

THE SECRETION OF FLUID BY RENAL CYSTS FROM PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

MIN YE, M.D., AND JARED J. GRANTHAM, M.D.

Abstract Background. The mechanism by which fluid accumulates in renal cysts of adults with autosomal dominant polycystic kidney disease is not known. This study was designed to determine whether transepithelial secretion of fluid may account for this accumulation.

Methods. We studied in vitro intact cysts that were excised from kidneys removed from three patients with end-stage autosomal dominant polycystic kidney disease. The cysts were loaded with natural cyst fluid or with a combination of Dulbecco's modified Eagle's medium and Ham's F12 medium (DME-F12) and incubated in DME-F12 for 24 hours. Fluid secretion, determined by the change in the weight of the cysts, was expressed as the rate of fluid secretion per square centimeter of surface area per 24 hours, to correct for the wide variation in the sizes of the cysts. To test for endogenous secretagogues, cyst fluid was added to confluent monolayer cultures of canine- and human-kidney cells.

Results. During the first 24 hours of incubation, the

mean (\pm SE) rate of fluid secretion in nine cysts containing natural cyst fluid was $20.8 \pm 5.6 \mu\text{l}$ per square centimeter of surface area per 24 hours, as compared with $2.3 \pm 3.6 \mu\text{l}$ per square centimeter per 24 hours in nine cysts containing incubation medium. Each group of cysts was then incubated with forskolin, a nonspecific stimulator of adenylyl cyclase activity, for an additional 24 hours. During this period the fluid-secretion rate of cysts containing natural cyst fluid did not change; however, the secretion rate of those containing incubation medium increased to $9.1 \pm 4.4 \mu\text{l}$ per square centimeter per 24 hours (mean change, 6.8 ± 1.1 ; $P < 0.001$). Cyst fluid stimulated fluid secretion by polarized monolayers of canine- and human-kidney cells.

Conclusions. Renal cysts from patients with autosomal dominant polycystic kidney disease can secrete fluid, and net fluid secretion can be increased by unidentified secretagogues in the cyst fluid. These results suggest that the process of cyst enlargement may be susceptible to pharmacologic intervention. (N Engl J Med 1993;329:310-3.)

IN 1969 Gardner¹ reported that fluid collected from cysts of a specimen of polycystic kidney obtained at nephrectomy had lower concentrations of sodium and chloride and higher concentrations of potassium and protons than did plasma. This evidence that steep electrolyte gradients were maintained across the walls of renal cysts indicated that the tubular epithelium composing the cysts was functional. These results were subsequently confirmed and reinforced by the finding that portions of cyst wall excised from a polycystic kidney generated lumen-negative electrical potentials and negative short-circuit currents that were inhibited by amiloride.^{2,3}

The evidence supporting continued active transepithelial transport of electrolytes indicated that the cysts functioned in some respects as giant nephrons.⁴ If, however, the cysts continued to absorb solute and water, the generally accepted mode of net transport by all segments of mammalian renal tubules, it was difficult to explain how fluid could steadily accumulate within the cavities. The origin of cyst fluid in patients with autosomal dominant polycystic kidney disease was ultimately revealed by an electron-microscopical study: 27 percent of the cysts were connected to glomeruli, and 73 percent were autonomous sacs.⁵ This finding meant that most of the cysts had to fill with liquid by transepithelial secretion, a transport process that had not been considered to have an important role in the processing of urine by mammalian kid-

neys.⁶ Recently, we removed intact cysts from the polycystic kidneys of three patients to determine whether transepithelial secretion of fluid is a plausible mechanism to account for the progressive accumulation of liquid in these cysts.

METHODS

In preparation for renal transplantation, polycystic kidneys from three women with end-stage autosomal dominant polycystic kidney disease were surgically removed because of pain or unusual size after each woman had given informed consent. The kidneys were sealed in a sterile bag, immersed in ice water, and shipped to the laboratory by overnight delivery. Individual surface cysts ranging in volume from 2 to 59 ml were dissected from the surrounding parenchyma and placed in chilled nutrient tissue-culture medium containing physiologic concentrations of electrolytes (a combination of Dulbecco's modified Eagle's medium and Ham's F12 medium [DME-F12]⁷) until used later that day.

