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The cellular basis of renal injury by radiation

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Some toxic agents have an acute and selective effect on part of the nephron. Histological studies then permit the localisation of damage: for example, chloroform and *cis*-platinum damage the renal tubule (Hewitt, 1957; Anon., 1982), and immune complexes induce glomerular injury (Ichikawa *et al.*, 1982). By contrast, the pathogenesis of radiation nephropathy has long been in dispute, with some authors favouring damage to the endothelial cells of the glomeruli (Glatstein *et al.*, 1977) or larger vessels (Hopewell, 1980) as the critical lesion, whilst others are persuaded that tubular cell injury is more important (Mason & Withers, 1985). This difference of opinion is a reflection of the division between those who believe that vascular injury provides a general explanation of the late effects of radiotherapy (Rubin & Casarett, 1968), and those who believe that parenchymal cell damage is more important (Withers *et al.*, 1980; Michalowski, 1981).

Structure and function of the kidneys

The kidneys preserve the constancy of the internal environment by the selective excretion or conservation of the constituents of plasma. They also produce the active derivative of vitamin D, the enzyme renin, and the hormone erythropoeitin, which stimulates red cell production in the bone marrow.

The glomerulus consists of a mass of capillaries which produces an ultrafiltrate of plasma. Filtration is influenced by physiological factors (Sullivan & Grantham, 1982) and by molecular weight and charge (Olson *et al.*, 1982). Molecular shape, flexibility and deformability are probably also important. Over 99% of the ultrafiltrate is reabsorbed as it passes down the renal tubule (Sullivan & Grantham, 1982). Accurate balance between glomerular filtration and tubular resorption is critical in the maintenance of salt and water balance. This control is mediated through the juxta-glomerular apparatus (JGA), which secretes renin and is important in the regulation of blood pressure and plasma volume. Glomerulotubular balance is maintained in the face of injury and if there is severe disruption, the nephron shuts down; functional abnormalities in damaged kidneys can be explained as the result of the increased load on remaining nephrons (Bricker & Fine, 1981).

Two types of nephron have been described. Cortical nephrons have short loops of Henle which reach only the outer medulla. They also have a JGA and their function varies according to physiological demand: after a meal they are selectively recruited from the quiescent state (Brenner *et al.*, 1982). By contrast, juxtamedullary nephrons are thought to be largely responsible for baseline renal function in the fasting animal. They have large glomeruli and are better adapted for the conservation of water and salt, with long loops of Henle which penetrate deep into the medulla.

Cell populations and kinetics

Studies with tritiated thymidine have shown that both tubular and endothelial cells have low labelling indices of 0.4 and 0.1% (Glatstein *et al.*, 1975; Denekamp *et al.*, 1979), indicating that the normal turnover of these cells is slow. The mitotic index increases dramatically within 36 h of nephrectomy (Phillips & Leong, 1967) and this kinetic change has recently been used to precipitate the expression of radiation-induced renal injury (Robbins *et al.*, 1985; Soranson *et al.*, 1985). This technique may permit identification of the critical target cell.

Histological studies

Most human material has been obtained at autopsy and there are few reports of the changes observed in biopsy material soon after the onset of radiation nephropathy. There is no agreement about whether the prime site of injury is vascular, glomerular or tubular (Redd, 1960; Rubin & Casarett, 1968; Mostofi & Berdjis, 1971; Mason & Withers, 1985).

Animal experiments permit histological observations at chosen times after a range of radiation doses, but this has not helped to resolve the problem of pathogenesis. Phillips *et al.* (1972) described early capillary endothelial damage at 2–4 months, with subsequent recovery; tubular damage appeared at 3 months and was progressive; the glomeruli showed only minor changes. By contrast, Glatstein *et al.* (1977) noted progressive thrombosis

