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CLIN. CHEM. 34/1, 91–97 (1988)

Liquid-Chromatographic Profiling of Solutes in Serum of Uremic Patients Undergoing Hemodialysis and Chronic Ambulatory Peritoneal Dialysis (CAPD); High Concentrations of Pseudouridine in CAPD Patients

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Using "high performance" liquid chromatography, we studied non-protein-bound fractions and total concentrations of 18 solutes accumulating in sera from a group of 12 patients who were undergoing chronic ambulatory peritoneal dialysis (CAPD) and in predialysis sera from a group of 15 hemodialysis (HD) patients. We monitored longitudinal changes in solute concentrations for two patients with respect to change of therapy between HD and CAPD. The concentrations of pseudouridine ($P < 0.001$), uric acid ($P < 0.001$), and an unknown fluorescent solute, "UKF3" ($P < 0.01$), differed in sera of HD and CAPD patients. When standardized with respect to serum creatinine concentrations, the concentration of the transfer-RNA catabolite, pseudouridine, was significantly ($P < 0.0001$) higher in sera of CAPD patients than in HD patients, suggesting an increase in turnover of transfer RNA. In stepwise linear discriminant analysis, the combination of pseudouridine and the probably biochemically related fluorescent unknown, UKF3, contributed most to the differentiation between sera from CAPD and HD patients.

Additional Keyphrases: *transfer RNA · protein synthesis · protein binding · metabolic status · peritonitis · peritoneal permeability · discriminant analysis · ultrafiltration · uremia · fluorometry*

During the last 10 years, chronic ambulatory peritoneal dialysis (CAPD) has more and more become an alternative to conventional hemodialysis (HD) in the treatment of end-stage renal patients (*1–3*).[4] Although recurrent peritonitis, with the accompanying decreased permeability of the peritoneal membrane, has limited the use of CAPD, this has become the method of choice for treating pediatric patients and (young) patients awaiting a kidney transplant. Problems of vascular access in hemodialysis, hypertension, and diabetes are also indications for treatment by CAPD. Homeostasis is better in CAPD-treated patients, because sudden fluctuations in fluid volume, electrolytes, and acid–base balance—as are seen in hemodialysis treatment—do not occur.

This, combined with the continuous removal of waste metabolites, leads to steady-state conditions in CAPD patients. Generally, these patients exhibit higher hematocrit values than do HD patients (*4*). A controversial property ascribed to CAPD treatment is the supposedly more efficient removal of so-called neurotoxic middle molecules (*5–8*). In the square meter–hour hypothesis introduced by Babb et al. (*5*) in 1971, these poorly dialyzable solutes are thought to be responsible for neurological disorders in patients undergoing regular dialysis. In kinetic studies (*6–9*) the ratio of predialysis solute concentration in HD to the steady-state concentration in CAPD plasma has higher values for solutes with higher molecular mass.

Hitherto, no evidence has been found that CAPD more efficiently removes those solutes that cause neurological symptoms. To measure the differences in solute concentrations in sera of patients treated with CAPD or with HD, we have used a screening method based on "high-performance" liquid chromatography (HPLC), as described previously (*10*).

Materials and Methods

Patients and Sera

Blood was sampled from 15 patients on HD treatment and 12 patients on CAPD. For hemodialysis we used Disscap 160 dialyzers (Cuprophan, Hospal, Meyzieu, France), a blood flow of 300 mL/min, and a dialysate flow of 500 mL/min. CAPD was performed with four 2-L exchanges within 24 h. HD samples were taken before dialysis. CAPD patients were sampled in the outpatient department, more or less randomly during their daily routine bag exchange. Table 1 summarizes patient characteristics.

None of the CAPD patients had symptoms of peritonitis. The two patients changing the mode of therapy volunteered to do so; they had no clinical indication, such as peritonitis or problems with vascular access. Although the group of CAPD patients had been receiving (any) dialysis treatment for a shorter time, we found no significant difference in residual creatinine clearance between this group and the HD group. All patients were treated at least for three months with the method specified.

Procedures

Protein binding. Protein binding was measured by ultrafiltration through "Centrifree" ultrafiltration units (Amicon Corp., Danvers, MA). We subjected 1-mL aliquots of serum to ultrafiltration by centrifugation in the units, at 22 °C, at an angle of 30°, and at 1900 × *g*, then determined the "free" (i.e., not protein bound) fractions of the solutes in the filtrate. To measure the total concentrations (i.e., bound and nonbound), we precipitated proteins by adding 50 μL of a 100 g/L solution of trichloroacetic acid (TCA) to 1 mL of serum, after treatment by ultrasonication. Then we centrifuged and ultrafiltered the samples, as described for the determination of free fractions. All Centrifree filtrates were analyzed by HPLC, after addition of an internal standard,

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[4] Nonstandard abbreviations: HPLC, "high-performance" liquid chromatography; HD, hemodialysis; CAPD, chronic ambulatory peritoneal dialysis; TCA, trichloroacetic acid; CREA, creatinine concentration in serum; PSI, pseudouridine concentration in serum; UKF, unknown fluorescent peak; and UK, unknown ultraviolet-absorbing peak.