The contents of each cyst were aspirated into a calibrated syringe to determine the volume. The volume was used to calculate the total surface area of the cavity. In some cysts approximately one third of the cyst fluid was reinjected into the cyst cavity; in the others the cavities were rinsed with DME-F12 supplemented with 5 percent fetal-calf serum, insulin, transferrin, selenium, hydrocortisone, triiodothyronine, penicillin, and streptomycin.⁷ After the cavity was rinsed, an amount of supplemented DME-F12 approximating one third of the original fluid volume was injected into the cyst. Each cyst was then blotted to remove adherent liquid and weighed on a Sartorius balance to the nearest 0.1 mg. The coefficient of variation of the blotting method, determined by repeating the immersion and blotting of single cysts 10 times, was 1.8 percent for cysts containing 1 ml of liquid and 0.3 percent for cysts containing 15 ml.

Eighteen excised cysts (six from each kidney) were selected for the principal study and divided into two groups; the cysts in the two groups were then matched according to weight. Nine cysts contained DME-F12 supplemented as described above, and nine cysts contained natural cyst fluid (Table 1). Each cyst was transferred into an individual plastic vial containing 30 to 40 ml of supplemented DME-F12. The 18 cysts were incubated for 24 hours in a 5 percent carbon dioxide atmosphere at 37°C (control incubation period) and then weighed. Forskolin (10 μM), which directly acti-

From the Division of Nephrology, Department of Medicine, University of Kansas School of Medicine, Kansas City. Address reprint requests to Dr. Grantham at the Division of Nephrology, Department of Medicine, Sudler 4015, Kansas University Medical Center, 39th and Rainbow Blvd., Kansas City, KS 66103.

Supported by grants from the National Institutes of Health (DK 45614) and the Polycystic Kidney Research Foundation.

Table 1. Characteristics of 18 Excised Cysts during Incubation in Vitro.*

FINAL CONTENTS OF CYSTS	INITIAL VOLUME <i>ml</i>	WEIGHT		CHANGE IN WEIGHT†		
		INITIAL	AFTER 24 HR IN CONTROL MEDIUM	AFTER 24 HR IN FORSKOLIN MEDIUM	CONTROL MEDIUM	FORSKOLIN MEDIUM
		<i>mg</i>				
Natural cyst fluid (n = 9)	15 (2–59)	6514±2258	7018±2395	7471±2535	504±232‡	453±152§
Incubation medium (n = 9)	12 (3–39)	4830±1636	4911±1690	5123±1758	81±78	212±109‡

*Plus-minus values are means ±SE. Ranges are given in parentheses. The cysts were incubated for sequential periods of 24 hours.

†Values are the means of the differences in individual cysts.

‡P<0.05 for the change in weight during incubation.

§P<0.01 for the change in weight during incubation.

vates adenylate cyclase, was then added to the external bath, and the cysts were reweighed after another 24 hours of incubation. Nine additional cysts containing natural cyst fluid were weighed before and after incubation with forskolin for 24 hours, after which ouabain (10 to 100 μM), an inhibitor of sodium transport, was also added to the medium for an additional 24 hours. There was a direct linear relation between the change in the weight of the cysts and the change in the volume of cavity fluid (weight change = $0.15 + 1.07$ times the change in volume in milliliters, $r^2 = 0.93$). The concentration of total protein, osmolality, and pH were determined in aliquots of the natural cyst fluids and in the fluids removed at the conclusion of the study.

The capacity of the original cyst fluid to stimulate net fluid secretion by renal epithelial cells was determined by a bioassay of pooled samples of cyst fluid from each of the three kidneys. Madin–Darby canine-kidney and human-kidney–cortex cells were cultured on specialized membranes to permit quantitative collection of fluid secreted from the basolateral to the apical surface of confluent monolayers of the cells.^{7,8} The rate of secretion across these cultured monolayers caused by cyst fluid (secretory activity) was expressed in relation to the effect of forskolin (10 μM).^{7,8}

RESULTS

Histologic examination confirmed that the epithelium lining the cavities of the excised cysts incubated in vitro remained intact for the duration of the study. The epithelial morphology was columnar or squamous, as described previously.⁹ The osmolality of the natural cyst fluid ranged from 287 to 332 mOsm per kilogram, and the pH ranged from 4.65 to 8.30 — values similar to those reported previously.² The osmolality of the culture medium was 323 mOsm per kilogram, and the pH was 7.31. The protein concentration of the natural cyst fluid ranged between 6.7 and 27.8 mg per milliliter; the protein concentration of the culture medium was 3.0 mg per milliliter. After 72 hours of in vitro incubation, the osmolality and pH of the cyst contents and the external bath were not appreciably different from one another.

