Bicarbonate/Lactate- and Bicarbonate-Buffered Peritoneal Dialysis Fluids Improve Ex Vivo Peritoneal Macrophage TNFα Secretion

RUTH K. MACKENZIE,* CLIFFORD J. HOLMES,† ANDREW MOSELEY,* JEAN P. JENKINS,* JOHN D. WILLIAMS,* GERALD A COLES,* DIRK FAICT,† and NICHOLAS TOPLEY*

*Institute of Nephrology, University of Wales College of Medicine, Cardiff, United Kingdom; and †Baxter Healthcare Renal Division, McGaw Park, Illinois, and Nivelles, Belgium.

Abstract. Peritoneal macrophage (PMØ) function was examined ex vivo after their in vivo exposure to either acidic, lactate-buffered solutions (PD4; 40 mM lactate, pH 5.2), bicarbonate/lactate-buffered solution (TBL; 25 mM/15 mM bicarbonate/lactate, pH 7.3), or bicarbonate-buffered solution (TB; 38 mM bicarbonate, pH 7.3), containing either 1.36 or 3.86% glucose. Initial experiments demonstrated that tumor necrosis factor-α (TNFα) release (assessed by TNF direct immunoassay [DIA]) from PMØ isolated from the peritoneal cavities of patients exposed to conventional fluid (PD4 1.36% glucose) was lowest after 30 min of intraperitoneal dwell (3591 ± 1200 versus 28,946 ± 9359 for 240-min dwell [pg/ml], n = 5, P < 0.05). Five patients were exposed on 3 successive days to PD4, TBL, and TB for 30-min acute dwells containing 1.36% glucose in the first week and 3.86% glucose during the second. PMØ TNFα release was assessed after ex vivo exposure to lipopolysaccharide (LPS). Exposure of PMØ to TBL or TB (1.36% glucose) resulted in a significant increase in the generation of TNFα (pg/2 × 10⁶ PMØ) compared with PD4. TBL: 68,659 ± 35,633, TB: 53,682 ± 26,536 versus PD4 17,107 ± 8996 (LPS 1.0 ng/ml, n = 5 patients, P = 0.043 versus PD4 for both). PMØ that were recovered from PD4 and TB dwells (3.86% glucose) showed no significant difference in TNFα secretion (21,661 ± 6934 and 23,923 ± 9147, respectively). In contrast, exposure to TBL resulted in a significant increase (41,846 ± 11,471) compared with PD4 (LPS 1.0 ng/ml, n = 5 patients, P = 0.043). These data demonstrate enhanced PMØ function after in vivo exposure to bicarbonate- and bicarbonate/lactate-buffered solutions. This response was sustained in TBL alone at the highest glucose concentrations. These results suggest that the newer solutions, and particularly bicarbonate/lactate, might improve host defense status in peritoneal dialysis patients.

Over the past 20 yr, peritoneal dialysis (PD) has become a major treatment for end-stage renal disease. More than 100,000 patients worldwide are currently treated with this type of renal replacement therapy (1). This increased use has focused attention on those factors that limit its long-term application. Recent data suggest that repeated intraperitoneal infection plays a significant role in reducing membrane function (2). In certain patients, however, membrane failure occurs without a history of peritoneal infection, which suggests that other factors such as uremia or long-term exposure to dialysis solutions may play a role in the process (3). These observations suggest that improving host defense and/or reducing the exposure of the peritoneum to unphysiologic dialysis fluids could, in the long-term, have a positive impact on peritoneal membrane function (2).

Despite significant in vitro and ex vivo data to indicate its bioincompatibility, lactate-buffered peritoneal dialysis solutions have been used almost exclusively in PD (4–6). The impact of lactate-buffered solutions on cell function has been assessed in many in vitro and ex vivo systems. These investigations have delineated the effects of these solutions on cell function (7) and have identified many of the cellular mechanisms by which these inhibitory effects occur (4, 8–15).

The potential in vivo consequences of repeated exposure to lactate-buffered solution have driven the development and introduction of potentially more "biocompatible" solutions. These have been designed to reduce some of the inhibitory components of lactate-buffered fluids (such as low pH, high lactate and glucose concentration, and glucose degradation products) (16,17).

