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Research Article

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ABSTRACT Patients with chronic uremia develop neurologic defects which are similar to the demyelinating lesions seen in thiamine deficiency. The present study describes inhibitory effects of uremic material on nervous tissue transketolase, a thiamine-dependent enzyme of the pentose phosphate pathway which has been reported to have functional importance in the metabolism of myelinated nervous structures.

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Hemodialysis markedly reduced the inhibitory effects of the patients' plasma and the data indicate that uremic patients who received effective long-term dialysis treatment show a parallel decline of transketolase inhibition and uremic neuropathy.

The findings demonstrate that in patients with chronic renal failure, low molecular weight factors accumulate and inhibit nervous tissue transketolase. This biochemical defect—uncorrectable by thiamine but reversible by dialysis—may interfere with the metabolism of myelin-supporting cells, and/or of the axonal metabolism of medullated structures, and may thus contribute to the degeneration of myelinated nerves seen with uremic neuropathy.

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INTRODUCTION

Patients with chronic renal failure are prone to develop neurologic abnormalities which often consist of symmetrical sensorimotor disturbances of the extremities and varying defects of mentation. These findings are usually accompanied by decreased motor nerve conduction velocity as well as by EEG changes.

Since prolongation of life for uremic patients has become possible by improved conservative treatment and chronic hemodialysis, the development of the slowly progressive neurologic lesions has, in recent years, been found with increasing frequency. Accordingly, researchers in some dialysis centers began to judge the indication and adequacy of dialysis treatment by the degree of uremic neuropathy and encephalopathy (1–4).

Histopathologic description of the nervous tissue in chronic uremia often reveals degenerative changes of the peripheral and, less frequently, of the central medullated structures (5–14). Although no definitive information exists as to the mechanism responsible for the morphological and functional changes in the nervous system there are reports which have shown the remarkable resemblance of uremic neuropathy to demyelinative lesions seen in patients with thiamine deficiency (5, 7, 14).

Evidence has been presented that a thiamine-dependent enzyme of the pentose phosphate pathway,

transketolase, is found predominantly in the myelinated structures of the nervous tissue (15–21). The transketolase activity (TKA),[1] as measured by the conversion of ribose into sedoheptulose or hexose, is low in brain and spinal cord of thiamine-deficient animals (18, 21, 22). Observations of depressed TKA in erythrocytes of patients with thiamine deficiency (23–25) or with uremia (26) have revealed a further similarity between the two diseases. These findings prompted us to examine the direct effect of uremic material on human nervous tissue TKA. Initial experiments showed impaired formation of sedoheptulose when human brain homogenate was incubated with uremic plasma, possibly due to suppressed transketolase activity.

Using modifications of an existing TKA assay, specific inhibition of normal brain and spinal cord transketolase by uremic material was then established, and the effect of thiamine pyrophosphate on the inhibition assay was examined. Suppression of nervous tissue TKA by plasma, cerebrospinal fluid, and dialysate fractions from patients with advanced chronic renal failure and varying degrees of uremic neuropathy were measured. These studies were performed before and after dialysis treatment of the patients. Attempts were made to relate the degree of TKA inhibition to the clinical and electrophysiologic extent of the uremic patients' neurologic deficits.

METHODS

Patients. 39 subjects with chronic renal failure were studied. The patients' glomerular filtration rates, as determined by endogenous urea clearances (27) were between 5 and 10 ml/min in 4, between 2 and 4 ml/min in 3, and less than 2 ml/min in the remaining 32 cases. At the time of study, 22 of the patients were being treated by intermittent hemodialysis (twice weekly 5–8 hr with the Kolff twin-coil hemodialyzer).[2] The diagnoses of the underlying renal diseases were based on morphological examinations of the kidney tissue obtained by biopsy or postmortem and/or by clinical and laboratory data. None of the studied subjects had a history, or clinical, laboratory, or histopathological evidence of diabetes mellitus, amyloid, heavy metal intoxication, malnutrition, vitamin B_1 deficiency, or primary neurologic diseases. All patients had an adequate caloric intake and were receiving vitamins, including thiamine, with their diet. Besides antihypertensive drugs and digitalis, taken by some patients, no other medications were used in the studied group for any length of time.

