Evolving Role of Growth Factors in the Renal Response to Acute and Chronic Disease

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ABSTRACT

The roles of growth factors in the pathogenesis of various forms of acute and chronic renal disease are largely putative. Nevertheless, there is a growing body of information that links specific growth factors to particular forms of renal injury. In all instances, it is supposed that such associations are not necessarily unique and that multiple cytokines probably interact to determine the pattern of injury or the regenerative response to such injury. Regeneration of tubular epithelium after acute tubular necrosis involves up-regulation of the epidermal growth factor (EGF) receptor. Early studies of exogenously administered EGF indicate that the severity and duration of renal failure may be attenuated by this growth factor. Thus far, the observed responses have been limited and the role of EGF as a therapeutic agent requires more study. The mechanism of generation of tubulointerstitial injury in most forms of renal disease is difficult to understand. Early in vitro studies of growth factor production by tubular cells (in the absence of any infiltrating cells) indicate that platelet-derived growth factor produced by the medullary collecting duct is mitogenic for renal medullary fibroblasts, suggesting a paracrine growth system in this region of the kidney. Insulin-like growth factor I has also been shown to be produced by collecting duct cells. Its production is increased by EGF, and its association with certain forms of renal hypertrophy, i.e., diabetes and hypersomatotrophic states, implies its participation in the hypertrophic growth response. Platelet-derived growth factor is a potent mitogen for glomerular mesangial cells, and its production is regulated by a variety of cytokines. In contrast, transforming growth factor β inhibits cell growth and stimulates extracellular matrix deposition in the glomerulus. Results of early studies with anti-transforming growth factor β antibodies have demonstrated suppression of the pathological changes in an experimental model of proliferative glomerular disease associated with increased transforming growth factor β production. Despite a long-standing search for a growth factor that could act as the initiating stimulus for compensatory renal hypertrophy, no such factor has been found. Indeed, studies of gene expression patterns suggest that hypertrophy is a form of growth that does not share early events with different models of cell proliferation, and the nature of this growth response remains an enigma.

Key Words: IGF-I, EGF, PDGF, nephrogenesis, renal hypertrophy, renal regeneration, glomerulonephritis

The study of cell growth control has come to affect all areas of renal pathophysiology and renal disease. Be it the effects of growth factors on resident cells in the glomeruli or tubules during the acute phase of injury, the proliferation or release of cytokines by infiltrating cells, or the regeneration and hypertrophy that attend the processes of repair and adaptation, growth control mechanisms now appear to be critical considerations in the understanding of disturbances of renal function and their resolution. Central to this field of investigation are the humoral substances, which either stimulate or inhibit cell growth and which usually act in concert to determine a specific pattern of growth. Recent reviews have provided an overview of growth factors relevant to
the kidney (1); therefore, their biochemical and biological effects will not be discussed in detail in this article. Instead, this review will focus upon distinct areas of growth control in which one or more growth factors are likely to play a role in the local response to acute or chronic disease.

PUTATIVE ROLE OF EGF IN THE REGENERATIVE RESPONSE TO ACUTE RENAL INJURY

ATN, which follows nephrotoxic or ischemic injury to the kidney, requires a regenerative or mitogenic growth response to restore the integrity of the renal tubular epithelium. It is only recently that this aspect of a relatively common clinical problem has received attention experimentally.

Of the numerous mitogens that are effective in stimulating the proliferation of tubular cells in vitro, epidermal growth factor (EGF) is one of the most potent (2). It increases thymidine incorporation into DNA of quiescent primary cultures of rabbit proximal tubular cells by at least 15-fold. The mammalian kidney itself produces EGF with the mRNA for the precursor molecule, preproEGF, being expressed predominantly in the ascending limb and distal tubule (11). EGF receptors are found on all segments of the nephron with the greatest density being in proximal tubules and collecting ducts (3). Autoradiographic studies of EGF binding to the normal mouse kidney show that receptor density is very low compared with that in the liver (4), which either bespeaks the fact that the kidney is a relatively quiescent organ in terms of cell turnover or that the high density of receptors in the liver represents a mechanism of clearing EGF from the circulation.

