AN ABSTRACT OF THE THESIS OF

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Title: Peritoneal Clearance of Vitamin B-6 and Evaluation of Vitamin B-6 and Vitamin A Status in Children Undergoing Continuous Ambulatory Peritoneal Dialysis (CAPD)

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The objectives of this study were to determine the peritoneal clearance of vitamin B-6 and to evaluate the vitamin B-6 and vitamin A status of children with chronic renal failure undergoing continuous ambulatory peritoneal dialysis (CAPD). Eight children aged 2 to 18 years (mean 12) were included in the study. Mean time on CAPD was 27.2 months. Vitamin supplements were discontinued 3 weeks prior to the study. Dietary intake of vitamin B-6, vitamin A and protein were estimated from a 3 day dietary record. Plasma was analyzed for total vitamin B-6 (total B-6), pyridoxal 5'-phosphate (PLP) and retinol. Dialysate and urine were analyzed for total B-6, PLP and protein. Mean total B-6 was 197, 34.7, and 257.7 nM for plasma, dialysate and urine, respectively. Mean plasma PLP was 25.5 nM while no PLP was detected in dialysate. Mean plasma retinol was 617 ug/dl. No retinol was detected in

the dialysate.

B-6 supplements are important in these children while vitamin A supplements should not be given and dietary vitamin A should be restricted.

PERITONEAL CLEARANCE OF VITAMIN B-6 AND EVALUATION OF VITAMIN B-6 AND VITAMIN A STATUS IN CHILDREN UNDERGOING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS (CAPD)

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I. INTRODUCTION

The technique of continuous ambulatory peritoneal dialysis (CAPD) for the treatment of end-stage renal disease (ESRD) was first described by Popovich, Moncrief, Decherd, Bomar and Pyle in 1976.(1)

CAPD was first used to treat children in Toronto in 1978. By the end of March 1982, it was estimated that over 200 children in the United States had been treated with CAPD.(2)

While the protein status of children undergoing CAPD has been described (3), no information is available concerning the vitamin status of CAPD-treated children. The literature suggests that certain water-soluble vitamins may be depleted in patients undergoing peritoneal dialysis.(4) Specifically, vitamin B-6 is known to be deficient in adults with ESRD (5,6), as well as in adults undergoing CAPD.(7) Vitamin A, on the other hand, is thought to accumulate to potentially toxic levels in children with ESRD.(8)

The objectives of this study were to determine the peritoneal clearance of vitamin B-6, and to assess the vitamin B-6 and vitamin A status of children undergoing CAPD.

II. METHODOLOGY

Patients

Eight children between the ages of 2 and 18 years (mean age 12) who were receiving regular medical care through the CAPD Clinic at The Oregon Health Sciences University (OHSU) in Portland were included in the study. All patients were medically stable and free of peritonitis at the time of the study. Informed consent was obtained from the children's parent or guardian, and the study protocol was approved by the Institutional Review Board of OHSU. A summary of the patients' characteristics is presented in Table 1.

TABLE 1. PATIENT CHARACTERISTICS

Pt.	Sex	Age (yrs)	Ht. (cm)	Wt. (kg)	Diagnosis	Length of time on CAPD	Alkaline Phosphatase* (IU/ml)
1	F	14	146	41.0	Ureteral reflux	1.5 yrs	277
2	M	10	123	22.0	Focal segmental sclerosis	9 mo	127
3	M	9	121	23.2	Hemolytic uremic syndrome	4.1 yrs	187
4	F	2	83	12.4	Hemolytic uremic syndrome	1.0 yrs	N/A
5	M	18	153	50.8	Dysplastic kidneys with ESRD	1.2 yrs	450
6	M	17	122	55.9	Henoch-Schonlein purpura nephritis	1.3 yrs	144
7	F	15	152	42.8	Chronic renal failure	2 yrs	59
8	M	11	124	25.7	Chronic renal failure	5 yrs	222

* Normal alkaline phosphatase values at OHSU:
6mo - 5yr: 80 - 310
5yr - 10 yr: 90 - 340
10yr - 16yr: 50 - 340
16yr - 19yr: 40 - 175
>19: 35 - 105

