

COMPOSITION OF FLUID IN TWELVE CYSTS OF A POLYCYSTIC KIDNEY*

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with comments by

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Abstract Analyses of the chemical composition of fluid in 12 cysts of a polycystic kidney disclosed marked variations in the concentrations of selected cations, glucose, urea and creatinine. The concentrations of sodium ranged from 3.1 to 150.0 mEq per liter, of potassium from 4.6 to 58.1 mEq per liter, of hydrogen from 13 to 9800 nanoEq per liter and of creatinine from 11 to 87 mg per 100 ml.

The concentrations of sodium varied directly with those of calcium and inversely with those of potassium, hydrogen, ammonium and magnesium, suggesting that the cyst walls were functioning as either proximal or distal nephrons. In cysts whose contents reflected distal tubular activity, amino acids contributed 50 to 100 mOsm per kilogram of water to the osmotic activity of the fluid.

IN 1943 Lambert¹ made known the results of his search for clues to the pathogenesis of adult polycystic kidney disease. Using chemical technics, he demonstrated that inulin, after systemic injection, appeared rapidly in cysts and that the concentrations of inulin, creatinine and urea often were higher in cyst fluid than in plasma. In 1955 Bricker and Patton² confirmed and extended these findings. They supported Lambert in a conclusion that many cysts in a polycystic kidney are connected dynamically to patent and functioning nephrons. They were unwilling to argue, however, that cyst fluid obtained from different depths in the kidney was analogous to urine from various levels of normal nephrons.

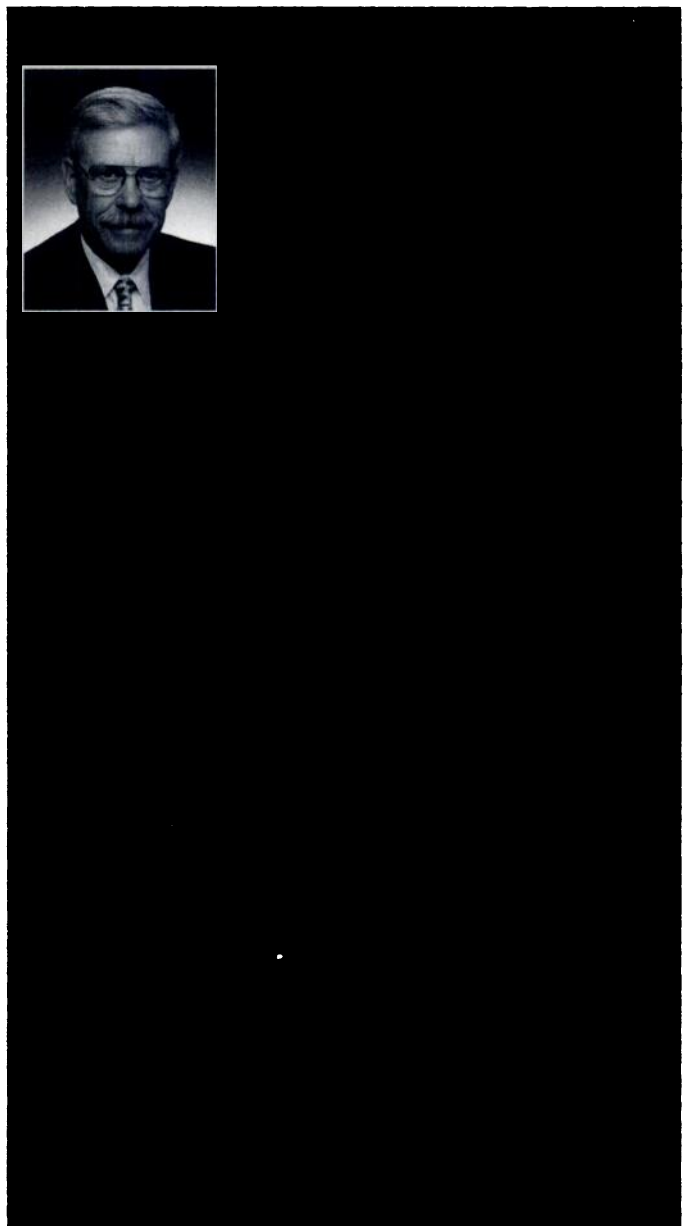
Recently, I encountered a polycystic kidney in which the composition of fluid from 12 cysts precisely reflected known activities of either proximal or distal tubular epithelium. Furthermore, detailed analyses of the samples disclosed that amino acids contributed significantly (50 to 100 mOsm per kilogram of water) to the osmotic activity of the fluid when its composition mirrored distal tubular activity.

