Many classes of pharmaceuticals and other chemicals (e.g., metals, pesticides, and solvents) impair the calcium messenger system (Pounds, 1984; Olorunsogo et al., 1985; Moore et al., 1986). Disturbances in intracellular Ca^{2+} homeostatis and sustained increase in cytosolic Ca^{2+} cause cell death by the disruption of the plasma membrane, cytoskeleton, endoplasmic reticulum, and mitochondria. In addition, chemicals (alkylating or arylating agents) can be toxic and may induce cell death through an initial DNA damage or by apoptosis (receptor-mediated programmed cell death). In cell injury caused by chemical toxicants, cellular accumulation of Ca^{2+} and the generation of oxygen free radicals damage cellular components, particularly mitochondrial membranes. Indeed Ca^{2+} potentiates oxygen free radical injury to renal mitochondria (Malis & Bonventre, 1986), and the result of this detrimental interaction could be due, in part, to the activation of phospholipase A2.

Lipid peroxidation has been suggested as one of the possible mechanisms whereby chemicals may produce membrane damage and cell death. Free radicals, generated either directly by the metabolism of a chemical or from the re-

duction of oxygen (forming O_2^-, H_2O_2 and OH•), can initiate lipid peroxidation via hydrogen abstraction from polyunsaturated fatty acids. This interaction will form lipid peroxyradicals and lipid hydroxyperoxides, propagating the chain reaction. Such a chain reaction may destroy cellular membranes and thereby result in increased plasma membrane permeability or altered fluidity and cell death. Lipid peroxidation may also cause cell death through the formation of potent toxic lipid metabolites (such as hydroxyalkenals). However, several lines of evidence indicate that lipid peroxidation is most often independent (or is a consequence rather than the cause) of cell death (Witschi et al., 1987). One or more of these mechanisms of cellular injury could closely interact.

Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals, owing to their high energy demand (such as reabsorptive and secretory functions). Redox-active agents may cause extensive oxidation of GSH to oxidized glutathione (GSSG). Under such conditions, often referred to as "oxidative stress", reduction of GSSG back to GSH by the NADPH-dependent GSSG reductase is lower than the rate of GSH oxidation. This may lead to gluthathione depletion and cause oxidation of cellular enzymes, depletion of cellular ATP, and loss of mitochondrial function (Trump et al., 1989).

Reactive electrophilic metabolites are known to bind covalently to tissue proteins, and it has been suggested that cell injury and death are a consequence of the interaction of such reactive intermediates with critical cellular molecules. Free sulfhydryl groups are involved in the catatylic activity of many proteins. Modification of such sulfhydryl groups by covalent binding or by oxidation may inactivate critical enzymes and lead to cell death. For some chemicals the loss of protein sulfhydryl groups results mainly from a reversible oxidative process, which leads to the formation of disulfide cross-links or mixed disulfides with another protein or GSH. Enzymes involved in Ca^{2+} homeostasis may be one example of such critical cellular target molecules for alkylating/arylating or oxidizing metabolites.

Studies on isolated cells have shown that exposure to lethal concentrations of some cytotoxic chemicals leads to a rapid and sustained rise in cytosolic Ca^{2+}. Ca^{2+}-

mediated cytotoxicity may at least in part be related to effects on cytoskeletal organization. High Ca^{2+} concentrations affect the regulation of the formation of actin bundles and tubulin polymerization. Activation, by high intracellular Ca^{2+}, of Ca^{2+}-dependent proteases that cleave cytoskeletal proteins has been proposed as one mechanism of cell death (Fig. 11). Activation of other enzymes, such as phospholipase A_2 (causing disruption of the plasma membrane and formation of toxic membrane breakdown products) and endonucleases (extensive DNA fragmentation), has also been associated with cell death (Trump et al., 1989).

Certain alkyl halides such as DBCP (Omichinski et al., 1987; Dybing et al., 1989) and reactive oxygen species cause single-strand breaks in DNA. Extensive DNA damage activates poly ADP-ribosyltransferase, which leads to a critical reduction in cellular NAD^+ levels, followed by depletion of ATP and eventual cell death.

The process by which glucocorticoids induce killing of immature thymocytes has been termed programmed cell death (apoptosis). Recent studies indicates that the environmental contaminant 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) causes a receptor-mediated influx of Ca^{2+} in thymic cells, which in turn activates endonucleases and thereby causes programmed cell death. The role of such a process in the chemically mediated killing of kidney cells has yet to be determined (McConkey et al., 1988).

4.5 Factors that modify cellular injury by toxins

4.5.1 *Cellular transport and accumulation*

Drugs and other chemicals including metals may be transported across proximal tubular cells, i.e., from renal capillaries across tubular cells to be excreted in the tubular lumen or vice versa (absorption). Many organic anions are excreted against concentration gradients at rates that exceed glomerular filtration. This implies an active carrier-mediated transport process. Such a process requires energy obtained from oxidative metabolism located in mitochondria. An active process for transporting solutes in renal tubular cells has certain implications concerning the susceptibility of tubular cells to effects

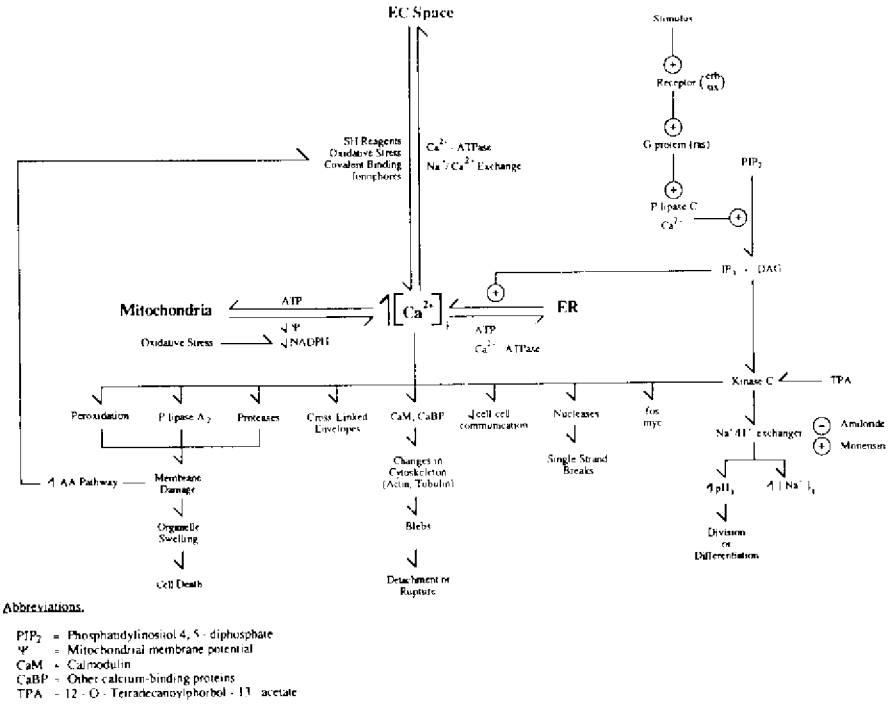


Fig. 11. Flowchart illustrating the relationships between ion deregulation, cell injury, and carcinogenesis. From: Trump et al. (1989).

of toxins (Berndt, 1989). If cationic drugs or chemicals are actively transported, there is the immediate problem of competition with the transport of essential cations. Active transport with the capability of concentrating absorbed material may concentrate potential nephrotoxins as well as essential solutes in the renal cortex. The same toxins that impair energy metabolism will impede the cellular transport of essential solutes (Rennick, 1978). Other toxic substances may be concentrated in the medulla or the papillae, probably as a consequence of the physiological mechanism that concentrates urine. The renal accumulation of chemicals such as gentamicin, cephaloridine, or cadmium is well documented.

4.5.2 *Metabolic degradation*

Metabolic degradation or transformation most often occurs in the liver, but many of the same enzyme systems are present in the kidney as well. The metabolism of drugs and chemicals within the kidney may result in substances that are either more or less toxic. Those drugs and chemicals that are metabolized by the mixed-function oxidase system have received the most attention. For example, several chlorinated alkyl hydrocarbons of low relative molecular mass, such as carbon tetrachloride and trichloromethane, may be transformed into reactive, toxic products that bind covalently to renal tissue, producing membrane injury. In addition, low-level exposure to other substances, such as polychlorinated biphenyls (PCBs), that activate the enzyme systems may enhance the production of toxic products (Kluwe et al., 1979). Similarly, pretreatment with phenobarbital enhances the activity of mixed-function oxidase enzymes and, hence, the toxicity of compounds like methoxyflurane whose metabolic products are fluoride and oxalate, two substances potentially toxic to the kidney. The fluoride ion is toxic to cell membranes, whereas oxalate may precipitate within the lumen of nephrons (Mazze, 1976). On the other hand, phenobarbital reduces the renal toxicity of DBCP due to an increase in its detoxication in the liver (Kluwe, 1983).

4.5.3 *Intracellular protein binding*

The intracellular concentration of toxins may be influenced by protein binding. The soluble cytoplasmic

protein, metallothionein, and insoluble acidic protein complexes forming nuclear inclusion bodies are examples of a phenomenon that concentrates two different groups of metals.

Metallothioneins are proteins of low relative molecular mass (6000-7000 Daltons) characterized by a high cysteine content (23-33%), a complete lack of aromatic acids, and a high content of heavy metals (7-12 metal atoms/molecule of protein). Metallothioneins can bind several essential or non-essential heavy metal ions including zinc, copper, cadmium, mercury, silver, gold, and cobalt (Goyer & Cherian, 1977). The metal ions are bound exclusively through thiolate coordination complexes, which involve all the cysteine residues (20 in rat liver metallothionein) located in two domains (α and β domains). Metal ions that can bind to metallothioneins can also, to variable extents, promote the transcriptional activity of metallothionein genes. In the kidney, Cd^{2+} and Hg^{2+} are the best inducers of metallothionein synthesis. Metallothionein synthesis can also be induced by various stresses (e.g., tissue injury, food restriction, infections). Not all the biological functions of metallothionein have been fully elucidated. They probably include protection against and detoxification of heavy metals, regulation of the metabolism and possibly the function of essential elements such as copper and zinc, and a protective response to various stresses by altering zinc distributions between tissues and within cells (e.g., macrophages) and by acting as a free radical scavenger (Dunn et al., 1987).

Lead and bismuth accumulate in renal tubular cells bound to a complex of acidic proteins that form morphologically discernible inclusion bodies (Goyer & Cherian, 1977). As with metallothionein, the sequestering of toxic metals by the protein complex is thought to reduce the intracellular toxicity of these metals.

4.5.4 *Membrane reactions and pinocytosis*

Macromolecular substances are transported by pinocytosis and included in intracellular vacuoles. Proteins that are normally in the glomerular filtrate are taken up by the cell membrane by pinocytosis. Such pinocytotic vesicles fuse with primary lysosomes, which contain lytic

enzymes. Secondary lysosomes are formed, and the macromolecular material is degraded or broken down. The products of low relative molecular mass then leave the lysosomes in order to prevent an increase in osmolality and lysosome swelling (Jacques, 1975).

Potential nephrotoxins that may be taken into renal tubular cells in this manner include chelating agents such as nitrilotriacetic acid, ethylenediaminetetraacetic acid (EDTA), and metallothionein. Membrane binding of EDTA administered as the calcium-EDTA chelate persists, the calcium but not the EDTA being dislocated to other cellular components. This suggests the manner in which EDTA may sequester cellular lead or other metals for excretion (Schwartz et al., 1970).

5. THERAPEUTIC AGENTS AND CHEMICALS THAT HAVE THE POTENTIAL TO CAUSE NEPHROTOXICITY

5.1 Therapeutic agents

Many therapeutic agents have been linked to clinically significant nephrotoxicity. At present much is known and understood about some of these agents, as there is a substantial amount of relevant animal toxicity and human data for comparison.

5.1.1 *Analgesics and non-steroidal anti-inflammatory drugs (NSAIDs)*

Analgesic nephropathy may be a consequence of the excessive consumption of mixed analgesics. Originally, phenacetin was common to all of these mixtures, which led to the conclusion that this drug was the only cause of "phenacetin kidney". Subsequently, a variety of analgesics, NSAIDs, and a number of industrial and environmental chemicals have been shown to have the potential to cause RPN and interstitial nephritis (Burry et al., 1977; Schwarz, 1987).

The prevalence of analgesic-associated nephropathy varies worldwide and is probably related to patterns of analgesic use. It has been found most often in women aged 45–55 as a result of a high incidence of analgesic abuse, and has been reported more frequently in Australia and Switzerland and less frequently in the USA, Canada, and Germany. It is estimated that more than 37 million people in the USA have arthritis and use these drugs. This is indeed a very large population at risk. Analgesic effects are said to be a factor in as many as 20–30% of cases of interstitial nephritis in the south-east of the USA (Murray & Goldberg, 1975).

Diagnosis is often made by coupling the history of analgesic abuse with morphological evidence of renal papillary necrosis and chronic interstitial nephritis. The resultant lesions can be recognized by radiological examinations or ultrasonography and consist of calcifications along the line of Hodson, shrinking of the kidneys resulting in irregular contours, and decreased length of

both kidneys. Necrotic papillae may be voided in the urine and this is occasionally observed.

At least two countries have legislated to prevent phenacetin from being sold over the counter. This legislation has had the effect of a change in analgesic formulation, usually towards that of a single drug such as aspirin or paracetamol (acetaminophen). In Sweden, the removal of phenacetin resulted in the progressive decline in the incidence of analgesic nephropathy. This began approximately 6 years after the removal of phenacetin and the major impact was seen after 12 years. In Australia, phenacetin was removed from combination analgesics by 1976 and replaced with acetaminophen (the principle metabolite of phenacetin); by 1979 legislation prohibited the over-the-counter sale of combination analgesics. According to the Australia-New-Zealand Dialysis and Transplant Registry, this has resulted in a progressive decline in the incidence of analgesic nephropathy in patients presenting with end-stage chronic renal failure in dialysis and transplant programmes. In 1982, 22% of patients in dialyses and transplant programmes had analgesic nephropathy; by 1988, the incidence had declined to approximately 13%. By contrast, the sale of phenacetin-containing analgesics declined to 21% (in 1976) and 9% (in 1983) of the total volume of analgesics sold in Belgium, but the prevalence of analgesic nephropathy remained unchanged over this period. Retrospective and prospective studies in Belgium have shown decreased renal function in analgesic abusers who never took analgesic mixtures containing phenacetin (Elseviers & De Broe, 1988). Paracetamol, which largely replaced phenacetin in analgesic mixtures), is assumed to be involved in the pathogenesis of RPN. Recently, an increased risk of renal disease was found in daily users of paracetamol in North Carolina, USA (Sandler et al., 1989). Other therapeutic agents (mostly analgesics and NSAIDs) have been implicated in RPN, and a number of industrial and environmental chemicals also have the potential to cause this lesion (Bach & Bridges, 1985a).

Most patients deny analgesic abuse, thus making epidemiological studies that attempt to identify the causative agent difficult. Most of the epidemiological data that has been reported over-reflected the intake of phenacetin at the expense of other agents that could equally

be implicated. The estimate of the total lifetime dose of analgesic that produces papillary necrosis varies from < 1 to 35 kg (but this was based only on phenacetin). No data was given in the epidemiological studies on what quantities of other analgesics were also taken or on exposure to papillotoxic chemicals. Thus the etiology of RPN has been complicated by poly-pharmacy, lack of documentation on the intake of other analgesics and NSAIDs, exposure to other chemicals, and the difficulty in diagnosing RPN.

Analgesic abuse (Nanra et al., 1978; Nanra, 1980; Prescott, 1982; Bach & Bridges, 1985a; Schwarz, 1987) and addiction have been linked to co-formulation with caffeine, but there is no firm supporting evidence. Few analgesic abusers take the drugs for appropriate indications. The origins of abuse are usually psycho-social and represent neurotic, dependent, immature, introverted, anxious, or depressed individuals, up to 20% of whom also smoke and abuse alcohol, psychotropic drugs, and sleeping tablets. Most analgesic abusers are women (over 30 years of age) from lower socioeconomic/education groups, who have taken these mixtures for 5-30 years. Several factors such as dehydration, secondary to high ambient temperatures and bacterial infection, have been implicated in the development of renal failure (Kincaid-Smith, 1979). Renal function may be preserved and the progression to ESRD may be prevented by stopping analgesic exposure, but patients who continue to abuse analgesics have a poor prognosis (Schwarz, 1987). Analgesic abusers also have an increased incidence of anaemia, gastric ulcers, and cardiovascular heart disease (Dubach et al., 1978).

Clinical features of analgesic nephropathy include loss of urine-concentrating capacity (Bengtsson, 1962), electrolyte disturbances, (sodium wastage and hypocalcaemia), and defective urinary acidification after ammonium chloride loading (Bengtsson, 1962; Nanra et al., 1978). An increase in BUN or creatinine identifies incipient renal failure; at this stage papillary necrosis is well advanced and includes secondary degenerative changes. Radiology and ultrasound may identify irregular shrinking of the renal tissue and medullary calcifications, but these are advanced changes. Very early in the course of the injury histological changes are confined to the medulla (a region of the kidney that is not always

assessed at autopsy). This situation progresses to include other changes, especially in the cortex, that are easily biopsied to show interstitial nephritis, but they do not define the underlying cause.

The earliest degenerative changes begin at the papilla tip and affect interstitial cells, loops of Henle, capillaries, and the proteoglycan ground substance, and result in lipidosis. More advanced or intermediate RPN affects the outer medulla, as seen by atrophy, sclerosis, and inflammatory response and calcification of the necrosed papilla tip (Burry et al., 1977; Gloor, 1978). Total RPN affects the corticomedullary and cortical regions and is characterized by chronic interstitial nephritis, tubular dilatation, atrophy, basement membrane thickening, fibrosis, sclerosis, and inflammatory cell infiltration. Vascular degeneration such as suburothelial capillary sclerosis is pathognomonic for RPN (Mihatsch et al., 1984). Pelvic, ureteric, and bladder urothelia show thickening of capillary walls, sclerosis of lamina propria, altered fat and collagen deposition, and epithelial hyperplasia advancing to malignancy and tumours (Burry et al., 1977; Mihatsch et al., 1984).

The pathological changes in humans with RPN have been obtained from autopsy or postmortem tissues, where autolytic degradation may alter the appearance and make interpretation of the stage of the lesion difficult. Animal models of RPN in a laboratory situation have provided more detail on the focus of primary injury and the course of degenerative changes and also allow mechanisms to be studied, but they do not necessarily reflect the situation in humans.

Analgesics (e.g., aspirin, phenacetin, and paracetamol) do not always cause RPN in rats. Thus inappropriately high doses have sometimes been given for prolonged periods (Prescott, 1982; Bach & Bridges, 1985a). Biological variation within such groups is very high and experimental use of NSAIDs has caused fatalities due to extra-renal toxicity (gastric ulceration and perforation; see Kaump, 1966). This has produced experimental models that are of limited value for studying the course of the lesion or mechanism of RPN. The renal functional changes and the pathomorphological progression of the lesion in several

acute model systems show marked similarities with those reported for the analgesic-associated lesion in both experimental animals and man (Bach & Hardy, 1985; Bach & Bridges, 1985a).

The histological changes induced by experimental RPN follow a similar pattern of early, intermediate and total RPN to that described in man (Fig. 12), and are dose and time dependent. The earliest morphological changes induced by papillotoxins occur in the renal medullary interstitial cells. The medullary glycosaminoglycan matrix also undergoes changes, showing an increase and then a decrease in staining intensity. It is only subsequently that there are platelet adhesions, blocking of blood vessels, degenerative changes in the collecting ducts and proximal tubules, and the accumulation of lipid material in capillaries and epithelial cells. At the same time as repair and re-epithelization are taking place, there is an increase in the presence of tubular casts, proximal and distal tubular dilatation, and hyperplasia of the collecting ducts, and pelvic urothelia, and the suburothelial capillaries undergo sclerotic changes. When the repair phase is advanced or complete, there are also degenerative changes in the cortex, including fibrosis, glomerular sclerosis, and cystic dilatation. The histological and functional changes produced by model RPN are remarkably similar to those observed in human analgesic abusers. The use of high-resolution light microscopy and ultrastructural studies (in conjunction with histochemistry and immunohistochemistry) can help establish the changes in adjacent cells and link the "cause-and-effect" relationships in the sequence of degenerative events.

The mechanism of analgesic-induced renal papillary necrosis is still not fully understood. Progress in our understanding of the pathogenesis of the model lesions (Bach & Bridges, 1984, 1985a) has enabled some factors to be identified that may be involved in the molecular changes. There is no evidence to suggest that the model lesion has an early immunological basis, nor that it is a consequence of renal hypoxia or vasoconstriction, and there is no experimental basis to suggest that the altered intermediary metabolism is a critical factor (Bach et al., 1983). The concept that altered PG metabolism gives rise to vascular (or other) changes is an attractive one, but

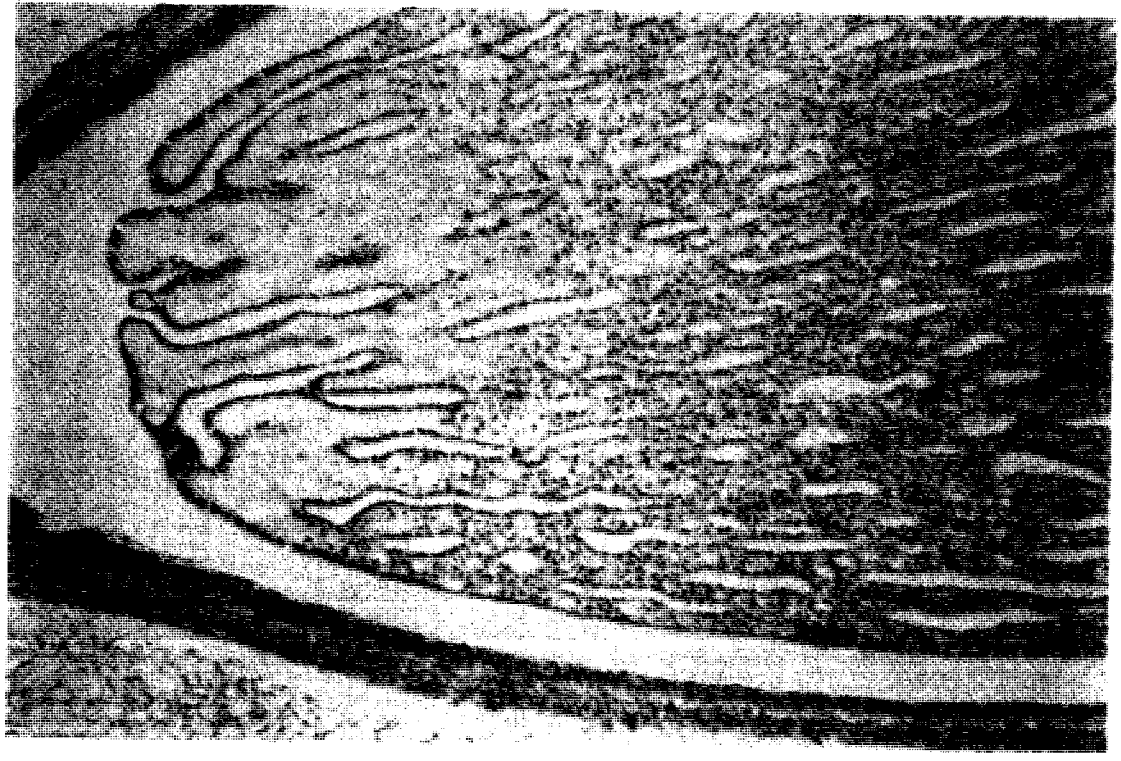


Fig. 12. Apex-limited focal RPN in animal dosed with 100 mg/kg BEA given orally; H&E stained wax section (Courtesy: P.H. Bach).

the exceptionally low levels of these hormones, combined with their instability, have made it very difficult to test this hypothesis. The countercurrent concentration mechanism is an important normal renal function and is thought to play an important role in concentrating chemicals to toxic levels within the medulla. One of the earliest changes in the development of RPN is the loss of concentrating processes, which detracts from this hypothesis. Furthermore, the concentrating of a compound in the medulla does not explain the molecular mechanism by which it causes RPN (Bach & Bridges, 1985a).

At present the most attractive explanation for the development of RPN relates to a metabolic activation within the kidney. There are two major oxidative systems for xenobiotic metabolism in the kidney. The cytochrome P-450 system is localized to the cortex, whereas the PG hydroperoxidase system (PGH) is located almost exclusively in the medulla. The reasons for the selective targeting of particular chemicals for the renal medulla interstitial cells are uncertain, but may relate to the absence of free radical scavengers or nucleophiles and/or to the presence of extensive numbers of lipid droplets containing polyunsaturated fatty acids within these cells. These would form an ideal substrate for extensive lipid peroxidation (Porter et al., 1980) within the renal medullary interstitial cells once a reactive species had been generated within these cells. The role of co-oxidation of substrates as a consequence of PG synthesis has become an attractive mechanistic basis on which to explain papillary damage (Fig. 13) and the activation of bladder carcinogens, and it may also be pertinent to other types of renal toxicity that are not associated with mixed-function oxidase activity (Bach & Bridges, 1984). Prostaglandin endoperoxide synthetase consists of two inseparable activities. Fatty acid cyclo-oxygenase catalyses the oxidation of arachidonic acid to PG hydroperoxy-endoperoxide (PGG_2), while the other activity, PG hydroperoxidase, reduces PGG_2 to PGH_2 and co-oxidizes another molecule. PGH_2 is the precursor for both PGs and thromboxanes (Davis et al., 1981). PG hydroperoxidase is inhibited by antioxidants and will reduce many different fatty acid peroxides, other organic peroxides (cumene hydroperoxide and *tert*-butyl hydroperoxide), and inorganic peroxides (hydrogen peroxide) and,

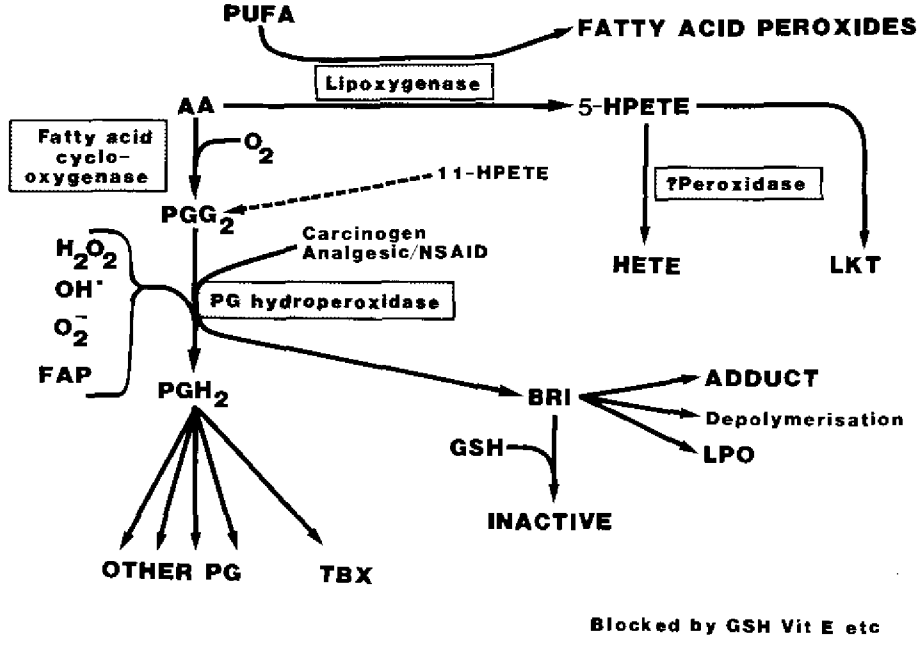


Fig. 13. Schematic representation for the arachidonic acid cascade including the proposed pathways for the oxidative activation of NSAIDs, analgesics and carcinogens, the generation of free radicals, and the oxidoreductive depolymerization of glycosaminoglycans by hydroxyl radicals. Abbreviations: AA = arachidonic acid; adduct = DNA, protein, or micromolecular adduct; BRI = biologically reactive intermediate; depolymerization = the oxidoreductive depolymerization of glycosaminoglycans by hydroxyl radicals or other reactive species; FAP = fatty acid peroxide; GSH = glutathione; HETE = hydroxyeicosatetraenoic acid; HPETE = hydroperoxyeicosatetraenoic acid; LPO = lipid peroxidation; NSAID = nonsteroidal anti-inflammatory drug; O_2^- = superoxide anion; $OH^{\cdot}$ = hydroxyl radical; PG = prostaglandin; PUFA = polyunsaturated fatty acid; TBX = thromboxane. From: Bach & Bridges (1984).

in the process, co-oxidize a range of substrates including several bladder carcinogens that produce free radicals.