Table I Histological assays of dose-response in nephrotoxic injury

Endpoint	*Nephrotoxic insult*	*Animal*	*Reference*
Tubule necrosis	Chloroform	Mouse	Hewitt, 1958
Tubular necrosis	Gentamicin	Rat	Wellwood *et al.*, 1976
Tubular atrophy	Bilateral irradiation	Mouse	Glatstein *et al.*, 1977
Tubular injury	Unilateral irradiation	Mouse	Jordan *et al.*, 1978
Tubular dilatation	Adriamycin	Rabbit	Fajardo *et al.*, 1980
Glomerular vacuoles	Adriamycin	Rabbit	Fajardo *et al.*, 1980
Fibrosis	Adriamycin	Rabbit	Fajardo *et al.*, 1980
Dilated tubules	Bilateral irradiation	Mouse	Meistrich *et al.*, 1984
Morphometry of tubule nuclei	Unilateral irradiation	Mouse	Jordan *et al.*, 1984
Tubular clones	Unilateral irradiation	Mouse	Mason & Withers, 1985

of glomerular capillaries at three months and considered tubular damage to be less severe.

Madrazo & Churg (1976) described both tubular and glomerular lesions in rats. Changes were detected earlier using electron microscopy, and the use of high doses merely accelerated the process without influencing the changes observed.

Only tubular damage has yielded dose-response information after irradiation. The scoring systems used are listed in Table I. Similar results seem to have been obtained by scoring slight or marked degrees of tubule enlargement, or by identifying surviving tubules. Glomerular damage was dose-responsive after adriamycin (Fajardo *et al.*, 1980), but after irradiation the changes were very variable even within different parts of the same section (Jordan *et al.*, 1978; Meistrich *et al.*, 1984). Radiation induced glomerular injury has *not* been shown to be dose-responsive, despite the use of scoring systems which can demonstrate temporal progression (Wacholz & Casarett, 1970; Glatstein *et al.*, 1977).

It is clear that the interpretation of subtle histological changes is subjective and it is unlikely that this approach will permit identification of the critical target cell for radiation nephropathy.

Clonogenic assays of tubular injury

Deschavanne *et al.* (1980) have reported an *in vitro* assay for clonogenic cells extracted from the kidney after irradiation *in situ*. Both fibroblast and epithelial cell colonies were identified in the resulting cultures. A plating efficiency of 8% and a D_0 of 1.7 Gy were reported.

Withers *et al.* (1985) have recently described an *in situ* assay of surviving tubules. Coronal cross sections of the kidney were prepared 30–70 weeks after unilateral irradiation, and tubules which abutted on the renal capsule were scored for viability (Hewitt, 1957). Normal looking tubules stood out clearly in the damaged kidney. There was clustering of surviving tubule cross sections as would be expected from the convoluted course of renal tubules. It is argued that these intact tubules arise by regeneration, because such localised sparing would be otherwise unlikely. Observation of these clones depends on irradiating only one kidney so that severe damage can be inflicted without killing the animal; a long latent period is also required to allow for cell death followed by repopulation. From single dose experiments a D_0 of 1.25 Gy was calculated (Mason & Withers, 1985). Results with fractionated doses (which will minimise the problem of intestinal injury) are awaited with interest.

Assays of renal function

Destructive assays

Destructive assays involve the death of the animal and therefore give information at only a single timepoint. Death is a poor endpoint as it can occur as a result of *intestinal* damage as long as 500 days after renal irradiation (Williams & Denekamp, 1983). Many better assays of renal damage are now available.

Assays of renal weight (Glatstein, 1973) and renal growth (Donaldson *et al.*, 1978) quantitate the overall response of the kidney. Estimates of blood flow using rubidium extraction (Glatstein, 1973) do not necessarily indicate vascular damage as intact tubular cells are needed to take up the isotope (Withers *et al.*, 1980). Similarly, hydroxyproline content shows little change on a per kidney basis and increased concentration is largely the result of nephron loss (Meistrich *et al.*, 1984).