Neurologic evaluations. The presence and extent of neurologic dysfunctions in the uremic patients were judged following the observations of Jebsen, Tenckhoff, and Honet (3), Tyler (28), and Kiley and Hines (4). Clinical evidence of uremic peripheral neuropathy was graded *mild* (1+): when mild paresthesias such as numbness, tingling, burning, etc. and diminution of vibratory sense in feet, grade 4 muscular strength in hands and feet (scale 0–5), and slightly reduced deep tendon reflexes were present; *Moderate* (2+): when the paresthesias were moderately extensive and there were deficits in perception of stimuli of vibration, pain and/or touch below knee, grade 3 muscular strength and moderately reduced deep tendon reflexes; or *severe* (3+): when severe paresthesias, hypesthesia to anesthsia in lower extremities and occasionally in hands, grade 0–2 distal muscular strength and advanced hyporeflexia to areflexia were found. Normal neurologic findings were indicated by 0.

Using a TECA model TE 3 electromyograph,[3] the mean motor nerve conduction velocities (MNCV) of 28 patients were determined unilaterally for the common peroneal, ulnar, and median or posterior tibial nerves following the techniques described by Jebsen et al. (3). The mean value for the patients' tested nerves was compared to the MNCV found in 20 healthy adults.

Clinical evidence of uremic encephalopathy was graded *mild* (1+): reduced concentration ability and memory, irritability, lethargy; *moderate* (2+): in addition to 1+, confusion, asterixis; or *severe* (3+): in addition to 2+, stupor, coma. Myoclonic movements, fascicular twitching and generalized jerks were at times seen together with 2+ and 3+ encephalopathy.

Collection and handling of plasma, cerebro-spinal fluid (CSF) and dialysate fractions. The patients' plasma was obtained from heparinized blood samples (50 U heparin per ml). No specimens were taken within 1 wk after the patient had received blood transfusions. Plasma from 20 healthy volunteers served as normal controls. Cerebrospinal fluid samples were collected from six uremic subjects by routine lumbar puncture technique. Timing of the spinal taps in relation to the onset of dialytic treatment varied, depending on the patients' clinical conditions. Control specimens of CSF were obtained from eight subjects with normal renal function. The samples were used for the incubation studies either immediately or after storage at $-70°C$ since it was found that storage did not change their effect on the TKA assay to be described later. Samples of dialysates resulting from 6 hr hemodialysis of uremic patients or of normal bank blood (BUN < 20 mg/100 ml), the latter serving as a control, were prepared as described previously (26). The 50-fold concentrated solution was desalted by passing through ion retardation resins[4] and separated by selective filtration through ultrafiltration membranes (Amicon UM-05, UM-2, and UM-10)[5] into three fractions: fraction I mol wt less than 500, fraction II mol wt 500–1000, fraction III mol wt 1000–10,000. Confirmation of the sieving process was obtained by passing known colored molecular weight markers through the filters: fluorescein sodium (mol wt 376), Arsenazo III (mol wt 818),[6] fluorescein-labeled insulin (mol wt 6000). The different fractions were used in TKA inhibition studies.

Preparation of brain and spinal cord suspensions. Samples of human brain were obtained from the frontal lobes of six patients who had died less than 8 hr previously from causes other than renal or hepatic failure and without evidence of malnutrition, vitamin deficiency, or brain diseases.

[1] *Abbreviations used in this paper:* CSF, cerebrospinal fluid; KRB, Krebs-Ringer phosphate buffer; MNCV, mean motor nerve conduction velocity; R5P, ribose-5-phosphate; S7P, sedoheptulose-7-phosphate; TKA, transketolase activity; TPP, thiamine pyrophosphate; Xu5P, xylulose-5-phosphate.

[2] Travenol Laboratories, Inc., Morton Grove, Ill.

[3] Teca Corp., White Plains, N. Y.

[4] AG 11 A8 Bio-Rad Laboratories, Richmond, Calif.

[5] Amicon Corp., Lexington, Mass.

[6] Mann Research Laboratories, Inc., New York.