Paracetamol forms a reactive intermediate that covalently binds to trichloroacetic-acid-precipitable macromolecules but is inhibited by aspirin and other inhibitors of fatty acid cyclo-oxygenase. Ethoxyquin, ascorbic acid, and glutathione also inhibit covalent binding of paracetamol during peroxidative activation by reacting with electrophilic intermediates generated by co-oxidation (Zenser et al., 1983).

Other non-steroidal anti-inflammatory agents may produce hypersensitivity reactions, lipoid nephrosis, and interstitial nephritis (Finkelstein et al., 1982).

5.1.2 *Paracetamol (acetaminophen) and para-aminophenol*

Large doses of paracetamol can produce acute proximal tubular necrosis, especially in male Fischer-344 rats (Mitchell et al., 1977; McMurtry et al., 1978; Hennis et al., 1981; Newton et al., 1983a,b). Microsomal cytochrome P-450 activation to a reactive arylating intermediate is thought to be an obligatory biochemical event in paracetamol-induced hepatic necrosis (Mitchell et al., 1973; Nelson, 1982). Nephrotoxic dosages of paracetamol bind covalently to renal protein (Mitchell et al., 1977; McMurtry et al., 1978; Nelson, 1982) by an NADPH-dependent, cytochrome-P-450-mediated process (McMurtry et al., 1978; Newton et al., 1983a,b). Alternatively, paracetamol is enzymically deacetylated to *para*-aminophenol, a potent selective nephrotoxin that damages the proximal tubule (Calder et al., 1979). *Para*-aminophenol produces acute necrosis of the proximal convoluted tubules in rats after a single injection (Green et al., 1969), and has been demonstrated to be a minor metabolite of paracetamol in the Fischer-344 rat and its isolated perfused kidney (Newton et al., 1982). Paracetamol (*N*-acetyl-*p*-aminophenol) is structurally closely related to *para*-aminophenol, and metabolites have been shown to be excreted by the biliary route in rats (Siegers & Klaassen, 1984) and mice (Fischer et al., 1985). These metabolites are the glucuronic acid and sulfate conjugates (Siegers & Klaassen, 1984) and the glutathione conjugate (Hinson et al., 1982). Toxicity arising from *para*-aminophenol has been previously

suggested to result from a dose-related depletion of kidney reduced glutathione and covalent binding to essential renal macromolecules (Crowe et al., 1977; 1979).

Both paracetamol and *para*-aminophenol deplete renal cortical reduced glutathione concentrations and arylate renal macromolecules (McMurtry, et al., 1978; Crowe et al., 1979). The changes produced by *para*-aminophenol are indistinguishable from those caused by paracetamol (Newton et al., 1983a,b). Mouse renal cortical slices and homogenates are capable of deacetylating paracetamol to *para*-aminophenol (Carpenter & Mudge, 1981), which has also been identified as a urinary metabolite of paracetamol in both hamsters (Gemborys & Mudge, 1981) and Fischer-344 rats (Newton et al., 1983a,b). Thus the rat is capable of deacetylating paracetamol to *para*-aminophenol. In the renal cortex, paracetamol deacetylation occurs primarily in the cytosolic fraction (Newton et al., 1983a). Similarly, metabolic activation of paracetamol to an arylating intermediate is dependent on the presence of a cytosolic deacetylase (Newton et al., 1983b).

Both *para*-aminophenol and bis-(*p*-nitro-phenyl)-phosphate (a carboxylesterase-amidase inhibitor) inhibit the covalent binding of paracetamol to renal macromolecules (Newton et al., 1983b). Conclusive evidence that paracetamol binds to renal macromolecules after deacetylation and metabolic activation to *para*-aminophenol has been provided by the demonstration of covalent binding of [ring-^{14}C]-paracetamol, but not [acetyl-^{14}C]-paracetamol, to renal protein (Newton et al., 1983b).

Thus, paracetamol activation by renal cortical tissue can occur in two different ways, i.e. either a microsomal cytochrome-P-450-dependent pathway or deacetylation to *para*-aminophenol and subsequent metabolic activation. The reactive intermediates formed by each pathway suggest that both mechanisms may play a role in paracetamol-induced renal cortical necrosis.

5.1.3 Antibiotics

Nephrotoxicity related to antibiotics is most often due to effects on transport, concentration, and excretory functions. All parts of the nephron or kidney may be

affected. However, there is usually some specificity in the site of action, particular toxins affecting specific portions of the nephron (Curtis, 1979).

Mechanisms of injury span a broad spectrum of potential lesions. The most common effect is direct toxicity to renal tubular cells manifested by cell injury and necrosis. Direct toxicity to glomeruli is not as conspicuous but does occur. Immunologically induced lesions in glomeruli and interstitial tissue may also occur.

5.1.3.1 *Aminoglycosides*

Nephrotoxicity is a common complication of aminoglycoside antibiotic therapy in man (Bennett, 1983; Matzke et al., 1983; Kahlmeter & Dahlager, 1984). Early signs of nephrotoxicity include increased urinary excretion of proximal tubular cell brush-border membrane enzymes such as alanine aminopeptidase, proteins of relative low molecular mass such as lysozyme and β2-microglobulin, and granular casts (Schentag, 1983). A urine-concentrating defect is usually evident and may explain the non-oliguric acute renal failure typically observed in these patients. Less common manifestations of tubular dysfunction include potassium, magnesium, calcium, and glucose loss in the urine. Azotaemia and elevation of the serum creatinine concentration are relatively late manifestations of nephrotoxicity and reflect depression of glomerular filtration rate consequent to extensive proximal tubular cell necrosis. Patients receiving standard doses of aminoglycoside antibiotics usually do not manifest depression of glomerular filtration until after seven or more days of drug therapy. However, pathological changes confined to the proximal tubule can be seen in renal biopsy material obtained before this time (DeBroe et al., 1984). At the light microscope level, these changes range from loss of brush-border membrane, apical blebbing, and prominent vacuoles to cloudy swelling, patchy cell necrosis, and sloughing of necrotic cells with cast formation in the lumen. Electron microscopy reveals the presence of multicentric multilamellar membrane structures known as myeloid bodies within distended lysosomes (Kosek et al., 1974). These lysosomal lesions can be seen within 1-2 days of drug treatment and they increase in size and number as therapy is prolonged.

The nephrotoxicity potential of aminoglycosides has been ranked as neomycin > gentamicin > sisomicin = kanamycin > tobramycin > netilmicin > streptomycin (Parker et al., 1982). The situation with amikacin has been somewhat controversial, but recent studies have suggested that it is less nephrotoxic, even in experimental animals, than the other clinically available aminoglycosides, except for streptomycin. Clear-cut therapeutic advantages of any particular aminoglycoside are not readily apparent in patients because of the serious nature of their underlying illness and concurrent therapy with multiple drugs. Furthermore, the relative nephrotoxicity is usually assessed by insensitive techniques, such as blood urea nitrogen, serum creatinine, and enzymuria, that do not give a quantitative representation of the extent of renal injury. In humans, few would argue that neomycin and gentamicin are much more nephrotoxic in therapeutic use than streptomycin, but there are also other important risk factors that relate to the clinical condition of the patient:

- dehydration, volume depletion, diuretic-induced volume depletion;
- advanced age;
- pre-existing renal disease;
- electrolyte imbalance (acidosis, hypomagnesaemia, hypokalaemia, hypocalcaemia);
- hypotension/renal ischaemia;
- extrarenal target organ disease such as cirrhosis of the liver;
- exposure to multiple nephrotoxins;
- frequent dose regimens as opposed to larger doses given less frequently;
- elevated aminoglycoside trough concentrations.

Current understanding of the pathogenesis of aminoglycoside nephrotoxicity has been derived primarily from studies in rats, which exhibit a pattern of renal injury indistinguishable from that observed in man (Kaloyanides & Pastoriza-Munoz, 1980; Humes et al., 1982; Bennett, 1983; Tulkens, 1989). The drug dose, in relation to body weight, required to induce injury in the rat is considerably larger than that required in man, whereas the dose is approximately the same when expressed in relation to body

surface area. From such studies has emerged unequivocal evidence that aminoglycoside nephrotoxicity is causally linked to the transport and accumulation of drugs by renal proximal tubular cells. Following parenteral administration, aminoglycosides are eliminated unchanged in the urine by glomerular filtration. A small fraction of the filtered drug is taken up by the renal proximal tubular cells via a low affinity, high capacity transport mechanism that exhibits saturation kinetics (Kaloyanides, 1984a; Giuliano et al., 1986). The first step in this transport process involves binding of the cationic aminoglycoside to apical membrane receptors, thought to be anionic phospholipids such as phosphatidylinositol (Sastrasinh et al., 1982). This is followed by uptake into the cell by adsorptive endocytosis (Silverblatt & Kuehn, 1979) with subsequent translocation and sequestration of the drug in high concentration within lysosomes (Morin et al., 1980; Josepovitz et al., 1985). In addition a small quantity of drug appears to gain access into the cell across the basolateral membrane (Collier et al., 1979). Following uptake into proximal tubular cells, aminoglycosides express their nephrotoxicity potential by disrupting one or more critical intracellular metabolic pathways.

Although these drugs have been shown to effect a variety of biochemical processes at several sites within proximal tubular cells (Kaloyanides, 1984b), it remains to be established which if any of these actions are causally linked to the cascade that eventuates in cell injury and necrosis. Prominent among the biochemical derangements is a disturbance of phospholipid metabolism reflected by an increase in renal cortical phospholipid enriched in phosphatidylinositol (Kaloyanides, 1984b). The phospholipidosis has been shown to be due primarily to the accumulation of lysosomal myeloid bodies (Josepovitz et al., 1985), which form as a consequence of the inhibition of lysosomal phospholipases (Laurent et al., 1982; Carlier et al., 1983) by the high concentration of drug within the lysosomal compartment (Ramsammy et al., 1989a). The mechanism of inhibition is thought to be related to an electrostatic interaction between the cationic aminoglycoside and anionic phospholipid. Another example of an adverse interaction between aminoglycosides and phospholipid is the observation that gentamicin inhibits agonist activation of

the phosphatidylinositol cascade (Ramsammy et al., 1988a), an effect that localizes the site of interaction at the cytoplasmic surface of the plasma membrane and most likely reflects binding of the polycationic gentamicin to the polyanionic phospholipid, phosphatidylinositol-4,5-bis-phosphate. This effect may also explain the observation that aminoglycosides inhibit phosphatidylinositol-specific phospholipase C in renal brush-border membranes (Schwertz et al., 1984). Alterations of other biochemical processes associated with plasma membranes have been described, including depressions of Na^+-K^+-ATPase, adenylate cyclase, alkaline phosphatase, and calcium binding (Morin et al., 1980; Williams et al., 1981). Impaired mitochondrial respiration (Weinberg & Humes, 1980) and decreased incorporation of leucine into microsomal protein (Bennett et al., 1988) have also been observed prior to the onset of obvious irreversible cell injury. These findings emphasize that multiple sites serve as targets for drug-cell interaction. However, it remains uncertain which of these biochemical abnormalities are proximal events causally linked to toxicity.

One theory that attempts to integrate these diverse observations focuses on the lysosomal accumulation of aminoglycosides, with induction of a lysosomal phospholipidosis as the critical first step (Tulkens, 1989). If the injury threshold concentration of aminoglycoside is not reached, the lysosomal phospholipidosis regresses without any biochemical or morphological evidence of cellular necrosis and regeneration (Giuliano et al., 1984). However, if the injury threshold concentration is exceeded, the lysosomal phospholipidosis progresses and the overloaded lysosomes swell, resulting in the loss of integrity of the lysosomal membrane and the release of lysosomal enzymes, toxins, and large quantities of aminoglycosides into the cytosol. The extralysosomal aminoglycoside interacts with and disrupts the functional integrity of other subcellular membranes, thereby initiating the injury cascade that eventuates in cell death.

It should be emphasized that aminoglycoside-induced proximal tubular cell necrosis is accompanied by a conspicuous regenerative response (Parker et al., 1982; Toubeau et al., 1986). Thus, the clinical threshold for nephrotoxicity is determined by the balance between the

rate of necrosis and the rate of regeneration of proximal tubular cells (Soberon et al., 1979). If necrosis dominates, overt renal failure ensues.

Aminoglycoside nephrotoxicity is accompanied by increased generation of free radicals. Furthermore, nephrotoxicity is blocked with free radical scavengers/antioxidants such as dimethylthiourea, dimethyl sufoxide, sodium benzoate, or deferoxamine (Walker & Shah, 1987). However other studies have demonstrated that antioxidants such as vitamin E do not protect against aminoglycoside-induced injury (Ramsammy et al., 1986, 1987, 1988b). The reasons for this apparent discrepancy are not known, and the exact role of lipid peroxidation in gentamicin nephrotoxicity therefore remains unclear.

Polyaspartic acid has recently been shown to protect rats completely from developing aminoglycoside nephrotoxicity without inhibiting proximal tubular cell drug uptake (Williams et al., 1986; Gilbert et al., 1989; Ramsammy et al., 1989b). *In vitro* studies suggest that the protective effect of polyaspartic acid is due to the ability of this polyanionic peptide to bind the cationic aminoglycosides, thereby preventing these drugs from interacting electrostatically with various targets, presumably anionic phospholipids, within the cell.

5.1.3.2 *Cephalosporins*

The nephrotoxicity of cephalosporins was first noted when the drugs were used in combination with aminoglycosides, but it is now recognized that cephalosporins, particularly cephaloridine, may produce degeneration and necrosis of proximal tubular lining cells and acute renal failure. It has been suggested that the cellular toxicity is the result of metabolic activation of the five-member thiophene ring present in cephalothin and cephaloridine, the only two cephalosporins that seem capable of producing dose-dependent direct nephrotoxicity (Mitchell et al., 1977). More recently, it has been suggested that lipid peroxidation (Goldstein et al., 1989) and direct mitochondrial toxicity may be involved in the mechanisms of cephaloridine nephrotoxicity. Necrosis occurs when the concentration exceeds 1000 mg/kg wet tissue. The correlation between dose and response, as well as the localiz-

ation of the lesion in the proximal portion of the nephron, may be explained by a striking corticomedullary gradient in tissue concentration. The cellular uptake of cephaloridine and nephrotoxicity have been modified or eliminated in experimental animals by pretreatment with either probenecid or *p*-aminohippuric acid. The mechanisms of toxicity are complex (Wold et al., 1979; Tune & Fravert, 1980; Tune, 1986; Goldstein et al., 1987; Tune et al., 1988).

5.1.3.3 *Amphotericin B*

The increasing use of immunosuppressive therapy and the attendant systemic mycotic infections have resulted in an increase in the administration of amphotericin B. This drug is almost always associated with some degree of toxicity to the distal renal tubule and accompanying acidosis, hypokalaemia, and polyuria (Butler, 1966; Douglas & Healy, 1969). Reduced renal blood flow and glomerular filtration rate may also occur. Pretreatment of experimental animals with furosemide or sodium protects against decreases in renal plasma flow and glomerular filtration rate immediately following amphotericin B treatment. Salt loading also protects against amphotericin-induced decreases in renal plasma flow and glomerular filtration rate upon chronic drug administration in rats. However, it is important to note that tubular toxicity in these studies was not ameliorated by salt loading (Tolins & Raij, 1988). These data suggest that the tubular toxicity of amphotericin B is not secondary to renal vasoconstriction and ischaemia.

5.1.3.4 *Tetracyclines*

The nephrotoxicity of tetracycline incited considerable interest in the early 1960s, shortly after its introduction. People, particularly children, developed a reversible proximal tubular dysfunction after receiving outdated drugs. The nephrotoxicity was found to be due to a degradation product, anhydro-4-epitetracycline. The problem has disappeared with the substitution of citric acid for lactose as a vehicle (Curtis, 1979).

Other rare effects of tetracycline that have been reported are impairment of renal-concentrating ability by

demethylchlorotetracycline and occurrences of acute interstitial nephritis after minocycline treatment. More important to current usage is the awareness that the serum half-life of the two most commonly used drugs, tetracycline and oxytetracycline, is greatly prolonged in renal failure, and that the anti-anabolic effect of the tetracyclines, which inhibit the incorporation of amino acids into protein, may further contribute to negative nitrogen balance and uraemia by raising blood urea nitrogen (Curtis, 1979).

5.1.4 *Penicillamine*

Penicillamine (3,3-dimethylcysteine) was first used clinically as a copper-chelating agent to treat Wilson's disease. Because of the drug's potential for decreasing collagen formation, its use has been extended to a number of clinical disorders in which fibrosis is a major component, such as rheumatoid arthritis, pulmonary fibrosis, and liver disorders.

It has been suggested that the drug acts by reducing disulfide linkages. This inhibits polymerization of macromolecules and leads to impairment of collagen formation. Use of the drug has been tempered by the occurrence of side effects in as many as 30% of patients. The most important side effect (20% of cases) is proteinuria. The morphological appearance of the glomerular lesions is typically that of perimembranous glomerulonephritis with segmental subepithelial immune-complex deposits. These changes are best demonstrated as granular immunofluorescent deposits of IgG and C3. Withdrawal of penicillamine therapy results in disappearance of the proteinuria and repair of the basement membrane changes in 60% of cases (Gartner, 1980). Immune-complex glomerulonephritis with granular deposits along basement membrane and in the mesangium can be produced experimentally, confirming the role of an immunological mechanism in the pathogenesis of the nephropathy. In addition the drug is associated with the development of Goodpasture's syndrome with linear glomerular basement membrane deposits.

5.1.5 *Lithium*

Lithium salts, mainly lithium carbonate, have been used for 40 years to prevent relapses of maniac-depressive

illness. Impaired renal ability to acidify and concentrate urine is a common finding among patients given lithium (Batelle et al., 1982). It is usually regarded as a minor side-effect of the drug, i.e. a pharmacologically induced physiological impairment of distal tubules and collecting ducts, such a target-selective effect usually being reversible after discontinuation of the therapy. Since the polyurea is resistant to ADH, the effect has been characterized as resembling nephrogenic diabetes insipidus (Bendz, 1983).

Although several case reports of lithium-induced chronic renal insufficiency have been published (Hestbech et al., 1977; Hansen et al., 1979, 1981; Kincaid-Smith et al., 1979; Cohen et al., 1981; Walker et al., 1982,1986; Ottosen et al., 1984), the overall evidence suggesting progressive renal damage in patients taking lithium is rather limited because of methodological weaknesses in human studies (Lippmann, 1982). However, animal studies support the view that long-term treatment with lithium salts may lead to tubulo-interstitial nephropathies. Experimentally induced focal fibrosis, tubular atrophy, and cystic dilatation of distal tubules were obtained by exposing animals to toxic doses (Ottosen et al., 1984; Walker et al., 1986).

In addition to tubular effects, the occurrence of nephrotic syndrome in psychiatric patients has been attributed to long-term treatment with lithium salts (Richman et al., 1980; Depner, 1982). Thus, although case reports do not constitute evidence, there is some indication that lithium may adversely affect other segments along the nephron. A proportion of cases, ranging from 0 to 50% (median 8%) of patients on lithium, may eventually develop chronic renal insufficiency, evolving towards end-stage renal disease (Cohen et al., 1981). Such an increased risk may still be regarded as acceptable, especially when compared to the benefits of such a therapeutic approach to serious psychiatric problems. Thus, fear of renal disease may not require the therapy to be stopped. However, a close monitoring of renal function is strongly recommended. Once-daily dosing to maintain serum lithium levels at the lower therapeutic range, i.e. 0.4-0.7 mEq/litre, is advisable. Furthermore, it is critical to avoid salt depletion, which can disturb the equilibrium of serum lithium and induce acute intoxication.

5.1.6 Urographic contrast media (UCM)

Radiographic procedures are normally safe, but a small proportion of patients subsequently experience a transient or, very rarely, a permanent decline in renal function (Cedgard et al., 1986). There are various predisposition factors such as dose, age, multiple utilization of UCM, dehydration, diabetes (Taliercio et al., 1986), multiple myeloma (Harkonen & Kjellstrand, 1981), hypertension, atherosclerosis, prior kidney or liver diseases, the co-administration of nephrotoxic drugs, and kidney transplantation. Prospective studies suggest that diabetes *per se* is not a risk factor when matched for pre-existing renal disease (Teruel et al., 1981; D'Elia et al., 1982). This highlights pre-existing renal disease as a major risk factor. In view of the fact that there are several million radiological procedures each year, the number of patients at risk of developing adverse effects from the administration of contrast media is significant. Up to 10% of cases of acute renal failure in hospitalized patients may be due to intravascular urographic contrast medium administration (Hou et al., 1983).

The cause of such renal injury is not well understood, but hyperosmolality (e.g., with meglumine diatrizoate) has been claimed to be an important factor in renal damage (Forrest et al., 1981). New low-osmolar urographic contrast media (such as iopamidol) are being introduced, some of which are isotonic with plasma, but a progressive increase in the incidence of ARF from 0-12% up to 100% in high-risk patients has been reported (Eisenberg et al., 1980). About 65% of ARF follow intravenous urography, and 30% follow arteriography. The rest are associated with computerized tomography (Hanaway & Black, 1977; Harkonen & Kjellstrand, 1979; Fang et al., 1980). The reported increase in UCM-induced ARF could be due to better monitoring and awareness, higher health standards, or a prolonged survival of patients with critical illnesses (who would then be more prone to multiple X-ray contrast media examinations), or could represent other types of nephrotoxicity.

The pathophysiology of UCM-induced ARF is unclear, but may involve renal ischaemia and haemodynamic effects on glomerular function and/or intrarenal flow distribution.

Several hormonal systems may be activated prior to and/or during ARF (Caldicott et al., 1970; Chou et al., 1974; Katzberg et al., 1977). Thus, the effects of contrast media on kidney function continue to be conflicting and represent both glomerular and tubular dysfunctions (Milman & Gottlieb, 1977; Rahimi et al., 1981; Teruel et al., 1981; Khoury et al., 1983). It has been suggested, but not confirmed, that non-ionic low-osmolal contrast media have reduced nephrotoxicity (Gale et al., 1984; Spataro, 1984; Smith et al., 1985; Cedgard et al., 1986; Cavaliere et al., 1987).

Attempts to induce radiocontrast nephrotoxicity in animals have led to inconclusive or contradictory results. Intact hydrated rats with or without experimentally induced acute renal failure do not develop radiocontrast nephrotoxicity (McIntosh, et al., 1975; Moreau et al., 1980). Transient reductions in glomerular filtration rate and renal blood flow have been reported immediately following radiocontrast injection in rats and dogs (Norby & DiBona, 1975; Cunningham et al., 1986; Katzberg et al., 1986), but rarely has acute renal failure been studied or documented in the intact animal following these acute measurements. Nephrotoxicity may occur, however, when the radiocontrast agent is given in association with experimental manoeuvres designed to reduce renal function. These include repeated dehydration with furosemide injections, renal ischaemia (Schultz et al., 1982), and acute renal failure induced by mercuric chloride or glycerol (McLachlan et al., 1972).

5.1.7 *Anticancer drugs*

5.1.7.1 *Cisplatin*

Cisplatin (*cis*-diamminedichloroplatinum II) has become the chemotherapeutic agent of choice in the treatment of several solid tumours, particularly testicular and ovarian cancers (Einhorn & Donohue, 1977). Unfortunately cisplatin is also one of the most toxic anticancer drugs, its dose-limiting toxicity being nephrotoxicity (Madias & Harrington, 1978; Goldstein & Mayor, 1983; Safirstein et al., 1986). Despite the use of optimal methods for administering cisplatin, such as the use of active hydration

(Cvitkovic et al., 1977) or sodium chloride as the vehicle (Ozols et al., 1984), approximately 30% of patients will manifest nephrotoxicity.

Early clinical trials of cisplatin in cancer patients showed a striking incidence of persistent azotaemia and acute renal failure (Rossof et al., 1972; Lippman et al., 1973). In later studies serum creatinine levels increased within 6-7 days of treatment, and then apparently returned to pre-treatment levels by approximately 3 weeks (Hayes et al., 1977). Similar results were seen following the injection of cisplatin into rats (Ward & Fauvie, 1976; Chopra et al., 1982). Thus cisplatin-induced nephrotoxicity initially appeared to be an acute reversible condition. However, more recent findings suggest that cisplatin causes a permanent reduction in GFR (Dentino et al., 1978; Meijer et al., 1983; Fjeldborg et al., 1986), which may indeed be progressive in nature (Groth et al., 1986; Jaffe et al., 1987).

