OF HUMAN LOSS AND ERYTHROCYTE SURVIVAL: UREMIA AND ANEMIA IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Uremia has long been associated with shortened erythrocyte survival. Renal secondary hyperparathyroidism and increased serum parathyroid hormone concentrations have measurable effects on erythrocyte metabolism, osmotic fragility, shape, and deformability that may decrease survival and contribute to the anemia of chronic kidney disease. In addition, excess parathyroid hormone and decreased calcitriol levels can affect a patient's responsiveness to erythropoietin through direct and indirect inhibitory effects on erythropoiesis. Parathyroidectomy or calcitriol treatment for patients with uremia, especially those resistant to the effects of erythropoietin, may result in resolution of anemia. Eitan Bogin, a dedicated and talented biochemist, was one of the first to characterize the role of parathyroid hormone as a uremic toxin with deleterious effects on erythrocytes. The goal of this article is to focus on Eitan Bogin's role in the unfolding story of parathyroid hormone, erythrocytes, and anemia, and the legacy of his scientific contribution to current medical and veterinary treatment of chronic kidney disease.

INTRODUCTION

It is human loss that drives this story: the recent loss of Eitan Bogin, a dedicated scientist and valued colleague. It is survival, however, that begins this story: erythrocyte survival in chronic kidney disease. Erythrocyte research was a source of much shared scientific enthusiasm for Eitan and myself for many years.

As a PhD student at the University of Minnesota in the mid-1980s I studied Heinz body anemia in cats, the demise of erythrocytes under the influence of oxidative damage. My research included experiments on hemoglobin, membrane pathology, and erythrocyte life span, and included the role of biochemical changes on erythrocyte shape and function. As a budding and passionate 'red cell person' I avidly read every article I could glean from *Current Contents* about red cell morphology, biochemistry, and survival. One such paper that I read with interest was the "Effect of parathyroid hormone on osmotic fragility of human erythrocytes" published by Bogin et al in the prestigious *Journal of Clinical Investigation* [1]. The name Bogin meant nothing to me at the time, but his findings on the effects of uremia and parathyroid hormone (PTH) on erythrocytes were pertinent to ongoing studies by my thesis advisor, John Eaton, on oxidative damage of erythrocytes in uremic patients undergoing dialysis [2,3] and to my own observations on erythrocyte oxidative damage in cats with chronic renal failure [4].

In 1986, in the midst of my thesis research, a meeting poster for the IInd Congress of the International Society of Animal Clinical Biochemistry (ISACB, [5]) was taped onto the door of the –70° freezer in our research laboratory. Unbeknownst to me, the congress was being organized by Eitan Bogin. Every day, as I retrieved vials of NADPH and erythrocyte lysates from the freezer, I saw the poster's image of Jerusalem, domes gleaming in the sun, beckoning in that way of far-off places. Although I was unable to attend the Jerusalem meeting, I did travel to the Vth Congress of the ISACB in Parma Italy four years later, when I was an Assistant Professor at the University of Florida. It was in Parma, on a sunny afternoon on the Piazza Garibaldi, that I was first introduced to Eitan Bogin. I learned in the course of the conference that he was one of the founders of the ISACB, that he had a passion for bringing clinical biochemistry to developing countries, and importantly, that he was the author of that memorable article on PTH and erythrocyte fragility. The Parma meeting marked the beginning of my friendship with Eitan and with many others in the ISACB. The cultural appreciation and passion for global science nurtured by Eitan through the ISACB has had a long-term impact on my own career.

In this article, to acknowledge our mutual interest in erythrocytes, my goal is to focus on Eitan Bogin's role in the unfolding and sometimes controversial story of PTH, erythrocytes, and anemia, and to consider the legacy of his scientific contribution to current medical and veterinary treatment of chronic kidney disease. Rather than a comprehensive review, this narrated journey of Eitan's work will highlight and place into context his research on this important topic.