Sample Collection

For three weeks prior to a routine CAPD Clinic visit, subjects discontinued all vitamin supplements. All other routine medications were continued as usual. Vitamin A supplements had been previously discontinued in these patients because of concerns about potential toxicity. During the five day period before the clinic visit, a dietary record was obtained from the subjects or parents. The dietary record consisted of three days including one week-end day, the day of dialysate collection, and one other day prior to this collection. Dietary vitamin B-6, vitamin A and total protein intake were estimated from the dietary record using the Ohio State University Nutrient Database.(9)

For 24 hours prior to the clinic visit, subjects and/or their parents collected all spent dialysate in opaque, plastic containers and stored it under refrigeration. The total dwell time of the dialysate was recorded. The spent dialysate was then brought to the clinic and picked up by one of the investigators to be transported to Oregon State University in Corvallis. Total volume was noted and aliquots were frozen for subsequent analyses.

All urine, if any, was also collected during the 24 hours immediately preceding the clinic visit. This was also placed in an opaque plastic container and stored under refrigeration until brought to the clinic and picked up by one of the investigators. Total volume was noted. Toluene was added to the urine samples as a preservative. Aliquots of the urine samples were then frozen for subsequent analyses.

An 8 ml. fasting blood sample was obtained in a heparinized Vacutainer[R] at the time of the clinic visit. The sample, protected from light, was centrifuged immediately and the plasma collected. The plasma was then transported from Portland to Corvallis packed in ice. The mean time from plasma collection until the sample was frozen was 3.6 hours, the range being 2.75 to 5 hours.

<u>Analyses</u>

Analysis of dialysate, urine, and plasma samples for total vitamin B-6 (total B-6) was carried out via a microbiological method using <u>Saccharomyces uvarum</u>(10), and reflects the total content of pyridoxine, pyridoxal, and pyridoxamine. Using this method, all phosphorylated forms of vitamin B-6 are hydrolyzed when the samples are autoclaved before analysis. Determination of plasma and dialysate pyridoxal 5'-phosphate (PLP) was done using the tyrosine apodecarboxylase method.(11) Plasma vitamin A content was determined using a modified HPLC technique similar to that described by Bieri et al.(12) Briefly, plasma retinol was extracted with hexane which was then evaporated under a stream of nitrogen. After reconstitution with mobile phase, samples were injected onto a reverse-phase Nova-Pak C-18 column (Waters, Inc.). Detection was via fluorescence (excitation 390nM, emission 475 nM). Patient samples were analyzed in duplicate. Dialysate samples were also analyzed for total protein using the method of Lowry.(13)

III. RESULTS

Total Vitamin B-6

Mean plasma total vitamin B-6 was 196.8 nM with a range of 81.9 to 341.5 nM as shown in Table 2. Values in normal children have been reported to be in the range of 59.8 to 89.7 nM.(14) Unpublished data from Miller et al. indicate that mean normal plasma total B-6 in boys 23 to 60 months of age is 108.3 ± 51.4 nM, while the value for girls of the same age is 92.7 ± 38.9 nM.(15) The dialysate and urine (5 subjects only) total B-6 means were 34.7 and 273.3 nM, respectively, as can be seen from the data in Table 2. The mean daily loss of total vitamin B-6 through the dialysate was 0.03 mg. (3% of mean amount ingested daily), while through the urine the total amount lost was 0.01mg (1% of mean amount ingested daily). The mean 24 hour urine volume of the five subjects who had urine was 343 ml.

TABLE 2. TOTAL B-6 IN PLASMA, DIALYSATE AND URINE

PT.	Plasma (nM)	Urine (vol mls)	Urine (nM)	Dialysate (vol L)	Dialysate (nM)
1	269.7	36	299.0	7.0	28.1
2	156.1	-	-	4.7	54.4
3	341.5	-	-	5.7	22.7
4*	1048.4	-	-	2.1	12.6
5	230.3	100	331.3	6.0	57.4
6	182.4	840	242.2	8.5	31.7
7	119.0	290	413.9	9.6	27.5
8	81.9	450	80.1	5.2	17.3
Mean (SD)	197.3 (89.9)	343 (322)	273.3 (124.4)	6.1 (2.3)	34.2 (15.6)

* This patient was not included in the calculation of the means of total B-6 as she received only formula, so was therefore not entirely off vitamin B-6 supplements.