Hypomagnesaemia is frequently noted in patients receiving cisplatin (Buckley et al., 1984; Vogelzang et al., 1985), and is associated with inappropriately high levels of urinary excretion of magnesium. This deficiency in magnesium leads to hypokalaemia and hypocalcaemia. This selective renal loss of magnesium is not unusual and may be even more common than other renal abnormalities as an expression of cisplatin nephrotoxicity.

Light microscope studies of human kidneys have revealed focal acute tubular necrosis, affecting primarily the distal and collecting tubules, with dilatation of convoluted tubules and cast formation (Gonzalez-Vitale et al., 1977). More recently, Tanaka et al. (1986) reported sporadic degenerative lesions, necrosis, and regenerative changes in the S2 and S3 regions of the proximal tubule and also in the distal tubule and collecting duct. The glomeruli and vasculature appeared uninvolved. These observations are somewhat different to those seen in the rat, where cisplatin-induced damage is largely confined to the S3 segment of the proximal tubule, located in the outer stripe of the outer medulla (Chopra et al., 1982). With increasing time cystic tubules develop in this region (Dobyan, 1985). However, these cysts have not been reported clinically.

The activity of a number of urinary enzymes, including alanine aminopeptidase, *N*-acetyl-β-D-glucosaminidase, leucine aminopeptidase and β-glucurinidase, has been shown to be elevated as early as 36-48 h after cisplatin treatment (Kuhn et al., 1978; Jones et al., 1980). β2-microglobulin excretion has also been shown to be transiently increased after cisplatin treatment (Daugaard et al., 1988a,b). It is of interest to note that this proteinuria (involving proteins of low relative molecular mass), predominantly tubular in origin, was transient, whereas a persistent proteinuria consisting of proteins of high relative molecular mass, such as albumin and IGg, and glomerular in origin was seen after the completion of cisplatin treatment.

The pathophysiology of the GFR reduction remains ill defined. It is clear that cisplatin produces an acute, mainly proximal, tubular functional impairment within hours of administration (Daugaard et al., 1988a,b). It has been suggested that the former is a consequence of the latter. Thus, Groth et al. (1986) attributed the chronic reduction in GFR to increased intratubular pressure within damaged tubules. The glomerular proteinuria reported by Daugaard et al. (1988a) suggests that cisplatin may directly damage glomeruli. Cisplatin-induced glomerular lesions have been reported in the pig (Robbins et al., 1990).

5.1.7.2 *Adriamycin*

The anthracycline antibiotic Adriamycin is widely used in clinical oncology to treat several cancers, including breast carcinoma, malignant lymphomas, and sarcomas (Blum & Carter, 1974). Its clinical use is limited by its cardiotoxicity. Experimentally adriamycin has been shown to produce a nephrotic syndrome in rats (Young, 1975), rabbits (Fajardo et al., 1980), and pigs (van Fleet et al., 1979).

Rats treated with a single dose of Adriamycin exhibited a marked proteinuria evident within several days of treatment (Bertani et al., 1982). Maximal levels were seen after approximately 2 weeks, after which levels declined but remained significantly above control levels 10 weeks after treatment. Serum albumin levels were also

significantly reduced, whereas there was a concomitant hyperlipidaemia (Bertani et al., 1986). Adriamycin-induced changes in renal functional parameters are less well defined. Litterst & Weiss (1987) reported that BUN and serum creatinine values were either unaffected or only minimally increased. However, more recent studies indicate significant and progressive reductions in GFR (Hall et al., 1986). Single nephron glomerular filtration rate is reduced due to a decreased ultrafiltration coefficient (Michels et al., 1983).

Morphological damage is first seen in the glomerulus; ultrastructural examination reveals extensive damage to the glomerular epithelial cells occurring within 36-48 h of injection (Bertani et al., 1982). This leads to the eventual loss of the foot processes. Light microscope studies reveal the characteristic presence of vacuoles in the glomeruli; with time progressive glomerulosclerosis is seen. Associated with these glomerular changes are tubular changes; these consist of dilated tubules filled with casts, predominantly in the outer stripe of the outer medulla, and atrophic tubules associated with areas of interstitial fibrosis. It appears that Adriamycin primarily damages the glomeruli and that the tubulo-interstitial damage results from the proteinuria, which induces cast formation and interstitial inflammatory reaction. Fajardo et al. (1980) reported that juxtamedullary glomeruli were more sensitive than cortical glomeruli; micropuncture studies in the Munich Wistar Fromter rat confirm this observation (Soose et al., 1988).

Renal toxicity in patients appears rare, although there has been a report of acute renal failure following Adriamycin-treatment (Burke et al., 1977). This may reflect species differences in sensitivity or may reflect the use of inappropriate test protocols for detecting renal damage.

5.1.8 *Immunosuppressive agents*

5.1.8.1 *Cyclosporin A*

Cyclosporin A has been widely used for preventing organ rejection after transplantation and in auto-immune diseases, but it is highly nephrotoxic in the clinical situation (Kostakis et al., 1977; Calne et al., 1978;

Powles et al., 1978). Clinically, cyclosporin nephrotoxicity has been reported as an acute reversible renal dysfunction, an acute vasculopathy (thrombotic microangiopathy), and/or a chronic nephropathy with interstitial fibrosis (Mihatsch et al., 1985; Palestine et al., 1986). After cyclosporin A treatment there is an inverse linear relation between GFR and the severity of the lesions. During the first 6 months of treatment, renal fibrosis in patients given high doses of cyclosporin A shows a dose-dependent progression of increased severity (Klintmalm et al., 1981). The rat model of cyclosporin A nephrotoxicity may adequately represent the acute condition in man (Mihatsch et al., 1985, 1986) but does not represent that seen in the clinical situation. Although rats dosed with cyclosporin also develop renal surface changes that correspond to focal areas of collapsed proximal tubular regions with subcapsular fibrosis, degenerating tubular epithelium and thickening of the basement membrane, the chronic striped fibrosis and arteriolar lesions have not been reproduced experimentally.

Some animal data have shown increased blood urea nitrogen and creatinine, brush-border and lysosomal enzyme leakage, and vacuolation, necrosis, and regeneration of P3 cells in rats (Dieperink et al., 1983, 1985; Ryffel et al., 1983, 1986; Murray et al., 1985; Dieperink, 1989). However, most studies find no necrosis or enzymuria despite profound reductions in GFR. Functional changes in animals and man given cyclosporin A are similar. They represent haemodynamic changes such as an increased renal vascular resistance (Murray et al., 1985), proximal fractional reabsorption (Dieperink et al., 1983, 1985; Dieperink, 1989), and renal blood flow, plasma flow, and GFR decrease. The reduction in renal perfusion and filtration has been prevented experimentally by vasoactive α-adrenergic antagonists and renal denervation (Murray et al., 1985), but this has not been established in humans. Cyclosporin A appears to have a direct preglomerular vasoconstriction effect, decreasing the ultrafiltration pressure and increasing proximal fractional reabsorption. Presumably, tubular flow rates and end proximal tubular delivery decrease, and, due to varying tubular hypoperfusion, there is a focal tubular collapse, and degeneration and peritubular interstitial fibrosis develop. The precise relationship between renal vasocon-

striction and chronic tubulo-interstitial pathology is poorly understood.

5.1.9 *Heroin*

About 1% of heroin addicts develop haematuria, proteinuria, or the nephrotic syndrome (Cunningham et al., 1980). Morphologically, the renal lesion has been described as focal sclerosing glomerulonephritis. In the absence of proliferative lesions or immune deposits, a direct toxic effect of heroin or even a contaminant or solvent employed in the administration of heroin has been suggested as the major pathogenetic mechanism. However, heroin-related increases in IgM titres have been regarded as evidence that an immunological mechanism may play some role in this disorder.

5.1.10 *Puromycin aminonucleoside*

Direct toxicity of the puromycin aminonucleoside to glomerular components may also occur and may be a factor in renal failure. Experimental studies of the effects of puromycin have provided considerable basic information regarding the pathogenesis of direct chemical injury to glomerular structures. The effects are limited to rats and monkeys. Epithelial cells become swollen, and there is an increase in lysosome and pinocytotic activity, fusion and loss of foot processes, and a reduction in the number of filtration slits. As the lesion progresses, epithelial cells become detached, leaving "naked" basement membrane in direct contact with Bowman's capsule, which may account for the severe proteinuria (Caulfield et al., 1976). It is suggested that as the lesion progresses there is an increase in basement membrane synthesis, mesangial cell proliferation and fusion, and crescent formation leading to the light microscope appearance of focal glomerular sclerosis (Gartner, 1980). The glomerular lesions produced by puromycin do not appear to invoke an immunological response, so that the resulting alterations are entirely related to the direct toxicity of the drug.

5.2 Chemicals

The diversity of organic molecules is such that there are chemicals that are now known to adversely affect each

part of the kidney. This section will examine those chemicals that do not fit into any conventional section on therapeutically used agents.

5.2.1 Ethylene glycol

Ethylene glycol, a constituent of antifreeze, is occasionally ingested and causes severe acute toxicity to the brain and kidney. Acute tubular necrosis is followed by renal failure. Exposure often leads to permanent renal damage. A morphological feature of mild ethylene glycol toxicity is cytoplasmic vacuolation, which may suggest hypokalaemic nephropathy or osmotic nephrosis due to mannitol. Most ethylene glycol is excreted unmetabolized, but a small percentage is metabolized to oxalic acid. This is accompanied by deposition of calcium oxalate crystals in the kidney, which may contribute to a persistent inflammatory reaction and interstitial fibrosis. Excessive urinary excretion of oxalate and crystal formation may also be seen following administration of halogen-containing anaesthetic agents, particularly methoxyflurane and halothane (Roxe, 1980). Acute ethylene glycol toxicity is treated with ethanol, which competes as a substrate for alcohol dehydrogenase (Peterson et al., 1981).

5.2.2 Organic chemicals and solvents

5.2.2.1 Volatile hydrocarbons

Volatile hydrocarbons, particularly chlorinated compounds such as carbon tetrachloride and trichloroethylene, may produce glomerular lesions leading to nephrotic syndrome and renal failure. The relationship of volatile hydrocarbon exposure to the development of glomerulonephritis in populations is not clear. It has been found that among patients with glomerulonephritis there are more with a history of exposure to hydrocarbon solvents than would be expected. Attempts to reproduce in rats the glomerular lesions observed in patients have only been partially successful. Solvent-exposed rats had increased proteinuria and glomerular sclerosis, but proliferative lesions and significant immune deposits were not observed (Zimmerman & Norbach, 1980). Of 15 patients studied in Sweden with post-streptococcal glomerulonephritis, 6 had a

history of brief exposure to organic solvents before the development of their disease. This suggested to these investigators that solvent exposure may influence the outcome of an infection with streptococci. Prior exposure to hydrocarbon-containing solvents has been identified in a number of patients with Goodpasture's syndrome (Gartner, 1980). Apart from the pulmonary manifestation of cough, shortness of breath, and haemoptysis, there may be haematuria and proteinuria. Renal morphology consists of a proliferative glomerulonephritis with IgG and C3 in the glomerular basement membrane.

Studies conducted in Sweden (Askergren 1981; Askergren et al., 1981) and in Belgium (Viau et al., 1987) have reported a slight increase in the urinary excretion of albumin in groups of workers exposed to industrial solvents, particularly styrene. This effect probably reflects an enhanced glomerular permeability since the urinary output of markers of proximal tubular function (β2-microglobulin, retinol-binding protein) was not affected. In Italy, Franchini et al. (1983) also reported slight renal disturbances in workers occupationally exposed to solvents. These effects consisted of enhanced urinary excretion of total proteins, lysozyme, and β-glucuronidase and pointed to a tubular lesion, since they were not accompanied by a rise in albuminuria. It is at present impossible to relate nephrotoxic effects reported in these studies to exposure to one solvent or one class of solvents, although styrene has been incriminated by Askergren et al. (1981). One must also recognize that the renal effects reported in these studies are mild and do not appear to correlate with indices of exposure to solvents.

An auto-immune mechanism following chronic exposure is probably responsible for the glomerular lesions. In patients who have Goodpasture's syndrome, the primary site of damage may be the alveolar basement membrane of the lung, which is damaged by inhalation of the solvents, and antibodies to altered alveolar basement membrane may cross-react with glomerular basement membrane. Alternatively, auto-immunity may follow direct toxic injury to renal tubular or glomerular structures. Acute exposure to these solvents does produce acute tubular necrosis, and it is likely that prolonged exposure to low levels that do not result in cell necrosis produces cell injury suf-

ficient to damage renal cell membranes and provide the antigen for the immune reaction (Gartner, 1980).

5.2.2.2 Chloroform

In vitro exposure to chloroform has been shown to produce toxicity in kidney slices from male but not from female mice (Smith & Hook, 1984). Furthermore, ^{14}C-labelled chloroform was metabolized to $^{14}CO_2$, and the radioactivity was covalently bound by cortical microsomes from male but not female mice. The *in vitro* metabolism of chloroform by male, but not female, renal slices is consistent with reduced susceptibility of female mice to *in vivo* chloroform nephrotoxicity (Smith et al., 1983; Smith et al., 1984). Metabolism is dependent on oxygen, a NADPH-regenerating system, incubation time, microsomal protein concentration, and substrate concentration, and is inhibited by carbon monoxide (Smith & Hook, 1984). The negligible degree of chloroform metabolism and toxicity in female mice is consistent with a lower renal cytochrome P-450 concentration and activity in female mice than in males (Smith & Hook, 1984). Pretreatment of rabbits with phenobarbital, a renal cytochrome P-450 inducer in this species, enhances the toxic response of renal cortical slices to chloroform *in vitro* (Bailie et al., 1984). The rate at which deuterated chloroform is metabolized by the liver to phosgene is approximately half that of chloroform. Deuterated chloroform is also less hepatotoxic that chloroform since the C-D bond is stronger than the C-H bond. These data suggest that cleavage of the C-H bond is the rate-limiting step in the activation of chloroform. Deuterated chloroform is also less toxic to the kidney than chloroform (Ahmadizadeh et al., 1981; Branchflower et al., 1984). This deuterium isotope effect on chloroform-induced nephrotoxicity suggests that the kidney metabolizes chloroform in the same manner as the liver, e.g., by oxidation to phosgene. Indeed, rabbit renal cortical microsomes incubated in media supplemented with L-cysteine metabolize ^{14}C-labelled chloroform to radioactive phosgene-cysteine 2-oxothiazolidine-4-carboxylic acid (Bailie et al., 1984). These *in vitro* data collectively support the hypothesis that mouse and rabbit kidneys biotransform chloroform to a metabolite (phosgene) that mediates nephrotoxicity.

5.2.2.3 Halogenated alkenes

The nephrotoxin *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) is formed by trichloroethylene extraction of proteinaceous substances and was first identified in extracted animal food (McKinney et al., 1957, 1959). It has been widely used as a model compound in nephrotoxicity studies. DCVC is accumulated in the proximal tubules by an active carrier system for organic anions (Elfarra et al., 1986a,b). It is then activated by cysteine conjugate β-lyase to a reactive thiol (Bhattacharya & Schultze, 1967) and causes tubular damage (Terracini & Parker, 1965). DCVC is a potent specific nephrotoxin, which produces proximal tubular damage *in vivo* and *in vitro* (Elfarra et al., 1986a,b; Lash & Anders, 1986; Lash et al., 1986). *In vivo* DCVC causes its primary lesion in the straight segment (S-3) of the proximal tubule, and the molecule is also cytotoxic both for primary cultures of proximal tubular cells and for cell lines derived from this region of the nephron. There is a close correlation between the *in vivo* and *in vitro* effects of this compound with regards to its metabolism and effects on cells (Hassall et al., 1983).

Cysteine conjugates such as DCVC are metabolized by cysteine conjugate β-lyase to their ultimate toxic species i.e. pyruvate, ammonia, and a reactive thiol (Anderson & Schultze, 1965). This reaction plays a role in the nephrotoxicity of DCVC (Lash et al., 1986). β-lyase has been found to predominate in cytosolic and mitochondrial fractions (Lash et al., 1986) and has a requirement for pyridoxal phosphate. The enzyme activity can be inhibited by pyridoxal phosphate inhibitors such as amino-oxyacetic acid and propargylglycine (Elfarra et al., 1986a). In addition to monitoring enzyme activity, renal cortical slices can be utilized to assess the regulation of enzyme activity and the resultant effects on toxicity.

There is a greater sensitivity to DCVC-induced kidney damages in the adult mouse than there is in the newborn. Similar findings have been reported using cephaloridine, where the newborn animal is more resistant to nephrotoxicity than the adult rabbit (Tune, 1975). These finding for DCVC differ from those for hexachlorobutadiene (Kuo & Hook, 1983; Lock et al., 1984), where nephrotoxicity is greater in the young rat and mouse than in the adults.

Chlorotrifluoroethylene is a potent nephrotoxin (Potter et al., 1981) and is metabolized by hepatic cytosolic and microsomal glutathione *S*-transferases to *S*-(2-chloro-1,1,2-trifluoroethyl)glutathione (Dohn et al., 1985a), which is nephrotoxic in rats and cytotoxic in isolated rat kidney proximal tubular cells (Dohn et al., 1985b). The corresponding cysteine *S*-conjugate, *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine (CTFC) is also nephrotoxic in rats and cytotoxic in isolated kidney cells, and its bioactivation is dependent on metabolism by renal β-lyase (Dohn et al., 1985b). Pyruvate and hydrogen sulfide have been identified as metabolites of CTFC (Banki et al., 1986; Lash et al., 1986).

5.2.2.4 *Hydrocarbon-induced nephrotoxicity*

Inhalation of unleaded gasoline for 2 years produced renal tumours (adenomas and adenocarcinomas) in male Fischer-344 rats but not in female rats or mice of either sex (Kitchen, 1984; MacFarland, 1984; Mehlmann et al., 1984). Subchronic inhalation exposure increased protein (hyaline) droplets in proximal convoluted tubules (Halder et al., 1984), accumulated casts at the cortico-medullary junction and single cell necrosis and regeneration of the nephron (Short et al., 1986) in male rats. When different fractions of unleaded gasoline were screened for their specific effect on the male rat kidney, it was found that the branched-chain saturated hydrocarbon components (used as anti-knocking agents) caused hyaline droplet formation. A number of chemicals, such as 2,2,4-trimethyl-pentane (Phillips & Egan, 1984a,b; Halder et al., 1985; Viau et al., 1986a), decalin (Alden et al., 1984), 1,4-dichlorobenzene (NTP, 1987), and *p*-dichlorobenzene (NTP, 1986; Bomhard et al., 1988), have now been shown to cause such hyaline droplets in male rats. Although these chemicals cause minimal renal functional impairment, a protein droplet nephrosis develops, progressing to mild tubular degeneration, necrosis, and regeneration after several weeks of treatment (Phillips & Cockrell, 1984a,b).

Male mice excrete a sex-related protein, which results in a urinary protein level 2.5 to 3 times that of female mice. However, the male mouse sex-associated urinary protein hydrolyses readily and thus does not accumulate in

the proximal tubule (Alden et al., 1984; Alden, 1989). In humans, it has recently been reported that protein 1 (an α2-microglobulin of about 20 000 Daltons) has a sex-linked behaviour just like the androgen-dependent α2u-globulin. Protein 1 is excreted in greater amounts in the urine of males after puberty. In the age group 15 to 20 years, its concentration in the urine of males is on average fifty times higher than that in the urine of females (Bernard et al., 1989). The relevance of this observation in humans is unknown.

The basis for the marked sex dependence and species difference in the development of hyaline droplet deposition in male rats relates to the fact that they excrete the sex-hormone-related and therefore male-specific protein α2u-globulin (Stonard et al., 1986; Loury et al., 1987; Olson et al., 1987). This is also species specific to the rat and has not been reported in any other commonly used animals or man. α2u-Globulin is synthesized by the male rat liver and is an important constituent of the physiological proteinuria in adult male rats. At maturity the total urinary protein is 20-30% α2u-globulin and 10% albumin. At 160 days of age the excretion of albumin and total urinary proteins is markedly increased. By one year, albumin represents nearly 60% of the total protein while α2u-globulin is less than 10%. This reversal in relative content may be the consequence of a progressive glomerulonephrosis, associated with an apparent spontaneous accumulation of hyaline droplets. The nephrotic condition may be the consequence of the burden of excreting α2u-globulin. Its early onset is sex dependent; female rats do not exhibit the proteinuria until a later age. In both aging and the response to hydrocarbons, the common pathological factor may be the accumulation of α2u-globulin, via a susceptible pathway not shared with other proteins (Neuhaus, 1986; Stonard et al., 1986).

The chronic regenerative response subsequent to moderate proximal tubular damage in the kidney of male rats exposed to petroleum hydrocarbons may be an important stimulus in renal tumour formation caused by this group of chemicals (Short et al., 1986). Loury et al. (1987) have shown a 5- to 8-fold increase in S phase of renal cells induced by unleaded gasoline, and the renal proliferative effects of 2,2,4-trimethylpentane are localized to the

S_2 segment of the proximal tubule of the male rat (Short et al., 1986).

Administration of 2,2,4-trimethylpentane to male rats produces a dose-related increase in the concentration of 2,2,4-trimethylpentane-derived radiolabel in the kidney, which appears to parallel the dose-related accumulation of 2u-globulin (Stonard et al., 1986; Charbonneau et al., 1987). The reversible binding of a metabolite of 2,2,4-trimethylpentane to α2u-globulin in the male rat kidney (Lock et al., 1987) is thought to alter endocytosis or lysosomal handling of the α2u-globulin-2,2,4-trimethylpentane metabolite complex. This may increase cell turnover of the S_2 cells via lysosomal enlargement and/or instability, leading to cell death. It appears that lysosomal catabolism of the 2,4,4-trimethyl-2-pentanol-α2u-globulin complex compound to α2u-globulin causes lysosomal protein overload, resulting in cell necrosis (Swenberg et al., 1989).

There are other sex-related differences in the handling of 2,2,4-trimethylpentane by rats. Female rats rapidly metabolize 2,2,4-trimethylpentane and excrete it in urine, while in male rats the compound is eliminated more slowly and is retained in the kidneys (Kloss et al., 1985). Recent studies have also shown that 2,2,4-trimethylpentane is metabolized in male and female rats to trimethyl-pentanols, pentanoic acids, and hydroxypentanoic acids (Olson et al., 1986; Charbonneau et al., 1987).

5.2.2.5 *Bipyridyl herbicides*

Paraquat is a potent bipyridyl herbicide that has multiple organ effects. The kidney is frequently involved in serious cases of paraquat poisoning (WHO, 1984). The compound is actively secreted by the organic cation transport in the proximal tubule. Renal histological examinations in a variety of animals exposed to paraquat show vacuolation of the proximal convoluted tubules and proximal tubular cell necroses (Lock, 1979; Lock & Ishmael, 1979). Acute oliguric renal failure is common in severely poisoned patients. Less severe manifestations include impaired glomerular filtration, which often recovers after several days and before the paraquat induces severe pulmonary fibrosis. Other renal functional abnormalities

include proteinuria and haematuria. Tubular damage may be shown by the presence of glucosuria or all of the features of the Fanconi syndrome. The severity of the acute renal failure is a major determinant of the outcome of the poisoning (WHO, 1984).

The biochemical mechanism of nephrotoxicity has not been fully elucidated, but it is assumed to be identical to that seen in other tissues. Paraquat undergoes redox cycling in the presence of NADPH and oxygen with the generation of superoxide and subsequent development of lipid peroxidation and membrane damage. The development of hydroxyl radicals results in oxidative damage to nucleic acids, proteins, and polysaccharides (Autor, 1977).

Diquat is another bipyridyl herbicide that produces multiple organ toxicity. It undergoes active tubular secretion by the organic cation system in the proximal tubule (Lock, 1979; Lock & Ishmael, 1979). The histological lesion produced by diquat is necrosis of the proximal tubular cells and some distal tubular cells (Lock & Ishmael, 1979). Human cases of diquat poisoning result in acute renal failure. The mechanism of toxicity of diquat appears to be identical to that of paraquat (Autor, 1977; WHO, 1984).

5.3 Mycotoxins

A high frequency of endemic chronic nephropathy has been recognized in localized areas of Bulgaria, Rumania, and Yugoslavia since the 1920s. The affected people live in villages in valleys near the Danube (Hall & Dammin, 1978; WHO, 1979; Hall, 1982). The condition, known as Balkan endemic nephropathy (Fig. 14), is an interesting case study of an environmentally related chronic renal disease. The etiology is unknown at present. Mycotoxins, particularly ochratoxin A, have been implicated because of similarities with disease in animals and identification of the mycotoxin in food (Krogh et al., 1977; Pepeljnjak & Cvetnic, 1985; Petkova-Bocharova & Castegnaro, 1985) and in human tissues (Hult & Fuchs, 1986) where nephrotoxicity is most frequent. Silicates have been suggested because of the proximity of villages with affected families to streams and rivers containing silicon.

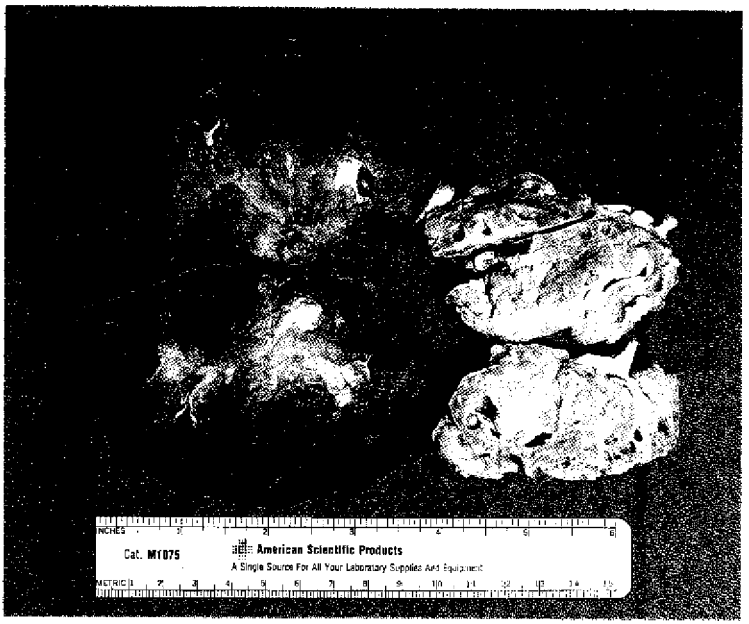


Fig. 14a. The cut surfaces of a normal kidney (left) and a BEN-compromised kidney (right). A marked reduction in size, a disappearance of normal kidney structures, and a pale greyish colour of the diseased kidney are evident (both specimens were fixed with formalin).