Biochemical Abnormalities and Erythrocyte Survival

Erythrocytes are itinerant workers, moving from tissue to tissue in the vital job of transporting oxygen and removing carbon dioxide from human and animal cells, the respiration

essential to life. It is estimated that erythrocytes travel a distance of more than 2 km daily through the circulation, which for a canine or human erythrocyte with a life span of 120 days is a journey of 250 kilometers [6], most of the distance from Jerusalem to Eilat! Throughout its lifetime, an erythrocyte passes repeatedly through the microvasculature of every tissue in the body, exposed to the effluent of physiological processes, the vagaries of biochemical fluxes, and the imperfections of endothelial surfaces, until finally it succumbs, senile and aged, to the immunologic and mechanical triggers that end its life.

Occasionally, disease processes such as antibody-binding, hemoparasites, or oxidative damage may intervene and abruptly end the life span of erythrocytes, resulting in severe hemolytic anemia. In many disease processes, however, more subtle biochemical changes occur in the plasma and tissues that have a more subtle effect on erythrocytes, shortening their life span only slightly or moderately and contributing to mild or moderate anemia, which may be compensated in patients with effective erythropoietic capability [7]. Such low-grade biochemical changes include oxidative stress, such as that seen in diabetes and cancer; abnormal phospholipids and excess cholesterol resulting from hepatic disease or diet; endotoxins and cytokines released from sites of inflammation; drugs, heavy metals, and uremic toxins; and changes in pH and albumin, calcium, phosphorus, and electrolyte concentrations [6,7]. These biochemical abnormalities may act by destabilizing the lipid bilayer of the erythrocyte, altering cytoskeletal integrity, altering red cell shape, decreasing deformability, and altering metabolic function in ways that gradually accelerate the removal of red cells from the circulation and bring their journey to a premature end.

Understanding the biochemical processes that shorten the survival of erythrocytes is critical to understanding how best to treat the underlying causes of anemia. Because chronic diseases often also depress bone marrow hematopoietic activity, the ability of patients to replenish damaged red cells may be impaired. Anemia is a debilitating complication of many chronic diseases and contributes to depression, weakness, and impaired cognitive function. Anemia also may impair the ability of patients to handle the drugs or rigorous treatment regimens required to treat the underlying disease.

Pathogenesis of Anemia in Chronic Kidney Disease

Nonregenerative anemia is a consistent sequel of chronic kidney disease, which results from the gradual and irreversible decline in the number of functional nephrons. Chronic kidney disease, a term synonymous with chronic renal failure, end-stage renal failure, and chronic renal insufficiency, is the most common renal disorder affecting dogs and cats and a major cause of morbidity and mortality [8]. Chronic kidney disease also affects an estimated 7.7 million adults in the United States [9] and appears to be increasing in prevalence in Europe [10]. In the United States alone, over 80,000 patients die each year from end-stage renal disease [11]. Decreased erythropoietin (EPO) production due to loss of renal functional mass is the primary underlying cause of anemia in chronic kidney disease. Hormone replacement therapy with human recombinant EPO is the treatment of choice for anemia in humans, dogs, and cats with chronic renal failure [8, 12]. In addition to loss of renal endocrine function, renal excretory function also is impaired in chronic kidney disease, leading to an accumulation in the blood of toxic substances normally cleared by the kidney. Calcium-phosphorus, acid-base, and electrolyte imbalances also result from impaired renal function. Many of these biochemical changes may have an effect on red cell shape and survival, which even though mild, can exacerbate the anemia of chronic kidney disease.

Uremia is the clinical manifestation of the accumulated biochemical compounds retained by the kidneys. These compounds include amino acids, phosphates, potassium, organic acids, aromatic compounds, guanidines, homocysteine, polyamines, and many others [8, 12]. These retained uremic toxins contribute to many clinical signs and symptoms of renal insuffiency, including anorexia, nausea, vomiting, stomatitis, oral ulceration, gastroenteritis, central nervous system depression, seizures, bleeding tendencies, hypothermia, and an impaired immune system. It had long been suspected that one or more of these uremic toxins might also have an effect on erythrocyte survival. As early as in 1958, erythrocytes from a uremic patient were found to have shortened life span but to survive normally when transfused into a healthy individual, suggesting that "extracorpuscular factors" decreased red cell survival in uremia [13]. Similar studies in the 1960s found an inverse relationship between the severity of loss of renal excretory function and erythrocyte life span, which was occasionally normalized by dialysis [6, 14]. Subsequent studies in the 1970s demonstrated that erythrocytes of uremic patients also had several metabolic defects that could result in their premature sequestration and destruction, including decreases in hexose monophosphate shunt, transketolase, and Na^+-K^+-ATPase activities [6,14]. Although the responsible substances were not identified, it was clear that the toxic biochemical environment of uremia was associated with erythrocyte abnormalities that could shorten erythrocyte survival and potentially contribute to anemia.