After acute injury induced by ischemia (5), expression of preproEGF and mRNA and excretion of EGF by the kidney fall dramatically. This important observation suggests that increased local production of EGF does not play a role in the regenerative response. On the other hand, after nephrotoxin-induced tubular injury, EGF immunoreactivity is redistributed from the inner stripe of the outer medulla towards the renal cortex (6). Whether this represents new expression of EGF by cortical cells or the fact that EGF receptors are up-regulated on these cells and hence bind more EGF has not been pinpointed. Observations do exist, however, which show that EGF receptor density increases within 24 to 48 h of injury, implying that surviving cells or partially damaged cells up-regulate their EGF receptors (4,5).

Given this enhanced ability to bind EGF, the effect of exogenous EGF on the rate and completeness of recovery from ATN has been examined (4,5,7,8). When EGF is administered s.c. or as a slow infusion into the renal arterial circulation (i.e., intra-aortic) in rats with severe ischemic injury, the rise in serum creatinine concentration is attenuated and its return to baseline is more complete than in sham-operated controls (4). A similar effect is seen after only a single s.c. dose of EGF in rats with milder ischemic injury (5,7). This is associated with an increase in nuclear labeling with [3H]thymidine of tubular cells. It is likely, therefore, that EGF, and possibly other mitogens that have yet to be studied, plays a role in the regenerative response. The source of EGF (or possibly another member of the EGF family, such as transforming growth factor alpha) remains to be determined.

TUBULO-INTERSTITIAL CROSS-TALK MEDIATED BY PDGF

Chronic interstitial fibrosis is a characteristic of many forms of glomerular and nonglomerular renal diseases. What causes the laying down of interstitial matrix material and the proliferation of fibroblasts is unknown. Certainly, cytokines and growth factors produced by infiltrating inflammatory cells may play a role in this process. We (L.G. Fine) have recently examined another possibility, i.e., that tubular cells produce and release growth factors that stimulate the growth of adjacent fibroblasts (9,10).

Our results reveal that the growth characteristics of rabbit renal fibroblasts are dependent upon the site in the kidney from which these cells are derived. Thus, papillary fibroblasts in secondary culture have a more rapid doubling time and undergo almost twice as many doublings before attaining a postmitotic state than do cortical fibroblasts. These two populations can be further distinguished by characteristic patterns on two-dimensional gel electrophoresis of proteins and peptides. Whether the differences in phenotype and growth patterns between the two fibroblast populations are due to intrinsic differences in vitro, or whether the methods of isolation and propagation in vitro favor younger mitotic fibroblasts (with more rapid doubling times) in the papilla than in the cortex, remains to be determined.

The mitogenic response to growth factors also differs in that only papillary fibroblasts respond strongly to platelet-derived growth factor (PDGF) (10). Of further interest is the fact that inner medullary collecting duct cells in culture produce PDGF and express the c-sts oncogene, which codes for the B chain of PDGF, whereas proximal tubular cells do not. Although these in vitro studies do not necessarily establish the existence of a similar in vivo system, the apposition of a tubular cell producing a growth factor to adjacent fibroblasts, which respond uniquely to that growth factor, is highly suggestive of the existence of a paracrine growth system in the renal medulla.

If such a system exists, it will become possible to postulate mechanisms of interstitial fibrosis on the basis of primary disturbances of tubular cell func-
tion. Chronic toxicity or low-grade ischemia, such as that caused by cyclosporine, may be the initiating cause of the severe, chronic interstitial fibrosis that characterizes its long-term use. It is also likely that a number of different paracrine systems will be defined in which different interstitial cell types show specific patterns of sensitivity to different growth factors.

**TUBULAR EXPRESSION OF IGF-I: RELATION TO CERTAIN FORMS OF RENAL HYPERTROPHY**

Insulin-like growth factor I (IGF-I) is the predominant growth hormone (GH)-responsive somatomedin. Circulating IGF-I is thought to interact as an endocrine hormone with receptors present on the plasma membranes of a variety of cells. In addition, IGF-I produced locally within a number of tissues acts as an autocrine or paracrine agent (11). The kidney is a site of IGF-I synthesis (12).

