The pathology of renal ischaemia

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The changes of renal ischaemia are amongst the commonest seen in renal pathology since they accompany a great variety of other diseases ranging from chronic glomerulonephritis to analgesic abuse, from irradiation to renal transplantation. Unfortunately it is not always easy to separate the changes of ischaemia from those of the underlying primary disease. However, renal artery stenosis provides a suitable model for this study. Many cases of renal artery stenosis are complicated by hypertension which itself produces secondary changes in the kidney and so some recognition of this must be given in any discussion of the pathology of renal ischaemia.

Renal artery stenosis may affect the main renal artery, one of its major divisions or one of the segmental arteries. If only part of the kidney is damaged nephrectomy specimens provide a useful comparison of an ischaemic segment and a control normal segment.

It is not the purpose of this study to consider the changes of acute ischaemia producing renal necrosis which will be dealt with elsewhere.

Gross appearance

Following ischaemia, the kidney is reduced in size. If a segmental artery is stenosed then the ischaemic area of the kidney supplied by the narrowed artery is depressed below the surface of the remainder of the kidney. In young individuals without intrarenal artery disease the capsular surface of the ischaemic part of the kidney is smooth but in older individuals with arteriolar sclerosis the capsular surface is often finely scarred. If a partial nephrectomy has been performed for segmental artery stenosis, the smooth surface of the kidney may make it appear deceptively normal unless an appreciable rim of non-ischaemic kidney is included with the specimen. If the patient has been hypertensive then the area of kidney not supplied by the stenosed artery may be finely scarred, even in young patients, due to the effects of hypertension. The narrowed renal artery protects the ischaemic area from the effects of this hypertension.

The cut surface of the ischaemic kidney shows narrowing of the cortex and there may be loss of demarcation of cortex and medulla.

Glomerular changes

Many of the glomeruli may appear remarkably normal in spite of severe damage elsewhere in the kidney. The changes of ischaemia in the glomerular tuft and Bowman's capsule were well described by McManus and Lupton (1960). Although the changes are distributed in the area of supply of the diseased artery they tend to be more severe in the outer cortex (fig 1). The vascular tuft of the glomerulus becomes compact (fig 2) due to collapse of capillaries. After staining with periodic acid-methenamine silver the basement membranes are seen to be wrinkled around the collapsed bloodless capillaries (figs 3 and 4), a pattern confirmed by electron microscopy (fig 5). The degree of collapse may vary from case to case. This is illustrated in the table where a comparison has been made between ischaemic and 'normal' glomeruli from two cases of segmental renal artery stenosis. In each case blocks were taken across the junction of ischaemic and normal kidney so that the tissues were subjected to identical processing and section cutting. In each area 20 glomeruli were measured using a Quantimet 720 image analyser. In one case the ratio of ischaemic glomerular tufts to normal tufts is not significantly reduced at 86 per cent, but in the other case the ischaemic tufts are only 60 per cent of the area of the normal tufts. In neither case did the normal zone appear damaged.

This collapse of the glomerular tufts is usually global in distribution but in some glomeruli it appears to be segmental (fig 6), suggesting a local factor within the tuft causing shutdown of capillary blood flow. There are various possibilities to account for the collapse of the glomerular tufts. Global collapse may be the result of low perfusion pressure between the stenosed renal artery and the glomerulus or it may be due to narrowing of the intrarenal

Fig. 1 Ischaemic changes in the kidney are most marked in the subcapsular zone (top) of the kidney. Note the tubular atrophy and crowding of glomeruli (H and E $\times 40$).

Fig. 2 Collapsed capillaries give the glomerular tuft a solid appearance. The apparent increased cellularity is due to concentration of cells into a smaller area (H and E $\times 400$).

Fig. 3 Wrinkled, collapsed capillaries of an ischaemic glomerular tuft. Bowman's capsule is thickened and split (silver methenamine $\times 400$).

Fig. 4 A normal glomerulus from the same kidney as in fig 3 for comparison showing well filled glomerular capillaries and smooth basement membranes (silver methenamine $\times 400$).

Fig. 5 Ischaemic collapse of glomerular capillaries outlined by dense wrinkled basement membranes. Capillary lumina are lost but epithelial, endothelial and mesangial cells survive (EM $\times 4930$).