Hemodialysis or peritoneal dialysis is the primary way to remove uremic toxins from the blood and alleviate the consequences of uremia [8, 12]. Worldwide, an estimated 1.5 million human patients are undergoing dialysis for end-stage renal failure [15]. Hemodialysis treatment for animals is available in only a few veterinary hospitals and clinics around the world. Although hemodialysis is routine for human patients with uremia, and more than 90% of adult human hemodialysis patients also receive treatment with EPO for anemia, more than half of them remain moderately to severely anemic [16]. Iron deficiency is an important cause of this persistent anemia, however, retention of uremic toxins also appears to play an important role [17].

Parathyroid Hormone and Erythrocytes in Uremia

In 1977, Dr. Shaul Massry, a professor of nephrology at the University of Southern California in Los Angeles,

hypothesized in an editorial that PTH was a uremic toxin [18]. Humans and monogastric animals often develop renal secondary hyperparathyroidism (HPTH) secondary to the sustained increase in PTH caused by phosphate retention and hypocalcemia in chronic renal failure. Although the pathogenesis of renal secondary HPTH is complex and remains somewhat controversial, common features usually include hyperphosphatemia, low blood calcitriol (1,25-dihydroxycholecalciferol, vitamin D) levels, hypocalcemia (low blood ionized calcium concentration), and skeletal resistance to the calcemic effect of PTH [8,12]. Recent attention has focused on altered vitamin D metabolism and the possibility that calcitriol deficiency plays a critical role in renal secondary HPTH. Metabolic changes associated with HPTH can also lead to renal osteodystrophy, including osteitis fibrosa (marrow fibrosis) in humans, and soft tissue calcification in animals.

Although kidney and bone are the main target organs for PTH, its effect on erythrocytes became a focus of study in the early 1980s. The primary ways in which PTH might be involved in the pathogenesis of the anemia of uremia were summarized by Massry as early as in 1983: 1) decreased erythrocyte survival; 2) inhibition of erythropoiesis; 3) induction of marrow fibrosis; and 4) blood loss, from the inhibitory effect of PTH on platelet aggregation (Figure 1) [19]. By 1983, Eitan Bogin, who had long been working in Massry's laboratory in southern California, engaged in some of the first research to address these important questions of pathogenesis.

Effect of PTH on erythrocyte survival

Osmotic fragility and the role of calcium

In his seminal study in 1982, Eitan Bogin tested the hypothesis that excess PTH increased the susceptibility of erythrocytes to osmotic lysis by facilitating the entry of calcium into the cells [1]. He incubated different portions of the PTH molecule—the amino-terminus (1-34 bPTH), the carboxy-terminus (53-84 PTH), and intact PTH (1-84bPTH)—with erythrocytes in vitro, and measured their tendency to lyse in solutions of increasing hypotonicity. He demonstrated a dose-response relationship between increased red cell fragility and the concentrations of both intact PTH and the amino-terminal fragment of PTH. Inactivation of the hormone eliminated the effect, indicating reliance on the biological activity of PTH. Importantly, he showed that the increase in osmotic lysis was dependent on the presence of calcium: it could be mimicked using a calcium ionophore and was partially blocked by the presence of verapamil. He also directly measured the increase in calcium uptake into erythrocytes using ^{45}Ca and showed it to be independent of glycolysis, potassium concentration, and the water content of the cell. He determined that the calcium influx was accompanied by marked and significant stimulation of Ca-ATPase, a membrane enzyme regulating the intracellular concentration of calcium. This effect on calcium was consistent with the known effect of PTH on other cell types. Thus, with this elegant set of experiments, Eitan and his colleagues were able to conclude that the red cell was a target organ for PTH, that the hormone directly increased osmotic fragility, and that the mechanism of the effect was enhanced calcium entry into the cells. This study was one of the first to identify PTH as a uremic toxin and a likely suspect for causing shortened red cell survival in the pathogenesis of anemia in uremic patients.