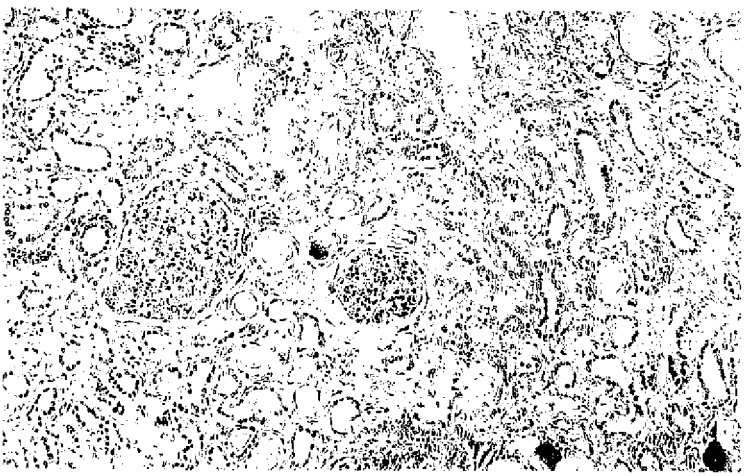


Fig. 14b. Photomicrograph of kidney from a BEN patient. Increased amount of interstitial, acellular tissue separating tubuli is visible. Some tubuli contain pink proteinaceous material within the lumen. The glomeruli are unremarkable. (H & E stain).

Although not clearly implicated in BEN, the fungal toxin citrinin has been suggested as a causative agent in porcine citrinin nephropathy and clearly has nephrotoxic effects in a number of species (Berndt & Hayes, 1977; Phillips et al., 1979; Phillips et al., 1980a,b; Lockard et al., 1980). Citrinin produces acute tubular necrosis primarily of the S_1 section of the proximal tubule. It is eliminated rapidly by the kidney and only metabolized to the extent of 10-15%, which suggests that effects are due to the parent compound. Little information is available concerning the cascade leading from the initial insult to the production of acute tubular necrosis 2-4 days after its administration. Citrinin has been shown to exert a synergistic effect on ochratoxin A toxicity in animal models. This is important because the same fungal species that synthesize ochratoxin A also produce citrinin. This was clearly demonstrated by the presence of citrinin in 19 out of 21 food samples contaminated with ochratoxin A. Both genetic and environmental factors, such as exposure to ochratoxin A, appear to be involved in BEN and the associated renal tract tumours (Castegnaro & Chernozemsky, 1987). In one endemic area in Bulgaria the relative risk of patients with BEN developing urinary tract tumours is 90-fold greater than in people from non-endemic areas (Castegnaro & Chernozemsky, 1987). Inhabitants of the 15 villages in the Vratza region of northern Bulgaria have a 30-40% mortality rate from chronic nephropathy, while urinary tract tumours comprise 25-30% of all neoplasms in males and females in these geographic areas (Markovic, 1972). In recent studies ochratoxin A has been found to induce renal adenomas and carcinomas both in mice (Kanisawa & Suzuki, 1978; Bendele et al., 1985) and in rats (NTP, 1988). Frequent metastases, mainly to the lung, were found in the rat study. The target of ochratoxin nephrotoxicity has been reported to be the S_2 and S_3 nephron segments (Jung et al., 1989).

Thus the animal and human data indicate that ochratoxin A is a risk factor for toxic nephropathies and in the etiology of human nephropathy and associated renal tumours.

5.4 Silicon

An association between occupational exposure to free silica (SiO_2) and chronic nephropathy has been suspected

for several years, but the number of reported cases is few. Clinically, lung fibrosis is the primary problem, but in an early study from Italy chronic renal failure was found in 40% and proteinuria in 20% of 20 patients with chronic silicosis. The renal silicon content of patients with proteinuria and chronic silicon exposure has been shown to be much higher than the normal level, and there appears to be a direct relationship between level of exposure and probability of renal disease. Animal studies have demonstrated that silicon is excreted by glomerular filtration, and a morphological study of experimental animals and human biopsy material has demonstrated silicon deposits in subepithelial and subendothelial areas of the basement membrane and in epithelial cells. Human biopsy materials show a mild focal or segmental proliferative glomerulonephritis and the absence of significant immune-complex deposits. These findings suggest a direct toxic effect on the glomerulus. These cases also have varying degrees of tubular cell degeneration. Animal studies demonstrate a dose-related nephropathy that is primarily tubular, with an interstitial inflammatory reaction and fibrosis. The proliferative glomerular lesions observed in humans are not seen in animals, but this difference in response may be related to dose or species (Hauglustaine et al., 1980).

5.5 Metals

Metals constitute some of the earliest recognized and the best investigated nephrotoxins. X-ray fluorescence gives a clear indication of the metal burden that an individual carries.

5.5.1 *Lead*

Lead has been a very common cause of acute or chronic renal failure in the past. Acute tubular necrosis has been described following accidental or intentional absorption of high doses of lead. Cases of chronic renal failure have been reported in adults who ingested large amounts of leaded paint during childhood (Queensland, Australia), in people who consumed alcohol illicitly distilled in lead-containing stills, and in workers with a long history of occupational lead exposure (Emmerson, 1973; Bennett, 1985).

Several epidemiological studies have consistently reported that workers with a heavy industrial exposure to lead experience an increased risk of death through chronic renal failure (Cooper & Gaffey, 1975; Malcolm & Barnett, 1982; McMichael & Johnson, 1982; Davies 1984; Selevan et al., 1985; Cooper et al., 1985). There is also some evidence that occult lead poisoning may contribute to renal insufficiency in patients with gout and essential hypertension (Batuman et al., 1981, 1983; Colleoni & D'Amico, 1986).

In adults, lead nephropathy occurs as an insidious progressive disease characterized by the absence of proteinuria, albuminuria, and urinary concentration deficit in its early phases (Wedeen et al., 1979). This renal disease can be diagnosed only by functional tests (e.g., estimation of GFR on the basis of blood urea nitrogen or creatinine clearance). Several cross-sectional studies have attempted to detect early renal effects in workers exposed to lead (Hammond et al., 1980; Buchet et al., 1980; Verschoor et al., 1987). These studies confirm that lead nephropathy in adults, even at an advanced stage (i.e. with decreased GFR), cannot be detected by the determination of urinary proteins of low or high relative molecular mass (e.g., β2-microglobulin, albumin). The only marker that seems to respond at an early stage of lead nephropathy is the urinary excretion of the lysosomal enzyme, *N*-acetyl-β-D-glucosaminidase (NAG) (Verschoor et al., 1987). However, the underlying mechanism of this renal effect remains to be elucidated. Increased urinary leakage of NAG might result from cell damage and exfoliation, but also from a stimulation by lead of exocytosis or of the renal activity of the enzyme.

The renal effects of lead are primarily tubular or tubulo-interstitial and they may be both acute and chronic. However, the acute effects of lead differ from those of most of the other metals in that cell injury is for the most part reversible and necrosis is uncommon. Cells of the proximal tubule are most severely affected, and this effect is characterized by a reduction in resorptive function leading to a generalized amino-aciduria, glycosuria, and hyperphosphaturia. These components of the Fanconi syndrome have been observed in children with acute lead toxicity and who also have overt symptoms of central

nervous system toxicity, and in rats exposed to lead. Proximal tubular dysfunction has been more difficult to demonstrate in workers with chronic lead nephropathy (Goyer & Rhyne, 1973).

The effects of lead on renal tubular cells and sodium reabsorption are less clear. Increase in plasma renin and aldosterone while a low-sodium diet is consumed has been observed in a group of men with a history of "moonshine" ingestion and occult lead toxicity (Sandstead et al., 1970). In contrast, studies on the effects of minimally toxic levels of lead exposure in rats showed a reduction in plasma renin activity in spite of a significant increase in blood pressure (Victery et al., 1982). These differences may reflect a difference in time-dose relationship.

The renal effects of lead may also be influenced by interactions with calcium. Decreasing dietary calcium increases lead retention, possibly because of a decrease in lead excretion. Increased blood lead in children is associated with decreased 1,2,5-dihydroxyvitamin D (synthesized in the kidney) and may reflect impaired synthesis (Mahaffey, 1980).

The renal proximal tubular cells of people and experimental animals with lead poisoning are characterized morphologically by the presence of intranuclear inclusion bodies. In conventional paraffin-embedded haematoxylin and eosin-stained sections of renal tissue, the inclusions appear as dense, homogeneous, and eosinophilic bodies, and at the electon microscope level they have a characteristic fibrillary margin around a dense central core. Morphologically they are always separate and distinct from the nucleoli and several may be found in the same nucleus (Fig. 15). The inclusion bodies contain a protein-lead complex, and they may be isolated by differential centrifugation. The protein is a non-histone protein rich in glutamic and aspartic acids and glycine, and may be a mixture of acidic proteins with similar physicochemical properties (Moore et al., 1973). The origin and nature of the protein has not yet been determined, but recent studies of formation of inclusion bodies in renal cell cultures suggest that they form initially in the cytoplasm and then migrate into the nucleus (McLachlan et al.,

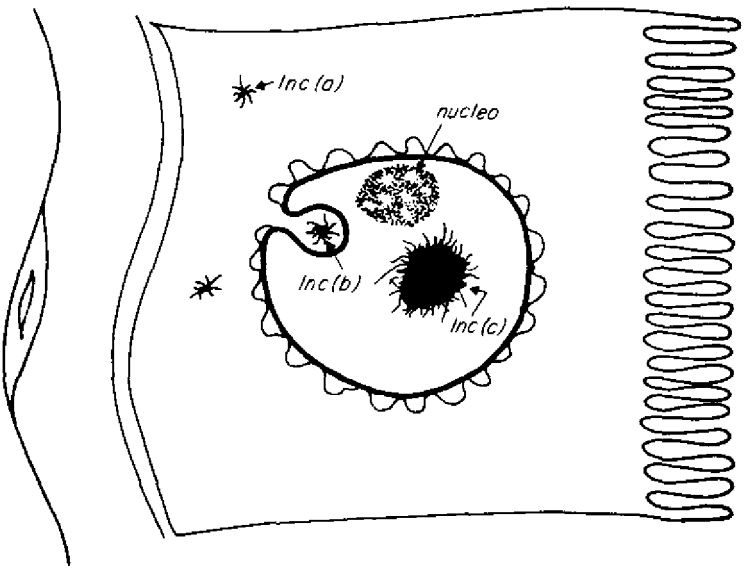


Fig. 15. Scheme for cytoplasmic-nuclear migration of inclusion bodies induced by exposure to lead in renal tubular cells. *nucleo* = nucleolus, *Inc (a)* = incipient fibrillary lead-protein complex in cytoplasm, *Inc (b)* = invagination of nuclear membrane around inclusion body, *Inc (c)* = aggregate of fibrillary lead-protein complex to form nuclear inclusion body. (From: Goyer, 1982).

1980). The major fraction of lead in the kidney during the acute phase of lead toxicity is bound in the inclusion bodies. For this reason, the inclusion bodies have been interpreted as serving as an intracellular depot for lead. Nevertheless, proximal renal tubular cells during the acute phase of lead toxicity are usually swollen, and the mitochondria show a decrease in matrical granules and altered cristae. Functional studies of mitochondria show reduced respiration and oxidative phosphorylation. Lysosomes do not seem to have a role in sequestering intracellular lead.

Chelation therapy following lead toxicity produces a marked increase in lead excretion. This is accompanied by reversal of the acute morphological effects of lead on proximal renal tubular cells, loss of inclusion bodies

from nuclei, and restoration of normal renal cell morphology and function (Goyer & Wilson, 1975).

Both experimental animals and people with chronic exposure to lead may develop a progressive interstitial nephropathy. In laboratory animals, progression from acute tubular to chronic tubulo-interstitial disease may be followed as a continuum. An increase in chronic interstitial renal disease has been reported in workers with long histories of occupational exposure, but the nonspecific nature of the morphological changes makes it difficult to identify lead as the etiological agent except by association. There is a progressive increase in fibrosis, beginning in peritubular areas extending into the interstitium (Cramer et al., 1974). Inflammatory cells are uncommon and are probably not a primary component of the process. There is eventual tubule atrophy and hyperplasia of surviving tubules. There is little evidence that the glomerulus is directly affected by excessive exposure to lead, except for some nonspecific swelling of mesangial and epithelial cells. In the terminal stage, glomeruli become sclerotic. An immunological basis for the progression of lead-induced nephropathy, as suggested following gold and mercury exposures, might be suspected. However, there is at present no published documentation of antirenal antibodies or immune-complex formation in the pathogenesis of lead nephropathy. One study suggested that lowered glomerular filtration rate occurs in occupational exposure to lead that does not produce clinical toxicity (Wedeen et al., 1979). The pathophysiological basis for this observation has not yet been determined but may be a consequence of direct toxicity to epithelial cells of the glomerular apparatus.

Intranuclear inclusion bodies are uncommon in the late stages of lead nephropathy, although they may be seen in renal biopsy or autopsy as a manifestation of a superimposed severe acute exposure. It has been shown that inclusion bodies may be found in the urine of workers with occupational exposure to lead, but their presence or absence in urine has not been related to the severity of lead nephropathy (Schumann et al., 1980).

5.5.2 *Cadmium*

Cadmium is an occupational and environmental contaminant that has received a great deal of attention. An

important toxicological feature of cadmium is its exceptionally long biological half-life in the human organism (10-30 years). Once absorbed, cadmium is efficiently retained in the organism and accumulates throughout life. In the newborn baby, cadmium is present only at very low levels, but by the age of 50 the cadmium body burden may have reached up to 20-30 mg and, in people occupationally exposed, it may reach values as high as 200-300 mg. Furthermore, cadmium concentrates in vital organs, particularly in the kidneys. At low levels of exposure, such as those prevailing in the general environment, 30-50% of the cadmium body burden is found in the kidneys alone (Nomiyama, 1980; Bernard & Lauwerys 1986; Friberg et al., 1986).

The accumulation of cadmium in the kidney, may give rise to a progressive form of tubulo-interstitial nephritis. In contrast to the situation with many nephrotoxins, including other heavy metals such as lead and mercury, there are virtually no acute effects of inorganic cadmium salts on the kidney, except perhaps for some nonspecific effects that have been seen in animals given near-lethal doses. One of the most challenging questions regarding the metabolism of cadmium has been the role of metallothionein in cellular metabolism and its potential toxicity. Metallothionein synthesized within the kidney protects from cadmium toxicity, but intravenously injected cadmium-metallothionein is more nephrotoxic than inorganic cadmium (see section 4.5.3.).

Cadmium nephropathy was first described by Friberg (1948, 1950) who studied a group of alkaline battery workers in Sweden during the late 1940s. Since these reports, a number of epidemiological studies have shown the occurrence of nephrotoxic effects in populations exposed to cadmium at work and in the general environment. These studies have demonstrated that the most prominent feature and probably the earliest sign of cadmium nephropathy is increased proteinuria.

Studies performed in the 1950s and 1960s (reviewed by Friberg et al., 1986 and Bernard & Lauwerys, 1986) showed that cadmium proteinuria is similar to the tubular-type proteinuria described by Butler & Flynn (1958) in patients with tubular disorders, and consists of unidentified

proteins of low relative molecular mass derived from plasma. Characterization of these proteins led to the discovery of β2-microglobulin, retinol-binding protein, and α1-microglobulin. Subsequent studies demonstrated that the increased urinary excretion of proteins of low relative molecular mass observed in cadmium nephropathy and other renal disease was due to the failure of the proximal tubules to reabsorb proteins filtered through the glomeruli.

The effects of cadmium on the excretion of β2-microglobulin have been extensively documented (Bernard et al., 1976, 1979a,b, 1982, 1987). Measurement of retinol-binding protein is much more reliable in acidic urine and detects tubular proteinuria with equal sensitivity (Bernard et al., 1982).

As cadmium nephropathy progresses, it increasingly presents the signs of a complete Fanconi's syndrome, i.e. aminoaciduria, glucosuria, increased urinary excretion of calcium, phosphorus, and uric acid, and decreased concentrating ability of the kidneys. In the most severe cases, the GRF decreases. The disturbances in calcium and phosphorus metabolism may lead to a demineralization of the bones and the formation of kidney stones (Friberg et al., 1974).

In cadmium-polluted areas of Japan, signs of renal dysfunction very similar to those observed in cadmium workers have been frequently found. A higher incidence of proteinuria, glucosuria, and aminoaciduria, and increased excretion of β2-microglobulin have been observed in the Zinzu river basin in Toyama where Itai-Itai disease was first seen (Fukushima et al., 1974; Kjellström et al., 1977; Shiroishi et al., 1977; Kjellström & Nordberg, 1978). In the endemic area of Toyama, the increased urinary excretion of β2-microglobulin was strongly related to the residence time in that area as well as to the purposes for which contaminated river water was used (Kjellström et al., 1977). In urine, β2-microglobulin concentration correlated with the cadmium level (Nogawa et al., 1979a,b).

Further investigations of the renal function of the inhabitants in this area revealed a significant decrease in both creatinine clearance and renal phosphorus reab-

sorption. Renal dysfunction due to chronic cadmium poisoning was also found in other areas of Japan where the rice was contaminated by cadmium (Saito et al., 1977; Kojima et al., 1977). Studies carried out in Belgium suggested that environmental exposure to cadmium in an industrialized area polluted by this metal may exacerbate the age-related decline of renal function in elderly residents (Lauwerys et al., 1980; Roels et al., 1981a,b). Since cadmium-induced nephropathy may occur within the general population, it is of major public health importance to know what level of cadmium exposure carries a risk of renal tubular dysfunction and cadmium nephropathy.

The concept of a critical concentration of cadmium has very important implications with regard to establishing maximum levels of cadmium that human populations may be exposed to with some margin of safety. From a comparison of the cadmium concentrations in the renal cortex of cadmium-exposed people with and without signs of kidney damage, Friberg et al. (1974) suggested that the critical level of cadmium in the renal cortex for the appearance of tubular proteinuria is around 200 mg/kg. With the development of neutron activation techniques allowing the *in vivo* determination of cadmium in tissues, the critical level of cadmium in the human kidney has been more precisely assessed.

Investigations conducted by Roels et al. (1981a) in Belgium and Ellis et al. (1981) in the USA have shown that when the concentration of cadmium in the kidney cortex reaches about 200 mg/kg, signs of renal dysfunction (e.g., increased urinary excretion of albumin and β2-microglobulin) develop in about 10% of male workers exposed to this metal. On the basis of the relationship between the concentrations of cadmium in the urine and renal cortex and the prevalence of renal anomalies, the critical concentration of cadmium in urine has been estimated to be 10 μg/g creatinine (Bernard et al., 1979a,b; Buchet et al., 1980; Roels et al., 1981a,b). Epidemiological studies of people living in cadmium-polluted areas of Japan have shown that β2-microglobulinuria occurs after a lifetime accumulation of 2000 mg cadmium or more (Nogawa et al., 1989).

Since several studies have shown that, in most cases, once cadmium proteinuria has developed, it is irrevers-

ible, the progression of renal dysfunction after cessation of exposure is very slow (Roels et al., 1982; Elinder et al., 1985a,b). Persistent proteinuria is found frequently among retired cadmium workers with no evidence of renal insufficiency. In a group of workers removed from exposure after the finding of microproteinuria (low or high relative molecular mass), the reduction in GFR during a 5-year follow-up was about five times greater than that accounted for by aging (Roels et al., 1989).

5.5.3 *Mercury*

It has been known for a long time that patients treated with mercurial compounds can develop a glomerulonephritis that is usually of the immune complex type (Becker et al., 1962; Druet et al., 1982). Cases of mercury glomerulonephritis have also been reported as a result of chronic exposure to high levels of mercury in industry (Tubbs et al., 1982). Patients with mercurial nephropathy usually present with a proteinuria and occasionally a nephrotic syndrome, but no renal insufficiency (Druet et al., 1982).

Mercury may produce different effects on the kidney depending on the biochemical form of the metal and nature of exposure. Inorganic mercury compounds are classic examples of agents that cause acute tubular necrosis. Mercuric chloride was used as a suicidal agent during the nineteenth and early part of the twentieth centuries but was unpopular for this purpose because of the painful accompanying corrosive injuries it produced.

Regardless of the route of administration, mercuric chloride produces acute tubular necrosis within hours of administration, resulting in anuria and death. If the patient can be maintained by dialysis, regeneration of tubular lining cells is possible. These may be followed by ultrastructural changes consistent with irreversible cell injury, including actual disruption of mitochondria, release of lysosomal enzymes, and rupture of cell membranes.

The necrosis of the epithelium of the pars recta following injection of mercuric chloride has been described in detail in the rat. Cellular changes include

fragmentation and disruption of the plasma membrane and its appendages, vesiculation and disruption of the endoplasmic reticulum and other cytoplasmic membranes, dissociation of polysomes and loss of ribosomes, mitochondrial swelling with appearance of amorphous intramatrical deposits, and condensation of nuclear chromatin. These changes are common to renal cell necrosis resulting from a variety of causes (Gritzka & Trump, 1968).

Mercury and its compounds are used widely, not only in various industrial processes but also in a number of other applications such as fungicides, contraceptive spermicides, and disinfectants. Several studies have been carried out to determine the extent to which current exposure of human populations to mercury can cause adverse renal effects. Foa' et al., (1976) reported an increased prevalence of glomerular proteinuria in workers exposed to mercury vapour in a chloralkali plant.

Studies carried out between 1979 and 1984 (Buchet et al., 1980; Roels et al., 1985) provided further evidence that occupational exposure to mercury vapour can lead to subclinical renal disturbances. These consisted of increased urinary excretion of proteins of high relative molecular mass (albumin, transferrin, and immunoglobulin G), lysosomal enzymes, and retinol-binding protein, which occurred at a higher prevalence in subjects who excreted more than 50 μg mercury/g creatinine. These observations were not confirmed by Stonard et al. (1983), who found only a slight increase in the prevalence of NAG and γ-glutamyltranspeptidase (an enzyme of the brush-border) in workers with urinary mercury levels higher than 100 μg/g creatinine.

Increased urinary excretion of NAG has also been found in workers involved in the production of various mercuric salts (Rosenman et al., 1986). Studies on patients with Minamata disease have provided inconsistent results regarding the induction of proximal tubular injury by methylmercury (Iesato et al., 1977; Ohi et al., 1982). By contrast, in a study of 509 infants exposed to phenylmercury fungicide on cloth diapers, Gotelli et al. (1985) clearly demonstrated that the kidney is a target organ during prolonged exposure to this compound. They showed that the urinary excretion of γ-glutamyl-transpeptidase

increased in a dose-dependent manner when urinary mercury exceeded approximately 220 μg/litre. This effect was, however, completely reversible and had disappeared when the infants were re-examined two years later.

Although exposure to a high dose of mercuric chloride is directly toxic to renal tubular lining cells, chronic low-dose exposure to mercuric salts or even elemental mercury vapour may induce an immunological glomerular disease. This form of mercury injury to the kidney is clinically the most common form of mercury-induced nephropathy. Exposed workers may develop a proteinuria that is reversible after they are removed from exposure. It has been stated that mercury-induced nephropathy seldom occurs without sufficient exposure to produce detectable mercury neuropathy as well.

Experimental studies have shown that the pathogenesis of mercury nephropathy has two phases: an early phase characterized by an anti-basement-membrane glomerulonephritis followed by a superimposed immune-complex glomerulonephritis (Roman-Franco et al., 1978). The pathogenesis of the nephropathy in humans appears similar, although antigens have not been characterized. Also, the early glomerulonephritis may progress in humans to an interstitial immune-complex nephritis (Tubbs et al., 1982).

5.5.4 *Gold*

The use of gold in the form of organic salts to treat rheumatoid arthritis may be complicated by development of proteinuria and the nephrotic syndrome (Hall et al., 1987). Morphologically, the kidney shows an immune-complex glomerulonephritis with granular deposits along the glomerular basement membrane and in the mesangium. The pathogenesis of the immune-complex disease is not known for certain, but gold may behave as a hapten and generate the production of antibodies with subsequent deposition of gold protein-antibody complexes in the glomerular subepithelium. Another hypothesis is that antibodies are formed against damaged tubular structures, particularly mitochondria, providing immune complexes for the glomerular deposits (Viol et al., 1977).

The pathogenesis of the tubular cell lesions induced by gold therapy is probably initiated by the direct toxicity of gold to tubular cell components. From experimental studies it appears that gold salts have an affinity for the mitochondria of proximal tubular lining cells. This is followed by autophagocytosis and accumulation of gold in amorphous phagolysosomes (Stuve & Galle, 1970). Gold particles can be identified in degenerating mitochondria, in tubular lining cells, and in glomerular epithelial cells by X-ray microanalysis (Ainsworth et al., 1981).

5.5.5 *Bismuth*

The effects of bismuth on the kidney are similar to those of lead, but it is a less frequent cause of renal disease. This is because bismuth is not present in such large amounts in the ambient environment, nor is it as important industrially. However, bismuth has been used therapeutically to treat a variety of ailments, most particularly syphilis. Bismuth administration results in the formation in proximal renal tubular lining cells of characteristic nuclear inclusion bodies that are similar to the lead-induced bodies and are composed of a bismuth-protein complex. The protein is acidic and has an amino acid composition similar to that forming the lead inclusion bodies. However, there is a slight difference in morphology between the lead- and bismuth-induced inclusion bodies. The bismuth-protein complexes are also observed in the mitochondria of proximal tubular lining cells (Fowler & Goyer, 1975). The bismuth content of the inclusion bodies has been confirmed by X-ray microanalysis of tissue sections. Whether bismuth produces a chronic interstitial nephropathy like lead has not yet been documented. However, bismuth inclusions have been found at autopsy more than 30 years after a course of bismuth therapy.

5.5.6 *Uranium*

Exposure of humans or experimental animals to compounds of uranium results in injury and necrosis of proximal renal tubules. The most sensitive site is the pars recta (as in the case of mercury), but, depending on the

dose, injury and necrosis may extend to other parts of the proximal tubule. Acute injury is followed by regeneration of tubular epithelial cells. Chronic effects have not been reported. An increase in the urinary excretion of β2-microglobulin and of specific amino acids has been reported by Thun et al. (1985) in uranium mill workers.