The introduction of bicarbonate, the body’s natural buffering system, has been suggested as the logical way to create a pH-neutral and more physiologic solution formulation (18). Its use was initially hindered by the problem of calcium and magnesium carbonate precipitation within dialysis bags and by
the difficulty of sterilizing glucose without caramelization (19,20). These problems have been largely overcome by the creation of a two-chamber bag that allows the glucose, calcium, and magnesium components of the solution to be separated from the neutral pH bicarbonate buffer. The two-chambered bag also allows the solution to be heat-sterilized in a manner identical to lactate-buffered solutions so that dextrose can be kept at an acidic pH, which limits the generation of glucose degradation products (20–22).

Biocompatibility assessments of bicarbonate-buffered solutions have concentrated on their effects on peripheral and peritoneal leukocytes, mesothelial cells, and fibroblast function after in vitro exposure (23–33). In general, cell function was improved in bicarbonate-buffered solutions (of neutral pH) compared with the corresponding lactate-buffered solution of pH 5.2 to 5.5. This improvement in cell function occurred in both low- and high-glucose solutions (23,24,32). Data from animal experiments have confirmed that bicarbonate-buffered solutions are less harmful to the mesothelium in vivo (34).

To date, biocompatibility studies of bicarbonate-buffered solutions have been performed using either in vitro or animal studies. No data exist on the potential impact of these solutions on parameters of peritoneal host defense or membrane longevity in PD patients. Initial clinical studies suggest that bicarbonate-buffered solutions are well tolerated and clinically efficacious (35–38), and reduce the pain on infusion suffered by many patients using lactate-buffered solutions (39).

The present study was designed to evaluate the impact of solution buffer (lactate versus bicarbonate versus bicarbonate/lactate) at two different glucose concentrations (1.36% versus 3.86%), using an ex vivo approach (40–43). Our data suggest that in low glucose solutions, in vivo exposure to bicarbonate- and bicarbonate-lactate-buffered solutions was associated with increased ex vivo peritoneal macrophage (PMØ) function. In the presence of 3.86% glucose, PMØ function was suppressed compared with 1.36% glucose. In this case, however, improved PMØ function was seen only after exposure to bicarbonate/lactate solutions. These data suggest that bicarbonate- and bicarbonate-lactate-buffered solutions might have a positive impact on peritoneal host defense status.

### Materials and Methods

#### Patients

Five patients who had consented to take part in the phase II bicarbonate/bicarbonate-lactate clinical trial were enrolled in the present study (35). All patients used System 2 Freeline disconnect Y-system (Baxter Healthcare, Newbury, United Kingdom). Informed consent was obtained, and the study was performed in accordance with local ethical approval guidelines. Patient characteristics are presented in Table 1. All patients had been on continuous ambulatory PD for at least 3 mo before the beginning of the study. Four men and one woman entered the study. Their mean (± SD) ages were 62.4 ± 11.2 yr. The mean (±SD) duration on PD was 17 ± 11 mo. During the study, there were no episodes of peritonitis in the study group. Patients received a standard dialysis regimen (4 × 2 L, 1.36% lactate-buffered peritoneal dialysis fluid, PD4 Baxter Healthcare, McGaw Park, IL) except for the 30-min acute dwell performed immediately after drainage of the overnight dwell effluent.

#### Study Design and Test Solutions

The protocol for the ex vivo evaluations is presented in Figure 1, and the test solutions are described in Table 2. On day 1 of the first week, all patients received PD4 (40 mM lactate, pH 5.2 to 5.3, 1.36% glucose) as their first acute dwell. On days 2 and 3, patients were randomized to either TBL, 25 mM bicarbonate/15 mM lactate, pH 7.3, or TB, 38 mM bicarbonate, pH 7.3. During the second week (after 4 d of regular dialysis), an identical protocol was performed using solutions containing 3.86% glucose. After each acute dwell, ex vivo PMØ function was assessed as described below.

#### Ex Vivo Evaluation of Cell Function

Initial experiments (using all five patients) established values for baseline and lipopolysaccharide (LPS)-stimulated synthesis of tumor necrosis factor-α (TNFα) from PMØ exposed in vivo to PD4 (1.36% glucose). There was a time-dependent increase in both constitutive and LPS (from *Escherichia coli* 055 B5, Sigma-Aldrich, Poole, United Kingdom) (100 ng/ml, 18 h)-stimulated TNFα synthesis from PMØ isolated from timed dwell effluents. TNFα levels (in LPS-stimulated PMØ) were 3591 ± 1200 (pg/ml) after a 30-min dwell and increased to 9156 ± 3645, 15,760 ± 4678, and 28,946 ± 9359 at 60, 90, and 240 mm, respectively (n = 5, P = 0.043 versus 30 min for all). TNFα values in PMØ isolated after a 30 min in vivo dwell were significantly lower than values obtained after all other dwell periods.

