Hyperfiltration and Glomerulosclerosis

By Thomas H. Hostetter

Nearly 70 years ago, the association between reduction in renal mass and subsequent glomerular injury was established. Later the pathologic changes were attributed to adaptive hemodynamic changes in the residual nephrons. This relationship seems to exist in a number of experimental animal species, as well as humans. Among the various hemodynamic changes that occur within residual nephrons, the increase in glomerular pressure is the most important in generating subsequent pathologic changes. The renin-angiotensin-aldosterone system seems to contribute to the intrarenal pressure. Both angiotensin and aldosterone participate. Increases in glomerular pressure appear to act on the mesangial compartment causing it to increase its cell number and matrix volume. Deleterious interactions between higher pressures, increased capillary volume, and a relatively fixed podocyte number also appear to contribute to the sclerotic process. Endothelial cell changes occur perhaps in response to direct hemodynamic stimuli, but their role in the sclerotic process is less clear than those of mesangial and glomerular epithelial cells.

IN 1932, CHANUTIN and Ferris[1] described proteinuria and progressive glomerulosclerosis after major reductions in renal mass in the rat. Subsequent investigators, notably Shimamura and Morrison,[2] further characterized the experimental disease of the remnant kidney using ultrastructural approaches and described fusion of epithelial cells as well as glomerular enlargement. Olson et al[3] further detailed the cellular and permselective responses, documenting detachment of podocytes from the basement membrane and loss of filtration size selectivity. These studies all used high degrees of subtotal renal ablation to induce injury in the remnant glomeruli. Others have shown a graded response to removal of kidney parenchyma with even lesser degrees of reduction accelerating damage but at a slower rate and with less severe damage.[4] For example, even simple unilateral nephrectomy leads to an increased pace of sclerosis in remaining kidneys. With loss of renal tissue and before they sustain pathologic changes, the remaining nephrons undergo a process conventionally termed *compensatory hyperfunction*, whereby their single nephron filtration rates increase and the nephrons grow.[5] This response mitigates to some degree the loss of whole kidney filtration, but at a cost. The notion arose that this increase in single nephron filtration or hyperfiltration, though compensatory in the short term, caused subsequent pathology.[6]

Not only does graded reduction in renal mass lead to graded increases in injury of the residual nephrons, but renal ablation also hastens injury in other experimental renal diseases. For example, diabetic animals have greater degrees of glomerular sclerosis if they undergo unilateral nephrectomy.[7] Imposition of nephrectomy on hypertension or chemically induced nephrosis accelerates glomerular sclerosis in these conditions as well.[8,9] In addition, concurrent dietary manipulations that modify renal hemodynamics also alter the degree to which any given level of reduction of renal mass provokes subsequent glomerulosclerosis.[4] In particular, dietary protein restriction lessens renal injury with reductions in renal mass. Because higher dietary protein elevates whole kidney glomerular and single-nephron filtration rate in its own right, its combination with compensatory hyperfiltration exaggerates disease and its restriction lessens disease.[4,6]

Systematic surveys of the compensatory response of various rat strains to reductions in renal mass are not available. However, certain rat strains seem to develop injury more readily within the given level of reduction than others.[10] For example, the Pvg strain studied by Weening et al[11] seems particularly prone to injury. Also, some investigators have observed that Sprague-Dawley rats seem more prone to hemodynamically mediated damage than strains based on the Wistar background.

Species other than the rat do develop proteinuria and sclerotic glomerular injury with renal ablation. Cats, like rats, not only sustain injury but the injury also is worsened by higher levels of dietary protein

From the National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD.

Address reprint requests to Thomas H. Hostetter, MD, National Institutes of Health, National Institute of Diabetes, Digestive and Kidney Diseases, 6707 Democracy Blvd, Room 645, Bethesda, MD 20892. E-mail: HostetterT@extra.niddk.nih.gov

0270-9295/03/2302-0009$30.00/0

doi:10.1053/snep.2003.50017

intake.[11] The responses of mice vary. At least one strain of mouse seems to be resistant remarkedly to this process. The glomeruli of the C57Bl6 strain undergo enlargement of the residual glomerular when renal mass is diminished.[12] However, this mouse does not develop hypertension, significant proteinuria, or accelerated glomerulosclerosis. Whether other mouse strains develop lesions more similar to the rat is unknown. The apparent variability within and between species suggest that even in the face of the hypertrophy and presumed increases in the single-nephron glomerular filtration rate, certain animals are more or less resistant to the forces inducing injury in the more susceptible.