MATERIALS AND METHODS

M.H., a 32-year-old man, had a documented 6-year history of rising blood pressures, diminishing renal function and enlarging flank masses. Intravenous pyelography in 1962 had led to a diagnosis of polycystic renal disease. His mother had died of the disease at age 39. On March 2, 1969, under sodium thiopental (Pentothal) anesthesia, bilateral nephrectomy was performed in preparation for renal transplantation. As the left renal hilus was clamped, blood was withdrawn from an indwelling venous catheter. No urine was present in the bladder.

After excision the left kidney was halved by a single cut along its midsagittal plane. Twelve intact cortical and medullary cysts protruding from the cut surfaces of the kidney were identified. The contents of these cysts were aspirated directly into individual clean glass syringes – a procedure that was completed 12 minutes after hilar clamping.

Aliquots of cyst fluid and serum were subjected to prompt analyses for pH (pH/mV Electrometer, model 245, Instrumentation Laboratory, Incorporated, Boston, Massachusetts), sodium and potassium concentrations (Flame Photometer, model 143, Instrumentation Laboratory, Incorporated, Boston, Massachusetts), osmolalities (Osmometer, Fiske Associates, Incorporated, Uxbridge, Massachusetts), and relative water content



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by weight (T/S Refractometer, American Optical Company, Buffalo, New York), and concentrations of urea and ammonia nitrogen by the microdiffusion method of Conway.³ Fluid and serum that remained were frozen and later analyzed for concentrations of calcium and magnesium (Atomic Absorption Spectrophotometer, model 303, Perkin Elmer Corporation, Norwalk, Connecticut), creatinine with the use of Lloyd's reagent,⁴ and glucose by means of an Auto-Analyzer (Technicon Instruments, Tarrytown, New York). Semiquantitative estimates of amino acid concentrations in selected cysts were determined by unidirectional descending chromatography against standards of known concentrations, a method modified from that of Katz, Dreyer and Anfinsen.⁵ Quantitative analyses of amino acid concentrations in 2 cysts were performed by column (Beckman AA Analyzer model 120C, Beckman Instruments, Incorporated, Palo Alto, California).

RESULTS

Table 1 indicates the patterns of solute concentrations that were found in the 12 cysts of this kidney. Arbitrarily, the cysts were arranged in an order of ascending sodium concentrations and designated 1 through 12. For each cyst the pattern of solute concentration was different. Wide ranges of solute concentrations were recorded. There were, for example, an eightfold difference in creatinine concentrations a 50-fold difference in sodium concentrations, and a 750-fold difference in hydrogen ion concentrations. These differences could not be related to the intrarenal location of the cysts (Table 1).

When ratios of cyst fluid to plasma were calculated for these substances, at least two populations of cysts were apparent (Table 2). In one, the concentrations of sodium, potassium, urea and creatinine differed but little from that of plasma (Cysts 5-12). In the second, significant and even dramatic differences were recorded (Cysts 1-4). Coefficients of correlation were determined between the concentrations of sodium and the concentrations of the other cations and molecules in the 12 cysts. Values shown in Table 3 were obtained. The concentrations of sodium varied in parallel to those of calcium and inversely to those of potassium, magnesium, ammonium and hydrogen. In addition, there were significant and indirect relations between the cyst-fluid concentrations of sodium and urea and creatinine.

The measurement of amino acid concentrations in the samples of cyst fluid was not originally considered. However, in some cysts marked differences were noted between the osmolalities measured directly by the freezing-point depression technic and those derived by calculation from known concentrations of cations, glucose and urea (Table 4). These differences existed even when all cations were assumed to be associated with monovalent anions in the cyst fluid and osmotic coefficients were ignored. In search of the missing milliosmoles it seemed reasonable to look at amino acid concentrations, since amino acids have significant but low magnitudes of osmotic activity in both the intracellular and the extracellular fluid compartments.⁶ Figure 1 shows a chromatograph of fluid from three cysts, together with standard concentrations of 18 amino acids that were run in conjunction with them. Both the semiquantitative and the quantitative analyses of amino acid concentrations in the fluid of selected cysts gave values of a magnitude sufficient to account for the missing milliosmoles (Table 4).

DISCUSSION

The array of solute concentrations observed in the cysts of this kidney was not unexpected. Bricker and Patton² found creatinine concentrations that varied from below that of plasma to more than 50 times higher in the cysts that they



TABLE 1. Locations of Cysts and Ranges of Concentrations of Selected Cations and Other Solutes in Their Fluid Contents.