Subsequent studies of erythrocyte osmotic fragility, PTH, and uremia have yielded conflicting results, likely due to differences in acute versus chronic exposure of erythrocytes to PTH and differences in methodology and treatment. In 1985, Docci et al [20] found significantly increased osmotic fragility in 35 uremic patients on hemodialysis, but the changes did not correlate with the severity of HPTH and did not improve with parathyroidectomy or treatment with 1,25-dihyroxycholecalciferol. A similar lack of correlation was observed in 20 pediatric patients on peritoneal dialysis [21]. In 1989, Foulks et al [22] also found no difference in erythrocyte osmotic fragility in patients with renal failure and HPTH and no relationship between PTH, osmotic fragility, and hematocrit (HCT). In 17 dogs with chronic renal failure, osmotic fragility also was not increased, but a control group was not clearly defined in the study and the dogs were heterogeneous with regards to type of renal disease and presence of anemia [23]. The results of other and more recent studies, however, strongly support a relationship between osmotic fragility and PTH. In 1996, Chen and Young [24] found significantly higher osmotic fragility in nephrectomized rats that was eliminated by thyroparathyroidectomy and re-occurred with administration of exogenous PTH. In 1998, Wu et al [25] found significantly higher osmotic fragility in uremic patients with intact PTH concentrations >100 pg/dL; red cell fragility was diminished following hemodialysis.

After his return to Israel, while working with colleagues at the Kimron Institute and at Tel-Aviv University Medical School, Eitan published follow-up experiments that confirmed his results on PTH-mediated osmotic fragility and calcium influx in a rabbit model. In experiments published in 1987 he found that erythrocytes from newborn rabbits were much more susceptible to PTH-mediated damage than those from adult rabbits, concomitant with greater stimulation of Ca-ATPase in erythrocytes from newborns [26, 27]. The importance of calcium in mediating the effect of PTH on erythrocytes was also gaining support from other investigators. In 1999, for example, using the fluorescent dye Fura-2, Soldati et al [28] found that uremic patients had higher cytosolic free calcium, compared with age-matched control subjects, and that high plasma levels of PTH augmented the entry of calcium into erythrocytes. Subsequent research has unequivocally asserted the importance of chronically increased PTH levels on calcium influx and sustained high intracellular calcium concentrations in the cells of many tissues in the body, including cardiac myocytes, pancreatic islet cells, and hepatocytes, contributing to the widespread deleterious effects of uremia on multiple organ systems [28].

Effect of PTH and calcium on erythrocyte shape and deformability

In his studies on PTH and osmotic fragility in erythrocytes

from humans [1] and rabbits [26,27], Eitan Bogin discovered, using scanning electron micrography, that PTH induced filamentous extensions on erythrocyte membranes that anchored the cells together. He surmised that these membrane abnormalities could hasten the demise of erythrocytes in vivo. Although the mechanism for the shape change was not determined, intracellular calcium and its many effects on erythrocyte membrane and metabolic function were suspected. Excess intracellular calcium has deleterious effects on the spectrin-actin cytoskeletal network and phospholipid bilayer, and causes other functional and shape abnormalities that alter the stability and integrity of the red cell membrane by increasing rigidity (decreasing deformability) and thereby increasing susceptibility to lysis [6]. Deformability is an important determinant of the survival of a red cell in the circulation.

In 1986, Eitan Bogin led an investigation on the effects of PTH and uremia on erythrocyte deformability [29], building on a previous study of erythrocyte sedimentation rate [30]. He found that PTH significantly decreased the ability of erythrocytes to pass through the pores of filter paper, a measure of their ability to deform. The decrease in deformability was calcium-dependent and correlated with the concentrations of both calcium and PTH. In addition, erythrocyte deformability decreased when erythrocytes were suspended in serum ultrafiltrate from patients with chronic renal failure and HPTH, but not in serum from patients following parathyroidectomy or healthy control subjects. These findings suggested that decreased erythrocyte deformability in uremic patients was caused, at least in part, by increased intraerythrocytic calcium mediated by high PTH concentrations. Ongoing work in Massry's laboratory suggested that phospholipid turnover in the erythrocyte membrane might be another mechanism underlying this effect [31]. In that study, calcium caused a significant increase in the content of phosphatidylserine, a procoagulant that could enhance aggregation and increase the rigidity of red cells, making them more susceptible to lysis.