Fortunately, in humans, simple unilateral nephrectomy in otherwise healthy individuals seems to lead to little in the way of adverse renal consequences. Perhaps the most complete study is one of men who lost a kidney owing to trauma during World War II.[13] When these people were followed-up 45 years later, no increase in renal disease, hypertension, or proteinuria could be discerned. Furthermore, in a subset for whom autopsy tissue was available, no increased prevalence of glomerular injury was notable. As the investigators cautioned, these servicemen were largely of European ancestry and healthy at the time of their initial loss of a single kidney. However, reviews of individuals who have donated a kidney for transplantation have in general revealed no substantial long-term consequences.[14] The donors were, of course, screened for serious underlying kidney disease or conditions predisposing to a renal injury. On the other hand, some suggestions that losses of renal mass perhaps at susceptible periods of development or in susceptible individuals may be associated with subsequent injuries have been recorded. For example, unilateral renal agenesis is a relatively rare congenital condition but it has been associated with serious proteinuria and sclerosis of the single kidney as an individual ages.[15] Conceivably in this circumstance, the solitary kidney has subtle developmental defects that render it susceptible to injury. Likewise, progressive damage to the remaining kidney after removal of a contralateral diseased kidney may just reflect unrecognized bilateral disease.[16] However, with more extreme renal surgery, injury may be seen in humans. One study of subtotal nephrectomy sustained owing to aggressive renal cancer surgery has suggested that sclerotic injury develops in the spared but hypertrophied glomeruli.[17] Perhaps as in the animal studies, some variations occur among different groups of people and susceptibility to loss of renal mass may be more pronounced in some individuals.

Fairly wide variation in the number of nephrons has been noted in human adult kidneys.[18] Given the general trend, at least in animal studies, for lesser nephron number to lead to greater single nephron filtration rate and greater propensity for injury, Brenner and Mackenzie[19] have argued that individuals with natively fewer nephrons are predisposed to renal disease, hypertension, and glomerular sclerosis. The concept fits in part with Osmond's and Barker's[20] hypothesis that lower birth weights predispose to cardiovascular disease in later life. However, to date, a relation between birth weight and nephron number has not been established, at least in Europeans.[21]

In summary, the degree of reduction in renal mass (and hence extent of compensatory response) dictates the degree of long-term injury. In addition, varying degrees of susceptibility occur between and within species and may vary between human individuals. The combination of an increased single-nephron filtration rate with other deleterious influences is likely to be especially damaging. Reductions in renal mass by a simple surgical ablation are rare clinical events but the simple remnant kidney model has its greatest use in explaining how residual nephrons, by themselves sufficient to sustain homeostasis, become injured after some initial injury such as hypertension, or immune disease destroys a portion of kidney. Furthermore, in these common clinical events the presence of concurrent tubulointerstitial disease and scarring is usual. Inflammatory and other mediators likely emanate from these areas adjacent to the hyperfiltrating glomeruli and may create an even more adverse intrarenal environment than with surgical ablation, especially simple nephrectomy.

HEMODYNAMIC MECHANISMS OF INJURY

If increased single-nephron filtration causes subsequent injury, the question arises as to what determinant of filtration is responsible for the damage. Filtration is governed by the imbalance of hydrostatic and oncotic pressures across the glomerular capillary wall. Studies of Baylis and Brenner[22] have analyzed the determinants of single-

nephron filtration rates into 4 parameters: single-nephron plasma flow, glomerular capillary pressure, the capillary ultrafiltration coefficient, and the systemic oncotic pressure. Increases in any of the first 3 and/or a decrease in the last may increase single-nephron glomerular filtration rate. Micropuncture studies in several models of hyperfiltration-associated injury have helped to dissect which of these elements is most important in predicting subsequent glomerular damage. The earliest studies involved the remnant model in the rat with surgical and infarctive reductions in renal mass.[6] In these studies, increases in glomerular capillary pressure and plasma flow rates were both associated with the increases in single-nephron glomerular filtration rate on the residual glomerular. Changes in oncotic pressure or ultrafiltration coefficient did not account for the increase in filtration rate. Restriction of dietary protein reduced single-nephron filtration to near control rates and also blunted the proteinuria and sclerosis. Reductions in both capillary pressure and plasma flow underlay the normalization of filtration. Subsequent studies pinpointed heightened capillary pressure rather than plasma flow as the harmful determinant.[23]