PLP

The mean plasma PLP was found to be 25.5 nM. The range was 9.7 to 56.2 nM. as seen in Table 3. Normal values in healthy children receiving no supplements have been reported to be 31.1 nM to 65.9 nM.(16,17) In the present study, no PLP was detected in the dialysate.

Retinol

The mean plasma retinol level was 617 ug/dl with a range of 342 to 1051 ug/dl as seen in Table 3. Casey et al. reported a mean value of 48 ug/dl. in 8 normal healthy children. Geigy (14) reports a mean value of 32.4 ug/dl in adults on a normal diet. There was a significant positive correlation (r = .72, P< 0.1) between dietary vitamin A intake and plasma retinol levels.

No retinol was detected in the dialysate.

TABLE 3. PLASMA PLP AND RETINOL IN CHILDREN UNDERGOING CAPD

Pt.	PLP (nM)	PLP* (nM)	Retinol (ug/dl)	Retinol* (ug/dl)
1	10.9	-	342	-
2	17.4	112.1	738	322
3	21.1	-	496	-
4	17.8	-	774	-
5	31.9	10.9	488	303
6	38.1	47.7	432	236
7	56.2	139.2	1051	459
8	9.7	-	612	-
9**	-	48.1	-	313
10**	-	11.8	-	229
Mean (SD)	25.5 (15.8)	61.6 (52.9)	617 (230)	310 (83)

* Plasma samples were obtained and analyzed for PLP and retinol approximately one year after the originial study. At this point patients were receiving a vitamin supplement that contained 1.05 - 10 mg vitamin B-6. Two subjects (5 and 7) were receiving a vitamin supplement that contained 2500 IU of vitamin A.

** Subjects who did not participate in the originial study.

Protein

The mean protein lost per liter of dialysate was 0.93 gm. The range was 0.6 to 1.27 gm/L. of dialysate. Total dialysate collected in these patients over approximately 24 hours ranged from 2.1 to 9.5 L, with a mean of 6.1 L. These results are displayed in Table 4. There was a significant positive correlation ($r=.78$, $P< 0.05$) between dialysate volume and grams of protein lost per hour in the dialysate.

Peritoneal Clearance

The peritoneal clearance of total B-6 was calculated using the formula:

$$Clp = \frac{C_d \times V}{C_p \times t}$$

where: Cl_p = peritoneal clearance in mls/min.

C_d = total B-6 concentration in dialysate in ng/ml

V = total volume of collected dialysate in mls

C_p = plasma concentration of total B-6 in ng/ml

t = total dwell time in minutes.

The peritoneal clearances of PLP and retinol were not calculated since neither of these substances were found in the dialysate. As shown in Table 5, mean peritoneal clearance of total B-6 was 1.03 ml/min with a range of 0.57 to 1.27 ml/min. No information is available in the literature regarding the renal clearance of total vitamin B-6 in normal healthy children.

Dietary Record

The data displayed in Table 6 reflects the average amount of each nutrient ingested per 24 hours based on the 3 day dietary record for each patient.

TABLE 4. TOTAL PROTEIN LOST PER LITER OF DIALYSATE PER 24 HOURS IN CHILDREN UNDERGOING CAPD

Pt.	Protein (gm/L)	Dialysate (L)
1	0.87	7.0
2	1.27	4.7
3	0.96	5.7
4	1.08	2.1
5	0.81	6.0
6	1.06	8.5
7	0.77	9.5
8	0.58	5.2
Mean (SD)	0.93 (0.21)	6.1 (2.3)

TABLE 5. PERITONEAL AND RENAL CLEARANCE* OF TOTAL B-6

Pt.	Peritoneal Clearance (ml/min)	Renal Clearance (ml/min)
1	0.57	0.03
2	1.27	-
3	0.26	-
4**	0.05	-
5	1.67	0.16
6	1.09	0.82
7	1.55	0.71
8	0.77	0.31
Mean (SD)	1.03 (0.52)	0.41 (0.34)

* Formula for calculation of peritoneal clearance:

$$Clp = \frac{Cd \times V}{Cp \times t}$$

where: Clp = peritoneal clearance in mls/min
Cd = total B-6 concentration in dialysate in ng/ml
V = total volume of collected dialysate in mls
Cp = plasma concentration of total B-6 in ng/ml
t = total dwell time in minutes.