		Total Area (To) (μ^2)	Tuft Area (Tu) (μ^2)	$\frac{Tu}{To}$ (%)	$\frac{ToI}{ToC}$ (%)	$\frac{TuI}{TuC}$ (%)
Case 1	Control (C)	14665 $\pm$ 2646	11499 $\pm$ 2626	78	65	60
	Ischaemic (I)	9525 $\pm$ 2575 (825)	6908 $\pm$ 2282 (778)	73		
Case 2	Control	17148 $\pm$ 5950	10373 $\pm$ 4416	60	80	86
	Ischaemic	13699 $\pm$ 2588 (1451)	8931 $\pm$ 2201 (1103)	65		

Table Effects of ischaemia on total glomerular area and tuft area¹

¹Average of 20 glomeruli in each group. Figures in parenthesis are the standard errors of the differences between the two means.

arteries and arterioles supplying the glomeruli. Alternatively there may be constriction of the arterioles at the hilum of the tuft, or of capillaries within the tuft. The juxtaglomerular cells of the afferent arteriole and the laci cells at the glomerular hilum are modified smooth muscle cells (Latta and Maunsbach, 1962a) as are mesangial cells within the glomerular tuft (Goormaghtigh, 1942; Tighe, 1975).

The glomerular mesangial cells react strongly with smooth muscle anti-actomyosin (Becker, 1972) and cells, presumed to be mesangial cells, grown in tissue culture have prominent intracellular fibrils containing actomyosin (Scheinman *et al*, 1976). The axial distribution of mesangial cells within the glomerulus and their close anatomical relation to capillaries (fig 6) would allow these cells to have an appreciable

Fig. 6 Segmental collapse of glomerular capillaries. A capillary in an adjacent segment (lower right) is widely patent and contains erythrocytes. Mesangial cells occupy the axial region of the ischaemic segment and are ideally placed to control capillary blood flow (EM $\times$ 3000).

effect upon glomerular blood flow. The segmental collapse of tuft capillaries without mechanical obstruction within the lumen is best explained by such mesangial contraction. The demonstration of angiotensin II receptors in mesangial cells (Osborne *et al*, 1975) points to these cells being liable to the vasoconstrictor effect of this hormone and is further evidence for their contractile role. Becker (1962) postulated that closing of peripheral tuft capillaries by mesangial contraction might result in shunting of blood through anastomoses within and between glomerular lobules (Hall, 1954; Boyer, 1956).

The cellularity of the collapsed glomerular tufts (fig 2) may appear somewhat increased but this is due more to the condensation of cells into a smaller area rather than to cellular proliferation. Hara *et al* (1975) reported that mesangial cell counts and total glomerular cell counts are not significantly increased in the ischaemic nephrosclerosis associated with hypertension. Although the mesangial area appears expanded this is, in part, due to condensation of capillary basement membranes about the axial core of the glomerular tuft.

As the basement membranes around the collapsed capillaries become thicker, the glomerular tuft becomes converted into a hyaline mass without identifiable capillaries (fig 5). In spite of the apparent loss of blood supply, a few cells remain viable within the hyalinized tuft.

Meanwhile Bowman's capsule shows changes which terminate in global sclerosis. Initially Bowman's capsule contracts around the shrunken glomerular tuft so that the urinary space is not greatly increased and the ratio of tuft area/total glomerular area remains remarkably constant (table). Only later may the urinary space increase due to disproportionate shrinkage of the tuft. Bowman's capsular basement membrane (fig 3) becomes thickened and duplicated (Jones, 1974). In some glomeruli, collagen is deposited internal to the capsular basement membrane. Initially this appears in the vicinity of the hilum (McManus and Lupton, 1960) but later spreads to fill the urinary space so that the hyalinized tuft and capsular fibrous tissue merge into one eosinophilic mass. However, using periodic acid-Schiff (fig 7) or silver-methenamine the tuft and capsular components of the glomerular mass are readily distinguished. Some cells, of capsular origin, are caught up in this proliferating fibrous tissue. This fibrosis internal to Bowman's capsule should be distinguished from the periglomerular fibrosis seen in chronic pyelonephritis (Heptinstall, 1974).

Late in the disease hyalinized glomeruli undergo resorption similar to that seen in chronic glomerulonephritis (Jones, 1963). There is fragmentation of

Fig. 7 The vascular tuft has collapsed to a solid mass of capillary basement membranes. Between the tuft and Bowman's capsule fibrous tissue has filled the urinary space (PAS $\times 400$).