In most disease models, the capillary hypertension can be attributed to decreases in afferent vascular resistance within the kidney, in most cases accompanied by arterial hypertension with resultant excess transmission of the arterial pressure to the glomerulus. The efferent vascular resistance often is maintained at a level close to normal. One can envision this latter phenomenon as also contributing to the maintenance of glomerular pressure in that postglomerular resistance fails to decrease in parallel with the preglomerular resistance. The remarkable efficacy of drugs that reduce angiotensin II (AII) level or action in models with glomerular hypertension have raised this view based on the following considerations.[24] This response to angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers suggests that AII may be in some fashion maintaining increased glomerular pressures. As one of its prime actions, AII preferentially constricts the efferent arteriole. On this basis, AII often has been invoked as maintaining efferent tone in the face of afferent vasodilation, the latter occasioned by less well-defined vasodilators, but prostaglandin species and nitric oxide may be disproportionately at play in this portion of the renal circulation. Glomerular hypertension results and is especially severe if arterial hypertension conspires with these intrarenal adjustments.

AII also has been accorded nonhemodynamic effects that may provoke injury on their own by inducing undue hypertrophy of various renal cells or stimulating production of cytokines such as transforming growth factor β, which in turn perpetuates scarring.[25,26] However, a simple unified description of the vascular endocrinology resting on the intrarenal AII is not consistently supported when several additional lines of evidence are considered in aggregate.

There is no doubt that many models of progressive renal injury, and, most importantly, most types of clinical progressive renal injury can be ameliorated by ACEIs or angiotensin receptor blockers (ARBs).[24] Although this suggests a particular role of the renin-angiotensin-aldosterone system across a wide range of hyperfiltering injuries, each element of the system likely can be removed, and injury can persist as long as pressure is excessive.

Measurements of AII levels within the simple remnant kidney model by Mackie et al[27] have failed to show any increase beyond those in the control animals. Thus, if AII is especially important in sustaining injury, either through increasing glomerular pressures or inducing nonhemodynamic tissue remodeling effects, these actions are not attributable to increases in levels of this hormone. Furthermore, acute blockade of AII by ARB does not acutely reduce glomerular pressures although these drugs, as well as ACEI when administered over several days, do reliably decrease glomerular pressures and mitigate injury.[28] These findings suggest that AII may not be a necessary component of hyperfiltration or glomerular capillary hypertension–induced injury or at least it does not exert ongoing minute to minute tonic action on the renal resistors. Studies of experimental mineralocorticoid hypertension also have shown increased glomerular capillary pressures, but in this instance neither ACEIs nor ARBs reliably reduce injury.[29] This is not an unexpected finding because levels of renin and presumably AII are essentially zero in mineralocorticoid-induced injury.[29] However, they show that AII is not necessary for capillary hypertension and injury. This raises the possibility that reductions in aldosterone may be a key mechanism of the efficacy of ACEI and ARB

because these drugs suppress aldosterone, and mineralocorticoid can increase systemic and glomerular pressures. Indeed, studies have shown that aldosterone is important to maintenance of arterial hypertension and glomerular injury in the remnant model.[30] However, even aldosterone itself may not be an absolutely necessary component of renal injury in this model. High-salt diets increase glomerular injury in the remnant kidney model, and though hormonal measurements have not been made with this dietary manipulation, one would predict that higher-salt diets would suppress not only AII, but aldosterone.[31] Although speculative, these results suggest that injury may be sustained as long as glomerular pressures are increased irrespective of whether the renin-angiotensin-aldosterone system is engaged. Other vasoactive systems such as prostaglandins, endothelin, and nitric oxide also may participate in propagating glomerular injury.[32] In summary, glomerular capillary pressure seems to be a dominant and necessary component of capillary injury, but the particular vasoactive system sustaining that pressure may be less relevant. Despite these considerations and for still poorly understood reasons, the renin-angiotensin-aldosterone system seems in most cases to sustain the arterial and intrarenal pressures.