Cervical spinal cord tissue from three of the patients was also used. These well-preserved samples were used as the source of enzyme for the inhibition studies. Frontal lobes from other nonuremic and uremic patients were also obtained at different times postmortem and examined for their transketolase activity. The frozen brain samples consisted of white and gray matter of approximately equal quantities and were devoid of meninges and visible blood vessels. They were homogenized individually in distilled water using the Sonifer Converter,[7] which was operated with the power set at position 7 for five bursts of 30 sec. This procedure was carried out at 4°C. After lyophilization of the homogenates the slightly yellow powders were stored at −70°C. Samples of brain or spinal cord suspension were freshly prepared for each TKA assay resuspending 75 mg of tissue powder in 1 ml of Krebs-Ringer 0.143 M phosphate buffer at pH 7.4 (KRB) and diluting this so that the protein content of the suspension was 20 mg/ml as determined by the method of Lowry, Rosebrough, Farr, and Randall (29).

Methods of analysis. To determine the effect of uremic material on the TKA of brain and spinal cord, a macromodification of the method by Dreyfus and Moniz (16) was used which measures the rate of sedoheptulose formation during the incubation of tissue suspensions with excess ribose-5-phosphate (R5P).[8] The procedure was carried out as follows: 0.2 ml of the tissue suspension was added to 0.2 ml KRB and 0.1 ml of uremic or normal plasma, CSF, or dialysate fraction or of buffer which served as a control. The pH of all incubation mixtures was identical with the buffer control, namely, pH 7.4. After preincubation at 37°C for 30 min and after introduction of 0.4 ml of 0.018 M R5P substrate, the reaction was immediately stopped in one of the tubes by adding 0.4 ml 15% trichloracetic acid (TCA) (zero time); the others were left at 37°C for another 30 min when TCA was added. Samples of 0.2 ml clear filtrate were used for spectrophotometric determination of sedoheptulose-7-phosphate (S7P) by Dische's sulfuric acid–cysteine method (30). Subtracting the optical density of the zero time sample from the value at 30 min and comparing the difference to a standard curve, the transketolase activity was expressed in μmoles S7P produced by 1 g brain or spinal cord protein per 30 min. An incubation period of 30 min was chosen because it was found that the sedoheptulose formation proceeded linearly for the first 40 min. All samples were assayed in triplicate. Reproducibility of the assay was shown when, in 83 determinations of an individual, stored lyophilized brain homogenate reconstituted in KRB brain TKA was 93.1 μmoles S7P ±4.1 SD and the mean TKA for one spinal cord homogenate determined on 49 occasions was 112.1 μmoles S7P ±4.4 SD. Using freshly prepared suspensions of the same stored tissue powders we found TKA to be stable for at least 8 months. Per cent inhibition was calculated by dividing the difference between the μmoles S7P formed in the buffer control and S7P formed in the presence of normal or uremic material by the value for the buffer control and multiplying this by 100. Although S7P formation by spinal cord specimens was higher than by brain specimens, it was found that the difference between both preparations as to the per cent inhibition by uremic material was within the experimental error of the colorimetic assay. The

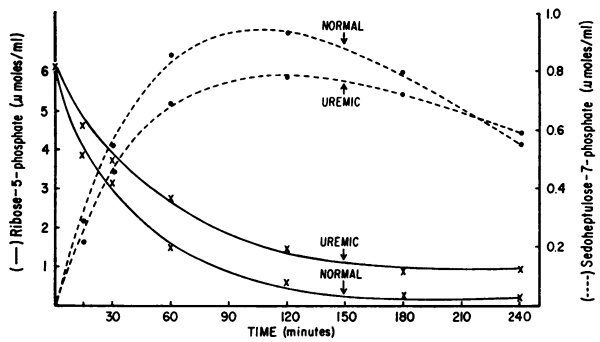

FIGURE 1 Sedoheptulose formation (– – –) and ribose consumption (——) by brain transketolase when incubated with normal or uremic plasma. The figure represents the typical results obtained when plasma samples from seven uremic and seven normal patients were individually incubated with brain homogenate and 0.018 M R5P at 37°C. Concentrations of R5P and S7P were measured at various times in the TCA filtrates. Each point represents the mean of triplicate determinations. Consumption of R5P parallels the generation of S7P; in the presence of uremic plasma the rates of R5P utilization and S7P formation are reduced when compared to the normal controls.

effect of a uremic or normal sample on the enzyme preparations was therefore expressed as the mean value of per cent inhibition of TKA from six cerebral and three spinal cord preparations. On 31 occasions uremic plasma from patient M. Ki was added to the assay and the inhibition of TKA was 55.2% ±3.7 SD. Further statistical analyses mentioned later were calculated by the standard techniques (31).