5.5.7 *Chromium*

The acute and chronic effects of chromium (mainly on the respiratory tract and skin) are due largely to hexavalent compounds. The acute tubular toxicity of chromate and dichromates salts in animals is well documented, and renal tubular necrosis has also been described in humans following acute poisoning (Langard & Norseth, 1986). Epidemiological studies have shown that chromium(VI) can produce slight tubular dysfunction in chronically exposed workers. Mutti et al. (1979) reported an increased prevalence of elevated β-glucuronidase and total protein levels in the urine of welders exposed to chromium. These observations have been confirmed by recent studies using more sensitive, reliable markers of tubular injury, such as β2-microglobulin (Lindberg & Vesterberg, 1983), retinol-binding protein, and the BB-50 renal antigen (Mutti et al., 1985).

Franchini & Mutti (1988) have studied dose-effect/response relationships between the urinary excretion of chromium and that of retinol-binding protein or the renal antigen BB-50. Most of the abnormal values were observed in subjects with urinary excretion of chromium greater than 15 μg/g creatinine; however, above this threshold the degree of tubular impairment was not related to urinary excretion of chromium. Franchini & Mutti (1988) explained this phenomenon by postulating that the tubular damage observed in chromium(VI)-exposed workers is transient and due mainly to acute exposure, and that workers become progressively resistant to the effects of more severe or prolonged exposure.

5.5.8 *Arsenic*

Acute arsenic poisoning may cause tubular necrosis. Acute or severe chronic poisoning is usually treated with

the chelating agent BAL (2,3-dimercaptopropanol). Inhalation of arsine may also produce an acute tubular necrosis as a result of intravascular haemolysis.

In a cross-sectional study, Foa' et al. (1987) failed to show significant differences between occupationally exposed workers and matched controls, with the exception of a slight increase in the urinary excretion of retinol-binding protein. However, owing to the small sample size and the low power of the study, no definite conclusion could be drawn from the slight increases in albuminuria, β2-microglobulin, and the brush-border antigen BB50. The authors concluded that extended population surveys would be desirable for a complete definition of such subtle effects.

5.5.9 *Germanium*

Germanium is naturally present in the diet, normal intake being about 1 mg per day. It is being used increasingly in the semiconductor industry. A recent report from Japan documented renal failure in ten individuals (including two deaths) among previously healthy individuals taking large doses of germanium (of the order of 50–250 mg per day) over periods of 4–18 months (Matsusaka et al., 1989). Renal biopsy or autopsy in seven cases showed degeneration of the renal tubular epithelium in all cases with or without interstitial fibroses or oedema. The glomeruli were only minimally effected in two cases.

6. RENAL CANCER

Tumours of the renal parenchyma, pelvis, and ureters are uncommon, accounting for less than 2-3% of all human cancers (DeKernion & Berry, 1980; Dayal & Kinman, 1983). The role of drugs, chemicals, and other environmental factors in the etiology of parenchymal and urinary tract tumours is unclear, but cancer of these sites is most common in certain industrialized nations (Sweden) and in people in the higher socio-economic groups (Rimpela & Pukkala, 1987). Other risk factors have been stratified (Selli et al., 1983). The ratio between tumours of the renal parenchyma and pelvis is fairly constant (about 5:1), and the parallel trends in increasing incidence argue for some commonality in the etiology of tumours at both sites, although some factors may be site specific. Tumours are nearly twice as common in males as in females. A compilation of trends in cancer rates in the USA indicates that the incidence of both kidney and bladder cancer is increasing (Pollack & Horm, 1980). A similar observation has been made with regard to renal parenchymal tumours in males in Scotland (Ritchie et al., 1984).

6.1 Renal tumour classification

The International Classification of Diseases for Oncology (code 189) divides tumours of the urinary system into five groups according to their size, i.e. parenchyma of the kidney (189.0), renal pelvis (189.1), ureter (189.2), urethra (189.3), and paraurethral gland (189.4) (Mostofi et al., 1981; WHO, 1990). These distinctions have only been made in recent years, so that many mortality studies of renal tumours have included this whole category. Of the five types of urinary tract tumours, about 90-95% of renal tumours in adults are adenocarcinomas arising from the renal parenchyma. Nephroblastoma (Wilm's tumour) is the second most common histological type of renal tumour and accounts for 2-4% of kidney cancer in Sweden and the USA. It is easily distinguished morphologically from renal adenocarcinoma and usually appears in the first five years of life; 95% of cases occur before the age of 15 years. Although nephroblastomas are the fourth most common tumour in childhood, they are relatively rare in adults.

6.2 Renal adenocarcinoma

Renal adenocarcinoma has been known under several synonyms (clear cell carcinoma, hypernephroma, Grawitz tumour) reflecting uncertainty about its origin, but immunological studies have established that renal adenocarcinomas arise from the proximal convoluted tubule (Wallace & Nairn, 1972). They tend to be circumscribed, ranging in size from microscopic lesions to large neoplasms (Hamilton, 1975). The spectrum from small benign lesions to clearly malignant lesions suggests a continuous pathological process, so that it is often difficult to label smaller tumours as benign or malignant. Hellsten et al. (1983) have defined all tumours of 2 cm or more in diameter as adenocarcinomas. Postmortem studies have shown that adenomas are present in approximately 25% of all males over 50 years of age, and it was found that 34% of 235 clinically unrecognized tumours present at autopsy were less than 3 cm in diameter. In the absence of invasion of surrounding tissue, features such as frequent mitotic figures, cellular pleomorphism, and haemorrhage and necrosis generally indicate a malignant potential regardless of size. Calcification may be detected by X-ray examination in about 15% of cases. Although 2-3% may be cystic, the commonest form is a solid tumour that is usually composed of clear cells rich in lipid, glycogen, or both, but may contain granular cells or even tightly packed eosinophilic cells referred to as oncocytes. The cell pattern may be trabecular, solid, or mixed, but it is doubtful that cell type or structural pattern has any clinical significance. Grading is difficult and has not been shown to be clinically useful. These tumours usually grow slowly, and overall survival is 20-25% after nephrectomy. The presence of multiple tumours, renal vein invasion, or regional lymph node metastases indicates a poorer prognosis. Hellsten et al. (1983) recorded metastasizing renal carcinoma as cause of death in 21% of a postmortem series, and in 33% a second malignant tumour was observed causing the death of 20%.

Immunological mechanisms are thought to determine the natural history of the disease. The development of monoclonal antibodies and flow cytometry have provided new methods for investigating immunological responses. Total

T lymphocyte counts were found in a study of 32 patients to be lower than in controls (Ritchie et al., 1984), due largely to a deficit of T helper cells but not T suppressor-cytotoxic cells. This effect of the tumour is reversed by removal of the primary tumour, and recurs with return of the tumour. These findings are believed to suggest that there is a systemic effect of the tumour acting at the level of the bone marrow or thymus to affect the production or maturation of T helper cells.

The role of specific environmental factors in the etiology of urinary tract tumours has been difficult to define (Newson & Vugrin, 1987). Apart from increases in renal tumours in asbestos workers and the identification of some occupationally related bladder tumours, there does not appear to be a clearly defined association with specific chemicals or environmental factors. However, there is an association with a combination of exposure to substances in the environment and life-style practices such as tobacco use. This suggests that there may be interactions between substances or that the urinary tract, like the lung, has to deal with a number of substances with promoter activity. Cigarette smokers have a 2-fold increase in risk of urinary tract tumours (Goodman et al., 1986), and an increase associated with alcohol and coffee usage has been suggested (Jacobsen et al., 1986). In addition, chronic interstitial nephritis may predispose to urinary tract tumours. People with endemic (Balkan) nephropathy have an increase in renal tumours and a possible relationship between chronic interstitial nephritis and renal neoplasia (see section 5.3). It is also noteworthy that human populations with excessive exposure to some known carcinogens (e.g., cycasin in Guam and aflatoxins in Africa and Asia) have not yet been shown to have an increase in kidney cancer (Sufrin & Beckley, 1980). Renal adenocarcinoma has been diagnosed with increasing frequency in patients with chronic renal failure, particularly in those patients treated with long-term dialysis (Dunhill et al., 1977).

An association between renal cancer and excess exposure to lead has not been clearly established, but a study of lead smelter and battery workers found a significant excess of malignancies at all sites, these being mostly lung tumours (Cooper & Gaffey, 1975). Case reports

of renal tumours in workers with lead nephropathy have appeared (Baker et al., 1980; Lilis, 1981).

6.3 Upper urothelial carcinoma (transitional cell carcinoma)

Tumours of the renal pelvis form a spectrum from benign papillomas to frank papillary carcinomas and, like bladder tumours, are generally low-grade cancers. However, they tend to recur regardless of their morphology. Upper urothelial carcinoma has been associated with RPN and analgesic abuse, but a cause-and-effect relationship between the two has not been proven (Bach & Bridges, 1985a). The incidence of upper urothelial carcinoma among analgesic abusers is very high, and females predominate in analgesic-associated upper urothelial carcinoma. The female:male ratio is 2.5 to 1 (Bengtsson et al., 1978), which is in keeping with the ratio for analgesic abusers. Analgesic abusers also develop upper urothelial carcinoma at a younger age than non-analgesic abusers (Mihatsch et al., 1980a,b,c). The distribution of urothelial carcinomas in analgesic abusers has a distinct pattern; tumours of the renal pelvis, ureter, and bladder are found 80 times, 90 times, and 7 times, respectively, more frequently than in non-analgesic abusers. The tumours are typically multiple, diffuse, and poorly differentiated, and spread rapidly (Mihatsch et al., 1980c).

Patients who discontinue abuse of the drugs are at a greater risk of developing upper urothelial carcinoma, often after a latent period of 10-20 years after initiating analgesic abuse. Greatly improved dialysis techniques may result in the survival of analgesic-abusing patients who would otherwise have developed end-stage renal disease and subsequently died (Mihatsch et al., 1980a). It has therefore been suggested that the incidence of upper urothelial carcinoma will increase.

The diagnosis of upper urothelial carcinoma is difficult in the clinical situation because of few specific clinical symptoms to indicate the malignant changes (Johansson et al., 1976; Bengtsson et al., 1978; Mihatsch et al., 1980a,b,c; Mihatsch & Knusli, 1982; Bach & Bridges, 1985a; Pommer et al., 1986). The prognosis is poor, and patients with upper urothelial carcinoma only have a mean survival time of 22 months (Mihatsch et al.,

1980a) owing to the difficulty of diagnosis, compromised renal function of patients with RPN, and multifocal sites of rapidly developing and widespread invasion and metastases (Johansson et al., 1976; Mihatsch & Knusli, 1982).

6.4 Experimentally induced renal adenomas and adenocarcinomas

Renal adenomas and adenocarcinomas may be induced in laboratory animals by various natural products and biological and chemical agents. However, linkage of exposure of these substances to renal cancer in humans is lacking in most instances, or, at best, is only suspected.

6.4.1 Background incidence of spontaneous tumours in experimental animals

The incidence of spontaneous renal parenchymal tumours in most commonly used strains of male rats and mice is in the region of 0.2%, whereas it is < 0.1% for females (Crain, 1958; Goodman et al., 1979, 1980; Ward et al., 1979; Maekawa et al., 1983). The incidence may be up to 2.7% (Pour et al., 1979) in hamsters, which is generally low enough for investigative studies.

Renal tumours have been induced experimentally by a large number of compounds including lead salts (Kilham et al., 1962; van Esch & Kroes, 1969; Goyer & Moore, 1974), nickel sulfides (Jasmin & Riopelle, 1976; Sunderman et al., 1984), methylmercury chloride (Mitsumori et al., 1981), *N*-(4′-fluoro-4-biphenylyl) acetamide (Hinton et al., 1980), trisodium nitrilotriacetic acid (Goyer et al., 1981), potassium bromate (Kurokawa et al., 1983), halogenated alkenes (Kociba et al., 1977; Reichert et al., 1984), and tris(2,3-dibromopropyl)phosphate (Reznik et al., 1979). Natural products include cycasin (Laqueur & Spatz, 1968), aflatoxins B_1 (Butler et al., 1969; Epstein et al., 1969), ochratoxin A (Kanisawa & Suzuki, 1978), citrinin (Arai & Hibino, 1983), the fermentation-derived anti-neoplastic agent daunomycin (Sternberg et al., 1972), and streptozotocin (Rakieten et al., 1968; Hard, 1985). Diethylstilbestrol (Horning & Whittick, 1954) and related estrogens (Li et al., 1983) produce a high incidence of parenchymal tumours in hamsters.

The classical two-stage model of carcinogenesis also applies to several of the nitrosamines, where promoters

include sodium arsenite (Shirachi et al., 1983), DL-serine (Hiasa et al., 1984a), folic acid (Shirai et al., 1984), lead acetate (Hiasa et al., 1983), nicotinamide (Rosenberg et al., 1985), trisodium nitrilotriacetate (Hiasa et al., 1984b), and citrinin (Shinohara et al., 1976). There are several factors that may affect the development of carcinomas, including diet (Hard & Butler, 1970; McLean & Magee, 1970; Hard, 1980, 1984; Swann et al., 1980), partial hepatectomy (Evarts et al., 1982), unilateral nephrectomy (Ito et al., 1969), and unilateral hydronephrosis (Ohmori & Tabei, 1983).

6.4.2 *Inorganic compounds*

Various inorganic compounds of lead have been investigated over the last 30 years (Van Esch & Kroes, 1969). The tumours arise from kidney tubular epithelial cells in kidneys and are similar to renal cortical tumours found in humans. Production of tumours requires continuous exposure to relatively high concentrations of lead in the diet or drinking-water for 1 to 2 years. The tumours occur in a background of severe interstitial nephritis characterized by tubular atrophy as well as focal areas of hyperplasia. They are usually multifocal and vary from microscopic adenomas to large renal adenocarcinomas that may invade contiguous structures or metastasize to the lungs. Intranuclear inclusions, which are usually present in proximal tubular epithelial cells in lead toxicity, are absent in neoplastic cells, and the tumors contain much less lead than adjacent renal parenchyma (Mao & Molnar, 1967). Tumor cells are pleomorphic, and ultrastructural studies have shown marked morphological alterations in mitochondria.

Renal cancer occurs after injection of crystalline nickel subsulfide (Ni_3S_2) into the kidney of rats, but not after treatment with amorphous nickel sulfide (NiS). No evidence indicates that nickel compounds are carcinogenic in experimental animals when administered by oral or subcutaneous routes (Sunderman, 1981).

6.4.3 *Organic molecules*

Nitrilotriacetic acid, a polyamino polycarboxylic acid with chelating properties similar to EDTA (used to treat

lead poisoning), produces chronic interstitial nephropathy in rodents. A spectrum of tubular cell histological changes occurs from hyperplasia to small adenomas to adenocarcinomas (Goyer et al., 1981). Renal adenocarcinoma has been induced in male rats by the chronic inhalation of unleaded gasoline vapour (MacFarland et al., 1984), but this relationship has not been supported by epidemiological studies on workers in the petroleum industry (Enterline & Viren, 1985).

6.4.3.1 *Nitrosamines and related compounds*

The nitrosamines represent one of the most widely investigated groups of model compounds. They include dimethylnitrosamine (Murphy et al., 1966; Mohr et al., 1974; Hard, 1984), which produces mesenchymal (connective tissue) tumours in young animals but adenomas and adenocarcinomas in mature animals (Hard, 1979). The co-administration of putrescine (Ohmori & Tabei, 1983) or *N*-3,5-dichlorophenyl-succinimide (Ito et al., 1974) with dimethylnitrosamine caused a dose-related incidence of up to 100% renal tumours after 100 weeks. *N*-ethyl-*N*-hydroxyethylnitrosamine (Hiasa et al., 1979) on its own, or, especially, when administered with basic lead acetate (Hiasa et al., 1983) or serine (Hiasa et al., 1984a) causes tumours in up to 95% of animals by 32-38 weeks. *N*-Nitrosomorpholine causes oncocytomas (Bannasch et al., 1978a,b, 1980). There are interesting differences between several of the model compounds, interspecies responses, effects of dose regimens, etc. However, the investigation of these models has generally permitted the progression of cellular injury to be described in terms of the acute effects, early hyperplasia, dysplasia, and different types of renal tumours.

6.4.3.2 *Morphological changes*

Many of these model compounds have been used to study the early, intermediate, and late changes at the light microscope and ultrastructural levels (Horning & Whittick, 1954; Butler, 1964; Butler & Lijinsky, 1970; Ertürk et al., 1970; Hard & Butler, 1971; Sternberg et al., 1972; Bennington, 1973; Hard, 1975, 1984, 1985; Bannasch et al., 1978a,b, 1980; Dees et al., 1980a,b; Ohmori et al., 1982;

Tsuda et al., 1983; Eble & Hull, 1984; Hard et al., 1984; Hiasa et al., 1984a,b,c). The phenotypic changes associated with loss of normal growth control have concentrated on the focal preneoplastic changes in heterogeneous cells. These undergo slow changes (where it is highly desirable to define the origins of the neoplasm) in a limited number of enzyme markers and in lipids, and carbohydrates. In addition, an increase in cytoplasmic RNA (shown by enhanced cytoplasmic basophilia or numerous ribosomes at the ultrastructural level) has been observed as a marker for hyperbasophilic and basophilic preneoplastic foci in the epithelium of the renal tubular system (Hard, 1986; Bannasch & Zerban, 1986).

The nomenclature of renal parenchymal tumours is based on several criteria, including the size of the tumour and the morphological-histochemical characteristics of cells and their organization. The progression in tissue mass from hyperplasia, through dysplasia, to adenoma, adenocarcinoma, and carcinoma is a continuum, although the earliest changes, particularly hyperplasia, may be reversible. Cells may have no cytoplasmic staining (clear cells) or granular acidophilic or basophilic cytoplasm staining, and tumours may have a mixture of cells with different staining characteristics. Where adenomas contain a uniform population of finely granulated eosinophilic cells, they are termed renal oncocytomas. Tumours can also be classified as tubular, solid, lobular, disorganized, invasive, papillary or cystadenoma, or by a composite of such terms based on their appearance (Hard, 1987).

Clear and acidophilic (granular) cell kidney tumours induced by limited exposure of rats to *N*-nitrosomorpholine are associated with a transient storage of glycogen (Bannasch et al., 1978a) and closely parallel the most common malignant renal neoplasm in man. The tumours originate from segments of the collecting duct system storing large amounts of glycogen (Nogueira et al., 1989). However, when microadenomas develop, the clear (glycogenotic) cells loose glycogen and acquire an acidophilic (granular) cytoplasm, although both cell types can coexist in large tumours. Lipid-storing cells are often found in the clear cell tumours, but the significance is not known. By contrast, there are no such relationships between cells storing glycogen and the so-called renal oncocytomas also

found in NNM-induced rats (Bannasch et al., 1978b; Nogueira et al., 1989), although rat renal oncocytomas also originate from the collecting duct system (Nogueira & Bannasch, 1988). They are, however, benign end-stage lesions where the cytoplasm is crowded with pathologically altered mitochondria (Krech et al., 1981). A temporary focal storage of glycosamino-glycans has been reported in chromophobic rat renal cell tubules and tumours (Bannasch et al., 1980, 1981) and in the corresponding type of human tumour (Thoenes et al., 1985).

6.4.3.3 *Biochemical changes in cells*

An immunohistochemical increase in glucose-6-phosphate dehydrogenase is associated with basophilic renal cell tumours (Tsuda et al., 1986) and nephroblastomas (Moore et al., 1986). The pentose phosphate shunt provides sugars for RNA and DNA synthesis, and the activation of this pathway is probably closely related to certain phenotypic changes, such as an increase in ribosomes and an enhanced cell proliferation in preneoplastic and neoplastic lesions. In line with this interpretation, rat renal oncocytic tubules and tumours, which are poor in ribosomes and grow very slowly, usually show a normal or even decreased activity of glucose-6-phosphate dehydrogenase (Tsuda et al., 1986). However, in some experimental models a reduced amount or activity of glucose-6-phosphate dehydrogenase was found in the more malignant populations, suggesting involvement of the enzyme in other metabolic aberrations relevant to tumorigenesis (Moore et al., 1986).

Alterations in drug-metabolizing enzymes (see below) during carcinogenesis have been detected by immunohistochemical methods in various tissues, especially in the renal tubular system, but they have not been correlated to the same extent with the respective enzyme activities and with other changes in the cellular phenotype as have those of carbohydrate metabolism. By contrast, *N*-ethyl-*N*-hydroxyethylnitrosamine-induced renal carcinomas show opposite alterations in drug-metabolizing enzymes in preneoplastic and neoplastic lesions of these tissues (Tsuda et al., 1987). Reduced activities of γ-glutamyl-transpeptidase (Ohmori et al., 1982; Tsuda et al., 1986), succinate

dehydrogenase (Tsuda et al., 1986), and alkaline phosphatase (Tsuda et al., 1986) are seen as early changes during the development of basophilic cell tumours from hyperbasophilic segments of the proximal nephron. In contrast, however, there are no similar changes in γ-glutamyltranspeptidase or alkaline phosphatase activity (but there is an increase in succinate dehydrogenase activity) in oncocytic tubular lesions seen in these animals (Tsuda et al., 1986). The increased binding of anti-cytochrome c oxidase to the oncocytic lesions in both man (Ortmann et al., 1988) and rat (Mayer et al., 1989) may be a useful marker for preneoplastic renal changes.

6.4.3.4 The mechanistic basis of renal carcinoma

The mechanistic basis for the development of renal carcinoma may be genotoxic or non-genotoxic. The common feature to all genotoxic agents is the generation of a reactive electrophilic (electron-deficient) species which is capable of binding to nucleophilic (electron-rich) sites on cellular macromolecules including proteins, lipids, RNA, and especially DNA (Miller & Miller, 1981). For example nitroso-compounds alkylate DNA in the N-7 and, especially (due to its prolonged stability), O-6 positions (Nicoll et al., 1975).

Genotoxic compounds are either direct alkylating agents (requiring no activation) or they require one or more biotransformation steps by the P-450-dependent monooxygenases (e.g., chloroform) (Bailie et al., 1984; Smith & Hook, 1984). However, hexachloro-1,3-butadiene is transformed by β-lyase (Elfarra & Anders, 1984) or prostaglandin hydroperoxidase-mediated co-oxidation (Davis et al., 1981), which may also be involved in *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide transformation (Zenser & Davis, 1984). In addition, there may be several other renal and extra-renal metabolic steps. There are also some molecules that cannot be reliably classified into either group. Diethystilbestol is a very weak alkylating agent (Lutz et al., 1982), and its mechanism of action is thought to be mediated by renal estrogen receptors (Li & Li, 1984). However, some metabolic component may be involved, e.g., hepatic mixed-function oxidase (Metzler, 1981) and peroxidative activation (Metzler & McLachlan, 1978).

6.5 Experimentally induced upper urothelial carcinomas (transitional cell carcinomas)

There is experimental evidence to connect analgesic exposure to the development of urothelial tumours (Johansson & Angervall, 1976; Bengtsson et al., 1978; Bach & Bridges, 1985a). While bladder tumours have been studied extensively in animal models, the practical difficulties of looking for malignancies in the ureter or pelvis has limited studies in this area. Based on long-term carcinogenicity studies, there are few data to establish a clear experimental relationship between analgesic exposure and upper urothelial carcinoma. There is, however, experimental evidence to suggest that upper urothelial carcinomas can be induced using a classical two-stage initiation/promotion regimen (Bach & Gregg, 1988; Gregg et al., 1989). These data suggest that localized injury associated with papillary necrosis adjacent to urothelium that has already been initiated will result in a proliferation of changes that lead to malignancy. At present the full significance of these findings in terms of the human analgesic problem is not clear.

7. ASSESSMENT OF NEPHROTOXICITY

No single *in vivo* or *in vitro* method of studying the nephrotoxicity of chemicals can address all of the questions that must be asked. It is therefore inadvisable to separate mechanistic research into target cell toxicity from the screening of novel compounds for their potential nephrotoxicity. The holistic approach to nephrotoxicity assessment also demands that *in vivo* investigations are not separated from *in vitro* studies, and that data continue to be derived from several different animal species and related to accurately conducted epidemiological and clinical studies (where these data are available).

Present data suggest that most *in vitro* methods can provide information on the mechanism of primary insult and the effect on cell viability. However, there appears at present to be little place for *in vitro* techniques in the assessment of secondary renal changes, as the factors that contribute to the cascade of degenerative changes that follows renal insult are largely obscure. Thus there is inherent uncertainty as to what should be studied in *in vitro* systems. There are, however, several approaches that can be used to provide a better understanding of the contribution to degenerative changes *in vivo*. These include harvesting tissue at different time points following an insult and using this tissue to study function in different cell types, or studying the effects of chemicals on target and non-target cells using pure and mixed cell cultures in the presence and absence of cells that are related anatomically. It is also possible to exchange culture media between cells that have been insulted, and those that have not, in order to assess the release of factors that may be toxic to other cells. Different cells can be studied in the same media to define cell-cell interactions and how chemical insult affects this process.

7.1 *In vitro* studies

Despite the complexity of the kidney and nephrotoxicity, and the difficulty in defining what any one *in vitro* system achieves, there are several ways to progress in the use of screening methods.

One such rational approach could include:

- the careful identification of compounds with well-documented *in vivo* nephrotoxicity, including the sequence of pathological and functional changes, the metabolites formed, quantities excreted, and the cellular pharmacodynamic effects;
- the choice of chemicals that target specifically for one anatomically discrete cell type *in vivo*;
- the use of both more and less nephrotoxic analogues of the chemical for determining structure-activity relationships and computer-based simulations;
- the systematic study of these compounds by several different *in vitro* methods, and the use of several criteria for assessing *in vitro* nephrotoxicity for each.

7.1.1 Choice of chemical concentrations for in vitro studies

The validity of using the cytotoxicity *(in vitro)* for a given concentration of a chemical as a likely indicator of a toxicological effect *in vivo* can be very difficult to establish. It may be impossible to assess this in the kidney, because this organ is highly compartmentalized. In the intact and functioning organ, it is currently not possible to establish the concentrations of a xenobiotic (or its metabolites) associated with any one cell type. This uncertainty relates, for instance, to the different transport systems that are distributed in discrete parts of the nephron, transcellular pH gradients, and the selective accumulation of certain chemicals in cell organelles (which have heterogeneous distribution in different types of cells). Thus some chemicals can be selectively concentrated in a discrete area of the kidney to several times the plasma concentration. Alternatively, certain chemicals may be actively or selectively excluded from some cell types (Mudge, 1985).