Received June 25, 1987; accepted October 21, 1987.

Table 1. Some Characteristics of the Groups of Patients Studied

Variable	CAPD (n = 12)			HD (n = 15)		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
RCC, mL/min[a]	2.3	2.2	0–7.0	1.7	2.0	0–6.5
Albumin, g/L	32.1	5.4	20.6–38.2	34.0	3.4	30.1–40.9
TTOD, months[b]	9	8	3–25	46[c]	40	6–146
Age, y	48	17	18–67	60	10	37–72
Sex ratio, F/M	3/9			9/6		

[a] Residual creatinine clearance. [b] Total time on (any) dialysis. [c] Significantly ($P < 0.001$) different from CAPD group.

naphthalenesulfonic acid. We checked for any decomposition of uremic solutes caused by the added TCA and found the following losses, which we took into account in the calculations: pseudouridine 3.6%, tyrosine 3.9%, indoxyl sulfate 6.7%, an unknown fluorescent peak UKF7A (see Figure 1 below) 10%, and indole-3-acetic acid 10%. Concentrations of all solutes in subsequent ultrafiltrates (collected at 20-min intervals) were constant, indicating that protein binding was maintained at equilibrium during ultrafiltration. This was not completely true for tryptophan and indoxyl sulfate, however, the concentrations of which increased slightly during ultrafiltration. For these solutes, we used the values obtained by extrapolation to zero ultrafiltration time. Protein binding (PBL, %) was calculated as follows:

$$\text{PBL, \%} = [1 - (C_f/C_t)] \cdot 100$$

where C_f is the concentration of free solute, and C_t is the total concentration.

HPLC. HPLC analysis was performed as described previously (*10*). We used a 4.6 mm (i.d.) × 25 cm Ultrasphere Octyl (C_8-modified silica) column, packed with 5-μm (average diameter) particles, in conjunction with a 2 mm (i.d.) × 3 cm Ultrasphere Octyl guard column, packed with 10-μm particles.

The solvent gradient was linear from ammonium formate buffer (50 mmol/L, pH 4) to methanol/buffer (60/40 by vol) within 45 min. The flow rate was 1 mL/min.

The temperature of the solvent and separation columns was kept at 25 °C by means of a thermostated bath and column water jacket. The chromatograph consisted of a Model 421 controller, two Model 100A double-piston pumps, a Model 160 fixed-wavelength ultraviolet-absorbance detector (wavelength 254 nm, range 0.05 *A* full scale), and a Model 500 autosampler (all from Beckman Instruments, Berkeley, CA). For fluorescence detection we applied excitation at 280 nm and measured emission at 340 nm in a Model RF530 double monochromator fluorescence detector (Shimadzu, Tokyo, Japan).

Gas chromatography/mass spectrometry. For gas chromatography/mass spectrometry analysis we used a Model HP 5970A mass-selective detector coupled to an HP 5790A gas chromatograph (both from Hewlett Packard, Avondale, PA), equipped with a 40-m CPSil5 capillary column (Chrompack, Middelburg, The Netherlands).

The fraction corresponding to HPLC peak 2 (Figure 1) was isolated by repeated HPLC separation and collection, was lyophilized, then derivatized with bis(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co., Rockford, IL), and injected into the gas chromatograph through a falling-needle solids injector.

Data acquisition, handling, and statistics. We used Model 761S data interface and Model 2600 chromatography software (both from Nelson Analytical, Cupertino, CA), with an IBM PC/XT computer. Chromatographic data were read in SAS datafiles, and subsequently analyzed with SAS statistical procedures NPAR1WAY, REG, and STEPDISC for the nonparametric Wilcoxon's test, regression analysis, and stepwise discriminant analysis, respectively (*11*). With STEPDISC, significance levels were 0.05.

Results

The present study was divided into two parts. First, we analyzed and compared the concentrations of accumulated solutes in blood of two groups of patients, on CAPD (n = 12) and HD (n = 15). Second, we also determined the concentrations longitudinally in two patients changing therapy: one from CAPD to HD and the other vice versa. Characteristic HPLC profiles of the solutes are presented in Figure 1, both as absorbance and fluorescence traces. The unknown peak previously designated as UK6 (*10*) was identified by us by mass spectrometry as *p*-hydroxyhippuric acid.