Recent research using contemporary methods to measure erythrocyte deformability has supported many of Bogin's initial findings. Using blood from healthy volunteers, Mark et al [32] demonstrated that intracellular calcium, but not PTH, calcitonin, or extracellular calcium, led to a profound increase in blood viscosity at both low and high shear rates, due to decreased RBC deformability. In addition, changes in deformability were accompanied by severe echinocytic shape change of erythrocytes. Echinocytosis led to exovesiculation and loss of membrane phospholipid asymmetry, exposing phosphatidylserine on the red cell surface. Echinocytosis also increased membrane-bound hemoglobin, which alters membrane proteins and increases cell rigidity. These results not only verified Bogin's findings of a calcium-mediated decrease in red cell deformability, but also verified membrane phospholipid abnormalities and related these findings to important shape changes that could account for the increased osmotic fragility and decreased survival of erythrocytes in uremic patients.

Echinocytic transformation is one of the most consistent red cell shape abnormalities in people and animals with uremia and has been associated with increased intraerythrocytic calcium concentration as well as with altered electrolyte concentrations (Figure 1) [6, 33]. Because echinocytes are relatively rigid cells with less deformability, they have shortened survival compared with normal erythrocytes. A study of uremic patients undergoing dialysis demonstrated a transient increase in echinocytes and erythrocyte sedimentation rate [34]; a weak correlation also was found between the calcium content of erythrocytes and the percentage of echinocytes [35]. Echinocytes disappeared when erythrocytes from uremic patients were incubated in buffer, and control erythrocytes become echinocytic when placed in uremic patient plasma [36]. Also, as in the study by Marks et al, the degree of echinocytosis was related to increased blood viscosity at high shear rates [36]. The results of a 2007 study further documented increased exposure of phosphatidylserine, decreased membrane fluidity, cholesterol shedding, and echinocyte formation in erythrocytes from patients with varying severity of uremia, and relate these abnormalities to decreased erythrocyte deformability [37].

PTH and erythrocyte life span

Since the early studies done by Eitan Bogin, considerable evidence had accumulated to suggest that PTH and calcium influx caused increased red cell osmotic fragility, phospholipid abnormalities, shape changes, and decreased deformability. Additional studies were needed, however, to further correlate excess PTH with decreased erythrocyte survival in uremic patients. Akmal et al [38] demonstrated in 1985 that excess PTH levels and not other consequences of the uremic state contributed to shortened RBC survival in chronic renal failure in dogs. Using a nephrectomized dog model and using ^{51}Cr to measure red cell life span, they found that erythrocytes from dogs with renal failure had significantly shorter red cell survival, whereas, thyroparathyroidectomized dogs with renal failure had the same erythrocyte survival as control dogs. Saltissi and Carter [39] also found a marked difference in red cell survival, as measured by ^{51}Cr in human hemodialysis patients with secondary HPTH, although no difference was found in the severity of anemia.

Eitan Bogin contributed to several experiments that provided indirect evidence of shortened erythrocyte survival based on finding a higher proportion of younger erythrocytes in the circulation of uremic patients [40,41]. As uremic toxins cause erythrocytes to be removed prematurely from the circulation, the bone marrow responds by stepping up production of new erythrocytes, resulting in a generally younger erythrocyte population in the blood. In support of this hypothesis, aspartate transaminase (AST or GOT) activity and membrane sialic acid concentration were found to be higher in RBC populations from uremic-anemic patients, and AST was relatively higher in "older" erythrocytes (based on density centrifugation) from uremic patients compared with those from control subjects [41]. These results supported a shorter than normal erythrocyte life span and subsequent enrichment of younger erythrocytes in the circulation of uremic patients.

Effect of PTH on erythrocyte production

A second broad mechanism postulated to contribute to the

anemia of uremia was decreased erythrocyte production, through the direct and indirect effects of PTH on erythropoiesis. In 1981, even before completing his study on PTH and osmotic fragility, Eitan Bogin had participated in a study in Massry's laboratory in which the relationship between increased PTH and decreased erythropoiesis was evaluated in uremic patients [42]. Intact and partial molecules of PTH, in concentrations found in the plasma of uremic patients, were incubated with human peripheral blood and mouse bone marrow cells. Marked and significant inhibition of burst-forming units-erythroid (BFU-E) but not colony-forming units-erythroid (CFU-E) was observed. Inactivation of the 1-84 bPTH fragments abolished this action, suggesting PTH activity was necessary for the effect. Increasing the concentration of EPO in the cell growth media overcame the inhibition, raising the possibility of competitive interference by PTH in EPO action. This was one of the earliest in vitro studies to suggest an effect of excess PTH on erythrocyte production in the bone marrow.