Seven of the nine cysts containing natural cyst fluid increased in weight (mean [\pm SE] weight change for all nine cysts, 504 ± 232 mg; $P < 0.05$) during the first 24 hours of incubation (Table 1). The addition of forskolin (10 μM) to the medium bathing these cysts during a second 24-hour period of incubation resulted in a further weight gain (mean weight change, 453 ± 152 mg; $P < 0.01$). The rate of fluid secretion, normalized for the surface area of each cyst, was calculated on the basis of the assumption that a weight gain of 1 mg was equal to a gain of 1 μl (Fig. 1). There was no difference in the rate of fluid secretion between the control and forskolin incubation periods (20.8 ± 5.6

vs. 17.8 ± 3.2 μl per square centimeter of surface area per 24 hours; mean change in paired measurements, -3.0 ± 5.9).

In contrast to the cysts containing natural cyst fluid, three of the cysts containing incubation medium increased in weight and six lost weight during the first 24 hours of incubation (mean weight change, 81 ± 78 mg) (Table 1). The addition of forskolin to the medium bathing these cysts during a second 24-hour period of incubation caused all but one cyst to increase in weight (mean weight change, 212 ± 109 mg; $P < 0.05$). Forskolin increased the rate of fluid secretion, normalized for the surface area of individual cysts, from 2.3 ± 3.6 to 9.1 ± 4.4 μl per square centimeter per 24 hours (mean change in paired measurements, 6.8 ± 1.1 ; $P < 0.001$) (Fig. 1). The results indicated that the cysts contained material that stimulated net fluid secretion and that the effect of natural cyst fluid was mimicked by forskolin.

The effect of ouabain (10 or 100 μM in the external bath) was examined in three groups of cysts that contained natural cyst fluid (three cysts per group). In these nine cysts, fluid was secreted at a rate of

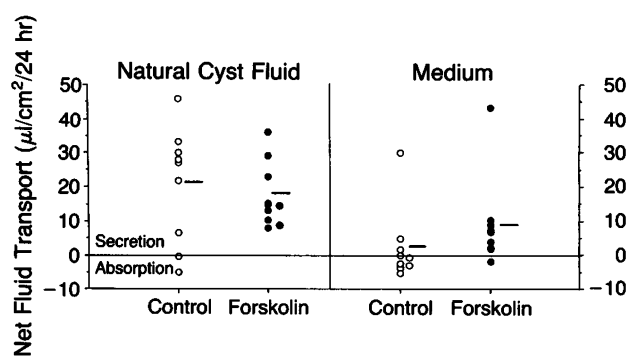


Figure 1. Secretion of Fluid by Cysts Containing Natural Cyst Fluid or Incubation Medium.

The cysts were loaded with natural cyst fluid or incubation medium equal to approximately one third the initial volume. The medium was supplemented DME–F12 during the initial 24-hour control period; forskolin (10 μM) was added during the second 24-hour period. Symbols represent different cysts from three kidneys. The mean (\pm SE) change caused by forskolin in the mean rate of net fluid transport was -3.0 ± 5.9 μl per square centimeter of surface area per 24 hours for cysts containing cyst fluid and 6.8 ± 1.1 μl per square centimeter per 24 hours for cysts containing DME–F12. Horizontal bars indicate mean values.

15.7±3.6 μl per square centimeter of surface area per 24 hours during incubation with forskolin for 24 hours. During incubation with ouabain in addition to forskolin, the rate of fluid secretion decreased to 7.2±1.5 μl per square centimeter per 24 hours (-54 percent); the effects of 10 and 100 μM ouabain were similar.

As reported in a previous study,⁸ cyst fluids from all the kidneys (diluted 20-fold with medium) stimulated the rate of net fluid secretion in Madin-Darby canine-kidney cells to levels well above the base line. The results from one of these kidney fluids is shown in Figure 2. This same cyst fluid also increased fluid secretion by human-kidney-cortex cells.