Detailed physiological experiments permit the study of specific glomerular and tubular functions at the level of the isolated nephron (e.g., Hayslett *et al.*, 1968; Allison *et al.*, 1974; Wilke *et al.*, 1979; Olson *et al.*, 1982). These assays have mainly been

Table II Non-destructive assays of renal damage

Assay	*Endpoint*	*Nephrotoxic insult*	*Animal*	*Reference*
Blood pressure	Elevation	Unilateral irradiation	Rat	Asscher, 1964
Renography	GFR, ERPF	Bilateral irradiation	Man	Avioli *et al.*, 1963
Tubular function	Resorbtion of PAH	Radiation	Rabbit	Hamada, 1964
Proteinuria	β_2-microglobulin	Chronic cadmium poisoning	Man	Peterson *et al.*, 1969
Renography	Functional index	Unilateral irradiation	Pig	Hopewell & Berry, 1974
Tubular damage	Urinary enzymes	Gentamicin	Rat	Wellwood *et al.*, 1976
Renography	Isotope clearance	Unilateral irradiation	Rat	Chauser *et al.*, 1976
Tubular damage	Urinary enzymes	Cis-platinum or amikacin	Man	Diener *et al.*, 1981
Urination	Frequency	Bilateral irradiation	Mouse	Williams & Denekamp, 1982
^{51}Cr-EDTA	Isotope retention	Bilateral irradiation	Mouse	Williams & Denekamp, 1982
Anaemia	Plasmacrit	Bilateral irradiation	Mouse	Alpen & Stewart, 1984
GFR & ERPF	Individual kidneys	None	Pig	Robbins *et al.*, 1984
Ultrasound	Kidney size and volume	Pregnancy	Woman	Cietak & Newton, 1985

used to study experimental nephritis induced by immune complexes, diabetes or reduction of renal mass. Their use might permit the early identification of specific sites of radiation injury.

Non-destructive assays

Non-destructive assays can be used to study renal injury sequentially in individual animals. This approach permits detailed study of latency and also of possible recovery of function. Steeply dose-responsive assays allow accurate comparison of different radiation fractionation regimes. Information about target cell response can then be deduced indirectly from tissue responses.

Table II lists some of the functional assays which have been described. In large animals such as the pig detailed physiological studies can be undertaken on individual kidneys (Robbins *et al.*, 1984). For the sequential study of large numbers of animals simpler techniques are available. In mice a single blood sample taken 60 min after the injection of ^{51}Cr-EDTA provides a steeply dose responsive index of renal function (Williams & Denekamp, 1983).

It is important to remember that the endpoint measured does not necessarily indicate the critical target cell. For example in mice an increase in urine volume has been quantitated using urination frequency (Williams & Denekamp, 1983). The urine passed was less concentrated than usual and there was a parallel increase in water consumption (Williams, 1984). The decline in urine concentration was not necessarily a consequence of direct tubular injury; it can instead be explained on the basis of an increase in the osmotic load on surviving nephrons (Bricker & Fine, 1981). The urine produced at the highest doses was still concentrated relative to plasma and diurnal variation was preserved (Williams, 1984).

Urinary enzymes provide a specific measure of tubular injury. Increased levels have been observed after treatment with gentamicin (Wellwood *et al.*, 1976), amikacin, *cis*-platin (Diener *et al.*, 1981) and many other nephrotoxins. The subject has been reviewed by Raab (1972). These techniques have not been applied to radiation nephropathy and might provide useful information about the time course of tubular injury.

Radiation dose per fraction

The kidney is a dose limiting tissue in radiotherapy (Luxton, 1961) and its response to dose fractionation is therefore of interest if novel regimens are to be applied in cancer treatment. This problem has been investigated using functional and histological assays.

Fraction size has a marked influence on the radiation tolerance of the kidney, as for other late responding normal tissues. If functional assays are used to determine an equal effect (E) then the dose effect curve underlying the observed effect can be described by the equation:

$$E = \alpha D + \beta D^2.$$

The ratio of the α and β parameters in this equation has dimensions of dose and describes the change in effectiveness of irradiation with dose per fraction (for discussion see Fowler, 1984). For mouse kidneys, α/β ratios of less than 6.0 Gy have been derived in two experiments (Williams & Denekamp, 1984*a*; Stewart *et al.*, 1984). The pig experiments of Hopewell and Wiernik (1977) yield an α/β ratio of 0.5–5.0 Gy (for discussion see

Williams & Denekamp, 1984*a*). These are all low values, consistent with those obtained for other late responding normal tissues (1–5 Gy) and lower than those for acutely responding tissues (8–14 Gy) (Fowler, 1984). These differences indicate that late responding normal tissues have a rapidly curving dose effect curve, i.e. that changing the size of the dose per fraction has a greater effect on isoeffect dose than it does for acutely responding tissues (see Fowler, 1984).