The effect of thiamine pyrophosphate (TPP)[9] was tested each time the assays were performed using in additional tubes Krebs-Ringer buffer which contained TPP (0.001–0.004 M) for the reaction. In some instances, the preincubation period in the presence of TPP was extended to 180 min to see if this would alter the effect of the cofactor on the transketolase reaction.

Specificity of the assay for TKA inhibition. Neither normal nor uremic plasma was found to contain measurable amounts of S7P or to generate S7P after incubation with excess R5P. Recovery of known amounts of sedoheptulose (50, 100, and 150 μg) in 12 instances added before beginning of the colorimetric assay was between 97.6 and 104.0% for uremic and nonazotemic incubation mixtures alike. The specificity of the spectrophotometric method for determination of S7P and R5P was further confirmed by thin-layer chromatography using aliquots from TCA filtrates of the nervous tissue—plasma incubation mixtures on cellulose precoated plastic sheets.[10] The ascending chromatograms were developed with an orcinol–TCA spray and heat (32, 33) and its was noted that the spots from the 30 min supernatants of the incubation mixtures were identical with those seen for the S7P[11] and R5P reference compounds. The finding that color intensity of the S7P spots

[7] Model L, Branson Sonic Power Co., Danbury, Conn.

[8] Disodium ribose-5-phosphate, Sigma Chemical Co., St. Louis, Mo.

[9] Thiamine pyrophosphate chloride salt, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[10] MN-Polygram CEL 300, Macherey-Nagel & Co., distributed by Brinkman Instruments, Inc., Westbury, N. Y.

[11] Kindly supplied by Dr. B. L. Horecker, Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, N. Y.

TABLE I

*Inhibition of Nervous Tissue Transketolase Activity by Plasma from Patients with Varying Degrees of Uremic Neuropathy**

Patient	Sex	Age	GFR‡	Underlying kidney disease§	Time on hemodialysis	Degree of clinical		MNVC‖	Inhibition of TKA
						Peripheral neuropathy	Encephalopathy		
			ml/min		*wk*			*m/sec*	%
L. S.	M	22	<2	CGN	3	0	0	49.1	4.8
J. F.	M	21	<2	CGN	4	0	1+	48.5	6.2
J. Fe.	M	21	<2	CGN	18	1+	1+		6.7
H. C.	M	33	<2	EKD	5	1+	2+		8.5
R. O.	M	15	2–4	CPN	6	0	1+	42.0	13.2
R. S.	F	52	<2	EKD	0	2+	1+	45.8	14.8
H. O.	M	30	<5–10	CGN	0	0	0	54.0	15.2
A. A.	M	59	2–4	CPN	15	2+	1+	38.9	16.0
A. B.	M	45	<2	PCD	6	0	2+	50.3	17.1
O. R.	F	35	5–10	EKD	0	1+	0	40.7	18.0
S. Ga.	M	42	<2	EKD	4	1+	2+	45.0	18.0
F. H.	M	49	5–10	PCD	0	0	0		18.3
R. F.	M	38	<2	CGN	12	3+	1+	28.1	19.6
E. J.	M	62	<2	EKD	0	3+	2+	34.6	20.5
S. G.	F	54	<2	HNS	0	1+	1+	54.2	21.8
B. Q.	F	34	<2	EKD	10	1+	2+	50.1	22.0
Ef. J.	F	22	<2	CGN	0	2+	1+	49.5	24.0
M. M.	F	60	5–10	EKD	0	1+	2+		25.8
M. J.	F	23	<2	PCD	24	1+	1+	33.5	25.9
W. W.	M	28	<2	CGN	11	2+	1+		27.4
C. A.	M	27	<2	CGN	9	2+	1+		29.3
L. G.	M	56	<2	CGN	0	1+	1+		30.8
J. Fi.	M	52	<2	CIN	8	1+	1+	36.5	31.3
E. R.	M	62	<2	HNS	0	3+	1+	42.5	34.3
J. K.	M	49	<2	CGN	0	3+	2+	35.2	37.5
N. S.	M	57	<2	CGN	4	2+	1+	37.5	41.0
A. F.	M	49	<2	CGN	0	3+	3+	27.7	41.9
C. W.	F	35	<2	EKD	0	2+	2+	49.1	42.0
L. F.	F	58	2–4	EKD	0	3+	2+	36.2	52.8
E. P.	M	25	<2	CGN	5	2+	2+	50.1	53.2
R. M.	M	23	<2	EKD	0	1+	2+		54.3
F. O.	M	43	<2	CGN	0	3+	2+	24.3	54.8
M. Ki.	M	30	<2	CGN	11	2+	1+	34.1	55.2
W. B.	M	37	<2	EKD	5	2+	2+	31.2	55.8
L. M.	M	42	<2	CGN	1	3+	3+		63.8
L. R.	M	51	<2	EKD	0	3+	3+		72.1
M. K.	M	50	<2	CGN	4	2+	1+	30.8	73.4
N. M.	F	35	<2	EKD	8	2+	1+	41.0	78.1
L. Fa.	M	61	<2	EKD	0	3+	3+		84.0
20 normal adults								49.2 ±4.7 SD	<6.7

