Bubble Cells: Renal Tubular Cells in the Urinary Sediment with Characteristics of Viability

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ABSTRACT
The urinary sediment was examined by light microscopy in 65 consecutive inpatients with renal insufficiency (not due to pre- or postrenal factors) referred to a nephrology consult service for evaluation. In the 60 patients in whom a single diagnosis was reached, the sediments of 34 (57%) contained an easily recognized cell, which we have called the "bubble cell". These cells were bizarre, large cells with a single nucleus, which appeared to contain one or more fluid-filled vesicles. Bubble cells were most prevalent in the sediment of patients with acute tubular necrosis but were also seen a variety of other renal diseases. In most patients with acute tubular necrosis, the sediment also contained "normal"-appearing renal tubular cells, muddy brown casts, and oval fat bodies which were indistinguishable from those seen in the nephrotic syndrome. By electron microscopy, the bubble cells appeared to be vacuolated renal tubular epithelial cells, which had characteristics of viable cells. Most bubble cells excluded the vital dye Trypan blue, whereas the normal-appearing renal tubular cells were typically strongly positive. It was concluded that bubble cells, often accompanied by oval fat bodies, are commonly present in the sediment of patients with acute tubular necrosis as well as many other types of renal disease. Most cells which would be classified as "normal" renal tubular cells in these sediments are dead.

In contrast, the findings suggest that the bubble cell represents an injured but viable renal tubular cell. The frequent finding of oval fat bodies in the same sediments suggests that the oval fat body is also produced by tubular cell injury.

Key Words: Urinalysis, urine sediment, urine cytology, cell injury, acute renal failure, oval fat body, nephrotic syndrome

Examination of the urinary sediment in acute renal failure is essential. A normal sediment suggests prerenal or postrenal failure, whereas the sediment is almost invariably abnormal in acute tubular necrosis (ATN) due to hypotension or acute nephrotoxic injury. The characteristic sediment in ATN is said to contain erythrocytes, leukocytes, muddy brown casts, and renal tubular epithelial cells. The epithelial cells are typically described as being either relatively "normal" in appearance (i.e., of normal size and without bubbles or membrane whorls) or necrotic. It is generally agreed that the segment of origin of these cells cannot be determined by routine light microscopy, although this can sometimes be surmised by using special stains (1) or by examination by electron microscopy (2). Segasothy et al. recently used monoclonal antibodies to identify the type of cells present in the urine of patients with acute renal failure and found large numbers of cells from all parts of the tubule (3).

There have been rare reports of an atypical vacuolated cell in the sediment of patients with tubular injury. For example, a vesicle-filled cell with ethylene glycol poisoning was observed by Haber (4; see Figure 2.17); a vacuolated cell has been seen with acute tubular necrosis by Schumann et al. (5; 6, see Figure 10.38) and Mandal et al. (2; see pp 349 and 353; 7, 8); and a similar cell has been described sporadically with "osmotic nephrosis" from mannitol (9). Malignant tubular cells may sometimes display a similar appearance (10). In one report, vacuolated cells were interpreted to be an artifact of preparation (11).

Although the literature would suggest that vacuolated cells in the sediment are highly unusual, it has been our impression that the sediment in patients with acute renal failure very commonly contains oval fat bodies and many bizarre, large cells which seem to contain intracellular clear vesicles. On the basis of its appearance by standard light microscopy, we have designated this the "bubble" cell. In this report,
we describe some characteristics of the bubble cell and examine the incidence and specificity of this finding based on a study of urine sediments in 65 consecutive patients referred to an inpatient nephrology service because of renal insufficiency.

METHODS

Freshly collected urine samples were centrifuged in 15-mL conical tubes at 2,000 rpm for 5 min. The supernatant was drained by inverting the tube for 10 s and tapping off the last drops. The sediment was suspended in the small amount of residual urine left in the tube (5 to 10 μL) and was placed between a glass slide and a 18- by 18-mm no. 1 coverslip. The volume used to resuspend the sediment is smaller than that used by many clinical laboratories and produces a relatively concentrated smear. Sediment smears were examined with low (10x)- and high (40x)-power plan objectives on the stage of an American Optical light microscope by using Kohler illumination.

Erythrocytes, leukocytes, oval fat bodies, and muddy brown casts were identified in the sediments by standard criteria (4,6,12). Sizes of other cells were estimated by reference to the size of leukocytes, which were assumed to be 11 to 14 μm in diameter. Normal-appearing renal tubular epithelial (RTE) cells were identified as 15- to 25-μm cells having a single eccentric nucleus which occupied 40 to 70% of the cell and which was bordered by a thick, dark nuclear membrane (1, see Figures 2-12 and 2-13). All sediments were classified by one examiner (MG).