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Time on PD (mo)</th>
<th>Previous Episodes of Peritonitis</th>
<th>Peritonitis Episodes During Study</th>
<th>No. of Ex vivo Dwell</th>
<th>Dialysis Regimen</th>
<th>Comorbidities</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>50</td>
<td>M</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4 × 1.36/2 L</td>
<td>PTH, IHD, hernia, PO₄</td>
<td>PKD</td>
</tr>
<tr>
<td>106</td>
<td>75</td>
<td>M</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4 × 1.36/2 L</td>
<td>BP, IHD, COAD, Pneumococcosis</td>
<td>Unknown</td>
</tr>
<tr>
<td>107</td>
<td>51</td>
<td>F</td>
<td>29</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>4 × 1.36/2 L</td>
<td>PO₄, CVA</td>
<td>GN</td>
</tr>
<tr>
<td>108</td>
<td>67</td>
<td>M</td>
<td>29</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>4 × 1.36/2 L</td>
<td>ESI, PO₄, IHD</td>
<td>Unknown</td>
</tr>
<tr>
<td>110</td>
<td>69</td>
<td>M</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4 × 1.36/2 L</td>
<td>Chest infection, PO₄, IHD, PTH, BP</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*PD, peritoneal dialysis; PTH, hyperparathyroidism; IHD, ischemic heart disease; PO₄, hypophosphatemia; PKD, polycystic kidney disease; BP, hypertension; COAD, chronic obstructive airway disease; CVA, cardiovascular accident; ESI, exit-site infection; GN, glomerulonephritis.*
Table 2. Experimental test solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Control</th>
<th>Bicarbonate (TB)</th>
<th>Bicarbonate + Lactate TBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (%)</td>
<td>1.36, 3.86</td>
<td>1.36, 3.86</td>
<td>1.36, 3.86</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>95</td>
<td>97</td>
<td>95</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>40</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>5.2</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>$P_{CO_2}$ at 37°C ± SD</td>
<td></td>
<td>77.0 ± 1.9</td>
<td>48.2 ± 3.5</td>
</tr>
</tbody>
</table>

To maximize the potential differences between control (acidic lactate-buffered peritoneal dialysis fluid) and test solutions and in accordance with previous studies (40–43), we selected a 30-min dwell period for subsequent evaluations of ex vivo PMØ function.

**Acute In Vivo Exposure Protocol**

Patients drained their overnight effluent, and immediately 2 L of appropriate solution containing 1.36 or 3.86% glucose was infused into the peritoneal cavity and allowed to dwell for a total period of 30 min from the time that the fill was completed. Dialysate was then immediately drained into a bag on ice (44–47).

**Isolation and Stimulation of Peritoneal Leukocytes Using TNFα-Dynamic Immunoassay**

Total leukocyte populations were isolated from chilled peritoneal effluents by centrifugation, as described previously (44–47). Total cell numbers were calculated using an automated cell counter (Coulter ZM, Coulter Electronics, Luton, United Kingdom). The cells were washed in phosphate-buffered saline, pH 7.3, and resuspended to a cell density of $1 \times 10^7$/ml in RPMI 1640 tissue culture medium (Life Technologies, Paisley, United Kingdom) containing 5% wt/vol human AB serum (Sigma-Aldrich). LPS-driven TNFα synthesis by PMØ was assessed using the TNFα-dynamic immunoassay (DIA) system (Bio-source Europe, Fleurus, Belgium) (48). Doubling dilutions of total peritoneal cells (final volume, 25 μl) were added to the DIA 96-well microtiter plate and overlaid with 100 μl of RPMI 1640 containing the appropriate doses of LPS. Initial experiments determined both the numbers of peritoneal leukocytes (50,000 to 200,000 cells/well) and the doses of LPS (0.1 to 10 ng/ml) required to generate dose-dependent effects within the effective range of measurement of the TNFα-DIA assay system (28 to 726 pg/ml).