* Formula for calculation of renal clearance is identical to formula for peritoneal clearance, substituting urine values for dialysate values.

** This patient was not included in the calculation of the mean as she received only formula, so was therefore not entirely off vitamin B-6 supplements.

TABLE 6. MEAN DIETARY INTAKE PER 24 HOURS IN CHILDREN UNDERGOING CAPD

Pt.	Prot. (gm)	Vit. A (RE)	Zn (mg)	B-6 (mg)	Ca (mg)	P (mg)	Fe (mg)	Cal	B6/Prot
1	17	603.9	2.7	0.62	223	354	4.4	1042	0.036
%RDA*	37	75	18	34	19	30	24		
2	32.2	1836	3.9	0.49	635	690	3.7	638	0.015
%RDA*	95	262	39	31	79	86	37		
3	42	437	4.3	0.57	738	1039	4.7	1253	0.014
%RDA*	124	62	43	36	92	130	47		
4	18.6	637	0.45	0.42	425	212	1.5	812	0.023
%RDA*	80	159	0.05	47	53	27	10		
5	48.9	774	9.9	1.34	677	929	9	1677	0.027
%RDA*	87	77	66	67	56	77	50		
6	88.1	1073	14.6	1.5	824	1537	12.6	1849	0.017
%RDA*	157	107	81	75	69	128	70		
7	106.2	1563	12.7	2	2131	2125	12.7	2524	0.019
%RDA*	231	195	85	111	178	177	71		
8	68.2	629	7.7	2	1247	1301	11.3	1795	0.029
%RDA*	152	63	51	111	104	108	63		

* Based on Food and Nutrition Board, National Academy of Sciences-National Research Council Recommended Daily Dietary Allowances Revised 1980

IV. DISCUSSION

Total Vitamin B-6

The plasma level of total vitamin B-6 for normal adults has been reported to be in excess of 300 nM.(18) A mean vitamin B-6 in serum of 215.3 nM was observed in a group of New York City school children ages 10 to 13 years by Baker et al. using a protozoological method.(18) Miller et al. (unpublished data) have found that the mean plasma total vitamin B-6 in normal children 23 to 60 months of age is 108.3 ± 51.4 nM and 92.7 ± 38.9 nM in boys and girls respectively.(15) The mean value obtained in our subjects was 196.8nM. It would appear that our subjects should have had enough total vitamin B-6 for conversion to PLP even though 5 of 8 children received 67% or less of the recommended dietary allowance (RDA) for vitamin B-6 in their diet.

The mean daily loss of total vitamin B-6 through the dialysate and urine was 0.04 mg. (4% of mean amount ingested daily). This would appear to not be a significant loss of total vitamin B-6.

PLP

Mean normal values of plasma PLP (no supplements) have been reported by Reinken and Zieglauer to be 31.1 nM in 15 children 17 to 104 months of age.(16) Barlow and Wilkinson reported a mean plasma PLP value of 65.9 nM in 19 healthy children.(17) Since the mean plasma PLP in our patients was 25.5 nM, this would indicate a deficiency of plasma PLP. The mean ratio of plasma PLP/total B-6 was 14.6%. This is a very low ratio of PLP to total B-6. A mean of 79.2% has been reported in normal adult males with a range of 64 to 92%.(10) This low proportion could have several explanations. Other investigators have reported a deficiency of PLP in uremic patients. Stone et al. found a PLP deficiency in both dialyzed and non-dialyzed patients with uremia.(5) They postulated that the deficiency could be due to impaired phosphorylation of pyridoxal or chemical inactivation of PLP. Spannuth et al. found an increased plasma clearance of PLP in uremic patients.(6) They concluded that the PLP deficiency in these patients appears to be due to a more rapid plasma clearance of this compound. Possible mechanisms for this loss of PLP, as suggested by Spannuth, could be accelerated degradation of the coenzyme or its greater extraction by the tissues of vitamin B-6 deficient patients. These authors also stated that they had found no correlation between increased plasma alkaline phosphatase activity and plasma PLP concentrations in uremia. We had only one patient (#5) whose alkaline phosphatase exceeded the upper limits of normal, so this seems to be an unlikely explanation for the decreased PLP concentrations found in our patients.