To define the incidence of various findings, urine sediments were examined on 65 consecutive inpatients referred to a Nephrology Consult Service of a 400-bed Veterans Administration Hospital because of an elevated serum creatinine concentration and in whom the urine sediment was abnormal. In each of these patients, the renal insufficiency had failed to resolve after intravenous volume expansion, and, in essentially all of the patients, urinary obstruction had been excluded by renal ultrasonography. Normal sediment was defined as one having \(<3\) erythrocytes or leukocytes and \(<1\) RTE cell per high-power field, with no oval fat bodies and no casts other than hyaline casts. Muddy brown casts, oval fat bodies, and bubble cells were defined as being present if at least five were detected per coverslip (approximately equal to one in every tenth low-power field). Clinical diagnoses were generally not confirmed by biopsy or autopsy. ATN was defined as acute renal failure, not due to obstruction or prerenal factors, occurring after a hypotensive episode or after exposure to drugs known to induce acute tubulointerstitial damage.

Special studies of the urinary sediment were performed in seven cases. In each of these sediments, bubble cells were a striking finding. The clinical diagnosis was ATN in six cases and acute interstitial nephritis in one. Two patients were oliguric. Plasma creatinine ranged from 2.4 to 11.3 mg/dL with a mean of 4.9 ± 1.1. Of the seven sediments, oval fat bodies were present in five, muddy brown casts in three, and normal-appearing renal tubular cells in five. To assess membrane integrity in these sediments, 0.4% trypan blue was added (1 cc/9 cc of urine) before centrifugation. To stain cell lipid, Nile red dye was added before centrifugation, from a 1- mg/ml stock solution in dimethyl sulfoxide at 0.1 cc/9.9 cc of urine. Fluorescence of Nile Red was observed with a Nikon Diaphot inverted fluorescence microscope by using a G filter cube and epifluorescent illumination. In samples to be examined by electron microscopy, glutaraldehyde was added to the urine to achieve a final concentration of 3%. Cell pellets were treated with 1% aqueous unbuffered osmium tetroxide, dehydrated in ethyl alcohol, and embedded in epoxy. Survey sections were cut from all zones of the pellet, and thin sections were stained with uranyl acetate and lead citrate. Micrographs were taken of all cells in two grid sections from each pellet zone. Dyes were obtained from Sigma Chemical Co., St. Louis, MO.

RESULTS

Of 65 patients referred for evaluation of parenchymal renal insufficiency, a single clinical diagnosis was established in 60 as being the most likely explanation for the major part of the renal insufficiency (Table 1). The overall incidence of urines having bubble cells was 34 of 60 = 57%. The highest incidence (76%) was seen in patients with ATN. The number of bubble cells per slip was also highest in the patients with ATN. In these sediments, bubble cells were typically present at counts up to 2 to 10/ high-power field, whereas the number of cells was typically in the range of 0.5 to 3/low-power field in the other groups in which bubble cells were detected. The urine was close to isotonic in essentially all of the patients with ATN, and there was no suggestion that any of the findings correlated with the presence of hypotonic final urine. None of the patients with ATN had more than +++ proteinuria, and, typically, the dipstick protein concentration was + or ++. Our patient population was too small to examine whether the bubble cells correlated with the residual urine volume (oliguric versus nonoliguric acute renal failure), the degree of renal insufficiency, or the clinical

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3 Five patients were excluded from further analysis because their acute renal failure occurred in a setting of documented preexisting nephrotic-range proteinuria. Of these patients, the sediments of all five had bubble cells and oval fat bodies in appreciable numbers, four had RTE cells, and two had muddy brown casts.
outcome. Bubble cells were also commonly detected in patients with nephrotic syndrome (4 of 9 = 44%), chronic renal insufficiency (9 of 22 = 41%), and hepatorenal syndrome (3 of 4 = 75%) and were especially dramatic in one case of acute interstitial nephritis.

The characteristics of the bubble cells appeared similar regardless of the clinical diagnosis of the patient. The bubble cells were typically large (20 to 30 μm) and were sometimes enormous (50 to 75 μm). In many bubble cells, a single large, clear bubble filled the cell, displacing the nucleus and cytoplasm to a thin rim around the periphery (Figure 1). In many other cells, multiple clear bubbles of various sizes were present (Figure 2A through C). When it could be seen, the nucleus resembled that of a renal tubular cell and multiple nuclei were never seen. The bubble cells sometimes appeared in clumps (Figure 2C) or as inclusions in cellular casts. The bubble cells were generally accompanied by (1) normal-appearing renal tubular cells which did not contain discrete bubbles or vesicles, (2) oval fat bodies, (3) muddy brown casts, (4) short, dark, granular casts resembling muddy brown casts but appearing to be composed of the same material as the oval fat bodies, and (5) cells containing refractile bodies appearing to be membrane whorls or aggregates of wrinkled membrane.