Tests for the fit of data to the linear quadratic model have been described by Tucker (1984), and applied by Lebesque *et al.* (1985) to data on mouse kidneys. They found that the data were not adequately described by the linear quadratic equation, and that at low doses per fraction the α/β ratio increased. This means that isoeffect dose does *not* increase as rapidly as the model would suggest. This is an important result which needs to be confirmed. Similar observations have been made on the spinal cord (Ang *et al.*, 1985).

Overall treatment time

In the kidney the dose increments which are required to counteract an increase in overall time are slight. Slow repair (as observed in the lung) provides an adequate description of the mouse data (Williams & Denekamp, 1984*b*; Williams *et al.*, 1985*a*, *b*). No significant dose sparing was seen in the rat (Rockwell & Moulder, 1985). The pig results are more difficult to interpret and may be complicated by the effects of continued renal growth in these immature animals (Robbins, 1984; Hopewell & Robbins, 1985). Stimulated repopulation, which requires early expression and recognition of injury seems unlikely in this slow turnover tissue.

Dose-latency

For many toxic agents cell death is a rapid consequence of exposure. Renal damage is manifest in mature male mice within 24 h of exposure to chloroform (Hewitt, 1957). Other toxins are less dramatic and renal injury becomes apparent within days or weeks after heavy metals or *cis*-platinum (Anon., 1982). If exposure is chronic and the dosage low then latency is prolonged as in the nephritis of human cadmium poisoning (Peterson *et al.*, 1969). These varied effects are the result of differences in the rate of cell death.

X-ray injury is clearly *inflicted* at the time of irradiation, but its major effects are *expressed* after a considerable delay. It is thought that cell death occurs at mitosis and thus reflects the slow turnover of cells in the kidney (Denekamp *et al.*, 1979; Michalowski, 1981). Dose-responsive latency after irradiation is characteristic of late responding normal tissues and is well established for the kidney (Asscher, 1964; Glatstein, 1973; Madrazo & Churg, 1976; Hopewell & Wiernik, 1977; Jordan *et al.*, 1978, 1984; Williams & Denekamp, 1983, 1984*a*; Williams *et al.*, 1985*a*; Stewart *et al.*, 1984; Lebesque *et al.*, 1985).

The observed latency of any response depends both on the sensitivity of the assay used and on the expression of injury. Michalowski (1981) has proposed that cells in Type F tissues (such as the kidney) can have the dual roles of function or division. Cell death occurs at attempted mitosis and the number of failed divisions will increase if the functional deficit can be recognised by a homeostatic sensor: this has been termed the avalanche phenomenon (Wheldon *et al.*, 1982). In irradiated mice the decline of renal function has been quantitated using an exponential rate constant. This analysis suggests that there is relentless progression from the time of irradiation even though this only becomes manifest to our assays after a 'latent period' (Lebesque *et al.*, 1985).

Some very early effects have been reported after renal irradiation: these may not have the same pathogenesis as do late effects. For example, in mice, renal wet weight increased dramatically within 24 h of irradiation (Glatstein, 1973; Glatstein *et al.*, 1977). It seems probable that this is an acute vascular effect akin to immediate erythema in the skin; at later times there is a dose responsive *reduction* in renal weight. Early alterations in tubular function have been described by some authors (Hamada, 1964; Coburn *et al.*, 1966), but others have described early alterations in ERPF and GFR (Avioli *et al.*, 1963; Raulston *et al.*, 1978; Robbins *et al.*, 1985). Further investigation of the relationship between early and late effects in the kidney is indicated.