Drug metabolism systems that alter the physicochemical characteristics of xenobiotics and their metabolites (that will facilitate the redistribution of chemicals within and between cells) are also heterogeneously distributed. Thus, certain xenobiotic products may be selectively concentrated in (while others are excluded from) specific cell

types. Therefore, even though arterial and venous blood and urinary concentrations of chemicals can be measured, there is no certainty that such data relate to the concentrations of any specific metabolite that reaches a target cell. Autoradiography may be very valuable in providing some idea on how chemicals are distributed, but it only shows the distribution of radiolabel-derived material, not its chemical nature. Once the anatomical integrity of the kidney has been altered, it may be difficult to relate the concentration of any chemical to the same cells *in vivo*. While structure is maintained in the perfused kidney, the functional changes impose a similar constraint. The uncertainty as to what concentrations of chemicals to use in *in vitro* systems is exacerbated by the undefined influence of extrarenal and renal metabolism on the delivery of the proximate and ultimate toxins to the target sites of injury *in vivo*.

7.1.1.1 *Proximate and ultimate nephrotoxicants* in vitro

In addition to the need to consider carefully the consequences of the changes in the route of chemical delivery when the anatomical integrity of the kidney has been disrupted (i.e. in all systems other than the isolated perfused kidney), it is important to consider how a chemical is delivered to the cells. This consideration should be expressed in terms of the chemical being free or bound (should the media contain protein or not?), the physicochemical characteristics of the solution in which the chemical is delivered (such as its pH, ionic concentration, and endogenous and exogenous micro- or macromolecules), and the kinetics of delivery (this is generally zero-order in most *in vitro* systems but follows first or second order kinetics *in vivo*).

Chemically induced nephrotoxicity may be the result of a direct action of the parent chemical in the kidney or may be due to an extrarenally formed metabolite. However, in some instances the chemical/metabolite has to be further metabolized *in situ* to form the ultimate nephrotoxic species. Assessing the role of *in situ* metabolism in nephrotoxicity from *in vivo* data may be difficult, since most nephrotoxic chemicals are also extensively metabolized in extrarenal tissue such as the liver. Experimen-

tal approaches using different species and strains of animals, inducers and inhibitors of drug metabolizing enzymes, and candidate proximate and ultimate nephrotoxic metabolites may give some evidence for the involvement of intrarenal metabolic activation (Rush et al., 1984). More direct evidence for a direct renal activation of a chemical has to come from *in vitro* studies or studies with perfused kidneys where extrarenal metabolism/activation can be excluded. *In vitro* studies may include experiments with isolated tissue preparation (kidney slices and tubules), cells (primary cells or cell lines) or subcellular fractions to assess chemically induced toxicity. In order to determine the role of extrarenal metabolism in the formation of nephrotoxic metabolites, co-culture systems using liver cells (as an activation system) and kidney cells (as target cells) may be very useful (Moldeus et al., 1978). The stability of a reactive metabolite, generated by liver cells, may be measured by transferring, after various time intervals, the incubation medium to the target cell population.

7.1.2 In vitro *investigations of nephrotoxicity*

In vitro techniques can be divided into those where the anatomical relationship between cells is maintained (perfusion, micropuncture, and slices), those where glomeruli and tubular fragments are isolated, and those where cells are isolated. The different techniques for assessing nephrotoxicity *in vitro* have been reviewed and the strengths and weaknesses of each presented in broad terms (Bach et al., 1985, 1986; Bach & Kwizera, 1988). Some methods are technically difficult, depend on sophisticated equipment, are subject to artefacts in inexperienced hands (perfusion and microperfusion), and are difficult to interpret.

7.1.2.1 *Perfusion and micropuncture*

Micropuncture methodology has not been widely used to assess the toxicological effects of chemicals (Bank et al., 1967; Biber et al., 1968) because the methodology is extremely complex. However, a few of the problem areas should be mentioned. Micropuncture procedures succeed only when the experimentalists can collect measured small

samples of tubular fluid and subject these to appropriate chemical analyses. These procedures are extremely complex, and, because of the small volumes involved, subject to considerable error. To assure that the micropuncture collections reflect the "physiological state", most workers attempt to collect only very small volumes at the "physiological" flow rate past the point of micropuncture. Stationary microperfusion has been used to assess tubular function in a restricted area of the nephron.

Micro-injection into a tubular segment has been used by many physiologists and may be of particular toxicological interest (Gottschalk & Lassiter, 1973; Roch-Ramel & Peters, 1979; Diezi & Roch-Ramel, 1987). With this technique, various renal function markers, as well as potential nephrotoxicants, may be injected into a segment of the proximal tubule during a free-flow situation. The urine from the injected kidney may then be collected to assess nephrotoxicant effects on the injected renal function markers. For micropuncture specialists, this is a relatively straight-forward technique and does not involve the problems associated with removal of tubular fluid by micropuncture. This procedure might permit the assessment of nephrotoxicant effects on membrane permeability by examining, for example, inulin excretion from the injected kidney.

The isolated perfused tubule technique represents the development of an *in vitro* procedure that permits the assessment of intact tubular function under carefully controlled *in vitro* conditions. Hence all of the advantages of *in vitro* methodology are available while intact tubular function is being studied (Diezi & Roch-Ramel, 1987).

Tubule segments can also be isolated by manual dissection for microperfusion, where they are attached to micropipettes suspended in a bathing solution and perfused with an artificial tubular fluid (Ullrich & Greger, 1985). Relatively few attempts have been made to apply this sophisticated methodology to the study of nephrotoxicants, where it would be possible to add chemicals to either the perfusate or the bathing solution and examine effects on either the tubular or basolateral side of the cell. This

technique has been used to demonstrate that organic anion transport across the tubular cell is active on the basolateral side but not on the luminal side (Tune et al., 1969).

The major advantage of this procedure is that it permits an *in vitro* assessment of renal function with a tissue segment that is essentially intact. The situation in an isolated nephron segment is obviously not identical to that in the intact kidney, but by carefully regulating the perfusion solution and the bathing solution one can approximate *in vivo* physiology. This is a potentially important procedure that needs to be assessed for its utility.

7.1.2.2 *Renal cortical slice*

These techniques have been reviewed extensively (Berndt, 1976,1987; Bach & Lock, 1982; Kacew, 1987) and used to show deleterious effects of chemicals and drugs on the kidney. Much of the published information has focused on renal tubular transport as the criterion for establishing the nephrotoxic potential of chemicals. The tests are based on measuring the accumulation of the organic ions *p*-aminohippurate (PAH) and tetraethyl-ammonium (TEA) (Hirsch, 1976) by renal slices. The organic anion transport is a sensitive indicator of aminoglycoside (Kluwe & Hook, 1978; Kaloyanides & Pastoriza-Munoz, 1980) and cephaloridine toxicity (Kuo & Hook, 1982; Kuo et al., 1982). Similarly, the effects of mercuric ions, chromate ions, hexachlorobutadiene conjugates, and other nephrotoxins appear readily detectable by this approach. Organic ion accumulation and gluconeogenesis in renal cortical slices may be poor indicators of early toxic effects to the kidney resulting from cisplatin administration because these parameters are not affected except at high doses. The poor sensitivity and delayed response of renal slice parameters indicate that membrane function and cell metabolism may not be early targets of cisplatin at the cellular level. Slices maintain β-lyase activity for up 12 h and have been used to study halo-alkene toxicity. Aminooxyacetic acid inhibits β-lyase activity almost completely. DCVC decreases PAH accumulation, but does not appear to use the same transport process.

7.1.2.3 *Isolated nephron segments*

Isolated tubules overcome many of the disadvantages of cortical slices, in that they remain in contact with substrates and toxins in the medium, whereas cortical slices may show lumen collapse within a short period (Chahwala & Harpur, 1986).

Freshly isolated tubule or cell suspensions offer an important way of studying the mechanisms of nephrotoxicity and screening novel compounds for their potential acute effects on the kidney. A limitation, however, is the short *in vitro* lifespan of isolated tubules prepared, by any technique, for studying early toxic effects. In general, most investigators limit incubations to no more than 2-4 h because of loss of viability and functional capabilities. Ormstad (1982) reported rapid loss of viability of isolated renal tubules and cells, more than 25% of the cellular lactate dehydrogenase (LDH) leaking to the medium during 1.5 to 2 h of incubation. Obatomi & Plummer (1986) observed a 40% loss in tubule cell viability during 3-h incubations of rat proximal tubules. Loss of renal function, such as O_2 consumption, has also been reported for isolated tubules (Harris et al., 1981).

A number of fresh tubular systems exhibiting high initial viability (> 90% by trypan blue exclusion) have been prepared from collagenase digests of rat cortical tissue (Cunnaro & Weiner, 1978; Belleman, 1980; Cojocel et al., 1983; Gstraunthaler et al., 1985; Obatomi & Plummer, 1986) using tubular fragments of proximal origin. The tubules can be used to study the metabolism of xenobiotics liable to be converted into compounds responsible for the alteration of normal renal metabolism (Jones et al., 1979). Tubular fragments obtained by collagenase treatment of dog (Baverel et al., 1978, 1980a), human (Baverel et al., 1979), baboon (Michoudet & Baverel, 1987), and guinea-pig (Baverel et al., 1980b) renal cortex have also been used for metabolic studies. These fragments retain the gluconeogenic capacity that is specific to the proximal convoluted tubule (Guder & Ross, 1984). Human, dog, and rat renal cortex tubules also release ammonia from glutamine, and any drug-induced disturbance of renal ammoniagenesis can be studied with these models (Martin et al., 1987, 1989, 1990).

Proximal tubules have been purified from the above preparations by centrifugation on a Percoll density gradient. The samples obtained exhibit enrichment in proximal tubule cells relative to cells from other areas of the nephron, as indicated by the distribution of alkaline phosphatase and hexokinase (Vinay et al., 1981).

Suspensions of thick ascending limb fragments have also been prepared from dog (Baverel et al., 1980a,b; Anand-Srivastava et al., 1986), rat (Trinh-Trang-Tan et al., 1986), and rabbit (Chamberlin et al., 1984) outer medulla. Suspensions of collecting tubules obtained from the inner medulla (Anand-Srivastava et al., 1986; Wirthensohn et al., 1987, 1989) may prove very useful in studies of the effect of nephrotoxic substances that interfere with the function of this renal zone.

Direct addition of different concentrations of nephrotoxic agents such as ochratoxin A, citrinin, furosemide, and potassium chromate to the suspension releases enzymes specific to the proximal tubule (Table 4). such as alanine aminopeptidase, leucine aminopeptidase and alkaline phosphatase, to the incubation medium in a dose-dependent manner (Endou et al., 1985). To characterize further the intrarenal site(s) and mode of nephrotoxicity, definite portions of a single nephron can be microdissected from the collagenase-treated kidney. There are two different methods available for the microdissection of individual nephron segments; one is from lyophilized kidney sections and the other is from collagenase-treated fresh kidneys (Morel et al., 1976). In nephrotoxicity studies, fresh individual nephron segments are used. The following segments can be isolated: the glomerulus, the proximal tubule (S_1, S_2, S_3), the thin descending limb of Henle's loop, the medullary and cortical thick ascending limb of Henle's loop, the distal convoluted tubule, the connecting tubule, and the cortical and medullary collecting tubules.

Several functional parameters can be studied in nephron segments. Gluconeogenesis is a unique function of the proximal tubule, within which the S_1 segment is most active (Maleque et al., 1980; Endou et al., 1985). Gluconeogenesis is strongly induced by metabolic acidosis or by α_1-adrenergic stimulation (Nakada et al., 1986a).

First-generation cephalosporins, cephaloridine and cephalothin cause a time- and concentration-dependent decrease in gluconeogenesis, and it is clearly indicated that the site of nephrotoxicity of these antibiotics is the proximal tubule (S_1, S_2, S_3). Ammoniagenic activity is distributed in all the nephron segments, but the highest production rate of ammonia from glutamine is observed in the proximal tubule (Nonoguchi et al., 1985). Ammoniagenesis via the purine nucleotide cycle from asparatate as a substrate is also high in the proximal tubule (Tamura & Endou, 1988). Ammonia production is increased in a similar way by metabolic acidosis or potassium depletion (Nonoguchi et al., 1986). Cisplatin nephrotoxicity is morphologically known to be focussed on the S_3 segment. However, this drug decreases ammoniagenesis from glutamine not only in S_3, but also in S_2, suggesting a discrepancy between morphological and biochemical evaluations, although cisplatin does not affect gluconeogenesis in isolated nephron segments (Nakada et al., 1986b).

The kidney possesses various active transport processes that consume ATP at a high rate. Individual nephron segments require their own particular substrates for synthesizing the necessary ATP: this has been shown in both mice (Uchida & Endou, 1988) and rats (Jung et al., 1989). The proximal tubule cannot use glucose to produce ATP, whereas the other nephron segments can use it. In general, pyruvate or lactate is the preferred substrate in all segments. Nephrotoxicity assessment by measuring cellular ATP content shows clearly that mercuric chloride decreases ATP content only in S_2 (Jung et al., 1989) and that ochratoxin A nephrotoxicity localizes in S_2 and S_3 (Jung & Endou, 1989). Thus, measurement of cellular ATP in specific nephron segments enables possible nephrotoxicants to be evaluated. A similar principle can be applied by measuring intracellular free calcium (Jung & Endou, 1990). From the biological point of view, it is essential to keep cellular ATP at a high level and to maintain a low concentration of intracellular free calcium for all living cells. It should, therefore, be reasonable and useful to introduce these sensitive parameters to nephrotoxicity assessment, although the methods require special techniques for microdissecting nephron segments or special instruments.

An advantage of the use of isolated tubules, as compared to *in vivo* experiments, is that it permits a cellular environment that is defined both quantitatively and qualitatively. This allows the relationship between the concentration of a nephrotoxin, exposure time, and effect to be studied. Extrarenal effects can be avoided, and so isolated tubules are very suitable for studying the effects of nephrotoxins that act directly at the tubular site. Owing to a lack of polarity (Koseki et al., 1988), isolated renal cell suspension may have limited usefulness.

There are several limitations associated with the use of freshly isolated or cultured renal cells. Cells released by enzymic digestion or fresh fragments can be cultured in the presence of serum-free, hormonally defined culture media. This prevents fibroblast proliferation and encourages epithelial cell growth (Chuman et al., 1982), but both cell preparations lack a brush border, which may be critical for the active uptake of drugs and chemicals.

Alternative approaches to obtain a preparation with an intact brush border include the use of different sized sieves to separate glomeruli and tubules (Bach et al., 1986), which may not result in a pure preparation of proximal tubular cells. The choice of method for monitoring cell viability may circumvent this, e.g., the use of prostaglandin synthesis (Sraer et al., 1980) to assess effects of chemicals selectively on glomeruli. The density gradient technique (Vinay et al., 1981) uses Percoll centrifugation to separate the different cell types to obtain a > 90% pure preparation of proximal tubular cells. These cells retain their viability and their GSH levels at > 50% for 2 h at 37 °C, and demonstrate cytochrome-P450-dependent mono-oxygenase activity profiles that are inducible only by 3-methylcholanthrene (3MC). This may be an appropriate preparation to study the effects of various drugs and chemicals, in both rats and man, that demonstrate nephrotoxicity to either the S_1, S_2, or S_3 regions after administration (Smith et al., 1986; Rosenberg & Michalopoulos, 1987).

7.1.2.4 *Primary cell cultures*

Cell to be cultured should be of well defined origin. For this, purification of a homogeneous glomerular or

tubular cell population can be achieved by several methods (Jakoby & Pastan, 1979), including sieving techniques (Striker et al., 1980), magnetic and mechanical techniques (Meezan & Brendel, 1973), density gradient centrifugation (Scholer & Edelman, 1979; Vinay et al., 1981), and collagenase digestion (Curthoys & Bellemann, 1979; Belleman, 1980; Ormstad et al., 1981). More recently, techniques such as immunodissection, cell sorting, free-flow electrophoresis, and microdissection have been used (Pretlow & Pretlow, 1982, 1983, 1984). The advantage of using primary cell cultures is that it allows long exposure to xenobiotics and the choice of appropriate metabolites. In addition, it is possible to monitor a variety of cell functional, biomedical, or morphological responses in a dose- and time-related manner (Fry et al., 1978; Belleman, 1980; Fry & Perry, 1981; Bach et al., 1986).

Mechanical or enzymic dispersal may damage cells, and once cells are dispersed it is generally difficult to establish their anatomical identity unless suitable markers are used. These markers include both the presence and absence of a range of functional and biochemical characteristics, such as transport systems, and an array of structural and functional molecules. These can best be assessed by a variety of histochemical and immunocytochemical methods (Bach et al., 1985, 1987). At present, isolated cells are generally mixtures (although they may be enriched) and must be used within a few hours. Primary cell cultures may rapidly dedifferentiate (Curthoys & Bellemann, 1979) or adapt to a new environment and change their characteristics as a result of the presence or absence of factors in the culture media, which may obfuscate their anatomic origins. More importantly, loss of a biochemical characteristic that is part of the molecular basis for target cell toxicity may invalidate *in vitro* studies. Changes in other aspects of cellular integrity can increase or decrease both the sensitivity and selectivity of screening methods used for cytotoxicity studies.

Two approaches have been used to modulate the expression of cell characteristics. The polarity of epithelial cells is better expressed when cell are grown on permeable supports such as collagen/filters (Jakoby & Pastan, 1979). Similarly, the appropriate modulation of culture media has been used to alter rabbit proximal

tubule cell metabolism to the gluconeogenic pathway and these cells then develop brush-border characteristics. Thus, media can be an important variable, especially because of the diverse combination of buffers and growth supplements used. There are major advantages in using fully defined culture media (Sato & Reid, 1978), but these have not been widely adopted.

Human proximal tubules have been shown to been sensitive to cyclosporin A (Trifillis et al., 1986), but there are no data on the mechanistic bases of these changes. Rat, rabbit, dog, and human glomerular mesangial and epithelial cells may be co-cultured or each type derived separately (Kreisberg et al., 1977, 1978; Foidart et al., 1979, 1980, 1981; Morita et al., 1980; Striker et al., 1980; Kreisberg & Karnovsky, 1983). Rat epithelial cells are more sensitive to puromycin aminonucleoside and Adriamycin than are mesangial cells, as is the case *in vivo*, but there is little mechanistic information. Rat medullary interstitial cells can be cultured at high osmolality and have been shown to be sensitive to a number of compounds that cause renal papillary necrosis.

7.1.2.5 *Established renal cell lines*

Several established renal cell lines have been studied that have properties reminiscent of specific parts of the nephron, such as LLC-PK1 (of proximal tubule type) and MDCK (of distal tubule type). The major disadvantage is that the exact site of origin, within the nephron, of each, is not known, and it may not totally represent the normal physiological state. However, these lines are often heterogeneous and there is a need to characterize them more systematically so as to establish where they may be useful in screening chemicals for toxicity or in understanding the mechanisms of target cell toxicity.

Differences exist between the apical and basolateral membrane transport of substances into cells, which may be central to the mechanism of nephrotoxicity. When cells are cultured on solid surfaces, only apical exposure to chemicals occurs, whereas *in vivo*, proximal tubule cells are exposed from the apical or basolateral sides or both. This disadvantage can be overcome by culturing renal cells on microporous membranes suspended in culture wells. These cells, which form a confluent single-cell monolayer

covering the membrane within some days, more closely mimic the *in vivo* state than those grown on plastic plates. They show anatomical and functional polarization. This culture technique allows access to the cell monolayer from both the apical and the basolateral sides, and apical and basolateral fluid may be studied simultaneously. This new experimental tool allows the study of transport and epithelial resistance across the cell monolayer and polarized uptake of various molecules, including potentially nephrotoxic drugs, as well as to perform a variety of analytical techniques.

The various cell lines used in nephrotoxicity studies have been reviewed by Wilson (1986). The LLC-PK1 cell lines have a typical epithelial polarity and have features similar to proximal tubular epithelium, such as transport systems (Handler, 1983) and the enzyme marker γ-glutamyltranspeptidase (Perantoni & Berman, 1979). Confluent LLC-PK1 cells cultured on a solid support form domes (due to transcellular transport), but monolayers grown on a porous membrane do not. More importantly these cells have polarity and have a well-developed brush border. Confluent LLC-PK1 monolayers exposed to PCBD-GSH from the apical side are more sensitive than when exposed from the basolateral side. This is due to the brush border localization of γ-glutamyltranspeptidase, which catalyses the first step of the breakdown of the conjugate to the ultimate reactive intermediate. Neither apical nor basolateral treatment with PCBD-NAC elicits any toxicity. It is assumed that the absence of an organic anion transporter from these cells could explain this finding, since it has been established that haloalkene conjugates enter cells via the basolaterally located anion transporters (Lock et al., 1986). The absence of an organic anion transport system limits the usefulness of LLC-PK1 cell lines for studying nephrotoxic compounds, such as PCBD-NAC, that need active transport to enter the cells. However, an active basolateral organic cation transport system (γ-glutamyltranspeptidase and dipeptidase) makes these cells especially useful for testing compounds that have a toxic action on these transport systems.

7.1.2.6 *Subcellular fractions*

It is also possible (and sometimes desirable) to use homogeneous or fractionated organelles, membranes, or

cytoplasm from defined cells for specific cell-free investigations. The constraints on the preparation of these systems should be apparent from the foregoing discussion. Subcellular fractions, such as vesicles, nuclei, lysosomes, and microsomes, can be used to study subcellular distribution, the interaction between a cellular compartment and a chemical, and the kinetics of binding or release of substances. It is also possible to study specific effects, such as enzyme inhibition, metabolic activation, covalent binding, or the modulation of lipid peroxidation, using purified or commercially available biochemicals with appropriate cofactors and suitable techniques for monitoring these interactions (Bach & Bridges, 1985b, 1987).

Many nephrotoxic agents interact with cell membranes, where they bind with receptors, effect transport systems, or disrupt structure and function *per se*. Thus, membrane vesicles may be useful for studying these interactions and the mechanisms of cell injury. It is possible to isolate vesicles from the brush border and basement membranes to study transport systems at each site *in vitro*. Williams et al. (1986) showed a very good correlation between the *in vitro* binding of aminoglycosides to brush-border membrane vesicles and their *in vivo* nephrotoxicity. Inhibition of aminoglycoside membrane binding by polyaspartate reduces nephrotoxicity and suggests that binding of these antibiotics to brush-border phospholipid may be a crucial event in nephrotoxicity.

7.2 *In vivo* experimental studies

Current methods for diagnosing renal injury and predicting the health significance are not sufficient to deal with the diversity of possible chemical injuries (for full discussion, see Bach et al., 1989). This is because the kidney can undergo substantial chemically induced injury without any clinical indication, since subtle injury may be buffered within the considerable functional reserve. This masks a substantial amount of renal degeneration (Friedlander et al., 1989). Thus, for example, the single cross-sectional measurement of GFR may only show incipient acute or chronic renal failure. Quantitative urinary enzyme excretion patterns cannot identify either the type or severity of renal injury, and often they do

not correlate with morphological and functional changes (Schentag et al., 1978).

There are a number of inherent difficulties in diagnostic procedures for nephropathy, which include the absence of standard diagnostic criteria and the inability to relate exposure to a given agent and the observed effect. In addition, renal functional reserve is a major factor that masks renal degeneration, as assessed by GFR, blood urea nitrogen, and creatinine, up to the point where over 75% of the functioning nephrons have been lost. Thus, it should be stressed that these factors measure incipient renal failure and that the fact that values are normal (something that is subject to age-related change and varies between the two sexes) does not signify the absence of renal dysfunction or even, in some cases, gross renal insufficiency. Therefore, cause and effect cannot be clearly established on the basis of available knowledge when the renal lesion results from a multifactorial process with a long latency. Part of this uncertainty can be addressed by studies on experimental animals.

7.2.1 *Methods for assessing chemically reactive nephrotoxic metabolites in animals*

It is known that many nephrotoxicities that follow the administration of inert, relatively nontoxic chemicals are related to the formation of reactive electrophiles during the metabolism of these chemicals (Ford & Hook, 1984). These electrophilic products can react covalently with nucleophilic sites on renal macromolecules such as protein, lipid, and DNA. The covalent binding may be measured by the use of radiolabelled forms of the chemical, by immunological detection of DNA/protein adducts (Harris et al., 1987), or by the ^{32}P-DNA postlabelling method (Reddy et al., 1984). Furthermore, chemicals that are metabolized to DNA-damaging intermediates may be detected *in vivo* by measuring the alkaline elution of isolated kidney nuclei (Omichinski et al., 1987; Brunborg et al., 1988) or unscheduled DNA synthesis in isolated kidney cells (Tyson & Mirsalis, 1985) after *in vivo* exposure of animals.

7.2.2 *Evaluation of glomerular function*

Evaluation of blood urea nitrogen is probably the most common procedure for indirectly evaluating GFR in exper-

imental animals. Although insensitive, this test may be sufficient to establish the time course of chronically developing renal failure in the experimental setting. Serum creatinine is also used for the same purpose. However, owing to interferences from nonspecific chromogens, this test is unreliable in most experimental animals and especially in the rat. Although this problem may be overcome, it has not been dealt with adequately in most available studies, thus generating wide scatter in "normal" ranges.

In animals, more subtle changes in GFR occurring during subchronic and chronic studies should be assessed by evaluating the clearance of exogenous substances such as inulin, EDTA, or iothalamate. The latter may be determined either by measuring the radioactivity of labelled material or by means of reliable HPLC methods (Prueksaritanont et al., 1984). Furthermore, the same HPLC method may be used to measure PAH and to assess other haemodynamic parameters. Two (or more) clearance periods should be calculated and averaged in order to ensure greater accuracy.

There is a growing body of evidence to suggest that reduced renal reserve due to hypertension/hyperfunction/hyperfiltration of remnant nephrons is important in the course towards end-stage renal disease. This can in part be lowered by reducing protein intake and blood pressure. The concept of renal functional reserve includes the evaluation of renal blood flow and GFR by measuring their increase after protein load or the administration of aminoacids, glucagon, or vasodilatory drugs. At present such tests do not have defined standardized stimuli, and there are no data on their use for detecting nephrotoxicity.

7.2.3 *Evaluation of tubular functions*

In experimental animals, tubular dysfunctions are usually detected through simple and inexpensive tests, such as those of glycosuria, enzymuria, and osmolality, which may provide other useful information. Some of these tests are sensitive enough to detect acute tubular damage, although caution must be exercised in predicting specific effects on transport processes or cell viability on the basis of data obtained from *in vivo* experiments (Berndt,

1981). More subtle renal changes occurring during chronic studies may be evaluated by measuring the renal clearance of lithium (Dieperink et al., 1983; Daugaard et al., 1988b). This non-invasive method is applicable both to human (Thompson et al., 1984) and animal studies. The loss of renal tubular functions can be assessed by test procedures that impose one or more stressing conditions to force compensatory changes, e.g., maximal urinary dilution/concentration or acidification/alkalinization, and maximal tubular reabsorption of glucose and phosphate. The value of these tests is limited for group studies by practical considerations but may be useful for individuals.