In many examinations, the bubble cells were the most striking feature in the sediment. Special studies of the bubble cells were performed in seven such cases. In four sediments, trypan blue staining revealed that most of the bubble cells excluded this dye (negative cells, 50, 80, 80, and 75%). In contrast, most leukocytes and normal-appearing renal tubular cells were strongly trypan blue positive (Figure 2D). The oval fat bodies could not be easily classified by trypan blue because the cytoplasmic volume was so small. In the bubble cells of two patients, Nile red fluorescence was found to be excluded from the large bubbles but the small vesicles of oval fat bodies were typically highly fluorescent.

Transmission electron microscopy of four sediments disclosed that the bubble cells contained filament bundles characteristic of renal epithelial cells, and, on some cell surfaces, microvilli were evident (Figure 3). The "bubbles" seen by light microscopy appeared to correspond to vacuolar spaces which were not lipid filled and which were typically surrounded by smooth membrane. The mitochondria of the bubble cells were typically intact, with compact structure and a normal inner-matrix density. None of the bubbles cells had ultrastructural characteristics of lymphocytes or macrophages.

### TABLE 1. Incidence of bubble cells

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No. of Patients</th>
<th>Bubble Cells</th>
<th>RTE Cells</th>
<th>OFB Cells</th>
<th>Muddy Casts</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATN</td>
<td>21</td>
<td>16 (76%)</td>
<td>16 (76%)</td>
<td>15 (71%)</td>
<td>13 (62%)</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>9</td>
<td>4 (44%)</td>
<td>2 (22%)</td>
<td>6 (67%)</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>22</td>
<td>9 (41%)</td>
<td>7 (32%)</td>
<td>10 (45%)</td>
<td>5 (23%)</td>
</tr>
<tr>
<td>Hepatic/renal syndromes</td>
<td>4</td>
<td>3 (75%)</td>
<td>3 (75%)</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
</tr>
<tr>
<td>RPGN and Acute GN</td>
<td>3</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Allergic interstitial nephritis</td>
<td>1</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
</tbody>
</table>

Abbreviations: OFB, cells resembling oval fat bodies; RPGN, rapidly progressive glomerulonephritis; GN, glomerulonephritis.

Figure 1. Light microscopic field of the urinary sediment in three patients with acute renal failure due to ATN. Each sediment contains a bubble cell, with a single large vesicle filling the cell and displacing the cytosol. Bubble cells containing multiple vesicles are also seen in panels A and C. Magnification, ×400.
The oval fat bodies which accompanied the bubble cells in the patients with ATN were indistinguishable, by both light and electron microscopy, from the oval fat bodies seen in patients with nephrotic syndrome. In the patients with ATN, the fully developed oval fat bodies were typically 20 to 50 μm and were completely filled with refractile spherical vesicular structures. Only the largest vesicles in the fat bodies displayed Maltese Cross patterns with polarized light, as was the case in the fat bodies of patients with classical nephrotic syndrome and no tubular injury.4

DISCUSSION

These results indicate that the urine sediment of most hospitalized patients with parenchymal renal insufficiency contains bubble cells which can easily be detected by standard light microscopy. The incidence (75% of patients) and the number of bubble cells present were both highest in patients with ATN. Bubble cells were also seen frequently in patients with the nephrotic syndrome and various types of chronic renal insufficiency. Regardless of the diagnosis, the bubble cells were almost always accompanied by normal-appearing renal tubular cells and cells which appeared to be oval fat bodies. In many cases, muddy brown casts were also present, as were casts which appeared to be composed of the same material as the oval fat bodies. Our results indicate that bubble cells are not a sporadic finding but instead are typical and characteristic findings in ATN, as are oval fat bodies. In our series, bubble cells were as frequent a finding as muddy brown casts, the hallmark of the ATN sediment.

We propose that bubble cells represent viable renal tubular cells which have been sublethally injured. Most of the bubble cells excluded trypan blue, indicating normal membrane integrity. In addition, the mitochondrial changes which characterize dead cells

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4 It should be emphasized that the ability to detect the Maltese Cross pattern, in addition to being dependent on the presence of anisotropic lipid in the sample, is largely determined by the quality of the polarizing elements of the microscope. To detect the Maltese Cross requires high-quality polarizing filters as well as strain-free objectives and internal elements to avoid degrading the polarization. The microscope used in our study performed relatively poorly in this regard, and, even in proteins with classical nephrotic syndrome, few of the oval fat body vesicles were positive for the Maltese Cross pattern.
were not seen in bubble cells examined by electron microscopy. Although these two findings suggest that these cells may be viable, substantiation of this hypothesis would require the demonstration that isolated bubble cells can be established in tissue culture. Racusen et al. have recently found that primary epithelial cell cultures can be established from the urine of the majority of patients with ATN (13), and it will be interesting in future studies to see whether this property correlates with the presence of bubble cells as our hypothesis would predict.