Mass-spectrometric investigation also showed that the peak previously identified tentatively as uracil (*10*) is identical to pseudouridine (PSI). A mass spectrum of the trimethylsilyl derivative of the silylated HPLC peak 2 (Figure 2) was found to be identical to the mass spectrum of a trimethylsilyl derivative of PSI standard. The retention times for the isolated HPLC peak 2 and the PSI standard, both by HPLC (underivatized, co-injected) and by gas chromatography (derivatized), were identical. A more detailed description of this identification will be published elsewhere. The differences between the HPLC profiles shown in Figure 1 will be discussed below.

Comparing the CAPD and the HD Patients

The free (non-protein-bound) fractions and the total concentrations of the solutes under study are summarized in Tables 2 and 3. The data for the HD group were measured before dialysis. Obviously, comparing these blood concentrations is not very informative because the HD group is sampled in a worst-case condition with respect to the high predialysis blood concentrations. Therefore, for statistical analysis, all concentrations in the individual samples were standardized with respect to the corresponding creatinine concentrations:

$$C_{i,st} = C_i \cdot 1000/\text{CREA} \quad (1)$$

where C_i is concentration of component i, CREA is the corresponding creatinine concentration (in μmol/L), and $C_{i,st}$ is the "standardized" concentration. Creatinine was taken as the reference because it is more "constant" than urea, and because it is widely used as a marker in clinical practice. For the sake of unambiguity, we report the original (nonstandardized) concentrations in Tables 2 and 3. However, we applied Wilcoxon's test to both the nonstandardized and the standardized data.