7.2.4 *Proteinuria*

Proteinuria is the loss of proteins following increased permeability of the glomerular barrier, reduced tubular reabsorption of filtered proteins, or shedding of specific constituents into the urine as a consequence of cellular turnover or selective tissue damage. Since pathological changes either at the glomerular or tubulo-interstitial level may occur even in the absence of a substantial reduction in GFR, the evaluation of proteinuria may also be useful in some circumstances to detect renal dysfunction occurring either at the glomerular or at the tubular level. Proteins may be measured by nonspecific assays, by immunochemical methods or by their enzymic activity. Sensitive methods have been developed to detect small amounts of proteins in microlitre quantities of unconcentrated urine.

7.2.4.1 *Total proteinuria and electrophoretic pattern*

Measurement of total proteinuria and electrophoretic separation of single proteins provide a comprehensive approach to chemically induced renal dysfunction.

The rationale for such an approach relies on the pathophysiological mechanisms controlling the renal handling of plasmaproteins. Proteins of high relative molecular mass (> 45 000 Daltons) are usually confined to the vascular compartment by basal membranes. Furthermore, the glomerular polyanion acts as a selective filter that retains negatively charged proteins, such as albumin,

because of electrostatic interactions. The glomerular pore size is thought to have an important role in retaining proteins of higher relative molecular mass (e.g., immunoglobulins). Proteins with lower relative molecular mass (< 45 000 Daltons) pass the glomerular barrier with sieving coefficients inversely related to their mass. Filtered proteins of low relative molecular mass are efficiently taken up by the proximal tubules (more than 99%). Even slight decreases in tubular fractional reabsorption due to tissue damage or dysfunction will result in increased low relative molecular mass proteinuria (Fig. 16).

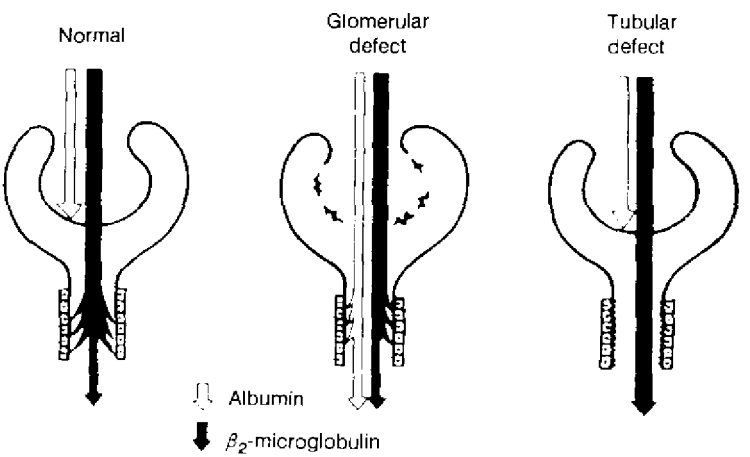


Fig. 16. Schemata of the mechanisms of glomerular and tubular proteinuria as expressed in the glomerular filtration and tubular reabsorption of albumin and β_2-microglobulin in normal subjects and in patients with glomerular and with tubular defects. In normal subjects 99.9% of the filtered β_2-microglobulin is reabsorbed by the proximal tubules. (Courtesy of P.W. Hall).

On the basis of the electrophoretic pattern, proteinuria may reveal glomerular damage, tubular dysfunction or mixed patterns. The glomerular damage may be selective (mostly due to a loss of glomerular polyanion) or unselective (involving more extensive damage, and glomerular hyperfiltration and hypertension). However, it should be

recognized that some features unique to experimental animals may account for large variations, which could lead to wrong conclusions. For instance, marked sex-, age-, and diet-related changes may occur, especially in the male rat (Neuhaus et al., 1981). Even if most of these changes have a counterpart in man, they are amplified by the lack of variations in housing conditions. As a result, the young male rat may physiologically show "tubular" proteinuria, whereas the aging rat displays "glomerular" patterns, owing to a spontaneous nephropathy, which can be prevented in part by reducing dietary protein content. Depending on other factors, especially diet and concomitant treatments, such electrophoretic patterns may be accounted for by underlying mechanisms and related morphological changes.

Owing to their limited affinity for most dyes, proteins of low relative molecular mass are better identified by the quantitative measurement of single components such as β2-microglobulin (Viau et al., 1986b). Because of its peculiar metabolism, the urinary excretion of α2u-globulin, a sex-related protein of low relative molecular mass, cannot be recommended as a marker of tubular damage. Furthermore, its renal handling and disposition may interfere with those of other proteins, accounting for some of the spontaneous changes occurring in the pattern of proteinuria from the male rat. Thus, when evaluating proteinuria, preference should be given to female rats, since in this case extrapolation to man seems to be less affected by species-related problems.

7.2.4.2 *Urinary excretion of single plasma proteins*

Very sensitive immunochemical methods are available for measuring the urinary excretion of single plasma proteins, such as IgG, albumin, β2-microglobulin and α2u-globulin in the rat (Bernard et al., 1988). In addition to better analytical features, in terms of sensitivity, specificity, accuracy, and reproducibility, the quantification of single urinary proteins has two other inherent advantages. Firstly, single proteins may be significantly increased without giving rise to pathological values in total proteinuria (Barratt, 1983). Secondly, the power of experimental studies is greatly increased by quantitative data making it possible to use parametric tests.

7.2.4.3 Enzymuria

Several different enzymes have been studied (Table 8), but none satisfies all the criteria for nephrotoxicity (Dubach et al., 1989). Enzymes are not uniformly distributed along or between nephrons. Although it should be possible to localize the area of kidney damage on the basis of the pattern of enzymuria, the site-selectivity of single enzymes is questionable. The failure to recognize selective damage by measuring enzyme activity may be accounted for by two factors. Firstly, it is possible that chemically induced early renal changes are less selective than advanced lesions preceding end-stage renal disease. Secondly, the poor analytical features of most enzyme measurements in urine may give rise to aspecific patterns. This important question may be addressed by measuring immunoreactive antigens, including enzymes, since the use of reliable immunochemical techniques would limit the effects of analytical problems.

Table 8. Some enzymes used as an index of nephrotoxicity

Enzymes	Cellular location
Alanine aminopeptidase	brush border
Alkaline phosphatase	
γ-Glutamyltransferase	
Maltase	
Trehalase	
Glutamic oxaloacetic transaminase	cytosol
Glutamic pyruvic transaminase	
Lactate dehydrogenase	
Malate dehydrogenase	
N-Acetyl-β-D-glucosaminidase	lysosome
Acid phosphatase	
β-galactosidase	
β-glucosidase	
β-glucuronidase	
Glutamate dehydrogenase	mitochondria

Most enzymes are stable over a narrow range of pH, and their activity may be affected by the presence of inhibitors such as urea (Price, 1982). It should be stressed

that all rat enzyme studies must be carried out under carefully controlled conditions, after an adequate period of acclimatization, and after changing to day instead of night feeding. Urine must be minimally contaminated with microorganisms. This is achieved by surrounding the urine collecting vessel with ice, so that bacteria cannot multiply as readily as in normal metabolic cages (Berlyne, 1984).

7.2.4.4 *Immunoreactive tissue constituents*

Tissue constituents may be released into urine due to increased cellular turnover or cell death and may be detected by immunochemical methods. Monoclonal antibodies have been produced against both rat (Tokoff-Rubin, 1986) and human brush-border antigens (Mutti et al., 1985; Mutti, 1989). In both cases, cross-reactivity between species has been shown (Mutti, 1987, 1989). The specificity of such an approach relies on the site-selectivity of target proteins and on the advantages of monoclonal antibodies, including monospecificity and reproducibility of reagents. Its sensitivity has been proved by comparison with other markers in various situations, but it needs further validation in carefully designed chronic studies, since the prognostic value of slight changes in such a sensitive test is currently unknown.

In general terms, it has been through the use of histopathological studies on kidney tissue that advanced renal lesions have been identified. This approach has many inherent advantages, and represents the method of choice, particularly if it can be used for the early diagnosis of renal lesions or dysfunction in experimental animals being used for nephrotoxicity screening studies. For clinical investigations in human and population studies, it is obviously the least desirable of the techniques that are available.

7.2.4.5 *Urinary excretion of prostaglandins*

The urinary excretion of PGs (mainly PGE_2) may reflect the rate of renal synthesis. This is modified in several nephropathies and may be affected by a number of nephrotoxic chemicals.

7.2.5 Clinical context

At present, the clinical diagnosis of toxic nephropathy still relies heavily on the case history of patients showing symptoms and/or laboratory abnormalities suggesting chronic renal failure without any obvious recent cause.

In such circumstances, history is the cornerstone of diagnosis. It can only be made by excluding other known conditions or risk factors that lead to renal insufficiency and assessing exposure to nephrotoxins, which has to be consistent with known dose-effect/response relationships and with the temporal sequence of events leading to the observed effect. For immune reactions, the role of individual susceptibility should also be considered. In some circumstances, kidney toxicity may be considered as a factor contributing to the clinical outcome in a multifactorial process. In this case, it is difficult to distinguish determinants from predisposing and/or aggravating factors.

There are two well-recognized clinical syndromes that result from immunologically mediated glomerular disease. These are the nephrotic syndrome and the nephritic syndrome. The nephrotic syndrome is characterized by massive proteinuria, hypoalbuminaemia (from proteinuria) and generalized oedema. The syndrome is the result of pathology that affects the integrity of the GBM. Although the cause of 85% of nephrotic syndrome is not known, it is the syndrome most frequently occurring with toxic nephropathies, it is generally dose related, and it is reversible. Glomerular pathology is most often membranous glomerulonephritis or circulating immune-complex disease. The nephritic syndrome is characterized by haematuria and a decreasing GFR and is likely to be accompanied by some degree of hypertension. The pathological changes in the glomeruli are usually caused by those diseases or toxic agents that produce an inflammatory proliferative response within the glomeruli. The proliferation may involve endothelial mesangial or epithelial cells and may be associated with an inflammatory cell infiltration.

7.2.6 Radiological techniques

Radiocontrast media can be used to study the kidney either by conventional or by retrograde pyelography. There

are a number of limitations to this technique, such as the need for adequate renal function by which to image the organ. There is adequate evidence that radiocontrast agents have a nephrotoxic potential. Media of high osmolality are especially likely to precipitate renal failure. In addition, those patients who are dehydrated or with reduced blood volume are at special risk. There is also evidence to show that patients who have multiple myeloma represent a special risk group.

7.2.7 *Other non-invasive renal assessment*

Whereas gamma camera renography and ultrasound are well-established techniques for the assessment of renal function, increasing use is being made of ultrasound linked with Doppler flow, nuclear magnetic resonance imaging, and spectoscopy; ureteral fibroscopy may be used to view the pelvis directly. Ultrasound evaluation of the kidneys provides a means of excluding obstruction as a cause of anuria or oliguria in ARF. It may also reveal papillary necrosis or perirenal haematomas and obviate the intrinsic dangers of pyelography. Radionuclide scans can be used to identify major atherothrombotic events and cortical necrosis. They can also be used to show reduced renal blood flow, which is usually preserved (some 50% of normal) in acute tubular necrosis, but is severely compromised in acute glomerulonephritis and vasculitis. Renal biopsy should not be used for ARF unless the duration exceeds three weeks and there is no obvious cause. It can then help diagnose glomerulonephritis, vasculitis, and AIN.

8. DETECTION OF NEPHROTOXICITY IN HUMANS

Traditional methods of diagnosing renal damage and predicting their health significance are not sufficient to deal with the diversity of chemically induced lesions (Bach et al., 1989). This is because the kidney can undergo substantial chemically induced injury without any clinical indication, owing to its considerable functional reserve. This masks a substantial amount of renal degeneration (Friedlander et al., 1989). Thus, for example, the single common measurement of GFR may only show incipient acute or chronic renal failure.

Most nephropathies in humans are of unknown origin. There is, however, some indirect evidence that toxicant exposure could be involved. For example, 80% of the cases of membranous nephropathy are of unknown origin. Among the remaining 20%, half of them have been found to be associated with drug exposure. It is likely, therefore, that a percentage of those cases of unknown origin are also related to toxic exposure.

8.1 Markers of nephrotoxicity

Over the last ten years, new biochemical and immunochemical methods have been developed for detecting early renal changes in humans. Their application has given rise to a number of markers, some of which also are available for studies on experimental animals and have been reviewed in section 7.2. Although some tests may open new perspectives in terms of selectivity, sensitivity, and specificity, most of the recently developed markers need further validation. Hence, some conceptual and methodological problems must be addressed and new methods critically evaluated. This is especially important when implementing screening programmes for the early detection of kidney damage and/or dysfunction in humans.

8.1.1 General requirements

Markers to be used for screening purposes in population groups should fulfill certain general criteria, including specificity, non-invasiveness, sensitivity in

detecting early renal functional changes, and predictive value for development of renal insufficiency. Analytical methods must be reproducible, easy to perform, and applicable to a large number of samples, and the samples must be stored appropriately to ensure stability.

The prevalence of true positive results among subjects who are actually ill indicates the sensitivity of the marker, whereas the prevalence of true negative findings among healthy individuals is a measure of its specificity.

Some markers meet the requirement of specificity, although it may be difficult to establish clear-cut distinctions in individual cases. For instance, whereas gross changes in proteinuria involving proteins of low and high relative molecular mass indicate tubular and glomerular damage, respectively, marginal increases over reference limits may be due to either condition (Mutti, 1989).

The predictive value of markers of nephrotoxicity has not been systematically tested, but it is usually assumed that such markers as the urinary excretion of single plasma proteins are highly sensitive but somewhat aspecific. This is due to the fact that they may be increased by a number of physiological conditions (e.g., physical workload, posture, pharmacological effects of exogenous substances, or even meat meals). On the contrary, other markers such as serum creatinine are considered to be relatively specific, but relatively insensitive, since they reflect late changes, occurring when more than 50% of the renal reserve has been lost. Within these extremes, there are various possibilities which should be carefully weighed according to the methodological context dealt with.

The predictive value of markers, in terms of sensitivity and specificity, should be taken into account when evaluating individual results. For some parameters (e.g., proteinuria involving proteins of low relative molecular mass) minor dysfunctions at the tubular level will result in deviations of several orders of magnitude from reference values, whereas the same relative change in other parameters (e.g., brush-border antigens, enzyme activities) may indicate severe tubular injury.

In the clinical context, it is often difficult to establish correct etiological diagnoses because of the

long latency between exposure and the development of overt disease. For the same reason, it is sometimes difficult in epidemiological work to assess the prognostic value of early changes. Nonetheless, it is clear that markers used in the clinical context must be specific enough to avoid further undue and sometimes invasive procedures, which tend to accumulate once a subject enters a diagnostic decision tree (Mold & Stein, 1986).

The quantification of any constituents in urine may only be obtained by assessing urine flow rate. The accuracy of timed urine samples (especially when obtained during epidemiological surveys) is unreliable and most analytes may be unstable. There are still a number of factors that can confound or modify the renal response, such as time of sampling (owing to spontaneous rhythms), posture, physical work load, and diet. Thus, there is a need to use standardized procedures to limit possible interferences that can increase the variability between and within subjects. To overcome these methodological problems, there is an increasing tendency to use spot samples, generally the second sample of the day, and to express results as a function of creatinine.

8.1.2 *Diagnostic value*

The predictive value of a marker only in part contributes to its diagnostic validity, which is defined in terms of the probability that the classification based on test values actually corresponds to the subject's condition. The diagnostic value may be both positive and negative, the positive diagnostic value being the probability that a subject classified as positive by the test is actually ill, and the negative diagnostic value being the probability that subjects negative at the test are actually healthy individuals. It must be stressed that the diagnostic validity is only marginally affected by the predictive value of the markers, since in most screening programmes a low prevalence of disease is the major determinant of pitfalls.

Thus, markers must be selected according to the condition under evaluation. When studying groups at risk, emphasis should be given to sensitivity, whereas specificity should receive adequate attention in the clinical setting.

8.1.3 Prognostic value

Another important property of markers, which has so far only been addressed in a few studies, is their prognostic value, i.e. their ability to predict the "natural" course of the disease. In most cases early functional changes may be compensated and structural damage may be repaired. However, both conditions may also trigger a cascade of events leading to end-stage renal disease. The ability to target groups actually at risk and to predict the "natural" evolution of the disease is part of effective prevention. This can be achieved by reducing exposure (primary prevention) or by establishing an individual diagnosis at reversible stages (secondary prevention).

Microscopic examination of urine sediment, although impractical in population studies, may help in establishing individual differential diagnosis. Even if it is insensitive for detecting nephrotoxicity, this test is suitable at the individual level to exclude confounding factors accounting for increased excretion of plasma proteins (e.g., leukocyturia, haematuria, or bacteriuria).

8.2 Screening for nephrotoxicity in humans

In the epidemiological context, it can be assumed that negative results are recorded in actually healthy subjects, whereas the probability of false positive values is rather high because of the low prevalence of the disease. Thus, health surveillance programmes should be mainly aimed at excluding harmful situations rather than at identifying ill people. This goal may be achieved using very sensitive markers of subclinical renal impairment. A clinical diagnosis in individuals showing abnormal test values should then be established only after repeated measurements and complementary confirmatory tests. Practical considerations are also important when planning population studies, where sampling procedures that are invasive or too elaborate cannot be included in the protocol.

8.2.1 Glomerular filtration

Clearance procedures can seldom be adopted when screening population groups, since only single blood and

spot urine samples are usually available. However, it must be stressed that some tests fulfilling the above objective have proved their diagnostic and prognostic validity and for this reason they are now also being employed in clinical practice.

An indirect method of assessing GFR by using a single blood sample is the measurement of serum creatinine and the expression of its reciprocal value adjusted to constant body surface area. This has been correlated with GFR (Siersbaek-Nielson et al., 1971). The same concept applied to β2-microglobulin would increase the sensitivity of such an approach, owing to the higher relative molecular mass and the use of more accurate and reproducible analytical methods (Wibell et al., 1973).

8.2.2 *Tests designed to assess selective dysfunction*

Serious renal diseases (e.g., nephrotic or Fanconi's syndromes) may occur in the absence of haemodynamic changes. In such circumstances, renal insufficiency is a late event that is preceded by earlier, though serious, selective dysfunctions occurring at the glomerular and proximal tubular level. These may be revealed by measuring single plasma proteins in urine (see also section 8.3.3.). The excretion in urine of proteins of high relative molecular mass generally reflects glomerular dysfunction, whereas urinary excretion of proteins of low relative molecular mass may be indicative of tubular dysfunction. The assessment of protein electrophoretic patterns has been discussed in section 7.2.4.

8.2.3 *Tests designed to assess tissue damage*

Tubular dysfunction is not necessarily associated with histopathological changes. It has been shown that pharmacological inhibition of tubular uptake may account for proteinuria (involving proteins of low relative molecular mass) observed after protein load (Buzio et al., 1989). Furthermore, highly selective damage to a tubular segment may be functionally compensated by other segments of the nephron. Methods aimed at detecting cellular damage may thus help both to show subclinical lesions and to interpret associated dysfunctions. The urinary excretion of

tissue constituents may be measured through the enzymic or immunochemical characteristics of material shed into the urine.

Renal functional changes may be reversible, depending on the efficiency of repair mechanisms and the cessation of exposure to the offending agent. Repeated monitoring may help to distinguish progressive renal disease from transient lesions (Thornley et al., 1985).

8.2.3.1 *Enzymuria*

The general principles describe in section 7.2.4.3. also apply to humans. Even when using freshly voided spot samples, urine is a hostile environment for most enzymes. Only a few enzymes (e.g., *N*-acetly-β-D-glycosaminidase) show acceptable stability and analytical precision (Price, 1982). Table 8 lists some human urinary enzymes that have been used in nephrotoxic studies.

8.2.3.2 *Immunoreactive tissue constituents*

Tissue constituents (including enzymes) are physiologically shed into the urine as a consequence of cell turnover and metabolism. When they are detected by immunochemical methods (e.g., immunofluorescence, enzyme-linked immunosorbent assay), they are referred to as antigens. Tamm-Horsfall glycoprotein is a specific renal protein, localized on the membrane of cells of the thick ascending limb of the loop of Henle, which is excreted in the urine at a relatively constant rate. This can increase following injury to the distal part of the tubule and is depressed when the renal mass is reduced (Thornley et al., 1985). Although increased excretion of proximal tubular antigens was reported twenty years ago in various clinical situations such as tubular necrosis, allograft rejection, and cephalotin/gentamicin-induced nephrotoxicity (Antoine et al., 1969; Rosenmann et al., 1971; Scherberich et al., 1976, 1984), only recently has it been possible to improve significantly the specificity and reproducibility of such an approach, relying on the properties of monoclonal antibodies.

Monoclonal antibodies to human brush-border antigens have been produced by Mutti et al. (1985). Monoclonal

antibodies may be conveniently employed in ELISA procedures set up to detect trace amounts of antigens in biological samples. Although the BB-50 brush-border antigen was also localized in peritubular capillaries (Mutti et al., 1985), subsequent work lead to the identification of a monoclonal antibody reacting with an antigen located specifically in the brush border and thus called BBA or brush-border antigen (Mutti et al., 1988). Promising results have been obtained in several cross-sectional investigations on groups at risk of chemically induced renal damage (for a review, see Mutti, 1989). Similar results were obtained with monoclonal antibodies to rat brush-border cross-reacting with the human kidney (Tokoff-Rubin et al., 1986).

Monoclonal antibodies have also been produced that react specifically with other segments along the nephron (namely S_3) where the intestinal isoform of alkaline phosphatase is located (Verpooten et al., 1989). They could be used to monitor the effects of chemicals (e.g., mercury) acting selectively on the straight part of proximal tubules. All of these recently developed tests need further validation in well-designed longitudinal studies, since their prognostic value is currently unknown.

8.3 Clinical investigations

The clinical diagnosis of toxic nephropathy frequently relies heavily on the history of patients who occasionally showed symptoms and/or laboratory abnormalities suggesting chronic failure without any obvious recent cause.

Diagnosis can only be made by excluding other known conditions or risk factors. This has to be assessed by estimating exposure to nephrotoxins in relation to the known dose-effect/response relationship and the temporal sequence of events that follow such exposure. The role of individual susceptibility should also be considered. In some circumstances, nephrotoxicity may be one factor in a multifactorial process leading to clinical renal disease. Retrospective data about exposure and early changes in renal function are usually not available.

Most progressive kidney diseases have a subtle onset. This is the reason why the markers designed for use in

epidemiological studies are becoming part of clinical investigations.

Owing to the short latency between exposure and the development of severe symptoms, acute renal failure should be accurately diagnosed in all cases. The simple evaluation of serum creatinine (increases of 3-5 mg/litre or 50% above baseline values) makes it possible to detect nephrotoxic reactions.

8.3.1 *Invasive techniques*

The use of invasive techniques is limited by ethical constraints.

8.3.1.1 *Biopsies from humans*

Renal biopsy is the only way to identify glomerular, tubular, or interstitial renal diseases. However, the risk-to-benefit ratio must be considered carefully in each individual patient being evaluated, and there is no universal agreement on the conditions in which percutaneous renal biopsy may be useful.

8.3.1.2 *Autopsy in humans*

Autopsies performed on patients with end-stage renal failure only reveal the etiology of the renal disease in a small percentage of cases.

8.3.2 *Tests designed to assess glomerular filtration and renal blood flow*

Traditional methods based on inulin and PAH clearances are progressively being substituted by more convenient methods such as the clearance of ^{51}Cr-EDTA and ^{99}Tc-DTPA to assess GFR or the clearance of ^{125}I-hippuran to measure renal plasma flow. These techniques are thought to be more sensitive than creatinine clearance and more accurate than colorimetric methods.

8.3.3 *Proteinuria*

Section 7.2.4. contains a detailed discussion of experimental studies involving proteinuria. Table 9 gives

Table 9. Excretion rates of common urine proteins

Protein	Relative molecular mass (Daltons)	Normal range
Albumin	68 000	< 30 mg/day
β2-microglobulin	12 800	< 0.3 mg/day
Retinol-binding Protein	21 400	< 0.3 mg/day
IgG	160 000	2-3 mg/day
α2-microglobulin	± 30 000	?

information on some proteins excreted in human urine. Values that are in excess of the normal range may be indicative of renal dysfunction.

8.3.4 *Tests designed to assess selective damage*

Tissue constituents may be shed into the urine following toxic damage to specific structures. All of these specific antigens may be detected by using immunochemical or biochemical methods designed to measure their concentration or enzymic activity, respectively. Although they have been designed expressly for evaluating population groups, these tests may also be useful in the clinical setting to monitor patients at risk. Even if they are very sensitive and relatively specific, they need further validation under carefully controlled clinical conditions, particularly in longitudinal studies.

9. SUMMARY AND CONCLUSIONS

The kidneys are the main organs of excretion and homeostasis for water-soluble molecules. The functional unit of the kidney is the nephron, essentially a continuous tube of highly specialized heterogeneous cells, which exhibits marked structural, functional, and biochemical organization. There are pronounced differences between the nephrons themselves, depending on the cortical localization of the individual glomeruli. This complex structural organization, combined with differences in regional vascularity arising from the specialized vascular arrangement, produces a highly complex heterogeneous organ.

Much of the anatomical and functional understanding developed from animals is directly applicable to the human kidney. However, the biomedical and metabolic processes in the human kidney, as well as the differences among animal species, have not been as thoroughly elucidated. Thus, the ability to extrapolate the effects of chemicals among species is limited.

Several chemicals (both therapeutic and non-therapeutic) have toxic effects on one or more anatomical elements of the kidney. Toxic effects may be acute or chronic, and they may be direct or mediated indirectly through immunological mechanisms. The health impact of nephrotoxic chemicals is related to risk factors, which include the intergrade of the renal functional reserve and factors such as pre-existing renal damage, disease, age, sex, and diet.

The epidemiology of chemically induced nephrotoxicity by individual chemicals or in mixed exposures has been inadequately studied. The contribution of chemicals to the overall incidence of nephropathy and of chronic renal failure is, with few exceptions, undefined. In the case of some occupationally exposed groups and analgesic associated renal disease, there has been extensive research that has shown variations in incidence between groups and countries. However, it is estimated that up to 18% of end-stage renal disease may be due to analgesic nephropathy and up to 5% to other toxic nephropathies. About 50% of

end-stage renal disease is of unknown etiology. A major problem in assigning a cause for end-stage renal disease is the long latency and/or slow development of chronic renal failure, which makes retrospective identification of the causative agent difficult. In only a few cases is measurement of tissue (body, kidney) levels of chemicals relevant to the diagnosis. A further problem has been the lack of consistency in diagnostic and pathological criteria and terminology.