The reason for the low PLP in our patients is unclear, although impaired phosphorylation seems a likely possibility. It also appears that the loss did not occur through the dialysate, since no PLP was found in the dialysate. PLP is known to not cross biological membranes, however. While the lack of PLP in the dialysate may have been due to its instability in dialysate fluid, stability studies using crystalline PLP in spent dialysate designed to mimic the collection procedure were carried out as part of this project. Had PLP been present in quantities greater than 5 ng/ml, it would have been detected by our assay procedure. It appears, therefore, that the dialysate is not a significant source of loss of PLP.

Approximately one year after the initial study was completed, we obtained plasma samples from 4 of the same patients that participated in the original study and two other patients. We found an increased level of plasma PLP in all except one patient. The children were receiving a daily multivitamin supplement that contained 1.05 - 10 mg. of vitamin B-6. There is a question of compliance in the patient whose B-6 level decreased with the supplementation.

Retinol

It has been known since the 1940's that retinol accumulates in patients with kidney disease.(22) A major pathway of vitamin A excretion is the conversion of retinol to retinoic acid in the kidney, which is then excreted in the urine. Patients with chronic renal failure lose this route of excretion. Casey et al. found a plasma retinol value of 48 ug/dl in 8 normal children, while children undergoing dialysis had retinol levels of 146.1 ug/dl.(8) Other studies conducted in adults have found control values of 28 to 40 ug/dl with values for patients on dialysis being 85 to 102 ug/dl.(20,21) One other study conducted in children with the nephrotic syndrome found levels similar to ours. Normal values were 37.5 ug/dl in 18 healthy children. Children suffering from nephrotic syndrome were found to have plasma retinol levels ranging from 50 to 500 ug/dl.(22) The retinol levels found in our patients were higher than those found by other investigators except for the study by Kagan.(22) The reasons for this are unclear. One possible explanation is that the other articles did not mention whether or not their assays had been corrected for endogenous retinol. Our assay was corrected for this. It is also interesting to note that the children with nephrotic syndrome lost significant amounts of protein, as did our subjects.

As with the PLP, we assayed plasma samples collected approximately one year after the initial study for retinol. Four children were receiving daily supplements that contained vitamin A. Four of the patients had been included in the original study and we analyzed plasma from two additional patients. Of the four patients in the original study, 3 had greatly decreased plasma retinol levels. Of these four, 2 were receiving a multivitamin preparation that contained vitamin A. The decrease in plasma retinol seen in these patients is therefore, difficult to explain. Perhaps the simplest explanation would be that the dietary intake of vitamin A had decreased. Since we have no diet histories for the second collection, it is not known whether this is the reason or not.

No retinol was found in the dialysate.

Protein

The protein lost through the dialysate ranged from 0.58 to 1.27 gm/L with a mean of 0.93 gm/L. With a mean daily dialysate volume of 6.1 L, this would amount to a mean protein loss through the dialysate of 5.7 gm/day. This is similar to other studies which found protein losses to be 3.24 to 5.92 gm/day.(4,24)

Peritoneal Clearance

The mean peritoneal clearance of total B-6 was 1.03 ml/min. The mean renal clearance of total vitamin B-6 was 0.41 ml/min. with a range of 0.03 to 0.82 ml/min. in the five subjects who made urine. It is interesting to note that the peritoneal clearance is greater than the renal clearance in these patients. No information is available concerning the renal clearance of total vitamin B-6 in normal children.

V. CONCLUSIONS

Our conclusions from this study are that children undergoing CAPD for end-stage renal disease need vitamin B-6 supplements. They do not receive an adequate amount in their diet. It also appears that plasma total vitamin B-6 is not a good indicator of B-6 status in these patients.

On the other hand, vitamin A supplements should not be given and dietary vitamin A should be reduced as there appears to be a positive correlation between dietary vitamin A and plasma retinol levels.

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