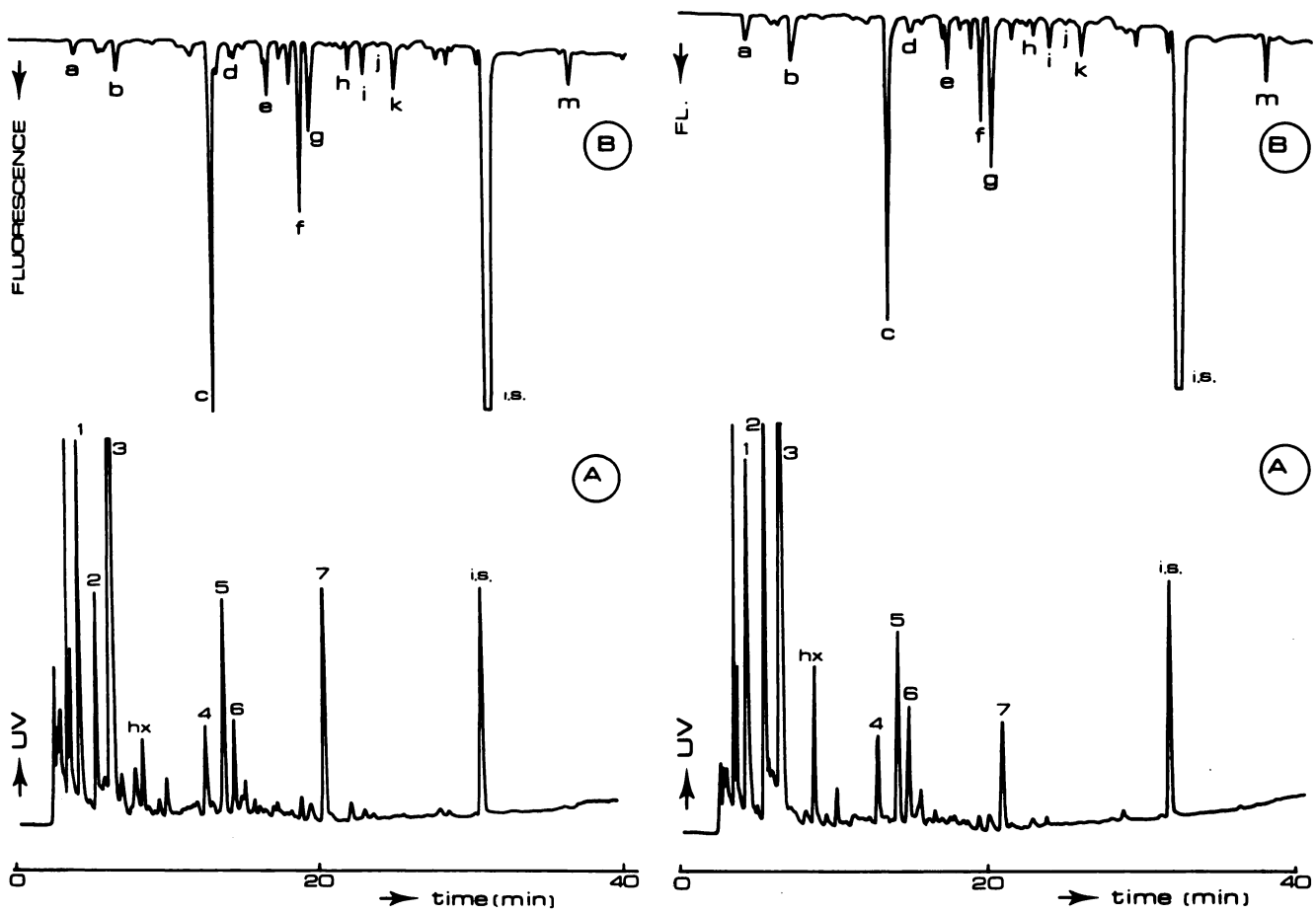

Fig. 1. HPLC-analysis of ultrafiltrated serum from patient R when treated by hemodialysis (*left*), and two months later by CAPD (*right*)

(*A*) absorbance at 254 nm, (*B*) fluorescence (280 nm excitation, 340 nm emission). The fluorescence trace was attenuated to keep solute peak UKF3 on-scale for comparison. Peak identification: *1*, creatinine; *2*, pseudouridine; *3*, uric acid; *hx*, hypoxanthine; *4* and *5*, unknown solutes UK4 and UK5; *6*, *p*-hydroxyhippuric acid; *7*, hippuric acid; *a*, unknown UKF1; *b*, tyrosine; *c*, unknown UKF3; *d*, unknown UKF4; *e*, unknown UKF5; *f*, indoxyl sulfate; *g*, tryptophan; *h*, unknown UKF6; *i*, unknown UKF7A; *j*, unknown UKF7; *k*, unknown UKF8; *m*, 3-indoleacetic acid

The standardized blood concentration of PSI is significantly higher ($P < 0.0001$) in the CAPD group than in the HD group, as is also true for UKF5 ($P < 0.025$) (Table 2). The standardized concentrations of PSI, calculated from the original data by using equation 1, are 76.3 and 38.7 mmol/mol creatinine for CAPD and HD patients, respectively. Although average concentrations of a number of solutes such as hippuric acid and *p*-hydroxyhippuric acid appear to differ, this difference was not significant for these numbers of patients and the observed among-patient variance.

For none of the solutes was protein binding significantly different between the HD and CAPD groups (Table 4), by bivariate statistical analysis.

To study the differences in the profiles by considering the relation of the solutes one to the other, we used a multivariate approach, stepwise discriminant analysis (*11*). By stepwise selection both for free and total concentrations of the solutes at a significance level of $P = 0.05$, only the variables PSI and unknown fluorescent compound UKF3 were selected. This means that the combination of these two variables contributed most to distinguishing the HD and CAPD groups; moreover, the influence of the combination was more than that of PSI alone, as was found by bivariate analysis of the standardized concentrations. Further analysis of the data showed that the serum concentrations of PSI and UKF3 were significantly correlated; in the 15 HD patients $r = 0.79$ ($P < 0.0005$), and in the 12 CAPD patients $r = 0.67$ ($P < 0.01$) by Spearman rank correlation analysis. The groups separation by these two variables is illustrated in the scatter plot of Figure 3. The separate regression lines through the CAPD and HD data points have significantly different slopes ($\alpha = 0.005$), possibly pointing to a biochemical or structural relationship of the two solutes.

A further indication for this relationship was found during experiments on selective isolation of PSI. Nucleosides (e.g., PSI) are known to be extracted selectively by solid-phase extraction on boronate gel (*12*). Performing this procedure, we extracted not only PSI, but also UKF3, with approximately equal recovery. This supports the suggestion that UKF3 is a fluorescent nucleoside, a carbohydrate, a glucuronide, or some solute with a *cis*-diol group. Moreover, the solute is present abundantly in normal urine, but has not hitherto been identified decisively.