Not all subsequent studies have supported a significant relationship between serum PTH concentration and inhibition of erythropoiesis [43]. Experimental and clinical studies in dogs did find evidence to support an erythrosuppressive effect of PTH, but did not find a correlation with anemia. In a dog model of terminal renal dysfunction induced by partial surgical ablation of the kidney, the erythroid regenerative capacity in dogs with renal insufficiency was comparable to that of control dogs when plasma PTH concentration was lowered by reducing the dietary intake of phosphorus [44]. Anemia was negatively correlated with plasma PTH and phosphorus concentrations, however, this correlation disappeared after controlling for serum creatinine concentration in a multiple linear regression analysis. A significant negative correlation also was observed between plasma PTH and serum EPO concentrations. In the study of 17 dogs with chronic renal failure, non-anemic dogs had only slightly increased PTH values, whereas most anemic dogs had extremely high serum PTH concentrations [23]. An increased in red cell 2,3-diphosphoglycerate (2,3-DPG) concentration also was found, which causes a right shift in the oxyhemoglobin dissociation curve and improves tissue oxygenation, but decreases the stimulus for erythropoietin synthesis, which may exacerbate anemia in dogs with renal failure.

Several studies suggest that PTH may suppress endogenous EPO synthesis or release and decrease bone marrow response to EPO, resulting in EPO resistance and persistent anemia in uremic patients. Marked increases in serum EPO concentration and reticulocyte counts were observed in uremic patients with HPTH who underwent parathyroidectomy, allowing the dosage of EPO to be decreased [45–47]. Decreased calcitriol levels also may reduce erythropoiesis. Bone marrow erythropoietic cells express calcitriol receptors, and calcitriol induces proliferation and maturation of erythroid progenitor cells [47]. As mentioned earlier, calcitriol deficiency is thought to play a determining role in the development of renal secondary HPTH. Calcitriol is a physiologic antagonist of PTH, such that high concentrations of PTH in severe primary or secondary HPTH cause downregulation of calicitriol receptors and attenuate the erythropoietic response to EPO. Treatment with alfacalcidol, a vitamin D3 derivative, was effective in reducing PTH levels and increasing hemoglobin concentration in patients with end-stage renal failure [17].

Induction of marrow fibrosis by PTH is also considered to be a factor in the decreased production of erythrocytes in uremic patients [12, 47]. Humans with severe secondary HPTH and osteitis fibrosis show considerable resistance to EPO, at least in part because of obliteration of the marrow space (myelophthisis) with fibrous connective tissue and interference with the erythropoietic response to EPO [12,48,49]. In a cross-sectional study of PTH-induced marrow fibrosis, mean serum PTH levels, evidence for bone destruction, and the severity of marrow fibrosis were significantly higher in patients responding poorly to EPO as compared with patients who responded well to EPO [49]. These findings indicated that the dose of EPO needed to achieve an effective HCT depends on the severity of secondary HPTH and the extent of marrow fibrosis.

Treatment of Anemia in Uremic Patients with Hyperparathyroidism

The research of Eitan Bogin, together with that of his mentor and colleagues in California and Israel, was critical in establishing that PTH is a uremic toxin with adverse effects on erythrocytes and erythropoiesis. It is also true that on several points, existing data are conflicting, and the clinical significance of these effects, in particular the extent to which PTH contributes to anemia, remains controversial [47,50]. Several reasons have suggested for disparities in the results of experimental studies: 1) in comparison with EPO, the role of HTPH in anemia is relatively minor, so its effects may be easily masked; 2) confounding factors, such as aluminum overload, also have a impact on anemia; 3) differences in the type and time course of medical treatment of chronic kidney disease may have important effects on experimental results; and 4) methodological differences exist in various studies [47]. Despite these discrepancies and continued debate, it is undisputed that some uremic patients—and their HCT—benefit significantly from medical and surgical intervention to decrease blood PTH and/or increase calcitriol concentration.