DISCUSSION

The results indicate that epithelial renal cysts from patients with autosomal dominant polycystic kidney disease can secrete fluid *in vitro* and that the rate of net fluid secretion can be augmented by forskolin, which activates adenylate cyclase. Net fluid secretion was apparently coupled to the active transport of solutes, since fluid was secreted in cysts in which no transepithelial concentration gradients were imposed in the initial study period. Ouabain, which inhibits the active transport of sodium, decreased the rate of net fluid secretion.

The average rates of fluid secretion in these excised cysts (17.8 and 9.1 μl per square centimeter of surface area per 24 hours for forskolin-treated cysts containing natural fluid and culture medium, respectively) may

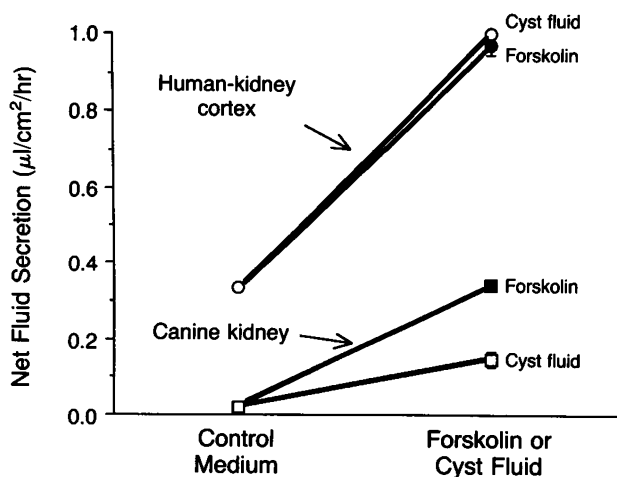


Figure 2. Effect of Cyst Fluid and Forskolin on Net Fluid Secretion by Cultured Renal Epithelial Cells.

Confluent human-kidney-cortex and Madin-Darby canine-kidney cells were cultured in supplemented DME-F12 for three days. Control measurements of net fluid secretion were made in the absence of agonists. Forskolin (10 μM) and cyst fluid (5 percent) were then added to the medium for 24 hours, and the measurements were repeated. Each symbol represents the mean (\pm SE) values for three cultures. Standard-error bars are enclosed by the symbols in most cases. The increases in fluid secretion caused by forskolin and cyst fluid in canine-kidney and human-kidney-cortex cells were significant ($P < 0.001$).

appear relatively low at first glance, given the usual pace of transepithelial fluid absorption in renal tubules. In cysts, however, the fluid collects in a closed compartment, and the secretion is cumulative. For cysts with volumes of 2 to 59 ml (the range studied), the corresponding range of surface areas is 7.9 to 73.3 cm^2 . Thus, fluid could accumulate in the cysts at rates ranging from 0.07 to 1.3 ml per day, or 26 to 475 ml per year.

The forskolin-induced increase in the secretion of fluid by excised cysts is consistent with the hypothesis that cyclic AMP is an important intermediary in the cellular mechanisms promoting the increased transport of fluid.^{10,11} Studies of cultured epithelial cells derived from cyst walls have demonstrated that forskolin, prostaglandin E_1 , methylisobutylxanthine, and 8-bromo-cyclic AMP stimulate the net secretory transport of sodium chloride and liquid for prolonged periods.^{7,11} Thus, cultured cyst epithelial cells appear to retain the solute- and liquid-transport phenotype of excised intact cysts. The relevance of the *in vitro* results to *in situ* cyst function is reinforced by a study showing that secretin, which also stimulates the formation of cyclic AMP by epithelial cells, increased the rate of fluid secretion in two intact liver cysts and one intact renal cyst in patients with autosomal dominant polycystic kidney disease.¹²

Renal cysts containing natural cyst fluid secreted fluid at higher rates than cysts containing culture medium. Renal-cyst fluid has higher concentrations of proteins than the supplemented DME-F12 in the external bath.^{12,13} Although this initial transepithelial oncotic gradient may have promoted the osmotic flow of liquid into the cyst cavities, it seems unlikely that this was an important means of generating secreted fluid, since the cysts accumulated liquid after stimulation with forskolin when the concentrations of protein (3 mg per milliliter) in the fluids bathing the cavity and basolateral surfaces of the epithelium were similar (Fig. 1). Ouabain also inhibited the net secretion of fluid by cysts that contained fluid with a higher protein concentration than the external bath, implying a role for primary or secondary active sodium transport in the process of fluid secretion. Additional evidence that cyst fluid contains secretagogues unrelated to the oncotic activity of proteins was suggested by the capacity of these fluids to cause net fluid secretion when they were applied to the basolateral surfaces of cultured renal epithelial cells (Fig. 2).⁸ The accumulation of endocrine, autocrine, or paracrine secretagogues by cysts or the synthesis of these products by cysts may contribute to the growth of the cysts *in situ*.