Effects of Changing Therapy

We monitored the concentrations of the uremic solutes in blood of two patients who were changing therapy. One patient (K) volunteered to go from HD to CAPD therapy, and the other (R) changed therapy from CAPD to HD for reasons of lifestyle. Blood was sampled from both patients

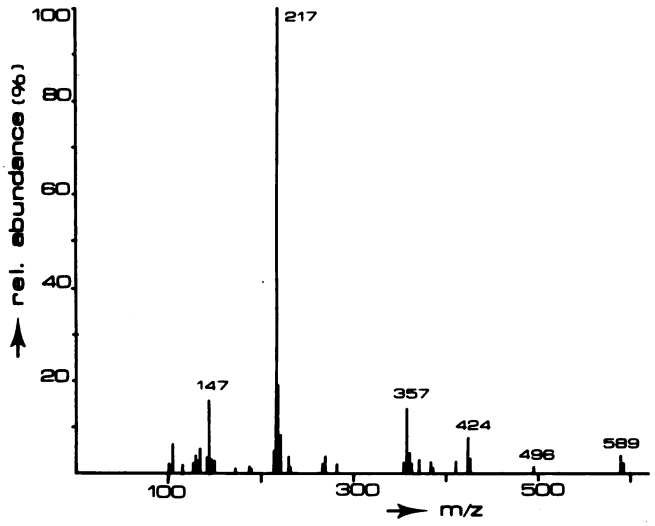

Fig. 2. Electron impact mass spectrum of trimethylsilylated, isolated HPLC peak 2 (see Fig. 1), which is identical to that of a similarly derivatized pseudouridine standard

one month before the changeover, during the equilibration period, and then for another several weeks. Representative HPLC profiles for the HD and CAPD periods in one patient are shown in Figure 1.

In accordance with the data on the patients shown above, the most striking and significant change was the high PSI/CREA ratio in the CAPD intervals of both patients. In addition, the UKF3/PSI ratio changed even more significantly, as illustrated in Figure 4 for patient K. A similar but opposite change was seen in patient R.

On the other hand, the hippuric acid concentration in patient R decreased significantly with onset of CAPD treatment (Figure 5), whereas the reverse was not observed for patient K, changing over from CAPD to HD. In both patients the concentration of indoxyl sulfate, a tryptophan metabolite formed by intestinal bacteria, and the indoxyl sulfate/tryptophan ratio were significantly lower in the CAPD period. These results are summarized in Table 5.

Discussion

The most striking difference between HD and CAPD sera demonstrated in the HPLC profiles centers around pseu-

Table 2. "Free" Concentrations[a] of Bound and Nonbound Solutes in CAPD and HD Patients

	CAPD (n = 12)			HD (n = 15)		
Solute	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Creatinine	1248	199	757–1511	1469	330	960–2112
Pseudouridine	94.9	40.0	49.8–170.5	55.9[c,d]	14.4	41.7–83.8
Uric acid	479.5	75.7	339.9–564.3	630.2[c]	144.2	455.2–1057.7
UK4[b]	0.56	0.43	0.08–1.29	0.46	0.31	0.07–1.00
UK5[b]	1.33	0.49	0.70–2.23	1.31	0.61	0.55–2.62
p-OH-hippuric acid	17.3	11.9	4.3–37.1	22.5	16.1	4.1–63.2
Hippuric acid	208.0	142.7	36.7–490.8	295.2	241.8	35.8–893.8
UKF1[b]	555	456	153–1812	514	329	43–1223
Tyrosine	75.4	29.1	41.4–138.5	75.2	19.0	40.2–116.7
UKF3[b]	6368	1682	4050–9756	8795[e]	3401	2982–14761
UKF5[b]	1859	965	734–3309	1276[f]	681	640–2879
Indoxyl sulfate	16.4	13.9	4.0–44.5	11.8	7.7	1.7–24.4
Tryptophan	10.7	3.2	5.9–16.1	11.9	3.7	5.9–18.4
UKF6[b]	703	527	152–1640	536	452	75–1926
UKF7A[b]	2.5	1.3	0.9–4.9	2.2	0.8	0.7–3.5
UKF7[b]	332	337	92–1305	333	192	68–828
UKF8[b]	3662	2826	1079–9125	4473	4064	559–16169
3-Indoleacetic acid	2.6	0.8	1.6–4.4	2.8	2.0	0.6–7.2

[a] μmol/L unless indicated otherwise. [b] Arbitrary units, used for unknown solutes. [c–f] Significantly different from CAPD values (by Wilcoxon's test) at: [c] $P < 0.001$, [d] $P < 0.0001$, [e] $P < 0.01$, [f] $P < 0.025$. [c, e] Significance of original data; [d, f] Significance after standardization in terms of serum creatinine concentration. All other comparisons not significant ($P \geq 0.05$).