Receptors for IGF-I are present on the renal glomerulus and the proximal tubule (13,14). IGF-I is known to exert several actions on the kidney. Administration of this peptide to rats (15,16) and humans (17) increases GFR and RPF and increases kidney size when infused directly into rats (18). States of GH excess (hypersomatotropic states) are characterized by elevations of circulating IGF-I levels and are accompanied by hypertrophy of the renal proximal tubule. Indeed, many actions of GH on renal size and kidney function appear to be mediated indirectly through the stimulation of synthesis and release of IGF-I.

We (M.R. Hammerman) have shown that the collecting duct is a major site of IGF-I synthesis within the rat kidney (19–22). IGF-I has been localized to the principal cells of the collecting duct by immunohistochemistry, and IGF-I mRNA colocalizes to this portion of the nephron (19). Administration of GH to normal (21) or hypophysectomized (18,19) rats results in enhanced immunostainable IGF-I in the collecting duct, as well as elevated levels of IGF-I mRNA. Incubation of collecting ducts isolated from rat kidney with GH similarly increases quantities of IGF-I present in suspensions and elevates levels of IGF-I mRNA (22). These findings demonstrate that GH enhances IGF-I gene expression via a direct action on the collecting duct.

The studies detailed above establish the presence of a GH-IGF-I axis within the kidney (23). Although IGF-I is present in urine, its concentration is low (approximately 10–11 M) (24). This suggests that IGF-I is not secreted into the collecting duct lumen but rather exits from the collecting duct across the basolateral membrane. Receptors for IGF are not present in the collecting duct (20), therefore, the IGF-I produced there must act at other sites in the kidney at which receptors are present, such as the glomerulus and proximal tubule, where IGF-I could function as a paracrine growth factor.

Agents that stimulate secretion of IGF by cells in culture have been shown in many cases to be distinct from those that increase levels of circulating IGF-I such as GH. Certain cell types respond to stimuli that are trophic for the organ of origin with an increase in IGF-I secretion. For example, gonadotropins and estradiol have been shown to enhance IGF-I production by ovarian cells _in vitro_ (25) and parathyroid hormone has been demonstrated to cause release of IGF-I from neonatal rat calvaria in organ culture (26).

To shed light upon the identity of agents other than GH that regulate IGF-I gene expression in kidney, we (M.R. Hammerman) incubated isolated rat renal collecting ducts with EGF. EGF, like IGF-I, is produced in the kidney. The sites of renal EGF synthesis are the thick ascending limb of Henle's loop and the distal convoluted tubule. Because it is synthesized in distal portions of the kidney, EGF may act upon cells in the collecting duct. Inclusion of EGF in suspensions of collecting ducts increased the production of IGF-I in a concentration-dependent manner (Figure 1). As is the case for GH, levels of IGF-I mRNA were increased by incubation of isolated collecting ducts with EGF (Figure 2). These findings demonstrate a direct action of EGF to enhance collecting duct IGF-I gene expression _in vitro_. Such enhancement is likely to reflect an effect of EGF to stimulate IGF-I production in the collecting duct of intact kidney. Because EGF is produced in the kidney, these observations are consistent with intrarenal paracrine regulation of IGF-I gene expression by EGF (an EGF–IGF-I axis) (27).

A role for renal IGF-I in the glomerular and proximal tubules has been suggested by the localized expression of IGF-I within these structures. This localization suggests a role for IGF-I in the maintenance of renal tissue homeostasis and/or the prevention of renal disease (28).

Figure 1. Levels of immunoreactive IGF-I in collecting duct suspensions. Collecting ducts were incubated in the absence of hormone (O) or in the presence of varying concentrations of recombinant human EGF for 2 h. Data are expressed as mean ± SE of three experiments. Reprinted from reference 25 with permission.

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mal tubular hypertrophy that occurs in hypersomatotropic states, in compensatory renal hypertrophy, and in diabetic renal hypertrophy is suggested by observations from several laboratories. First, IGF-I may be the major stimulus for renal cellular growth in hypersomatotropic states such as acromegaly (28), because GH administered in vivo or added to collecting ducts in vitro enhances IGF-I gene expression. Second, IGF-I could function as a hypertrophic stimulus in compensatory renal hypertrophy. We (20) and other investigators (29) have demonstrated enhanced IGF-I expression within the kidney in the setting of compensatory growth. Finally, levels of intrarenal IGF-I are increased in association with the renal hypertrophy that accompanies early diabetes mellitus (30).

