In patients on long-term continuous ambulatory peritoneal dialysis, the efficiency of dialysis declines because of peritoneal neovascularization and loss of peritoneal mesothelial cells. In this study, we investigated the influence of lactate and bicarbonate in peritoneal dialysis fluid on such changes of the peritoneum.

We studied the production of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which induce peritoneal neovascularization, by human peritoneal mesothelial cells (HPMCs) cultured with lactate or bicarbonate or both. Lactate dehydrogenase (LDH) was also measured to assess cell necrosis.

Levels of VEGF, bFGF, and LDH in the culture supernatant showed a significant decrease after incubation of HPMCs with 15 mEq/L lactate plus 25 mEq/L bicarbonate, or with 40 mEq/L bicarbonate, as compared with incubation with 40 mEq/L lactate. Levels of VEGF and bFGF showed a concentration-dependent decrease when the cells were incubated with lactate or bicarbonate; a concentration-dependent increase of LDH was simultaneously observed.

These results suggest that dialysis fluid containing 40 mEq/L bicarbonate is superior to fluid containing 40 mEq/L lactate with regard to its influence on the production of VEGF and bFGF, although lactate and bicarbonate are both toxic for HPMCs.

Key words
Fibrosis, vascular reactivity, growth factor

Introduction
Continuous ambulatory peritoneal dialysis (CAPD) has been used for more than two decades as a treatment for end-stage renal failure, but peritoneal dysfunction is still an important problem. Peritoneal neovascularization and vasculopathy are observed in patients with peritoneal dysfunction, and the extent of neovascularization correlates with the area of fibrosis, suggesting the involvement of angiogenesis in the progression of peritoneal fibrosis.

An increase in the rate of transperitoneal solute transport (for both creatinine and $\beta_2$-microglobulin) is associated with an increase in the surface area of peritoneal microvessels, especially in patients on long-term CAPD (1). Yoshio et al. reported that treatment with an antiangiogenesis compound could suppress submesothelial thickening and reduce type III collagen expression and angiogenesis (2). We previously reported that basic fibroblast growth factor (bFGF), a cytokine that participates in fibrosis and neovascularization, can cause peritoneal fibrosis in CAPD patients (3). However, the mechanisms involved in the initiation and progression of peritoneal dysfunction associated with CAPD remain to be elucidated.
Lactate has long been used as a buffer in peritoneal dialysate, but bicarbonate has been employed as a substitute in recent years. Lactate is a degradation product of glucose during anaerobic metabolism, and its accumulation causes fatigue and pain after exercise. As compared with bicarbonate-containing solutions, lactate-containing CAPD solutions induce a greater increase of microvascular flow and perfused capillary length per area of peritoneal membrane and also cause more marked functional impairment of human neutrophils (4). However, the effects of lactate and bicarbonate on the peritoneum in CAPD patients remain to be elucidated.

To clarify the role of lactate and bicarbonate in peritoneal fibrosis and neovascularization, we investigated the expression of vascular endothelial growth factor (VEGF) and bFGF, which are potent angiogenic factors (5), by human peritoneal mesothelial cells (HPMCs) cultured in lactate/bicarbonate–containing media. We also investigated the level of lactate dehydrogenase (LDH) as an indicator of HPMC necrosis, because loss of these cells is suggested to be the primary cause of peritoneal hyperpermeability and fibrosis in CAPD patients.

Materials and methods

Using pieces of human omentum (3 – 5 cm²) obtained at laparotomy, we isolated HPMCs according to the method of Stylianou et al. (6) and cultured them in M199 medium containing 10% fetal calf serum [FCS (Mitsubishi Kasei, Tokyo, Japan)]. In the experiments, cells from the first or second passage were used to test the effects of 10 different culture media.

Sodium bicarbonate–free M199 medium was supplemented with 10% volume/volume FCS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mmol/L L-glutamine before use. This medium was further supplemented with sodium lactate (Katayama Chemical, Osaka, Japan) or sodium bicarbonate (Katayama Chemical), or both. Lactate or bicarbonate alone was added at concentrations of 0, 15, 25, and 40 mmol/L; the combination media contained 15 mmol/L lactate and 25 mmol/L bicarbonate, or 25 mmol/L lactate and 15 mmol/L bicarbonate. All media were sterilized by filtration [0.22 µm Millex-GV filter (Millipore, Bedford, MA, U.S.A.)] and buffered to pH 7.4 with 1N sodium hydroxide (Katayama Chemical) or 1N hydrochloric acid (Katayama Chemical) just before use.

The levels of VEGF, bFGF, and LDH in culture supernatants were measured using sandwich enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, U.S.A.). Supernatants were harvested after HPMCs (1x10⁵/well) had been incubated for 24 hours with the various media (1.0 mL/well) in 24-well dishes coated with rat type I collagen (Becton Dickinson, Mountain View, CA, U.S.A.). Supernatants were stored at –30°C until assay.