Table 3. Total Concentrations[a] of Bound Solutes in CAPD and HD Patients' Sera

	CAPD n = 12			HD n = 15		
Solute	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
UK5[b]	1.45	0.49	0.69–2.23	1.45	0.63	0.57–2.70
p-OH-hippuric acid	19.4	12.8	5.3–39.6	27.4	16.2	4.8–66.4
Hippuric acid	307.0	184.0	71.3–686.5	432.4	287.5	78.7–1043.8
UKF1[b]	706	573	161–2271	681	407	56–1540
Indoxyl sulfate	96.7	52.1	6.3–168.7	94.0	44.3	2.3–152.3
Tryptophan	27.4	9.2	8.5–43.9	30.7	8.1	20.5–46.1
UKF6[b]	2687	1448	160–4517	2217	1108	228–3921
UKF7A[b]	2.7	1.3	1.1–4.7	2.3	0.8	0.6–3.6
UKF7[b]	509	316	140–1140	754	464	124–1794
UKF8[b]	3891	2875	1167–9575	4939	4216	1313–17015
3-Indoleacetic acid	5.6	2.7	2.8–12.8	8.7[c]	5.1	3.4–22.1

[a] μmol/L unless indicated otherwise. [b] Arbitrary units, used for unknown solutes. [c] Significantly different ($P < 0.025$) from CAPD value.

Table 4. Protein Binding of Uremic Solutes in CAPD and HD Patient's Sera

	Protein binding, %			
	CAPD (n = 12)		HD (n = 15)[a]	
Solute	$\bar{x}$	SD	$\bar{x}$	SD
Creatinine	—[b]	—	—	—
Pseudouridine	—	—	—	—
UK5	7	11	11	7
p-OH-hippuric acid	14	6	15	8
Hippuric acid	36	8	38	8
UKF1	22	4	25	7
Tyrosine	—	—	—	—
UKF3	—	—	—	—
UKF5	—	—	—	—
Indoxyl sulfate	87	8	90	5
Tryptophan	63	14	61	12
UKF6	79	9	74	18
UKF7A	18	8	12	10
UKF7	42	25	55	14
UKF8	8	5	9	5
3-Indoleacetic acid	64	20	71	10

[a] Predialysis. [b] Nonsignificant binding. All differences nonsignificant ($P < 0.05$, Wilcoxon's test).

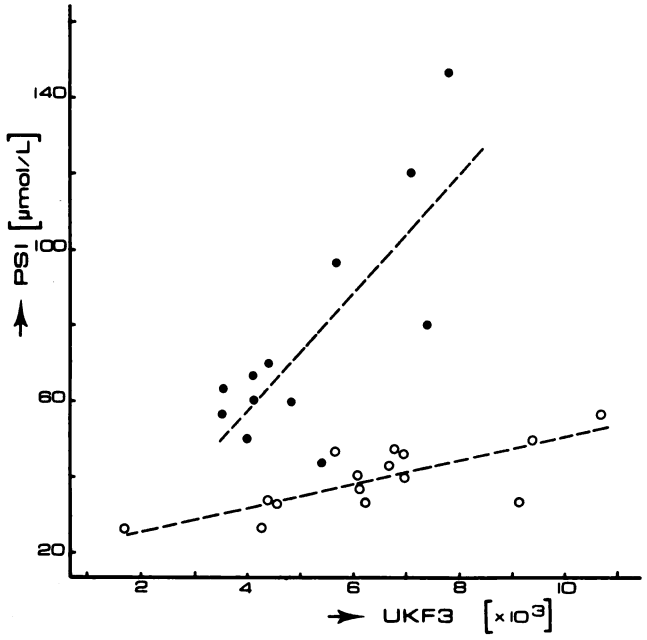

Fig. 3. Bivariate scatter plot of pseudouridine and UKF3 concentrations in sera of CAPD (●) and HD (○) patients
UKF3 concentration expressed in arbitrary units