CYSTS No.	LOCATION		WATER (Gm/100 Gm)	SODIUM (MEQ/LITER*)	POTASSIUM (MEQ/LITER*)	HYDROGEN (NANOEq/LITER*)	AMMONIUM NITROGEN (MG/100 ML*)	CALCIUM (MG/100 ML*)	MAGNESIUM (MG/100 ML*)	GLUCOSE (MG/100 ML*)	UREA (MG/100 ML*)	CREATININE (MG/100 ML*)
	CORTEX	MEDULLA										
	<i>no.</i>											
1-4	3	1	96.7-98.0	3.1-105.0	5.1-58.1	120-9760	4.5-21.7	1.0- 7.8	5.4-24.5	132-593	45-194	11-87
5-12	3	5	92.5-99.0	129.0-150.0	4.6- 8.6	13-42	None detected	5.7-14.7	2.3- 5.2	46-159	34-88	11-16

*Of cyst-fluid water.

studied. They noted sodium concentrations ranging from slightly above those of normal plasma to lows of 14 mEq per liter in the cysts of one kidney. As in the present study, they found no significant variation among the osmolalities of fluid from cysts in a given kidney.

From their study and the present one, it is evident that not every cyst in a polycystic kidney contains fluid with a unique composition and that the degree to which cysts can vary in their chemical composition within a kidney is inconstant among polycystic kidneys in general. Bricker and Patton² encountered a 10-fold difference in sodium concentrations among three cysts examined in one kidney but a variation of only 10 per cent among the sodium concentrations in six cysts of a second kidney. In addition to the kidney described here, I have examined cysts in the kidneys of two additional patients with polycystic disease and of two with medullary cystic disease. Limited volumes of fluid for analyses have precluded the detailed observations on cyst-fluid composition that are described here. Nonetheless, an occasional cyst has been found with concentrations of sodium and potassium that are lower and higher respectively than urine and plasma levels. In one polycystic kidney cation concentrations were similar to those of plasma in 11 of 12 cysts analyzed, but were reversed in the twelfth. Semiquantitatively, amino acid concentrations were higher in this cyst. In one with medullary cystic disease, fluid pooled from three cysts contained sodium and potassium in concentrations of 7 and 69 mEq per liter respectively.⁷

From their observations both Lamert⁸ and Bricker and Patton² argued that communications exist between functioning nephrons and cysts in the polycystic kidney. Morphologists support this contention.⁹ The data presented here are also consonant with it: concentrations of sodium in cyst fluid varied directly with those of calcium and indirectly with those of potassium, hydrogen, ammonium, magnesium and creatinine. This pattern of variation in chemical composition agrees precisely with the modifications known to be imposed on glomerular filtrate as it passes down the nephron in the normal kidney.¹⁰ To account for these findings, it could be argued that metabolically active proximal or distal nephrons emptied their contents into cystic lakes and that no further change in the composition of the fluid occurred while it lay in these reservoirs. However, the relative isosmolality of the

TABLE 2. Ranges of Cyst-Fluid to Plasma Ratios (CF/P) for Selected Solutes.

CYSTS No.	SODIUM	POTASSIUM	GLUCOSE	UREA	CREATININE
1-4	0.02-0.73	0.85-9.70	0.69-3.12	1.32-5.70	1.51-11.9
5-12	0.90-1.04	0.77-1.44	0.24-0.84	1.00-2.59	1.51-2.19

TABLE 3. Coefficients of Correlation between Sodium Concentrations and Concentrations of Other Solutes in Fluid from 12 Cysts of a Polycystic Kidney.

SODIUM CONCENTRATIONS VERSUS CONCENTRATIONS OF OTHER SOLUTE	R VALUE	P VALUE
Potassium	-0.91	<0.005
Hydrogen	-0.89	<0.005
Ammonium nitrogen	-0.94	<0.005
Calcium	+0.78	<0.01
Magnesium	-0.91	<0.005
Urea	-0.70	<0.01
Creatinine	-0.87	<0.005
Osmolality	+0.53	<0.05

cystic contents suggests that water was equilibrating among them. It seems unlikely that the steep chemical gradients (for example, 750-fold for hydrogen and 50-fold for sodium) could be maintained by a water-permeable but metabolically inactive cyst wall. It is more probable that these walls retain their metabolic activity and the functional characteristics peculiar to the segment of the nephron from which they arise.

The origin of the amino acids that were found in some cysts of this kidney is speculative. Several possibilities exist. The first is that they may have been filtered at the glomerulus, escaping proximal reabsorption and being concentrated by water reabsorption in cysts arising from distal nephrons. The fact that in one cyst (cyst 1), the amino acids were concentrated to a greater degree than creatinine (roughly 30:1 vs 10:1 respectively) argues against this possibility. Furthermore, no consistent pattern could be found among the concentrations of amino acids between cysts 1 and 2, implying that differences in their concentrations were not solely the result of a difference in the amount of water reabsorbed from the two cysts.

A second possibility considered to account for the presence of amino acids was that they came from the hydrolysis of filtered protein as it lay sequestered in the cysts, perhaps through the action of hydrolases from renal tubular epithelium or bacteria. A comparison of the relative concentrations of amino acids in the cyst fluid with the known frequency per mole and per gram of their occurrence in albumin¹¹ and "average" protein¹² failed to disclose any constant relation. Thus, although it could not be excluded, no substantiation for this possibility could be found.