CELLULAR MECHANISMS OF INJURY

The potential for glomerular capillary pressures to induce progressive sclerotic injury seems clear. This link raises the question of how increased glomerular pressure is translated into cellular pathology. Numerous studies have implicated all 3 of the major glomerular cell types in this process. Because of their similarities to vascular smooth muscle cells, which have vigorous responses to arterial hypertension, and because of the prominent mesangial abnormalities with hyperfiltration, investigators have focused on the mesangial cell's response to altered glomerular hemodynamics.

Mesangial cells, when grown in culture on a pliable matrix, proliferate in response to stretching.[33] This model simulating increased glomerular tension displays, in addition to cellular hyperplasia, increased production of matrix substances such as collagens, laminin, and fibronectin. Cells along the periphery of cyclically stretched membranes, where deformation is most pronounced, develop the greatest degree of proliferation and matrix production. This finding further supports the view that increased physical stretching of the mesangial cells contributes to responses reminiscent of the fundamental processes of sclerosis in vivo. Recently, the molecular mechanisms of the response to mechanical forces have begun to be dissected.[33] The interaction of the mesangial cell integrins with adjacent matrix seems to be crucial in their response to stretching. The connection of these integrins to focal adhesion kinases represents the next step in the signal transduction, which culminates in a series of complex intracellular events (recently reviewed in detail by Ingram and Scholey).[34]

A series of events involving podocyte failure have been proposed by Kriz et al.[37] These investigators have suggested that in the course of a hemodynamically induced sclerosis, the failure of the mesangial cells to provide a tethering function to the overlying basement membrane in conjunction with podocyte insufficiency bares basement membrane, which provides not only an egress for protein, but also a point at which the parietal epithelial cells may adhere to this exposed basement membrane. These investigators describe a process whereby this adhesion represents the point at which filtration then may occur, not into Bowman's space, but through the parietal epithelium into the adjacent interstitium. This mechanism supplies an attractive explanation of how glomerular disruption, of podocytes in particular, could incite adjacent tubulointerstitial disease.

The pathologic events proposed to follow podocyte loss or insufficiency have been developed mainly in animal models of sclerotic injury.[38] Perhaps the strongest evidence that podocytes are likely to be central to the sclerotic process in vivo is the finding that in the glomerular sclerosis attendant to diabetic nephropathy podocyte number is low.[40] This finding was reported from biopsy specimens obtained from Pima Indians, who have a strong predisposition to developing type 2 diabetes and associated nephropathy. Whether the reduction of podocytes was a consequence of the diabetic process or represented a characteristic of this group of people is unknown. In either case, the relative reduction of podocytes was associated with glomerular injury. Recently, this same finding of relative podocyte has been extended to immunoglobulin A nephropathy.[41] The clinical observations coupled with detailed animal studies discussed earlier point to podocyte insufficiency, perhaps in part as a result of the hemodynamic forces and unbri-

dled glomerular enlargement as a key component of sclerotic injury.

In addition to simple geometric mismatch, the presence of proteinuria itself may engender injury in the podocyte. The podocyte is anatomically continuous with the proximal tubular epithelium through the parietal epithelium, and the potential for excess protein load to damage the tubular epithelium has been discussed widely, especially in recent years. With excess endocytosis of abnormally filtered proteins, large numbers of cellular liabilities befall the proximal tubule.[39] These same liabilities may accrue to the presumably smaller, but nevertheless often detectable, increase of endocytosis by podocytes.

Endothelial cells have been studied least for their role in the sclerotic process, but several in vivo and many in vitro observations suggest that they too respond to altered hemodynamics and perhaps influence sclerosing. Lee et al[42] showed that angiotensinogen and transforming growth factor β began to be expressed in the glomerular endothelial cells of rats after subtotal ablation. Hence, endothelial cells may be a site for expression of compounds with the potential to amplify both glomerular pressure and fibrosis. Multiple studies on endothelial cells, largely outside of the glomerular microvasculature, have shown that shear stresses, occasioned by increased flow across the luminal face of the cell, alter cell morphology and signaling molecules.[43] Because plasma flow rates do occur in most instances of hemodynamically induced injury, these shear responses may play some as yet less well described part in the glomerular injury.