Discussion

Pathogenesis of renal injury by radiation

The pathogenesis of radiation nephropathy remains controversial. Histological studies have not revealed a single site of primary damage as has been observed with some other toxins. Withers *et al.* (1985) have provided convincing evidence that tubular clones can be observed at late times after high doses of X-rays. In order to establish the tubule cell as the critical target it will be necessary to use this technique to confirm that the influence of fraction size and overall treatment time are in broad agreement with the results of functional assays, or at least that any discrepancies are explicable (Wheldon *et al.*, 1982).

An alternative approach which might permit the identification of the critical target cell would be to seek very early functional changes. Tubular injury could be identified by an increase in urinary enzymes of tubular origin, and in low mol. wt proteinuria (e.g. β_2-microglobulin). By contrast, glomerular damage could be detected by early changes in GFR and ERPF and by changes in glomerular permselectivity with high mol. wt proteinuria (e.g. albumin). Such investigations could be performed in man as well as in animals.

The progression of renal injury

In man and laboratory animals normal ageing is associated with a fall in renal blood flow and glomerular filtration. Renal function can also be profoundly influenced by diet: as aggregate protein intake increases there is a sustained increase in both renal blood flow and filtration rates. The result is glomerulosclerosis, which can either be slowed by dietary restriction or accelerated by overeating (see review by Brenner *et al.*, 1982).

Nephron loss can also cause glomerular sclerosis. It is well known that compensatory hypertrophy occurs after nephrectomy. Overall renal function returns almost to normal, but there is an increase in blood flow and glomerular filtration on a per nephron basis. As a result, removal of a large proportion of the renal mass can cause glomerulosclerosis, hypertension and renal failure. Only three months after five-sixths nephrectomy, it is possible to identify ultrastructural changes which progress to glomerular hyalinization and sclerosis (Shimamura & Morrison, 1975).

Brenner *et al.* (1982) have proposed that there is a final common pathway for the progression of renal injury as shown in Figure 1. The key abnormality is chronic renal vasodilation with resultant elevation of glomerular pressure and flow. These changes have been shown by micropuncture studies in the remnant kidney (Hostetter *et al.*, 1981) and in experimental nephritis (Olson *et al.*, 1982). No such studies have been undertaken in radiation nephritis, but both a high protein diet (Mahler *et al.*, 1982) and unilateral nephrectomy (Rosen *et al.*, 1968) hasten the progression of glomerular sclerosis after renal irradiation.

The role of hypertension

There are clear species and strain differences in the development of hypertension after renal irradiation. The untreated kidney can exert a protective influence in some species (Redd, 1960), but the mechanism is unknown. Hypertension is rare after unilateral irradiation in man, but this may in part reflect inadequate follow-up, because a mean latent

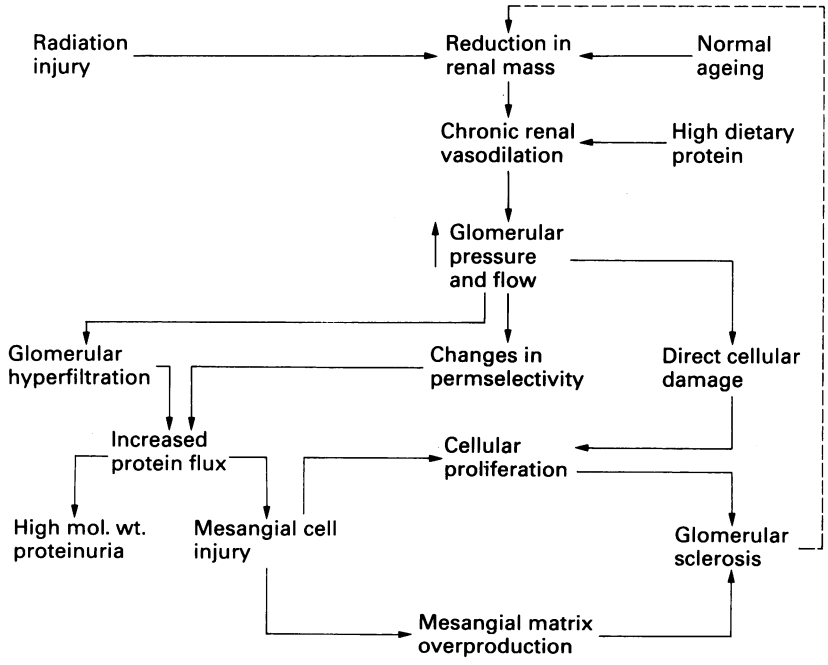

Figure 1 Influence of pattern of dietary protein intake on the progression of renal damage (redrawn after Brenner *et al.*, 1982). Reduction of renal mass results in compensatory vasodilation. This has damaging effects on the glomeruli, resulting in sclerosis and further loss of renal mass.