Levels of IGF-I mRNA extractable from the kidney are elevated in hypersomatotropic states (21), consistent with increased transcription of the IGF-I gene being causative of enhanced IGF-I expression in this setting. In contrast, levels of extractable IGF-I mRNA are not increased in compensatory hypertrophy (20) or in diabetic hypertrophy (30). Increased translation of IGF-I mRNA could underlie enhanced IGF-I expression in these conditions. The differences between hypersomatoplasia, compensatory hypertrophy, and diabetic hypertrophy suggest that the stimulus/stimuli for enhancement of renal IGF-I gene expression differs in the former condition from one or both of the latter. Clearly, GH cannot be the stimulus in compensatory hypertrophy because compensatory renal growth occurs in the hypopituitary state (35). Similarly, GH cannot be the stimulus in diabetes mellitus because levels of circulating GH are depressed in this condition in the rat (31). The identity of stimuli for enhancement of IGF-I gene expression in compensatory renal hypertrophy and in diabetic hypertrophy is yet to be determined.

**PDGF, TGF-β, and Their Interactions With Other Growth Factors in the Glomerular Response to Injury**

The glomerulus is invariably involved in the course of many types of immune- or nonimmune-mediated kidney disease. The involvement of the glomerulus manifests as hypercellularity because of intrinsic cell proliferation, infiltration by inflammatory cells (32–34), or both. Subtle morphological evidence of injury to one of the glomerular cell types may also be a presenting manifestation. Glomerular enlargement, with or without hypertrophy or proliferation of cells (e.g., mesangial cell), may occur. Changes in capillary permeability and hemodynamic abnormalities (including an increase in glomerular blood flow, filtration rate, and hydrostatic pressure) are additional manifestations of glomerular disease. Both the hemodynamic abnormalities and glomerular hypertrophy may precede the advanced lesions that lead to matrix expansion and eventual sclerosis of the glomerulus. Considering the diverse biological effects of polypeptide growth factors, it is clear why they have potential involvement in glomerular pathology (35). These peptides participate in such diverse processes as growth (hypertrophy or proliferation) regulation of matrix synthesis and degradation, development and differentiation, immunoinflammatory responses, and importantly, regulation of vascular tone.

Although the systemic circulation is a potential source of peptides such as EGF and IGF-I, mounting evidence suggests that they do not primarily function in an endocrine manner but rather in an autocrine or short-loop paracrine fashion. Thus, an important source of growth factors in the glomerulus is intrinsic glomerular cells themselves, as well as infiltrating inflammatory cells and platelets (34,36,37). Mesangial cells synthesize several peptide growth factors such as interleukin 1 (IL-1), PDGF, IGF-I, macrophage and granulocyte-macrophage–colony-stimulating factors (Table 1).

**TABLE 1. Potential sources of growth factors in the glomerulus**

<table>
<thead>
<tr>
<th>Mesangial cells</th>
<th>PDGF, IL-1, IGF-I, TNF-α, TGF-β, IL-6, MCSF, GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial cells</td>
<td>PDGF</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>Heparin</td>
</tr>
<tr>
<td>Infiltrating cells</td>
<td>IL-1, PDGF, TGF-α, IGF-I</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td>PDGF, EFG, TGF-β</td>
</tr>
<tr>
<td>Platelets/lymphocytes</td>
<td>TGF-β</td>
</tr>
</tbody>
</table>

*MCSF, macrophage colony-stimulating factor; GM-CSF, granulocyte-macrophage–colony-stimulating factor.*
There are several mechanisms by which glomerular and, specifically, mesangial cell proliferation could affect glomerular function. Proliferating cells may undergo phenotypic modulation that results in enhanced matrix production and secretion. Up-regulation or down-regulation of receptors for cytokines or vasoactive hormones may be a manifestation of proliferating cells. Secretion of cytokines or growth factors may also be modulated in such cells. Finally, simple mechanical clogging of the glomerular capillaries may also result from overgrowth of these cells.

A wide range of growth factors has been associated with the glomerulus. Ballerman (38) showed that glomerular endothelial cells secrete a protein that competes for the binding of labeled PDGF to mesangial cells. Glomerular epithelial and endothelial cells have been shown to release heparin-like material, which inhibits mesangial cell proliferation. PDGF stands out as the most potent mitogen followed by basic fibroblast growth factor, EGF, and transforming growth factor alpha (TGF-α). Some peptides inhibit mesangial cell proliferation. Prominent among these is transforming growth factor beta (TGF-β), which also inhibits epithelial and endothelial cell proliferation as discussed below.