What are the Bubbles?

The clear, homogeneous, spherical appearance of the bubbles by light microscopy suggests that they are membrane-delimited fluid-filled spaces. As additional evidence that the bubbles represent an aqueous space, they did not stain with a lipophilic dye (Nile Red), they did not exhibit a Maltese Cross pattern in polarized light, and their appearance by electron microscopy was that of a nonlipid aqueous space. Definitive proof that the bubbles are fluid filled will require direct isolation and analysis of the bubble contents. The electron micrographs of Mandal support the concept that at least some of the bubbles represent dilated endoplasmic reticulum (2,8), and dilation of the endoplasmic reticulum is thought to be a characteristic change in dying cells (14). An alternative explanation is that the reincorporation of brush border or plasma membrane might give rise to the membrane comprising the bubbles or oval fat body vesicles.

It is interesting that cells in tissue culture injured by ischemia or ATP depletion also develop vesicles. LeMasters, Herman, and their associates have defined three stages of cell injury in these models (15,16). In the first and earliest stage of injury, the cells begin to develop clear vesicles. In the second stage, the vesicles enlarge and coalesce. Despite the gross distortion of cellular morphology at this time, these stage 2 cells appear to be viable as judged by their exclusion of trypan blue, and the vesicles resorb if the ischemia is relieved. Sudden rupture of one of the surface vesicles is the cellular equivalent of death, mediating an irreversible transition from stage 2 to stage 3, after which the cell freely admits trypan blue.

A vacuolar change is also described in the tissue of both human ATN and animal models. In humans, dramatic vacuolization of the proximal nephron is seen in the acute renal failure induced by mannitol (17). This change, initially referred to as "osmotic nephrosis," has now also been described in the acute renal failure seen after radiographic contrast agents (18) and burns (19). Solez, with biopsy material provided by Mandal, has noted a similar lesion in patients with the hepatorenal syndrome (20). A dramatic vacuolization is similarly seen in animal models of tubular injury, and, in all of these cases, the lesion is typically seen only in the proximal tubule (18,21–24). For example, in a rat model of ischemic ATN, the proximal tubular cells undergo a striking toxic change characterized by both shedding and reincorporation of the brush border microvilli, producing whorls of membranous material and a prominent vacuolization of the subapical regions of the cell (23,24). Although we feel it is likely that such cells are the in situ precursor of the bubble cells we observe, this remains speculative because we do not have biopsy material on any of our patients and none of these tissue studies has correlated the in situ morphology with urinary findings.

Oval Fat Bodies

The notion that oval fat bodies are a specific finding in the sediments of patients with the nephrotic syndrome is probably incorrect (2,25–27). For example, Duncan et al. reported that 60% of patients with polycystic kidney disease shed oval fat bodies in the urine (25). Our observations that oval fat bodies were common in the urinary sediments of patients with tubulointerstitial types of renal failure are similar to those of Braden et al. (27). In their study, 15% of patients with ATN shed oval fat bodies in the urine, as did 33% of patients with acute interstitial nephritis (27). Our limited observations from light microscopy, special stains, and electron micrographs revealed no differences between the oval fat bodies seen in association with the bubble cells and those present in classical nephrotic syndrome. We therefore speculate that oval fat body, like the bubble cell, may be a nonspecific manifestation of tubular cell injury.

The relationship between the bubble cells and the oval fat bodies is also not clear. One possible explanation is that the oval fat bodies and bubble cells are the same cells but at different stages of injury. For example, collapse of the bubble cell vesicles might produce compact membrane droplets, accounting for the appearance of the oval fat bodies. Alternatively, the oval fat bodies may represent cells arising from a different nephron segment than the bubble cells, which would imply that the same ischemic/toxic insult produces different patterns of cellular injury in different segments.

In summary, we report that bubble cells, often accompanied by oval fat bodies, can be seen in the majority of patients with ATN, as well as in many other conditions producing renal insufficiency. We propose that the bubble cell represents an injured but viable renal tubular epithelial cell. Clarifying how the bubble cells develop and how they are related to oval fat bodies may produce new insights into the etiology of acute renal failure at the cellular level.
REFERENCES