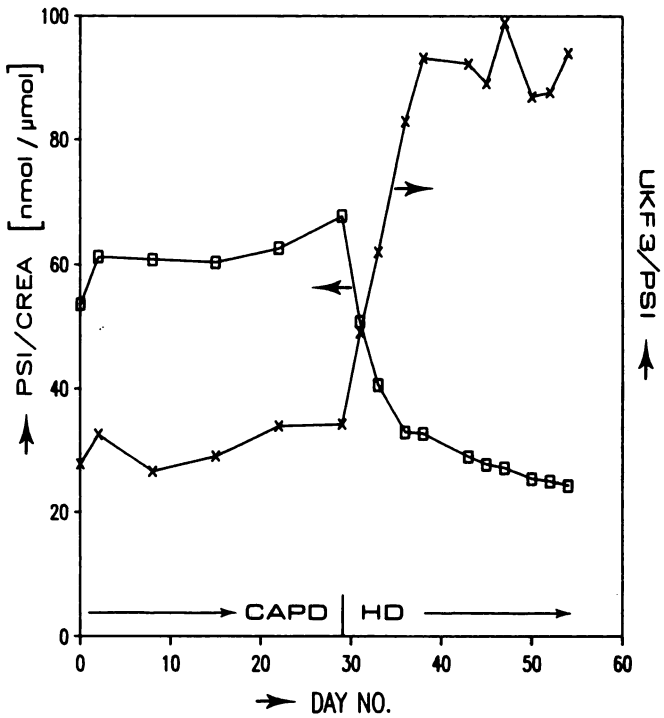

Fig. 4. Longitudinal plot of creatinine-standardized pseudouridine (PSI/CREA □), UKF3/PSI ratio (×) in patient K, as treatment changed from CAPD to HD
UKF3/PSI ratio expressed in arbitrary units

douridine, a modified nucleoside, and an unknown fluorescent solute UKF3, both of which also occur in normal urine. Pseudouridine is reportedly increased in uremia (*13*); however, the authors did not establish whether the higher concentrations originated from decreased excretion, increased generation, or both. Pseudouridine is a rare nucleoside found predominantly in transfer RNA (tRNA). It may be present also in messenger RNAs and ribosomal RNAs, but only in much lower proportions (*14*). Formation of PSI in tRNA takes place at the macromolecular level. Thus far there is no evidence that PSI phosphates are synthesized for use during transcription of tRNA in humans, although PSI monophosphate synthetases have been isolated from certain bacteria (*15*).

Therefore, PSI in normal urine and in uremic serum results predominantly from tRNA turnover. Because the PSI generated, unlike the regular nucleosides, is not phosphated and re-utilized and because it is chemically stable, PSI is excreted unmodified in the urine. Turnover of tRNA occurs during protein synthesis (*16*). tRNA plays a key role in protein synthesis, although it also has various other regulatory functions (*17*). High concentrations of urinary tRNA catabolites, such as PSI and methylated nucleosides, are found in patients with different forms of cancer or various other diseases (*18*).

It is unlikely that the high concentrations of PSI (M_r 244) we found in sera of CAPD patients are related to a less efficient removal of PSI than of creatinine (M_r 113). Indeed, the reverse would be expected, because CAPD clears larger

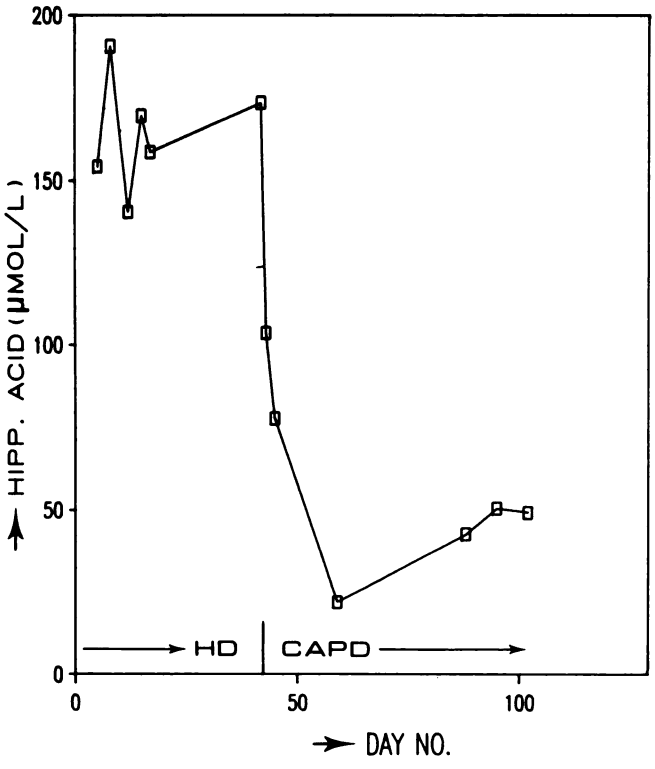

Fig. 5. Longitudinal plot of hippuric acid concentration in serum of patient R, as treatment changed from HD to CAPD

molecules more efficiently than does conventional hemodialysis when both methods show equivalent urea removal on a weekly basis (*6, 9*). However, there are no published data on the permeability of the peritoneal membrane to PSI.

In normal healthy adults the urinary PSI/CREA ratio is higher in women: 26.7 (SD = 4.5) vs 22.4 (SD = 2.1) mmol/mol in men (*18*). Although the patient groups studied here are asymmetric with respect to sex, this could not explain the difference because there were more females in the HD group, which had a lower mean value.