That abnormal growth of glomerular cells may lead to progression of glomerular disease has been suggested by observations of transgenic mice (39). Certain peptide growth factors may also be involved in glomerular hypertrophy, as has been suggested for IGF-I (39). Recent studies with mice transgenic for GH, GH releasing factor (GHRF), and IGF-I demonstrated that only mice transgenic for GH and GHRF develop hypertrophy and sclerosis (39,40). Mice transgenic for IGF-I did develop some degree of hypertrophy but no sclerosis. This occurred even when circulating levels of IGF-I were higher than those observed in GH and GHRF transgenics. (It should be emphasized, however, that glomerular levels of IGF-I were not reported and that a local increase of IGF-I may have been responsible for the hypertrophy.)

There is accumulating evidence that growth factors may be involved in the pathogenesis of glomerular disease. As pointed out above, PDGF is the single most potent mitogen for mesangial cells (37). It is synthesized by glomerular mesangial cells as well as by microvascular endothelial cells isolated from whole kidney tissue (36). Mesangial cells express PDGF A and B chain mRNAs that are regulated in vitro by a number of peptide growth factors as well as by phospholipids and vasoconstrictors (37). The potential involvement of PDGF in proliferative glomerular diseases in humans and experimental animals is suggested by the studies of Iida and colleagues, who recently reported that PDGF may be an important factor in the pathogenesis of mesangial proliferative nephritis (41). These authors found increased expression of PDGF and PDGF receptors, as well as their respective mRNAs, in glomeruli of a rat model of mesangial proliferative nephritis induced with an antibody to Thy-1 antigen present on mesangial cells (41).

TGF-β is another peptide that appears to be involved in mediating glomerular pathology. TGF-β binds to isolated mouse glomeruli as well as to glomerular epithelial mesangial and endothelial cells. Jaffer et al. (42) and MacKay et al. (40) demonstrated an inhibitory effect of TGF-β on all glomerular cell types. TGF-β in vivo is a potent stimulus of the synthesis of proteoglycans, fibronectin, and other matrix components in cultured mesangial and epithelial cells (44). A potential role of TGF-β in mediating matrix expansion in proliferative glomerular disease has recently been demonstrated by Border and colleagues (43,44). These investigators demonstrated an increased production and activity of TGF-β1 in isolated glomeruli from rats with a proliferative glomerular disease induced by the injection of anti-Thy-1 antibody. Most recently, these investigators demonstrated that the administration of anti-TGF-β1 at the time of the induction of glomerular disease suppresses the increased production of extracellular matrix and dramatically attenuates histological manifestations of the disease.

It should be emphasized that glomerular growth may be mediated by other cytokines such as interleukin 6 (45) and by vasoactive compounds such as angiotensin II, serotonin, and arginine vasopressin. Many of these compounds have been shown to induce metabolic changes consistent with hypertrophy or proliferation in cultured smooth muscle cells and in mesangial cells. Delineating the precise roles of these compounds and their relations to growth factors in mediating pathological changes in the kidney, and specifically in the glomerulus, is an area of increasingly fruitful investigation.

COMPENSATORY RENAL HYPERTROPHY: A GROWTH FACTOR-INDEPENDENT FORM OF TUBULAR GROWTH?

The hallmark of adaptive growth in the diseased kidney is an enlargement of the nephron, which occurs predominantly by a process of hypertrophy rather than hyperplasia (46). (This statement is not absolute, because in young animals and after extensive renal ablation, there appears to be significant hyperplasia of cells in addition to their hypertrophy.) How this growth process proceeds is entirely unknown because the described effects of growth factors have been confined largely to stimulation or inhibition of mitogenesis rather than to control of cell size. A key question thus becomes: Is the initiation of renal hypertrophy and, in particular, tubular cell hypertrophy under the control of growth factors?
To answer this question, it is relevant to attempt to separate a direct hypertrophic effect of one or more growth factors from a permissive effect on the growth process. An example of this is illustrated by the role of IGF-I in compensatory hypertrophy. If renal ablation is performed in GH-deficient animals, which have low levels of IGF-I in the kidney, there is blunting of, but not ablation of, the hypertrophic response (47). This illustration regarding a single growth factor probably applies more broadly. Thus, it is likely that an array of circulating growth factors and growth inhibitors establishes a permissive steady state for controlling both cell turnover rate and cell size and that superimposed upon this are adaptive responses to acute and chronic injury.