Renal anatomical and physiological differences make direct extrapolation from experimental systems *(in vivo* and *in vitro)* to humans difficult. There are very few examples of nephrotoxic chemicals where there are adequate comparable data from animals and humans to form a firm basis for the assessment of potential human health risk.

Chemicals may damage selectively vulnerable kidney structures or activate immunological mechanisms. Mechanisms of renal injury fall generally into two categories: (a) immunologically induced disease of acute interstitial nephritis; (b) those that primarily affect the glomerulus by either anti-GBM-mediated antibodies or immune complexes. Another major group is composed of diseases initiated by chemicals or their metabolites that interfere with cellular biochemical and haemodynamic effects, etc. Factors that can modify cellular injury by toxicants include cellular transport systems, pinocytosis, metabolic degradation, and interaction with cellular proteins, lipids, membranes, DNA, and perhaps other cellular constituents.

The increasing use of therapeutic agents and chemicals increases the possibility of nephrotoxicity. Nephrotoxicity induced by therapeutic agents depends on the dose and duration of exposure (e.g., combination analgesics leading to renal papillary necrosis). Nephrotoxic effects of analgesics, antibiotics (such as the aminoglycosides), anticancer agents (such as *cis*-platinum), and a variety of other agents have been investigated extensively. Chemicals frequently used in industry or the home, e.g., chlorinated hydrocarbons and ethylene glycol, also have the potential to produce renal damage. Environmental chemicals such as lead and cadmium are capable of inducing nephrotoxicity. These agents act as toxicants after

intracellular accumulation of the parent compound or after renal or extrarenal biotransformation. Multichemical exposure may result in antagonistic or synergistic responses.

Tumours of the kidney and urinary tract account for less than 2-3% of all human cancers, but the frequency of these tumours is increasing, suggesting a role for environmental factors. Although many drugs and chemicals have been shown to be carcinogenic in experimental models, only a few specific substances have been related to tumours in man. These include asbestos (renal adenocarcinoma), analgesic abuse (transitional carcinomas), and lifestyle factors (tobacco use, alcohol, coffee). There are population groups with urinary tract cancer of as yet undetermined etiology. Occupational chemicals have been related to the etiology of cancer of the urinary bladder. Many drugs and chemicals cause interstitial nephritis, which may in itself be a factor in developing urinary tract cancers.

No single *in vivo* or *in vitro* method is sufficient to assess chemically induced renal dysfunction. Therefore, it is advisable not only to screen compounds for nephrotoxic potential, but to incorporate mechanistic studies of target cell toxicity into the experimental protocols. To accomplish this, both *in vivo* and *in vitro* investigations should be utilized in concert. *In vitro* studies involve those where anatomical cellular relationships are maintained (e.g., isolated perfused kidney and tubules, renal cortical slices, and isolated nephron segments) and those where renal cells are used (e.g., cell suspension, primary cell cultures, established cell lines, and subcellular fractions). *In vivo* studies utilize both invasive and non-invasive techniques. Invasive procedures include histopathological and routine renal function measurements. Non-invasive procedures permit repeated assessment of renal function in animals through the measurement of an array of renal function tests (glomerular filtration, tubular function, proteinuria, enzymuria, etc.). Specialized biochemical tests should be used where relevant. The appropriate mixture of *in vivo* and *in vitro* experiments will reveal whether or not chemicals are nephrotoxic and give insights into potencies, sites, and mechanisms of toxicity.

Traditional methods for the assessment of renal function in humans are inadequate for the timely diagnosis of chemically induced renal dysfunction and prediction of its health significance. The lack of specific, early markers for nephrotoxicity is particularly troublesome. Non-invasive assessment of nephrotoxity should employ markers of high specificity, sensitivity for detection of early renal changes, and predictive value for the development of renal insufficiency. Present techniques for monitoring glomerular or tubular function are useful only when severe renal damage has developed. Although immuno-reactive tissue constituents are being suggested as appropriate markers, their suitability needs to be validated in well-designed longitudinal studies.

10. RECOMMENDATIONS

1. A continued effort should be made to develop and validate more selective and specific markers, including monoclonal antibodies, for assessment of renal dysfunction in animals. These markers may be applicable to humans.

2. The data base for predicting the potential of chemicals for human nephrotoxicity should be extended. This includes development and validation of experimental animal approaches (*in vivo* and *in vitro*), alternative methods for studying nephrotoxicity, information on interspecies differences, and experience from the preclinical evaluation of new therapeutic agents in humans.

3. Epidemiological studies (i.e. prospective studies in occupational and general population groups exposed to nephrotoxic chemicals or involved with analgesic abuse) should be reinforced.

4. More effort should be made to establish the role of chemicals in the etiology of renal disease at the earliest diagnostic stage (e.g., work history, tissue monitoring for nephrotoxins).

5. Understanding of the mechanisms of action of nephrotoxicants will be helpful in the prevention and clinical management of unwanted renal effects, and may help in predicting the nephrotoxic potential for new drugs and chemicals. Areas of particular importance for further research are:

- immunological mechanisms;
- direct effects of chemicals on membranes, including mechanisms of lipid peroxidation, membrane/chemical interaction, ion shifts, and receptor-mediated events;
- activation of proto-oncogenes and cell differentiation;
- regulation of cellular metabolism.

6. There is a need to identify and correlate specific functions with discrete anatomical locations within the kidney.

7. The role of genetic variation and susceptibility to the toxic effects of drugs and chemicals should be studied further.

8. The relationship between nephrotoxicity and renal carcinogenesis (e.g., mycotoxins and Balkan nephropathy) needs further study.

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RESUME ET CONCLUSIONS

L'excrétion et l'homéostase des molécules hydrosolubles sont principalement assurées par les reins. Ceux-ci sont constitués d'unités fonctionnelles appelées néphrons, qui consistent essentiellement en un tube continu formé de cellules hétérogènes hautement spécialisées qui présentent une organisation structurale, fonctionnelle et biochimique poussée. Il existe des différences marquées entre les néphrons eux-mêmes, qui dépendent de la localisation plus ou moins profonde du glomérule dans le cortex. C'est l'agencement complexe de cette structure qui, joint à des différences régionales de vascularisation tenant à la disposition particulière des vaisseaux, constitue cet organe hétérogène et hautement organisé qu'est le rein.

Une grande partie des connaissances sur l'anatomie et le fonctionnement du rein, obtenues grâce à l'expérimentation animale, est directement transposable à l'homme. Toutefois, les processus biologiques et métaboliques qui se déroulent dans le rein humain ne sont pas encore complètement élucidés, non plus que les différences constatées d'une espèce animale à une autre. Dans ces conditions, on comprend qu'on ne puisse pas toujours extrapoler d'une espèce à une autre les effets toxicologiques observés.

Plusieurs substances (qu'elles aient ou non un effet thérapeutique) exercent des effets toxiques sur les divers éléments anatomiques du rein. Les effets toxiques peuvent être aigus ou chroniques; ils peuvent être directs ou se produire par l'intermédiaire de mécanismes immunologiques. L'impact qu'une substance néphrotoxique peut avoir sur la santé dépend de plusieurs facteurs de risque: état fonctionnel du rein, lésion préexistante, maladie, âge, sexe, régime alimentaire, etc..

On n'a pas suffisamment étudié l'épidémiologie des cas de néphrotoxicité imputables à des diverses substances chimiques agissant seules ou en association. A quelques exceptions près, la contribution des produits chimiques à l'incidence globale des néphropathies et de l'insuffisance rénale chronique reste mal définie. D'importantes recherches ont été consacrées à certains groupes exposés

de par leur profession ainsi qu'aux néphropathies résultant de la consommation d'analgésiques : elles ont montré l'existence de variations entre les différents groupes et les différents pays. Toutefois on estime que jusqu'à 18% des néphropathies terminales pourraient être dues à la prise d'analgésiques et jusqu'à 5% avoir une origine toxique quelconque. Environ 50% des néphropathies terminales sont d'origine inconnue. L'un des principaux problèmes qui se posent dans la recherche de l'étiologie des néphropathies terminales tient à la longue période de latence ou à l'évolution lente de l'insuffisance rénale chronique, qui rendent difficile l'identification rétrospective de l'agent causal. Ce n'est que dans quelques cas que le dosage de certaines substances dans les tissus (organisme, rein) peut conduire au diagnostic. Par ailleurs on se heurte également à un certain manque de cohérence dans les critères et la terminologie utilisés sur le plan diagnostique et anatomopathologique.

Les différences anatomiques et physiologiques rendent difficile une extrapolation directe à l'homme des résultats obtenus sur des systèmes expérimentaux (*in vivo* ou *in vitro*). On ne connaît que très peu de cas où les données de néphrotoxicité sont comparables chez l'homme et chez l'animal et permettent d'évaluer de façon fiable le risque pour la santé humaine.

Certaines substances chimiques peuvent provoquer des lésions sélectives au niveau des structures rénales vulnérables ou déclencher des mécanismes immunologiques. On considère qu'il existe en général deux types de mécanismes à la base des lésions rénales : a) les mécanismes immunologiques qui conduisent à la néphrite aiguë interstitielle et b) les mécanismes qui affectent principalement le glomérule par l'intermédiaire d'anticorps anti-GBM ou d'immunocomplexes. Il existe un autre grand groupe de maladies dues à des substances chimiques ou à leurs métabolites qui perturbent l'activité biochimique cellulaire ou modifient les propriétés hémodynamiques, etc. Parmi les facteurs susceptibles d'influer sur les lésions cellulaires provoquées par les produits toxiques figurent les systèmes de transport cellulaire, la pinocytose, la dégradation métabolique, l'interaction avec les protéines, les lipides, les

membranes et l'ADN cellulaires et éventuellement avec d'autres constituants de la cellule.

Plus on utilise de produits pharmaceutiques et de substances chimiques, plus on accroît les risques de néphrotoxicité. La néphrotoxicité des médicaments dépend de la dose et de la durée du traitement (par exemple dans le cas des associations d'analgésiques qui conduisent à une nécrose papillaire). On a beaucoup étudié les effets néphrotoxiques des analgésiques, des antibiotiques (comme les aminoglycosides), des agents anticancéreux (comme le *cis*-platine) et de divers autres agents. Nombre de substances fréquemment utilisées dans l'industrie ou à la maison comme les hydrocarbures chlorés ou l'éthylèneglycol peuvent également avoir une action toxique sur le rein. D'autres substances, présentes dans l'environnement comme le plomb ou le cadmium sont effectivement néphrotoxiques. L'action toxique de ces substances intervient après accumulation intracellulaire du produit initial ou métabolisation intra- ou extrarénale. L'exposition à plusieurs substances chimiques peut entraîner des réactions antagonistes ou synergistiques.

Les tumeurs du rein et des voies urinaires représentent moins de 2 à 3 % de l'ensemble des cancers humains, mais leur fréquence est en augmentation, ce qui incite a penser que des facteurs environnementaux pourraient être à l'oeuvre. Nombre de produits pharmaceutiques et de substances chimiques se sont révélés cancérogènes sur des modèles expérimentaux mais seuls quelques-uns d'entre eux sont responsables de l'apparition de cancers chez l'homme. Il s'agit de l'amiante (adénocarcinome rénal), de l'abus d'analgésiques (carcinomes de type transitionnel) et de facteurs tenant au mode de vie (tabagisme, consommation d'alcool et de café). Il existe des groupes de population chez lesquels certains cancers des voies urinaires ont une étiologie encore inconnue. Certaines substances chimiques utilisées dans le cadre professionnel sont à l'origine de cancers de la vessie. De nombreuses substances chimiques ou pharmaceutiques peuvent entraîner une néphrite interstitielle, affection qui serait susceptible de favoriser l'apparition de cancers des voies urinaires.

Il n'existe pas de méthode qui permette d'évaluer *in vivo* ou *in vitro* dans des conditions satisfaisantes une

insuffisance rénale provoquée par des substances chimiques. Aussi faut-il non seulement contrôler l'activité néphrotoxique des produits chimiques, mais également inclure dans les protocoles expérimentaux l'étude du mécanisme toxique au niveau des cellules cibles. Pour y parvenir il faut faire appel tant à l'expérimentation *in vivo* qu'à des études *in vitro*. Les études *in vitro* doivent porter sur des éléments où les relations anatomiques et cellulaires sont maintenues (par microperfusion de reins et de tubules isolés, coupes de cortex, fragments isolés de néphrons, etc.) et sur des cellules rénales (par exemple suspensions cellulaires, cultures cellulaires primaires, lignées cellulaires et fractions infracellulaires). Pour les études *in vivo*, on peut faire appel à des techniques effractives ou non effractives. Parmi les techniques effractives figurent les examens histopathologiques ainsi que les techniques classiques de bilan de la fonction rénale. Les techniques non effractives permettent un bilan répété de la fonction rénale chez l'animal par la mesure d'une série de paramètres fonctionnels (filtration glomérulaire, fonction tubulaire, protéinurie, enzymurie, etc.). Des épreuves biochimiques spécialisées devront être effectuées le cas échéant. En associant de façon convenable les épreuves *in vivo* et les épreuves *in vitro* on pourra déterminer si telle ou telle substance chimique est néphrotoxique et avoir une idée de son activité, de son site d'action et du mécanisme de son action toxique.

Les méthodes habituellement utilisées pour l'exploration fonctionnelle du rein chez l'homme ne permettent pas un diagnostic suffisamment rapide d'une insuffisance rénale due à des substances chimiques ni d'en apprécier le retentissement sur la santé. L'absence de marqueurs spécifiques précoces de la néphrotoxicité est particulièrement gênante. Les méthodes non effractives devront faire appel à des marqueurs très spécifiques et sensibles, qui permettent de déceler rapidement les modifications qui se produisent au niveau rénal afin de prévoir l'apparition éventuelle d'une insuffisance fonctionnelle. Les techniques actuelles de contrôle de la fonction glomérulaire ou tubulaire ne sont utilisables que lorsque les lésions sont déjà très importantes. On a proposé comme marqueurs l'utilisation de constituants

tissulaires immunoréactifs mais encore faut-il en confirmer la valeur au moyen d'études longitudinales bien conçues.

RECOMMANDATIONS

1. Il faut faire un effort soutenu pour développer et valider des marqueurs plus sélectifs et plus spécifiques et notamment des anticorps monoclonaux en vue d'étudier l'insuffisance rénale chez l'animal. Ces marqueurs pourraient être utilisables chez l'homme.

2. Il faudrait compléter la base de données utilisables pour déterminer le potentiel néphrotoxique des substances chimiques pour l'homme. A cet effet, on développera et on validera diverses méthodes d'expérimentation animale (*in vivo* et *in vitro*), on mettra au point des méthodes nouvelles d'étude de la néphrotoxicité, on étudiera les différences interspécifiques et on prendra en compte l'expérience tirée des essais précliniques de nouveaux médicaments chez l'homme.

3. Il faudrait renforcer les études épidémiologiques (c'est-à-dire les études prospectives sur des groupes professionnels ou des groupes de la population générale exposés à des substances néphrotoxiques ou qui font une consommation excessive d'analgésiques).

4. Des efforts plus importants devront être consentis afin d'établir le rôle de certaines substances chimiques dans l'étiologie des maladies rénales au stade le plus précoce possible (antécédents professionnels, recherches de néphrotoxines dans les tissus).

5. L'élucidation du mode d'action des substances néphrotoxiques peut être utile à la prévention et au traitement des effets rénaux indésirables et pourrait contribuer à la prévision du pouvoir néphrotoxique des nouveaux médicaments et des nouveaux produits. Parmi les secteurs de recherche particulièrement importants on peut citer :

- les mécanismes immunologiques
- les effets directs des substances chimiques sur les membranes et notamment les mécanismes de peroxydation des lipides, les interactions membranes/substances chimiques, les déplacements d'ions, les événements au niveau des récepteurs

- l'activation des proto-oncogènes et la différenciation cellulaire
- la régulation du métabolisme cellulaire.

6. Il faut également préciser la localisation anatomique de certaines fonctions rénales.

7. Il faudrait étudier de façon plus approfondie le rôle des variations et de la prédisposition génétiques aux effets toxiques des médicaments et des produits chimiques.

8. La relation entre néphrotoxicité et cancer (mycotoxines et néphropathie des Balkans par exemple) doit être étudiée plus à fond.

RESUMEN Y CONCLUSIONES

Los riñones son los principales órganos de excreción y homeostasis de las moléculas hidrosolubles. La unidad funcional del riñón es el nefrón, que consiste esencialmente en un tubo continuo de células heterogéneas sumamente especializadas, y que exhibe una notable organización estructural, funcional y bioquímica. Existen diferencias pronunciadas entre unos nefrones y otros, atendiendo a la localización de los glomérulos individuales correspondientes en la corteza. Esta compleja organización estructural, combinada con las diferencias en la vascularidad regional debida a lo especializado de la disposición vascular, da lugar a un órgano sumamente complejo y heterogéneo.

Gran parte de los conocimientos anatómicos y funcionales obtenidos en los animales pueden aplicarse directamente al riñón humano. No obstante, los procesos biomédicos y metabólicos del riñón humano, así como las diferencias entre las especies animales, no se han elucidado de forma tan detallada. Así, los efectos de las sustancias químicas sólo se pueden extrapolar hasta cierto punto de unas especies a otras.

Varias sustancias químicas (tanto terapéuticas como no terapéuticas) tienen efectos tóxicos en uno o más elementos anatómicos del riñón. Los efectos tóxicos pueden ser agudos o crónicos, y pueden ser directos o estar indirectamente mediados por mecanismos inmunológicos. El impacto que tienen en la salud las sustancias químicas nefrotóxicas está relacionado con los factores de riesgo, entre los que figuran el estado de la reserva funcional del riñón y factores como las lesiones renales ya existentes, las enfermedades, la edad, el sexo y la dieta.

No se ha estudiado bastante la epidemiología de la nefrotoxicidad inducida con sustancias químicas aisladas o en exposiciones mixtas. La contribución de las sustancias químicas a la incidencia global de la nefropatía y del fallo renal crónico está, salvo raras excepciones, sin definir. En el caso de algunos grupos expuestos por su profesión y de enfermedad renal asociada a los analgésicos, se han hecho amplios estudios que han demostrado la

existencia de la incidencia entre grupos y países. Sin embargo, se estima que hasta el 18% de las enfermedades renales en fase terminal pueden deberse a nefropatía por analgésicos y hasta el 5% a otras nefropatías tóxicas. Alrededor del 50% de las enfermedades renales en fase terminal son de etiología desconocida. Uno de los principales problemas para atribuir una causa a la enfermedad renal en fase terminal es la larga latencia y/o la lenta evolución del fallo renal crónico, lo que dificulta la identificación retrospectiva del agente causal. Sólo en algunos casos, la medida de los niveles tisulares (organismo, riñón) de sustancias químicas es útil para el diagnóstico. Otro problema ha sido la falta de coherencia en la terminología y los criterios patológicos y de diagnóstico.

Las diferencias anatómicas y fisiológicas del riñón dificultan la extrapolación directa al ser humano a partir de los sistemas experimentales *(in vivo* e *in vitro)*. Existen muy pocos ejemplos de sustancias químicas nefrotóxicas para las que se disponga de datos adecuados y comparables entre los animales y el hombre como para formar una base sólida sobre la que evaluar el riesgo potencial para la salud humana.

Las sustancias químicas pueden dañar de modo selectivo las estructuras vulnerables del riñón o activar mecanismos inmunológicos. Los mecanismos de lesión renal pueden dividirse generalmente en dos categorías: a) nefritis intersticiales agudas inmunológicamente inducidas; b) aquellos que afectan primordialmente al glomérulo por conducto de anticuerpos mediados por la anti-GBM o por conducto de complejos inmunes. Otro grupo principal está compuesto por las enfermedades desencadenadas por sustancias químicas o sus metabolitos que interfieren con los efectos bioquímicos y hemodinámicos en la célula, entre otros. Entre los factores que pueden modificar la lesión celular producida por sustancias tóxicas figuran los sistemas de transporte celular, la pinocitosis, la degradación metabólica y la interacción con las proteínas, los lípidos, las membranas y el ADN celulares, y tal vez otros constituyentes de la célula.

El uso cada vez más extendido de agentes y sustancias químicas terapéuticas aumenta las posibilidades de

aparición de nefrotoxicidad. La nefrotoxicidad inducida por agentes terapéuticos depende de la dosis y del tiempo de exposición (por ejemplo, analgésicos de combinación que producen necrosis papilar del riñón). Los efectos nefrotóxicos de los analgésicos, los antibióticos (como los aminoglucósidos), los agentes anticancerosos (como el *cis*-platino), y varios agentes más han sido objeto de amplios estudios. Las sustancias químicas de uso frecuente en la industria o el hogar, como los hidrocarburos clorurados y el etilenglicol, también tienen potencial para producir lesiones renales. Las sustancias químicas del medio ambiente como el plomo y el cadmio son capaces de inducir nefrotoxicidad. Estos agentes tienen efectos tóxicos tras la acumulación intracelular del compuesto original o tras la biotransformación renal o extrarrenal. La exposición multiquímica puede dar lugar a respuestas antagonistas o sinérgicas.

Aunque los tumores del riñón y del tracto urinario representan menos del 2-3% de todos los cánceres humanos, la frecuencia de esos tumores está aumentando, lo que indica que los factores ambientales ejercen cierta influencia. Si bien se ha demostrado que muchos fármacos y sustancias químicas son carcinogénicos en modelos experimentales, sólo algunas sustancias concretas se han relacionado con tumores en el hombre. Entre ellos figuran el amianto (adenocarcinoma renal), el uso indebido de analgésicos (carcinomas de transición), y los factores relacionados con el modo de vida (uso de tabaco, alcohol, café). Existen grupos de población con cáncer del tracto urinario de etiología aún por determinar. Las sustancias químicas presentes en el medio profesional se han relacionado con la etiología del cáncer de la vejiga urinaria. Muchos fármacos y sustancias químicas provocan nefritis intersticial, que en sí misma puede ser un factor desencadenante de cánceres en el tracto urinario.

No hay ningún método *in vivo* o *in vitro* que por sí solo baste para evaluar la disfunción renal químicamente inducida. Así pues, es aconsejable no sólo estudiar compuestos en busca de su potencial nefrotóxico, sino incorporar a los protocolos experimentales estudios mecanicistas sobre la toxicidad para células diana. Para conseguirlo, deben realizarse investigaciones concertadas *in vivo* e *in vitro*. Los estudios *in vitro* son aquellos

en los que se mantienen las relaciones anatómicas entre células (por ejemplo, riñón y túbulos aislados y perfundidos, secciones de la corteza renal y segmentos aislados de nefrones) y aquellos en los que se utilizan células renales (por ejemplo, suspensión de células, cultivos de células primarias, líneas celulares establecidas y fracciones subcelulares). En los estudios *in vivo* se utilizan técnicas tanto invasivas como no invasivas. Los procedimientos invasivos comprenden las mediciones histopatológicas y ordinarias de la función renal. Los procedimientos no invasivos permiten evaluar de modo repetido la función renal en los animales midiendo una serie de pruebas de la función renal (filtración glomerular, función tubular, proteinuria, enzimuria, etc). Cuando convenga, deben realizarse ensayos bioquímicos especializados. La combinación adecuada de experimentos *in vivo* e *in vitro* revelará si las sustancias químicas son nefrotóxicas o no y dará idea de la potencia, la localización y los mecanismos de la toxicidad.

Los métodos tradicionales de evaluación de la función renal en el hombre no bastan para diagnosticar de modo oportuno la disfunción renal inducida por sustancias químicas y la predicción de su importancia para la salud. La falta de marcadores específicos y precoces en la nefrotoxicidad resulta particularmente problemática. La evaluación no invasiva de la nefrotoxicidad debe hacer uso de marcadores de gran especificidad, sensibilidad para la detección de las alteraciones renales precoces y valor predictivo para la evolución de la insuficiencia renal. Las técnicas actuales de seguimiento de la función glomerular o tubular sólo son útiles en los casos en que se ha producido una lesión renal grave. Aunque actualmente se señalan los constituyentes tisulares inmunorreactivos como marcadores apropiados, es preciso validar su idoneidad en estudios longitudinales bien diseñados.

RECOMENDACIONES

1. Debe hacerse un esfuerzo continuado por desarrollar y validar marcadores más selectivos y específicos, inclusive anticuerpos monoclonales, para evaluar la disfunción renal en animales. Es posible que esos marcadores sean aplicables al hombre.

2. Debe ampliarse la base de datos para predecir el potencial nefrotóxico de las sustancias químicas para el hombre. Ello comprende el desarrollo y la validación de criterios experimentales en animales *(in vivo* e *in vitro)*, métodos alternativos para estudiar la nefrotoxicidad, información sobre diferencias interespecíficas, y experiencia de la evaluación preclínica de nuevos agentes terapéuticos en el hombre.

3. Deben reforzarse los estudios epidemiológicos (es decir, estudios prospectivos en grupos profesionales y de la población general expuestos a sustancias químicas nefrotóxicas o que hagan uso indebido de analgésicos).

4. Deben intensificarse los esfuerzos por establecer el papel de las sustancias químicas en la etiología de las enfermedades renales en la etapa más temprana de diagnóstico (por ejemplo, pasado laboral, vigilancia de los tejidos en busca de nefrotoxinas).

5. La comprensión de los mecanismos de acción de los nefrotóxicos ayudará a prevenir y gestionar desde el punto de vista clínico los efectos renales no deseados, y puede ayudar a predecir el potencial nefrotóxico de nuevos fármacos y sustancias químicas. Los aspectos de particular importancia para las investigaciones futuras son:

- los mecanismos inmunológicos;
- los efectos directos de las sustancias químicas en las membranas, inclusive los mecanismos de peroxidación lipídica, la interacción membrana/sustancias químicas, los cambios de iones, y los procesos mediados por receptores;
- la activación de los proto-oncogenes y de la diferenciación celular;
- la regulación del metabolismo celular.

6. Es necesario determinar y correlacionar las funciones específicas con localizaciones anatómicas discretas dentro del riñón.

7. Debe estudiarse más a fondo el papel de la variación genética y la susceptibilidad a los efectos tóxicos de fármacos y sustancias químicas.

8. Debe estudiarse con más detalle la relación entre la nefrotoxicidad y la carcinogénesis renal (por ejemplo micotoxinas y nefropatía de los Balcanes).

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