Bowman's capsular basement membrane and fading of the tuft basement membranes with replacement by collagen.

Juxtaglomerular apparatus

Much of the interest in ischaemic renal disease stems from the work of Goldblatt *et al* (1934) who produced hypertension in dogs by constriction of the renal arteries. Goormaghtigh (1940) drew attention to the hyperplasia of the juxtaglomerular apparatus and the increased granularity not only of the juxtaglomerular cells but also of interlobar and interlobular arteries following clamping of the renal arteries in dogs. Since that time there has been much evidence relating renal ischaemia to hyperplasia of the juxtaglomerular apparatus and hypertension (Tobain *et al*, 1958; Heptinstall, 1965).

The juxtaglomerular apparatus at the hilum of the glomerulus consists of the afferent and efferent

Fig. 8 A tangential cut of the afferent arteriole as it enters the glomerulus (left) reveals hyperplastic, large, pale, epithelioid, smooth muscle cells, the juxtaglomerular cells (EM $\times$ 2530).

Fig. 9 The juxtaglomerular cells surrounding the afferent arteriole are hyperplastic, causing increased thickness of the arteriolar wall. Note the increased granularity of the JG cells (EM $\times$ 1800).

arterioles, the macula densa of the distal convoluted tubule and, between these, the laci cells which are continuous with mesangial cells within the glomerular tuft (Latta and Maunsbach, 1962b). The modified smooth muscle cells of the afferent arteriole shortly before it enters the glomerulus become large, pale and epithelioid (fig 8). Within these cells are found the juxtaglomerular granules which are now recognized as containing renin (Tobain *et al*, 1959; Cook and Pickering, 1959; Edelman and Hartroft, 1961; Cook, 1968). It is these large epithelioid cells which show the initial hyperplasia (fig 9) resulting in appreciable thickening of the arteriolar wall. Turgeon and Summers (1961), studying patients with hypertension associated with renal artery stenosis, found that there was an inverse correlation between the degree of hyperplasia and the duration of the disease, the most prominent juxtaglomerular cells being found in young patients with hypertension of short duration. This hyperplasia also affects the laci cells, so that it may be difficult to distinguish the two types of cells.

Juxtaglomerular granules are rarely as numerous in the human as in laboratory animals. This needs to be considered when comparing illustrations of the granularity of hyperplastic juxtaglomerular cells in man with those of laboratory animals. The juxta-

Fig. 10 Type 1 dense JG granules in a hyperplastic juxtaglomerular cell. These granules are regarded as specific for this site. Note the dilated rough endoplasmic reticulum (EM $\times 25\,650$).

Fig. 11 Type 2 JG granule in a juxtaglomerular cell. Similar granules containing lipid droplets or myelin are also seen in interlobular arteries and in the mesangium (EM $\times 31\,250$).

Fig. 12 Type 2 JG granule showing laminar and lattice crystalline structure in a coarsely granular matrix (EM $\times 150\,000$).

Fig. 13 Autofluorescent granules in hyperplastic juxtaglomerular cells at the pole of the glomerulus, extending back into the afferent arteriole (unstained $\times 400$).

glomerular granules are of two main types (Biava and West, 1966) both of which can be demonstrated with Bowie's stain. The first consists of granules bounded by a single membrane and filled with homogeneous or finely fibrillary material (fig 10). Some of these assume a rhomboidal shape. These granules are usually found in the vicinity of Golgi cisternae of the juxtaglomerular cells. The second resembles an autophagic vacuole or residual body and consists of a membrane-bound granular matrix with included lipid droplets (fig 11). The granular matrix is sometimes arranged in a laminar or hexagonal lattice form (fig 12) and may contain myelin figures. Biava and West (1966) regarded the first variety as a specific juxtaglomerular granule and the latter as non-specific lipofuscin-like, being found in both the juxtaglomerular cells and in the smooth muscle cells of arteries. However, the latter granules increase progressively in number in arteries of decreasing size and are most numerous in afferent