SUMMARY

Reductions in nephron number cause increases of filtration rate in residual nephrons—the greater the degree of ablation, the greater the compensatory increase in the function of the residual units. After these seemingly adaptive increases in function, pathologic changes appear, eventuating in glomerular sclerosis. Among the determinants of increased single-nephron filtration after renal mass reduction, the increase in glomerular capillary pressure seems to be pre-eminent in aggravating the progressive sclerotic changes. Actions of the renin-angiotensin-aldosterone system probably underlie many of these hemodynamic changes. Other vasoactive systems also are likely to exert actions in the process but no one element of these systems has yet proven to be a necessary component of heightened pressures. Various cellular responses follow from these higher glomerular pressures, especially in association with the hypertrophy of the glomerular unit. Changes in mesangial cell function, relative deficiency of podocytes, and perhaps endothelial generation of vasoactive and fibroproliferative cytokines, all have been linked in the chain connecting hemodynamic changes with glomerular sclerosis.

REFERENCES

1. Chanutin A, Ferris EB: Experimental renal insufficiency produced by partial nephrectomy. I. Control diet. Arch Intern Med 49:767, 1932

2. Shimamura T, Morrison AB: A progressive glomerulosclerosis occurring in partial five-sixths nephrectomized rats. Am J Pathol 79:95-106, 1975

3. Olson JL, Hostetter TH, Rennke HG, et al: Altered glomerular permselectivity and progressive sclerosis following extreme ablation of renal mass. Kidney Int 22:112-126, 1982

4. Hostetter TH, Meyer TW, Rennke HG, et al: Chronic effect of dietary protein on renal structure and function in the rat with intact and reduced renal mass. Kidney Int 30:509, 1986

5. Kaufman JM, Siegel NJ, Hayslett JP: Functional and hemodynamic adaptation to progressive renal ablation. Circ Res 36:286-293, 1975

6. Hostetter TH, Olson JL, Rennke HG, et al: Hyperfiltration in remnant nephrons: A potentially adverse response to renal ablation. Am J Physiol 241:F85-F93, 1981

7. Steffes MW, Brown DM, Mauer SM: Diabetic glomerulopathy following unilateral nephrectomy in the rat. Diabetes 27:35-41, 1978

8. Grond J, van Goor H, Erkelens DW, et al: Glomerular sclerotic lesions in the rat. Histochemical analysis of their macromolecular and cellular composition. Virchows Arch 51: 521-534, 1986

9. Dworkin LD, Feiner HD: Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. J Clin Invest 77: 797-809, 1986

10. Weening JJ, Beukers JJ, Grond J, et al: Genetic factors in focal segmental glomerulosclerosis. Kidney Int 29:789-798, 1986

11. Adams LG, Polzin DJ, Osborne CA, et al: Influence of dietary protein/calorie intake on renal morphology and function in cats with 5/6 nephrectomy. Lab Invest 70:347-357, 1994

12. Kren S, Hostetter TH: The course of the remnant kidney model in mice. Kidney Int 56:333-337, 1999

13. Narkun-Burgess DM, Nolan CR, Norman JE, et al: Forty-five year follow-up after uninephrectomy. Kidney Int 43:1110, 1993

14. Miller JJ, Suthanthiran M, Riggio RR, et al: Impact of renal donation: Long-term clinical and biochemical follow-up of living donors in a single center. Am J Med 79:201, 1985

15. Kiprov DD, Colvin RB, McCluskey RT: Focal and seg-

mental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. Lab Invest 46:275-281, 1982

16. Zucchelli P, Cagnoli L, Casanova S, et al: Focal glomerulosclerosis in patients with unilateral nephrectomy. Kidney Int 24: 649, 1983

17. Novick AC, Gephardt G, Guz B, et al: Long-term follow-up after partial removal of a solitary kidney. N Engl J Med 325:1058, 1991