Statistical analysis was performed using the Mann–Whitney U-test, as appropriate. Results are expressed as mean ± standard deviation, with each experiment being performed in triplicate on cells obtained from each of three donors. Values of $p < 0.05$ were considered statistically significant.

Results

The levels of VEGF, bFGF, and LDH in the culture supernatant showed a significant decrease (Figure 1) after incubation of HPMCs with 15 mEq/L lactate plus 25 mEq/L bicarbonate (VEGF: 44.7 ± 5.6 pg/mL, $p < 0.05$; bFGF: 27.8 ± 3.6 pg/mL, $p < 0.05$; LDH: 19.8 ± 4.6 IU/L, $p < 0.05$) or with 40 mEq/L bicarbonate (VEGF: 35.2 ± 6.0 pg/mL, $p < 0.01$; bFGF: 18.1 ± 7.0 pg/mL, $p < 0.05$; LDH: 13.4 ± 5.2 IU/L, $p < 0.01$) as compared with 40 mEq/L lactate (VEGF: 51.0 ± 8.9 pg/mL; bFGF: 31.9 ± 4.6 pg/mL; LDH: 27.5 ± 6.1 IU/L).

![Figure 1](image-url) Levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and lactate dehydrogenase (LDH) in the culture supernatant of human peritoneal mesothelial cells incubated with lactate (Lac) or bicarbonate (Bic) or both at a total concentration of 40 mEq/L. * $p < 0.05$; ** $p < 0.01$; † $p < 0.05$; ‡ $p < 0.05$; ‡‡ $p < 0.01$. 

![Image](image-url)
The levels of VEGF and bFGF showed a concentration-dependent decrease when cells were incubated with lactate (40 mEq/L: VEGF, p < 0.05; bFGF, p < 0.05) or bicarbonate (40 mEq/L: VEGF, p < 0.05; bFGF, p < 0.05), while LDH showed a concentration-dependent increase (40 mEq/L lactate: p < 0.01; 40 mEq/L bicarbonate: p < 0.01). The p values are compared with 0 mEq/L lactate or bicarbonate [lactate: VEGF, 68.9 ± 14.9 pg/mL; bFGF, 40.3 ± 11.1 pg/mL; LDH, 2.75 ± 2.1 IU/L; bicarbonate: VEGF, 52.9 ± 25.2 pg/mL; bFGF, 28.3 ± 14.2 pg/mL; LDH, 4.4 ± 5.6 IU/L (Figure 2)].

Discussion
We investigated the levels of VEGF, bFGF, and LDH in the culture supernatant when HPMCs were incubated with bicarbonate or lactate or both. Our results suggest that 40 mEq/L bicarbonate is superior to 40 mEq/L lactate as an additive to CAPD fluid with respect to the effect on VEGF and bFGF, which have a role in peritoneal fibrosis and neovascularization. However, lactate and bicarbonate are both toxic for HPMCs, as shown by the increase of LDH, indicating cellular necrosis.

We previously reported the possibility that bFGF released by HPMCs may participate in the development of peritoneal neovascularization, leading to peritoneal dysfunction, and we showed that lower levels of glucose, advanced glycation end products, and lactate in the culture medium, plus a higher bicarbonate level, improves the viability of HPMCs and prevents secretion of bFGF (3,7,8).

Muscle catabolism and high turnover bone disease are exacerbated by metabolic acidosis, and these abnormalities can be ameliorated by increasing serum HCO₃⁻ to the normal range in patients with end-stage renal disease (9,10).

Conclusions
There is no consensus about the correct concentrations of lactate and bicarbonate for CAPD fluid. However, 40 mEq/L lactate is currently the most widely used buffer in CAPD solutions. During a 6-hour exchange period, approximately 75% of the lactate in the CAPD fluid is absorbed by the patient and is rapidly converted to bicarbonate. When the fluid itself contains no bicarbonate, a continual loss of bicarbonate from the body into the dialysis fluid simultaneously occurs, with the rate of bicarbonate loss being determined by the dialysate and the transmembrane concentration gradient. Bicarbonate that enters the dialysis fluid from the patient is not metabolized, and its concentration rises to approximately 80% of serum level after a 6-hour exchange (11).

Although our in vitro data support the benefits of a high bicarbonate solution without lactate, further investigation is still required to determine the ideal dialysate for CAPD patients.

![Figure 2](image-url)

**Figure 2**: Levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and lactate dehydrogenase (LDH) in the culture supernatant of human peritoneal mesothelial cells incubated with various concentrations of lactate or bicarbonate. * p < 0.05; † p < 0.05; ‡ p < 0.05; ‡‡ p < 0.01.


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