Two possible explanations of the high concentrations of PSI in serum from CAPD patients will be discussed here. First, asymptomatic, non-clinical peritonitis accompanied by cell death occurs in all CAPD patients, which could result in generation of PSI (*19*). Second, protein synthesis may be increased in these patients for various reasons. The possibly more efficient removal of certain unknown uremic toxins that might be inhibiting protein synthesis (*20–22*) and (or) the generally better homeostasis in CAPD may result in a more anabolic state (*23*). On the other hand, hemodialysis has been described as a catabolic process (*24*), the etiology of which is not yet clearly understood. Or protein synthesis may be induced by the combination of protein loss, via the peritoneal dialysate, and the availability of essential amino acids such as tryptophan (*25*), as a result of a more free diet. Although amino acid loss in peritoneal dialysis has been reported (*26*), these losses probably do not decrease the amino acid concentrations in plasma of stable patients who are eating well (*27*).

If the second explanation is valid, perhaps the serum PSI/CREA ratio can serve as an indicator of metabolic and nutritional status in dialyzed patients in addition to methods described elsewhere (*27*). Urinary PSI/CREA ratio has been proposed as an indicator of nutritional status in "healthy" persons, as an alternative to the determination of nitrogen balance, which is a very complicated procedure (*16*). Children demonstrate a strong age dependence of the excretion of creatinine-normalized RNA catabolites, reflecting age-dependent growth velocity (*29*). The PSI/CREA ratio in anabolic as well as catabolic processes was evaluated, and children with "failure to thrive" showed a marked depression of tRNA catabolites (*30*). Perhaps the higher serum PSI/CREA ratio in CAPD patients than in HD patients indicates a better noncatabolic status in CAPD. CAPD treatment reportedly is more beneficial to growth of children than is HD (*31, 32*), probably as a result of better homeostasis and more free diet, despite the risk of undernutrition from treatment-induced anorexia.

It has been reported that urinary PSI excretion is positively related to protein intake (*33*). However, the PSI/CREA ratio in normal individuals is closer to that in HD patients than to that in CAPD patients. Normal concentrations of PSI in serum have been reported as 2.48 (SD 0.13) μmol/L (measured by HPLC) (*34*) and 1.72 (SD 0.77) μmol/L (measured by RIA) (*35*). For a normal serum creatinine value of 80 μmol/L, a normal serum PSI/CREA ratio would be 20–30 mmol/mol, similar to the normal value for this ratio in urine. However, because healthy individuals ordinarily have neutral or positive nitrogen balance and nonrestricted protein intake, the foregoing seems to invalidate the suggestion that the high PSI/CREA ratio in CAPD is related to protein intake or protein synthesis, or both. Nonetheless, secondary effects associated with the derangement state of uremia are not unthinkable. No hard data were available on differences in diet between the groups of patients. To assess the metabolic state and (or) the influence of diet, careful clinical control of the different variables in a further study is indicated.

Less significant was our observation of the somewhat decreased concentrations of hippuric acid and *p*-hydroxyhippuric acid in CAPD patients than in HD patients. In a preceding pilot study involving seven patients on CAPD and three on HD, a similar observation was made. In the present study, hippuric acid decreased very significantly with the onset of CAPD for a patient changing therapy.

Table 5. Significance of Differences in Various Analytes for HD Interval vs CAPD Interval

Variable	*P*[a] for Patient K (CAPD → HD)	Variable lower in CAPD	*P*[a] for Patient R (HD → CAPD)	Variable lower in CAPD
Pseudouridine/creatinine	<0.01	no	<0.01	no
Indoxyl sulfate	<0.02	yes	<0.01	yes
Indoxyl sulfate/tryptophan	<0.02	yes	<0.01	yes
UKF3/pseudouridine	<0.01	yes	<0.01	yes
Hippuric acid	n.s.	—	<0.02	yes

[a] By Wilcoxon's test for five samples each taken during the stable HD and the stable CAPD periods. Results for samples from the one-month equilibration period are not included.

In conclusion: we found that pseudouridine concentrations in serum of CAPD patients are significantly higher than in HD patients. This difference is even more significant when concentrations are standardized to serum creatinine concentration. Further study is needed to answer the question whether low peritoneal membrane permeability, specific tRNA turnover, asymptomatic peritonitis accompanied by cell death, or another as-yet-unrecognized cellular process is the origin of the high pseudouridine concentrations in CAPD.

This study was supported in part by a grant from Travenol B. V., Utrecht, The Netherlands.

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