18. Nyengaard JR, Bendtsen TF: Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 232:194-201, 1992

19. Brenner BM, Mackenzie HS: Nephron mass as a risk factor for progression of renal disease. Kidney Int Suppl 63: S124-S127, 1997

20. Osmond C, Barker DJ: Fetal, adult, and childhood growth are predictors of coronary heart disease, diabetes, and hypertension in adult men and women. Environ Health Perspect 108:545-553, 2000

21. Jones SE, Nyengaard JR, Flyvbjerg A, et al: Birth weight has no influence on glomerular number and volume. Pediatric Nephrol 16:340-345, 2001

22. Baylis C, Brenner BM: The physiologic determinants of glomerular ultrafiltration. Rev Physiol Biochem Pharmacol 80: 1-46, 1978

23. Dworkin LD, Hostetter TH, Rennke HG, et al: Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. J Clin Invest 73:1448-1461, 1984

24. Taal MW, Brenner BM: Renoprotective benefits of RAS inhibition: From ACEI to angiotensin II antagonists. Kidney Int 57:1803-1817, 2000

25. Ma LJ, Fogo AB: Role of angiotensin II in glomerular injury. Semin Nephrol 21:544-553, 2001

26. Cao Z, Cooper ME: Role of angiotensin II in tubulointerstitial injury. Semin Nephrol 21:554-562, 2001

27. Mackie FE, Campbell DJ, Meyer TW: Intrarenal angiotensin and bradykinin peptide levels in the remnant kidney model of renal insufficiency. Kidney Int 59:1458-1465, 2001

28. Baboolal K, Meyer TW: The effect of acute angiotensin II blockade on renal function in rats with reduced renal mass. Kidney Int 46:980-985, 1994

29. Sorooshian M, Olson JL, Meyer TW: Effect of angiotensin II blockade on renal injury in mineralocorticoid-salt hypertension. Hypertension 36:569-574, 2000

30. Hostetter TH, Rosenberg ME, Ibrahim HN, et al: Aldosterone in progressive renal disease. Semin Nephrol 21:573-579, 2001

31. Daniels BS, Hostetter TH: Adverse effects of growth in the glomerular microcirculation. Am J Physiol 258:F1409-F1416, 1990

32. Benigni A, Peerico N: Research on renal endothelin in proteinuric nephropathies dictates novel strategies to prevent progression. Curr Opin Nephrol Hypertens 10:1-6, 2001

33. Riser BL, Cortes P, Zhao X, et al: Intraglomerular pressure and mesangial stretching stimulate extracellular matrix formation in the rat. J Clin Invest 90:1932-1943, 1992

34. Ingram AJ, Scholey JW: Stress-responsive signal transduction mechanisms in glomerular cells. Curr Opin Nephrol Hypertens 9:49-55, 2000

35. Riser BL, Cortes P, Heilig C, et al: Cyclic stretching force selectively up-regulates transforming growth factor-beta isoforms in cultured rat mesangial cells. Am J Pathol 148:1915-1923, 1996

36. Keane WF, Raij L: Relationship between altered glomerular barrier permselectivity, angiotensin II and mesangial uptake of macromolecules. Kidney Int 25:247, 1984

37. Kriz W, Gretz N, Lemley KV: Progression of glomerular diseases: Is the podocyte the culprit? Perspectives in Basic Science. Kidney Int 54:687-697, 1998

38. Fries JW, Sandstrom DJ, Meyer TW, et al: Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. Lab Invest 60:205-218, 1989

39. Ruggenenti P, Remuzzi G: The role of protein traffic in the progression of renal diseases. Annu Rev Med 51:315-327, 2000

40. Pagtalunan ME, Miller PL, Jumping-Eagle S, et al: Podocyte loss and progressive glomerular injury in type II diabetes. J Clin Invest 99:342-348, 1997

41. Lemley KV, Lafayette RA, Safai M, et al: Podocytopenia and disease severity in IgA nephropathy. Kidney Int 61: 1475-1485, 2002

42. Lee LK, Meyer TW, Pollock AW, et al: Endothelial cell injury initiates glomerular sclerosis in the rat remnant kidney. J Clin Invest 96:953-964, 1995

43. Fisher AB, Chien S, Barakat AI, et al: Endothelial cellular response to altered shear stress. Am J Physiol 281:L529-L533, 2001