The observation that excised cysts may absorb as well as secrete fluid *in vitro* is consistent with an earlier study of the bioelectrical properties of excised patches of cyst wall studied *in vitro*. Perrone³ reported that cyst-wall epithelium could generate lumen-negative electrical potential differences and negative short-circuit currents that were diminished by

amiloride, indicating that sodium was actively transported from the cavity to the basolateral side of the cyst wall. That finding, which seems to be inconsistent with the current results, may be explained by the fact that the electrical measurements were made in the absence of stimulation by cyclic AMP agonists or cyst fluid. In the absence of stimulation by adenylylate cyclase agonists, the epithelium of cyst walls may absorb sodium and liquid, as did several of the cysts (Fig. 1).

The finding that cysts may absorb as well as secrete fluid implies that the rate of cyst enlargement reflects an imbalance between two opposing transport processes. It is not unrealistic to suppose that strategies linked to the abnormalities in solute and fluid transport may be useful to reduce the growth of renal cysts in situ. One strategy would be to inhibit the specific cellular processes that promote increased rates of net fluid secretion; another would be to promote the absorption of liquid from the cysts by eliminating the effect of endogenous secretagogues. Pharmacologic modification of net fluid secretion would seem to be a reasonable therapeutic strategy to control the enlargement of cysts, but at the moment, no such agents are available.

REFERENCES

1. Gardner KD Jr. Composition of fluid in twelve cysts of a polycystic kidney. *N Engl J Med* 1969;281:985-8.
2. Huseman R, Grady A, Welling D, Grantham J. Macropuncture study of polycystic disease in adult human kidneys. *Kidney Int* 1980;18:375-85.
3. Perrone RD. In vitro function of cyst epithelium from human polycystic kidney. *J Clin Invest* 1985;76:1688-91.
4. Grantham JJ. Polycystic kidney disease: a predominance of giant nephrons. *Am J Physiol* 1983;244:F3-F10.
5. Grantham JJ, Geiser JL, Evan AP. Cyst formation and growth in autosomal dominant polycystic kidney disease. *Kidney Int* 1987;31:1145-52.
6. Grantham JJ. Fluid secretion in the nephron: relation to renal failure. *Physiol Rev* 1976;56:248-58.
7. Neufeld TK, Grant ME, Grantham JJ. A method to measure the rate of net fluid secretion by monolayers of cultured renal epithelial cells. *J Tissue Cult Methods* 1991;13:229-34.
8. Ye M, Grant M, Sharma M, et al. Cyst fluid from human autosomal dominant polycystic kidneys promotes cyst formation and expansion by renal epithelial cells in vitro. *J Am Soc Nephrol* 1992;3:984-94.
9. Cuppage FE, Huseman RA, Chapman A, Grantham JJ. Ultrastructure and function of cysts from human adult polycystic kidneys. *Kidney Int* 1980;17:372-81.
10. Mangoo-Karim R, Uchic M, Lechene C, Grantham JJ. Renal epithelial cyst formation and enlargement in vitro: dependence on cAMP. *Proc Natl Acad Sci U S A* 1989;86:6007-11.
11. Mangoo-Karim R, Uchic ME, Grant M, et al. Renal epithelial fluid secretion and cyst growth: the role of cyclic AMP. *FASEB J* 1989;3:2629-32.
12. Everson GT, Emmett M, Brown WR, Redmond P, Thickman D. Functional similarities of hepatic cystic and biliary epithelium: studies of fluid constituents and in vivo secretion in response to secretin. *Hepatology* 1990;11:557-65.
13. Ye M, An S, Jiang H, Sheng S, He S. Clinical analysis of 141 cases of adult polycystic kidney disease. *Chin J Surg* 1986;24:73-6.