* Plasma of 39 uremic patients obtained at the time of their initial neurologic evaluation inhibited individually TKA of six normal brain and three spinal cord samples. Per cent inhibition was calculated by comparing S7P formation in incubation mixtures containing uremic or normal plasma to buffer controls. The data are mean values of triplicate determinations using the nine preparations. (See under Methods for details of calculation and variance). The patients were separated into group I—inhibition 0–10%, group II—10–30%, and group III—greater than 30%. For grading of the clinical neurologic findings, see under Methods.

‡ GFR, glomerular filtration rate, as determined by endogenous urea clearance.

§ CGN, chronic glomerulonephritis.
EKD, endstage kidney disease of unknown etiology.
CPN, chronic pyelonephritis.
PCD, polycystic kidney disease.
HNS, hypertensive nephrosclerosis.
CIN, chronic interstitial nephritis.

‖ Mean motor nerve conduction velocity calculated from four tested peripheral nerves.

from eight uremic samples was much less and of the R5P spots greater than in eight normal controls indicated diminished S7P formation by transketolase in the presence of uremic material. Another approach for studying the transketolase reaction was to measure simultaneously, R5P consumption (phloroglucinol method) (34) and S7P formation in the assay as a function of time. Fig. 1 shows the typical results of a comparison between the R5P and S7P levels at sequential incubation times when brain homogenates were incubated with excess R5P. TKA inhibition by uremic plasma is borne out by the correlation of slower R5P utilization to less S7P formation when compared to normal controls. In eight control experiments it was found that addition of 0.018 M xylulose-5-phosphate (Xu5P),[12] the other substrate of the transketolase reaction, with the 0.018 M R5P solution to the assay did not alter the S7P formation in the presence of uremic or normal plasma by more than ± 3.2% of the values read without added Xu5P. This indicated that the usually high activities of pentose phosphate isomerase and epimerase in nervous tissue are not affected in the presence of uremic material and do not assume a rate-limiting role for S7P formation normally attributed to transketolase (35, 36). After inactivation of transaldolase by preincubation of the nervous tissue suspensions for 4 hr at 43°C and pH 8.4, the inhibition of cerebral TKA by 11 examined uremic samples differed from the results obtained by the standard technique by less than ± 4.8%. This would rule out the possibility that the observed difference in measured S7P may be due to more rapid elimination of S7P from the assay system by enzymatic reactions subsequent to the transketolase step.

A suspension of commercial partially purified bakers' yeast transketolase[13] (0.045 mg protein/ml) served in a few instances instead of nervous tissue preparations as the source of enzyme for incubation with the aforementioned amounts of Krebs-Ringer buffer (which regularly contains Mg++), TPP, Xu5P, and plasma or with buffer controls. Significant suppression of the activity of this enzyme preparation by plasma from five uremic patients confirmed the selective inhibition of transketolase.