To establish whether growth factors are involved in renal tubular cell hypertrophy, we (L.G. Fine) initially tested the hypothesis that elaboration of a growth inhibitor could arrest the growth of cells responding to mitogens and, because the arrest would occur after the entry of quiescent cells into the cell cycle when early accumulation of protein and an increase in size take place, that the result would be the conversion of hyperplasia to hypertrophy. Indeed in an in vitro system, this could be shown to occur. When the growth inhibitor TGF-β was added to the mitogenic combination of insulin and hydrocortisone, DNA synthesis by proximal tubular cells in culture was inhibited but protein content per cell was increased (48).

To determine whether this applies in vivo after partial renal ablation (uninephrectomy), we used the strategy of asking whether the early events in hyperplasia are shared by cells undergoing early hypertrophy. If they are, it would indicate that both processes follow the same initial pathway but that hypertrophy deviates from this at a later stage because DNA synthesis does not occur. On the other hand, if the early events in hypertrophy and hyperplasia are totally dissimilar, the former presumably cannot be initiated by growth factors that cause mitogenesis. To test this, we compared the regenerative responses to folic acid injection (which causes self-limiting acute tubular injury) to unilateral nephrectomy (49). Within a few hours of folic acid injection, the pattern of expression of proto-oncogenes (c-fos, c-myc) and genes encoding for structural proteins (β-actin, vimentin) and transport proteins (NaKATPase, etc.) was entirely consistent with the pattern seen in cultured cells after the addition of growth factors or tumor promoters, i.e., an early and transient increase in the expression of these genes over the first 12 h. In contrast to this response, no increase in mRNA levels for these proteins above sham-operated control levels was observed after unilateral nephrectomy (49). Thus, the early events in in vivo compensatory hypertrophy appear to differ fundamentally from those that occur in regenerative hyperplasia.

To further explore this comparison, we have exploited the recent elucidation of a family of primary response genes that are rapidly induced by growth factors and tumor promoting agents, i.e., their expression is the hallmark of early mitogenesis. These genes, which encode for transcription factors or transcriptional activators, cytokines, etc., are activated without the need for intervening protein synthesis (and, indeed, may show superinducibility when cells are treated with the protein synthesis inhibitor cycloheximide). mRNA levels reach a peak at 30 to 60 min after the addition of a mitogen (50). Of further importance is the fact that the same family of genes is activated when certain cell types are induced to undergo differentiation, e.g., in the PC12 rat pheochromocytoma cell line, which is stimulated to become neuron-like upon treatment with nerve growth factor (51). If hypertrophy is a form of cell differentiation, i.e., "amplified differentiation," it is feasible to expect the same family of genes to be expressed early in hypertrophy. We have been unable to find any evidence for the expression of TPA-inducible sequences (TIS genes) after uninephrectomy in the mouse, whereas these genes are strongly induced after the administration of folic acid (52).

Taken together, these findings argue for the fact that hypertrophy is initiated by mechanisms that are fundamentally different from those that occur after growth factor-induced mitogenesis or differentiation. Alternative explanations for the initiation of the cell enlargement and protein accretion must therefore be sought.

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"Like many good things, this term (clearance) was born of necessity. In 1926 (D.D.) Van Slyke had been on his way to Baltimore to give an address on kidney function, and on the train his courage failed him when he thought of facing an audience again with a mathematical equation. He had learned what every lecturer must ultimately learn, that only experts can visualize and comprehend the true realities which the unreal symbols of a mathematical equation are intended to represent: the simplest equation has the fearsome power of completely dispelling the comprehension of an audience, at least in the fields of medicine. As Van Slyke sat on the train seeking a solution of how to dispense with mathematics for the benefit of the medical profession, it occurred to him that all that the equation for high urine flows said was that in effect some constant volume of blood was being "cleared" of urea in each minute's time."