**TNFα-DIA Protocol**

After the addition of cells and LPS, the microtiter plate was incubated for 2 h at 37°C in an atmosphere of 5% CO2. The plates were subsequently washed (3×) with assay buffer, once with cell lysis buffer, and again (3×) with assay buffer. The TNFα assay was performed according to the manufacturer’s instructions. All determinations were performed using assay kits from the same batch. Minimum detectable concentration was 8 pg/ml (zero standard ± 2 SD), intra-assay coefficient of variation was 10%, and the interassay precision was between 5 and 11%. Each data point was calculated from the mean of triplicate determinations of TNFα synthesis with each cell and LPS concentration. All data are presented as pg/2 × 10^6 PMØ after correction of the data for the percentage of PMØ present in the total peritoneal leukocyte populations.

**Cell Differentials**

Before assessment of peritoneal leukocyte function, cytopin preparations of peritoneal leukocytes were prepared (Cytopin II, Life
Scientific, Basingstoke, United Kingdom), air-dried, and stained with Neat Differential Haematology Stain (Guest Medical, Seven Oaks, United Kingdom). Differential cell counts were performed by at least two independent observers.

Statistical Analyses
All statistical analyses were performed using the Wilcoxon signed rank test (StatView 512+, Apple Macintosh, Cupertino, CA) for nonparametric data. All data are presented as mean ± SEM.

Results
Cell Differentials
Total cell numbers and the percentage of PMØ within the isolated peritoneal cell populations showed a large degree of variability both with time and between and within individuals during the study. However, there was no consistent relationship with time in any of the patients. No significant differences were observed between acute dwells with PD4, TBL, or TB (Figure 2). There was no significant variation in the numbers of PMØ, lymphocytes, erythrocytes, or mesothelial cells either within an individual, between individuals, or between test solutions during the six acute dwells performed in each patient (data not shown).

Constitutive PMØ TNFα Synthesis
The mean constitutive ex vivo generation of TNFα by PMØ after acute dwells with solutions containing 1.36% glucose were 7413 ± 5515, 9921 ± 3526, and 11,057 ± 7675 (n = 5, pg/2 × 10⁶ PMØ) for the PD4, TBL, and TB groups, respectively. These differences were not statistically different from each other. The mean constitutive ex vivo PMØ TNFα generation in the acute dwells with solutions containing 3.86% glucose were 4372 ± 2703, 6371 ± 3199, and 4261 ± 1673 (pg/2 × 10⁶ PMØ) for the PD4, TBL, and TB groups, respectively. These values were not statistically different from each other. There was no significant difference in the constitutive ex vivo PMØ TNFα generation levels between any of the 1.36 or 3.86% treatments (Figure 3, A and B).

LPS-Stimulated TNFα Synthesis by PMØ
After LPS stimulation, ex vivo PMØ TNFα generation values were all significantly increased above constitutive levels in a dose-dependent manner regardless of solution or its glucose content (Figure 3, A and B).

Acute in vivo exposure to TBL and TB, containing 1.36% glucose, was associated with increases in stimulated ex vivo PMØ TNFα synthesis at all doses of LPS tested, compared with the values obtained in the same patients after acute dwells...
with PD4 (Figure 3A). These differences were statistically significant at all LPS doses \( (n = 5, P = 0.043 \text{ for all}) \). At an LPS dose of 1.0 ng/ml, the mean \( \text{ex vivo} \) PMØ TNF\( \alpha \) levels were 16,155 \( \pm \) 9995, 59,499 \( \pm \) 33,938, and 27,924 \( \pm \) 8855 for PD4, TBL, and TB, respectively \( (n = 5, P = 0.043 \text{ versus PD4 for both}) \).

For solutions containing 3.86% glucose, PMØ TNF\( \alpha \) synthesis was only significantly elevated with TBL. At all doses of LPS there was significantly enhanced function compared with PD4 \( (n = 5, P = 0.043 \text{ for all}) \) (Figure 3B). A comparison between the individual LPS-stimulated \( (0.1 \text{ ng/ml}) \) PMØ TNF\( \alpha \) values is presented for PD4 and TBL \( (1.36 \text{ and } 3.86\% \text{ glucose}) \) in Figure 4, A and B.

At each dose of LPS, the mean response was lower in PMØ exposed to TB than to TBL; this difference, however, was only significant after stimulation with 1.0 ng/ml LPS. At the other doses of LPS, the TNF\( \alpha \) values were higher in four of the five patients exposed to TBL compared with TB.