RESULTS

Transketolase activities of brain, spinal cord, and plasma of normal and uremic subjects. The six homogenates of frontal brain lobes which were assayed within 8 hr postmortem using control buffer had comparable TKA values ranging between 90.8 and 99.1 μmoles S7P/g brain protein per 30 min (average 94.6 μmoles). The results for the three spinal cord suspensions were 108.9, 112.1, and 114.8 μmoles S7P/g protein per 30 min. These nine preparations served individually as the source of enzyme for the inhibition studies. Two uremic brains which were assayed within 8 hr following the patients' deaths had TKA values of 62.0 and 68.1 μmoles S7P, while TKA of another uremic sample obtained 30 hr postmortem was 53.7 μmoles S7P. Three nonuremic brains were received 23, 33, and 37 hr after the patients' deaths and had TKA values of 85.3, 88.9, and 81.3 μmoles S7P, respectively.

Effect of uremic plasma on TKA. The initial plasma samples from 35 out of 39 uremic subjects inhibited the TKA of six brain and three spinal cord suspensions more than 10% as compared to buffer controls (Table I). Mean inhibition of cerebral and spinal cord TKA was in 4 cases less than 10% (group I), in 17 cases 10–30% (group II), and in 18 cases more than 30% (group III). Plasma from 20 healthy volunteers showed less than 6.7% inhibition.

Neurologic deficits. The degree of clinical peripheral neuropathy found for group III patients was in eight instances severe (+++), in seven moderate (++), and in three mild (+), for group II patients in two instances severe, in five moderate, in six mild, and four patients no clinical evidence of peripheral neuropathy. From the four subjects of group I, two had mild abnormalities.

MNCV was reduced in 11 of 13 studied cases of group III, in 6 of 13 group II patients and in none of the 2 tested group I cases as compared to the normal values determined in 20 healthy adults. Patients of group III had more extensive slowing of the MNCV than those of groups II and I of whom the majority had normal values. There was a significant correlation between MNCV and the inhibition of TKA ($r = 0.468$, $P < 0.05$, the 95% confidence interval was −0.11 to −0.71) as shown in Fig. 2. Three of four group II patients (A.A., R.F., E.J., M.J.) whose MNCV was markedly reduced had already received dialysis treatment for more than 10 wk when their initial plasma sample was tested and found to have only moderate TKA inhibition.

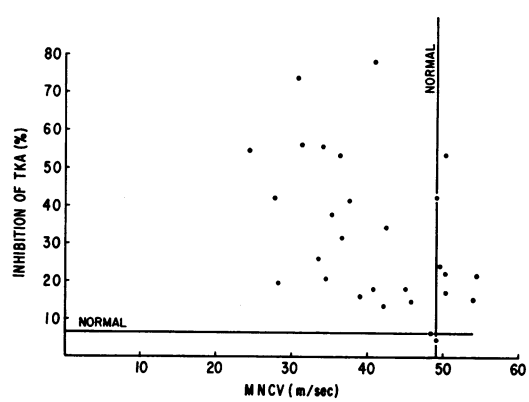

FIGURE 2 Relationship between motor nerve conduction velocity (MNCV) and inhibition of cerebral and spinal cord transketolase activity by plasma of uremic patients. The two solid lines (labeled Normal) indicate that 20 healthy adults had a mean MNCV of 49.2 m/sec (±4.7 SD) and that their plasma inhibited TKA less than 6.7%. Coefficient of correlation (r): −0.468, $P < 0.05$; the 95% confidence interval was −0.11 to −0.71.

[12] Sodium xylulose-5-phosphate, Sigma Chemical Co., St. Louis, Mo.

[13] Sigma Chemical Co., St. Louis, Mo.

Four of the group III patients were stuporous or comatous due to chronic uremia at the time their plasma was collected (encephalopathy+++). Seven subjects of group III, five of group II, and one of group I had evidence of moderately extensive uremic encephalopathy (++). Seven group III patients had only mild central nervous system disturbances (+), which were also shown by nine of group II and two of group I.

Effect of CSF on TKA. The studied CSF samples from six uremic patients had slightly elevated protein levels but were, by routine examination, otherwise within normal limits. When they were incubated with brain suspensions, significant suppression of cerebral TKA was found in all but one case as compared to buffer controls and spinal fluid from eight nonazotemic patients (Table II). CSF specimens from four patients had been collected after several dialyses and their inhibition values were lower than those of the plasma samples of these patients obtained and tested before onset of dialytic treatment. In one of the other two cases (R.M.) where spinal fluid and plasma had been secured at the same time comparable inhibition of TKA was found (Tables I, II).