The third possibility considered was that the amino acids entered the tubular or cyst fluid through their epithelial



TABLE 4. Measured and Derived Osmolalities for Fluid from Five Representative Cysts.

CYST No.	SODIUM (mEq/LITER x 2)	POTASSIUM (mEq/LITER x 2)	AMMONIUM (mEq/LITER x 2)	CALCIUM (mEq/LITER x 3)	MAGNESIUM (mEq/LITER x 3)	GLUCOSE (MM/LITER)	UREA (MM/LITER)	TOTAL (mOsm/Kg)	OBSERVED TOTAL (mOsm/Kg)	DIFFERENCE*	AMINO ACIDS (mOsm/Kg)	
											SEMI-QUANTITATIVE	QUANTITATIVE
1	6	90	25	1	25	10	36	193	287	+94	107	80†
2	9	92	31	2	32	34	41	241	286	+45	57	79
3	100	116	6	1	9	10	71	313	286	-27	37	Not measured
4	210	10	6	6	8	7	17	264	287	+23	49	Not measured
10	292	10	0	7	3	7	17	336	290	-46	10	Not measured

*Observed - calculated.

†Leucine reported as "too high to measure."

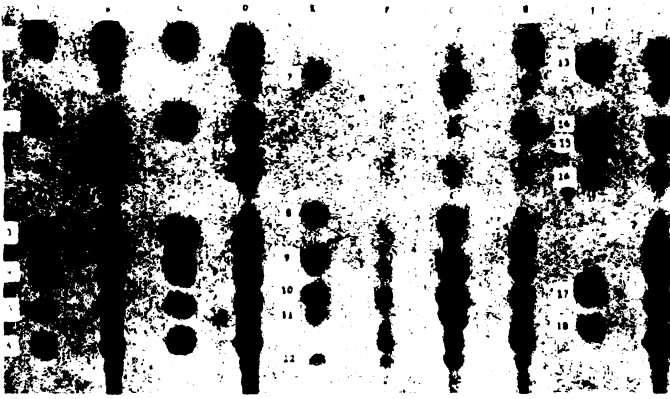


Figure 1. One-Dimensional Chromatography of Amino Acid Standards and Fluid from Cysts 1, 3 and 4.

The photograph is oriented so that amino acids migrated from its lower toward its upper edge. Standard concentrations are 1 $\mu\text{g}/\mu\text{l}$. Columns: (A) standard A, 10 λ ; (B) cyst 1, 10 λ ; (C) standard A, 20 λ ; (D) cyst 1, 20 λ ; (E) standard B, 10 λ ; (F) cyst 3, 10 λ ; (G) cyst 3, 20 λ ; (H) cyst 4, 10 λ ; and (I) standard C, 20 λ ; (J) cyst 4, 20 λ ; Numbers (amino acid identification): (1) leucine; (2) valine; (3) alanine; (4) glutamic acid; (5) aspartic acid; (6) lysine; (7) phenylalanine; (8) proline; (9) threonine; (10) serine; (11) arginine; (12) cystine; (13) isoleucine; (14) methionine; (15) tryptophan; (16) tyrosine; (17) glycine; and (18) histidine.

walls. That they did so down an electrical gradient seems unlikely since the hydrogen ion concentrations recorded in these cysts (pH 5.0 to 5.9) brackets the isoelectric points of almost all the amino acids present.¹³ It also seems unlikely that they entered along a chemical gradient since amino acids normally total 3.2 mM per liter in the extracellular compartment and 14.7 mM per liter in the intracellular space in mammals.⁶ No evidence was found of increased amino acid levels in the plasma of the patient whose kidney is under discussion here. On the other hand, selected amino acids may apparently be secreted by proximal and distal tubular epithelium,^{14,15} possibly by active transport. Such a process could account for their appearance in the cysts of this kidney.

Of these possibilities protein hydrolysis and amino acid secretion are the most intriguing. Large cysts are absent from the typical polycystic kidney during the first two or three decades of its existence.⁹ Only with advancing years does evidence of cyst expansion appear. This process has not been explained but could be accounted for if osmotically active substances were generated in or added to the cystic contents. Amino acids are not thought to undergo detectable reabsorption in the distal nephron.¹⁶ Therefore, they meet a necessary criterion for osmotic effectiveness in cysts derived from that segment of the nephron. To speculate further, the gradual dilatation of strategically located cysts could obstruct adjacent nephrons and lead to their subsequent dilatation and the final appearance of the polycystic kidney. Further careful studies of the composition of cyst fluid in the polycystic kidney and in other forms of renal cystic disease seem warranted.

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