period of 9 years has been reported (Thompson *et al.*, 1971). It is now established that the hypertension of radiation nephritis results from renin secretion from the JGA (Shapiro *et al.*, 1977) and that most of the vascular injury observed in the irradiated kidney is a secondary effect of hypertension (Wacholz & Casarett, 1970). In man acute radiation nephritis can develop with a normal blood pressure, and hypertension is probably a consequence rather than a cause of the nephritis (Luxton, 1961).

Asscher *et al.* (1961) showed that radiation sensitized mesenteric vessels to hypertensive damage. He proposed that a similar mechanism might accelerate damage in irradiated kidneys if hypertension occured either spontaneously or as a consequence of the irradiation (Asscher, 1964). In radiation nephropathy, as in chronic renal failure of any cause, blood pressure should be carefully controlled. If the renal damage is unilateral and renin levels are elevated, nephrectomy should be advised (Shapiro, 1977).

Conclusion

Even if there were a single critical cell type involved in the pathogenesis of radiation nephritis, it is clear that injury to any part of the nephron can cause secondary changes elsewhere and that the picture can be complicated by hypertensive injury. Figure 2 shows a model of radiation nephropathy. It is proposed that radiation injury will result in a reduction of functioning nephron mass by primary damage to the tubules or glomeruli. Compensatory renal vasodilation would close the positive feedback loop described in Figure 1. Detailed physiological assays are now available and could be used to test this hypothesis as in other nephropathies. Radiation could also cause direct vascular injury: *decreased* renal perfusion and hypertension would result. Again sensitisation to hypertensive vascular damage would close a positive feedback loop.

If this model is correct, it is perhaps not surprising that physiological and histological studies have not permitted the identification of a single target cell, nor any consensus about the pathogenesis of radiation nephropathy. The tubular clone technique (Withers *et al.*, 1985) should clarify the role of tubular cells in late renal injury by radiation. This will be important as the first such assay for a late responding tissue. It may provide important support for the concept that late effects reflect parenchymal rather than vascular damage.

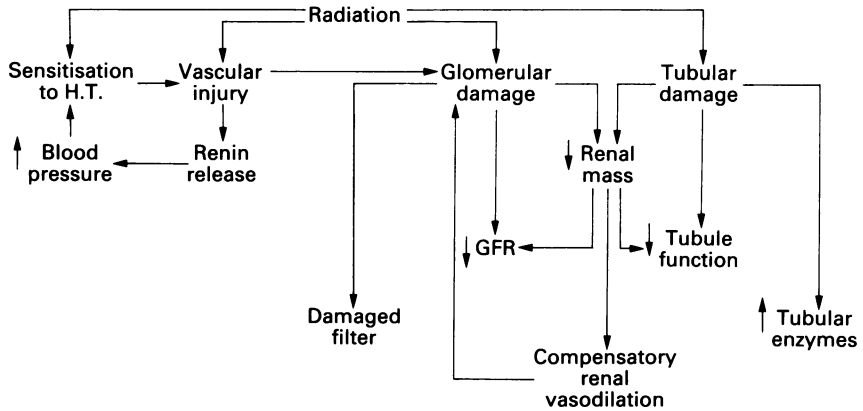

Figure 2 A model for the progression of radiation nephropathy. The link between glomerular and tubular function obscures the primary site of injury. Nephron loss leads to glomerular sclerosis. The resulting reduction in renal mass closes a positive feedback loop. Radiation both causes hypertension and sensitises the vasculature to further injury – this can also result in rapid deterioration.

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