Because of the clear benefit in the resolution of anemia in some patients with excess PTH, a combined therapeutic approach that includes calcitriol or its analogs and parathyroidectomy is recommended [12, 51]. In any case of unexplained resistance to EPO, secondary HPTH should be investigated. A body of supporting evidence finds that patients with parathyroidectomy develop dramatic improvements in HCT and concomitant reduction of required EPO doses [45–47]. The clinical benefit of parathyroidectomy in dogs remains uncertain [8]. Experimental studies on the effect of parathyroidectomy in dogs did not demonstrate a beneficial effect on soft tissue mineralization; however, a potential effect on anemia was not evaluated [52, 53]. The effectiveness of calcitriol therapy in treating renal secondary HPTH in dogs and cats is well recognized [8, 54]. Nagode et al found a high level of enthusiasm among veterinarians for the clinical benefit of calcitriol treatment in

dogs and cats, but controlled studies that include an assessment of its effect on anemia are needed [8,54]. Clearly, the clinical importance of PTH and renal secondary HPTH on anemia in both humans and animals warrants further study.

Eitan Bogin: A Surviving Legacy

Like erythrocytes transversing the microvasculature, life's journey has its perils and risks, which—abruptly or gradually—can shorten our life span. The loss of Eitan Bogin reminds us of this finite journey; his research, however, reminds us of survival, that of ideas, concepts, and controversies that form the core of the scientific process. Research done by Eitan decades ago survives today as new ways of thinking about PTH and uremic toxins and a better understanding of how to treat anemia in uremic patients. His research formed the basis for many promising new avenues of investigation that continue to this day; and although emphasizing human disease, we have seen how his work also surfaces, survives, and stimulates new ways of thinking about animal disease. Eitan Bogin's work on PTH and erythrocytes is only one small part of what he accomplished in his lifetime, but like so much else in his life, it will survive as an important legacy to inspire future generations.

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Figure Legend

Figure 1. Pathogenesis of parathyroid hormone–mediated effects on erythrocytes and anemia. Adapted from reference 12.

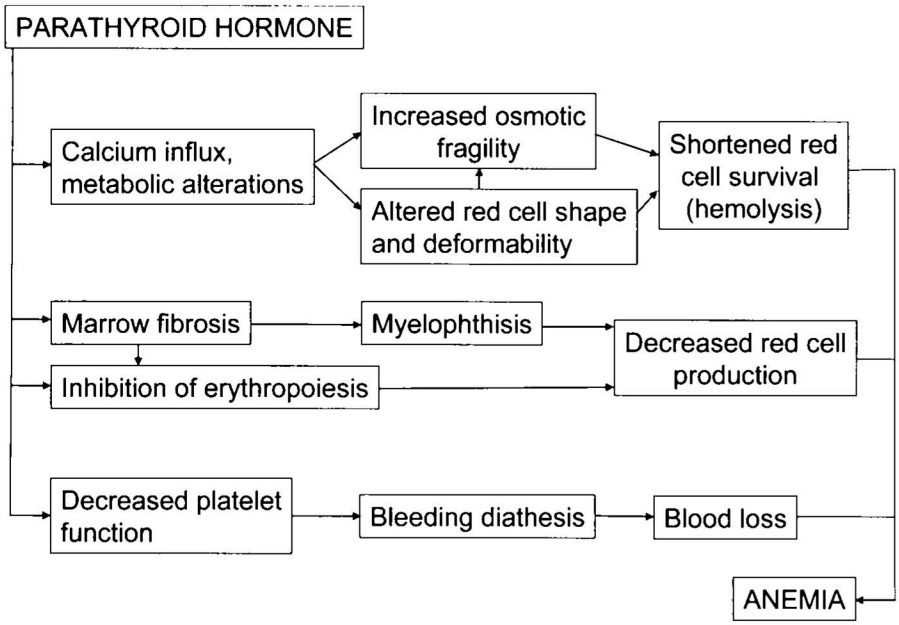

Figure 2. Echinocytosis in a dog with chronic renal failure and uremia. Wright's-Giemsa, bar = 10 µm.