Acute exposure to solutions containing 3.86% glucose was associated in general with a reduction in \( \text{ex vivo} \) LPS-driven PMØ TNF\( \alpha \) synthesis. There was no apparent difference between the LPS-stimulated TNF\( \alpha \) synthesis levels in the PD4 1.36 or 3.86% acute dwell groups. Acute exposure to TBL or TB containing 3.86% glucose was associated with a reduction in mean PMØ response compared with cells exposed to 1.36% glucose-containing solutions \( (39.06, 40.99, \text{ and } 58.4\%) \text{ reductions for TBL and } 55.5, 14, \text{ and } 49.3\% \text{ reductions for TB at LPS doses of } 0.1, 1.0, \text{ and } 10 \text{ ng/ml, respectively}) \). These reductions, however, were only significant with TBL at an LPS dose of 1.0 ng/ml \( (n = 5, P = 0.043 \text{).}) \)

Effect of Repeated Acute Dwells on PMØ TNF\( \alpha \) Synthesis

In a single patient (number 106), 30-min acute dwells were carried out on 5 successive days using PD4 containing 1.36% glucose (Figure 5). The percentages of PMØ in the total leukocyte population were 44.6, 49.9, 38.9, 40.32, and 45.2% on days 1 through 5, respectively. The mean constitutive and LPS \( (1.0 \text{ ng/ml}) \)-stimulated TNF\( \alpha \) values were 6820 \( \pm \) 1906 and 11,698 \( \pm \) 1747, respectively \( (n = 5, P = 0.043 \text{ versus control}) \). Although the stimulated values were always above

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Production of TNF\( \alpha \) by PMØ acutely exposed (30-min dwell) to peritoneal dialysis solutions (PD4 and TBL) containing 1.36% glucose (A) and 3.86% glucose (B). Data presented are the individual values for LPS \( (0.1 \text{ ng/ml}) \)-driven TNF\( \alpha \) release from PMØ exposed to PD4 and TBL. Thick horizontal bars represent the mean value for each treatment.
control, the day-to-day variability in the difference between control and stimulated values was 45.6%.

**Discussion**

Investigations over the past 15 yr suggest that repeated intraperitoneal exposure to dialysis fluids might reduce the host’s resistance to infection and thus potentially have an impact on the rate or severity of peritonitis (49). To circumvent these potentially negative effects, alternative fluid formulations have been introduced (33,50,51). The most recent of these has been the introduction of neutral pH solutions buffered with bicarbonate or combinations of bicarbonate with lactate or glycyl-glycine. *In vitro*, these solutions have been shown to have a less detrimental effect on cell function when compared with the equivalent lactate-buffered solutions (23,24,32,33,52,53). However, these data are exclusively based on *in vitro* observations, and their relevance to the clinical situation remains to be established (54).

In the present study, we have used the same *ex vivo* approach described by de Fijter *et al.* (41,42) to compare the effects of a newly formulated solution buffered with bicarbonate or a combination of bicarbonate and lactate on PMØ TNFα secretion after *in vivo* exposure. As our functional readout, we chose to examine the production of TNFα by PMØ. This proinflammatory cytokine is known to play a central role in the host’s response to peritoneal infection, and previous observations have confirmed its presence in the peritoneal cavity early during the course of CAPD peritonitis (55).

*In vivo* exposure of PMØ to lactate-buffered solution was associated with a dwell time-dependent increase in the constitutive and stimulated *ex vivo* secretion of TNFα. These data parallel observations made in previous *ex vivo* studies, in which PMØ function was lowest after a 30-min dwell and increased with dwell time (40–43). That this reduction in cell function represents true inhibition is based on the fact that identical lactate-buffered solutions of neutral pH showed significantly higher values for PMØ functional parameters. These data appear to suggest that despite the *in vivo* equilibration process that occurs rapidly for pH, cell function is still modified by acute *in vivo* exposure to acidic lactate-buffered solutions (54). Previous studies have suggested that both acidic pH and, in high glucose concentrations, osmolality are primarily responsible for the observed modulation of PMØ functions (41,42). All subsequent experiments, therefore, used a 30-min *in vivo* dwell period with the test solution before the assessment of *ex vivo* function.