arterioles (Biava and West, 1965). In spite of the morphological differences, both types of granule are lysosomal (Lee *et al*, 1966; Fisher, 1966; Cantin *et al*, 1974; Nemes, *et al*, 1976). The lipofuscin-like granule is autofluorescent (fig 13) and may also be demonstrated using thioflavine T (Janigan, 1965). Both types of granule increase in number in ischaemic kidneys in juxtaglomerular cells and in lacis cells. The autofluorescent granules are also increased in number in small arteries and the granules extend into the proximal mesangium within the glomerular tuft. It is worth recalling that Goormaghtigh (1940) noticed the increased granularity of interlobular arteries in his models of experimental renal ischaemia. Heptinstall (1965) has demonstrated in the rat that the increased granularity of juxtaglomerular cells is restored to normal when the ischaemia is relieved. The increased granularity is also associated with increase in rough endoplasmic reticulum within the juxtaglomerular cells (fig 10), a change found in cells

concerned with protein synthesis and secretory activity.

Tubules

Ischaemic tubular atrophy is disproportionate to the glomerular damage. The reduction of renal parenchyma caused by this tubular atrophy results in crowding of glomeruli (fig 1). Counts of glomeruli/mm² have shown an increase from the normal mean of 65 to a mean of 300 in ischaemic areas.

The severity of the tubular atrophy prevents easy identification of proximal and distal tubules in the cortex (fig 14) but electron microscopy reveals that most of the tubules are proximal. The tubular lumina are reduced in diameter and in this respect they differ from the tubular atrophy of chronic pyelonephritis or of hydronephrosis where there is frequently gross

tubular dilatation. Occasionally some ischaemic tubules contain protein casts and the tubular lumen here is more obvious. The peritubular basement membranes are thickened (fig 15) and may be demonstrated using periodic acid-Schiff. This is a valuable way of detecting small foci of ischaemia for the thickened membranes draw attention to the atrophic tubules.

Interstitial tissues

The degree of interstitial fibrosis in the ischaemic areas is often surprisingly small in spite of the severe tubular atrophy. Where there is longstanding arteriosclerosis, as in elderly patients with renal ischaemia, there may be more severe interstitial fibrosis.

Fig 14

Fig 14 An ischaemic glomerulus with hyalinization of part of the glomerular tuft. The surrounding tubules are atrophic and cannot be identified as proximal or distal tubules (H and E $\times 400$).

Fig 15

Fig 15 Atrophic tubules are surrounded by thickened basement membranes. Interstitial fibrous tissue separates adjacent tubules (PAS $\times 400$).

Fig. 16 Subendothelial hyaline in an arteriole. A few coarse lipofuscin-like granules can be seen in the smooth muscle cells surrounding the hyaline deposit (toluidine blue $\times 1000$).

Interstitial inflammation is also usually slight, being limited to focal collections of lymphocytes. Inflammatory cells in tubules are not a feature of uncomplicated renal ischaemia.

Arterial disease

In elderly patients both the ischaemic areas of kidney and that part of the kidney not affected by renal artery stenosis may show fibroelastic intimal proliferation of interlobar, arcuate and interlobular arteries. Although this change may be more noticeable in the ischaemic area due to the crowding of vessels following tubular atrophy and parenchymal loss, analysis of individual arteries shows little variation between the ischaemic and less damaged areas of kidney. Arterioles may show hyaline change (fig 16); granules similar to those in juxtaglomerular cells may be found in the smooth muscle cells. In young patients arterial disease is often more severe in

the 'normal' kidney due to the protective effect of the renal artery stenosis in the ischaemic kidney preventing the arteries becoming damaged by the associated hypertension. This is seen particularly in patients with malignant hypertension secondary to renal artery stenosis. The changes of fibrinoid necrosis and mucoid intimal proliferation are confined to those vessels not protected by the arterial stenosis.

Conclusion

Examination of ischaemic kidneys reveals changes which generally correlate well with the pathophysiology of renal ischaemia. Although there is an association between renal ischaemia, juxtaglomerular granulation and hypertension, notable exceptions are seen from time to time. Improved methods of identification of the contents of juxtaglomerular granules may provide better methods of prediction of those patients likely to benefit from nephrectomy for hypertension associated with renal ischaemia. Further studies of the function of mesangial cells and of the smooth muscle cells of the juxtaglomerular apparatus promises to provide information to explain the collapse of glomerular tufts, and hence of impaired glomerular filtration, following ischaemia.

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