The observed significant increases in PMØ TNFα generation in the test solutions represented increases in each of the five patients studied. In a single patient, repeated acute dwell experiments (over 5 d) showed large variability in day-to-day responses when tested with the same solution on each day. In each case, however, the stimulated levels of TNFα were above that of the control. These data suggest that despite the large degree of variability in PMØ function (even within individuals), a measurable and significant increase in all patients studied is indicative of a consistent effect mediated by any particular dialysis solution.

In cells exposed to acidic lactate-buffered solutions, there was no difference between PMØ function regardless of whether 1.36 or 3.86% glucose was used for the acute dwell. This finding is perhaps not surprising because both solutions were infused at acid pH. In the studies of de Fijter *et al.* to demonstrate the effects of glucose/osmolality, solutions were neutralized before infusion (42). These data indicate that the acute effects of low pH (presumably combined with high lactate concentration) (13,14) are the major inhibitory factor(s) modulating cell function after an acute *in vivo* dwell with acidic lactate-buffered solutions.

The improvements in *ex vivo* cell function in low glucose-containing bicarbonate- and bicarbonate/lactate-buffered solutions parallel *in vitro* observations (23,24,32), in which leukocyte and mesothelial cell function was improved in bicarbonate-buffered solutions, regardless of glucose concentration. Although the data showing improved cell function in bicarbonate/lactate-buffered solution containing 3.86% glucose were predictable from previous experiments, the lack of significant effect with bicarbonate-buffered solution containing 3.86% solutions was unexpected. Previous *in vitro* experiments have demonstrated that cell function is better preserved in all bicarbonate-buffered solutions containing 3.86% glucose (26,29,33,52,56). Compared to bicarbonate-containing solutions with 1.36% glucose, cell function was depressed in identical solutions containing 3.86% glucose. The level of cell functional inhibition, however, was significantly less than that observed in equivalent acidic or neutral pH lactate-containing solutions (23,32).

The reason(s) why the predicted improvement in cell function was not observed in neutral pH bicarbonate-buffered solution containing 3.86% glucose are unknown. The only differences between the solutions tested was their bicarbonate concentration (25 mM versus 38 mM), their Pco₂ (48.2 ± 3.5 versus 77 ± 1.9), and the presence of 15 mM lactate. An earlier *in vitro* study designed to test the impact of buffer bicarbonate concentration and elevated Pco₂ on neutrophil and mesothelial cell function, however, failed to demonstrate significant dif-
ferences between the solutions tested in terms of their in vitro effects on cell function (24).

The observed increases in cell function (as evidenced by increased TNFα secretion) were always most marked with bicarbonate/lactate solution regardless of glucose content. These data suggest that, for whatever reason (physiologic bicarbonate concentration, lower Pco₂), the combination of bicarbonate and lactate provides some benefit as far as ex vivo cell function is concerned. For the first time, these data demonstrate differences in observations made on the same test solutions, between in vitro and ex vivo test systems. As mentioned previously, solutions instilled into the peritoneal cavity are equilibrated, whereas almost all in vitro test systems have used static experimental design in which cells are exposed to unused solution for varying periods of time (5,54). Changes induced by the in vivo equilibration process therefore might serve to unmask differences between solutions not evident in in vitro test systems. In this respect, recent observations suggest that although pH and lactate have the most profound effects on cell function in vitro, in vivo the major modulatory factor (in identical solutions) is glucose (34). These findings emphasize the fact that extrapolation of data from in vitro test systems needs to be exercised with caution.

The increasing use of peritoneal dialysis has increased awareness of those factors that limit its long-term application. This, in turn, has identified the need to reduce the cumulative exposure of the peritoneal membrane to inflammatory episodes (peritonitis). Hand in hand with this has been the desire to reduce the cumulative effects of repeated exposure to unphysiologic acidic dialysis solutions (23). The introduction of neutral pH bicarbonate and particularly bicarbonate/lactate-buffered solution appears to offer potential advantages and might serve to better preserve the function of both leukocytes and peritoneal membrane cell populations, all of which contribute to the complex series of interactions that control peritoneal homeostasis and resistance to infection (49,57).

Acknowledgments

Drs. Topley and Mackenzie are supported by the Kidney Research Unit Foundation. This study was supported by an educational grant from the Renal Division of Baxter Healthcare. We are grateful to Suzanne Jones for technical assistance.

References

24. Topley N, Kaur D, Petersen MM, Jöres A, Williams JD, Faict D, Holmes CJ: In vitro effects of bicarbonate and bicarbonate-