Effect of dialysate fractions on TKA. Three molecular weight fractions were obtained individually from the dialysates of eight patients on their initial hemodialysis. Fraction II (500–1000 mol wt) and fraction III (1000–10,000 mol wt) did not inhibit cerebral or spinal cord TKA more than 12%. Fraction I (0–500 mol wt) inhibited in all eight instances more than 65%. None of the three fractions when obtained from dialysates of normal bank blood inhibited the TKA more than 12%.

Effect of hemodialysis. When patients with uremic neuropathy were new on the dialysis program it was found that a single 5–7 hr dialysis reduced the inhibition of cerebral and spinal cord TKA by their plasma significantly (Table III). The inhibition reappeared within the next 2–3 days although generally to a somewhat lower level. The inhibitory effects of predialysis plasma on TKA were clearly decreasing as the period of maintenance dialysis of the patients progressed (Table IV). Eight subjects who were on the dialysis program longer than 40 wk were studied and it was found that the predialysis inhibition of TKA continued to diminish as the time on dialysis increased.

TABLE II

Effect of Cerebrospinal Fluid from Patients with Chronic Uremia on Brain Transketolase Activity

Patient	Inhibition*
	%
O. R.‡	10.1
L. F.	32.2
L. R.	36.0
R. O.	44.4
L. Fa.	52.4
R. M.	60.0
8 Nonazotemic CSF controls	<9.6

* Data are mean values of triplicate determinations using six brain preparations (see under Methods).
‡ Only patient O. R. had a GFR >4 ml/min (8.7 ml/min).

TABLE III

*Inhibition of Cerebral and Spinal Cord TKA by Plasma Obtained from Uremic Patients before and after Single Hemodialysis**

Patient	Inhibition‡	
	Predialysis	Postdialysis
	%	%
A. B.	17.1	2.9
O. R.	18.0	4.8
S. G.	21.8	1.7
B. Q.	22.0	5.8
A. A.	24.7	2.2
R. M.	28.9	6.1
C. A.	29.3	13.3
L. G.	30.8	3.7
C. W.	42.0	5.9
E. P.	53.2	21.3
M. Ki.	55.2	17.3
W. B.	55.8	6.4
M. K.	73.4	4.9
N. M.	78.1	5.5

* 5–7 hr dialysis with a twin-coil hemodialyzer. No blood transfusions were given for at least 1 wk before sampling of plasma.
‡ Data are expressed as in Table I.

Two examples of detailed electrophysiologic evaluations which paralleled the testing of TKA inhibition gave the following results: M.K., a typical case of those dialyzed patients who have been observed for more than 1 yr, initially had TKA inhibition of 73.4% and MNCV of 30.8 m/sec. After 18 wk of dialysis, he no longer showed inhibition of TKA, while his MNCV improved more gradually and was after 14 wk 33.9 m/sec and after 54 wk 43.7 m/sec. Another patient (M.Ki.) who was successfully transplanted after 22 wk dialysis, had after 10 wk dialysis TKA inhibition of 55.2% and MNCV of 34.1 m/sec; after 26 wk dialysis the values were 16.2% and 38.4 m/sec, and 30 wk following transplantation 3.8% and 46.8 m/sec.

Absence of a TPP effect on the inhibition of cerebral and spinal cord TKA. Use of buffer containing excess TPP in concentrations of 0.001–0.004 M did not change TKA of the tested brain or spinal cord suspensions in the presence of normal plasma or CSF or buffer controls

TABLE IV

*Influence of Long-Term Intermittent Hemodialysis on Inhibition of Cerebral and Spinal Cord TKA by Predialysis Plasma**

Duration of dialysis	Inhibition of TKA‡							
	M. K.	A. A.	C. W.	E. P.	W. B.	B. Q.	R. M.	M. Ki§
wk				%				
0–5	73.4	24.7	61.1	87.3	55.2	16.9	54.3	
5–10	49.7		38.7	50.1	36.7	22.0	28.9	55.2
10–15	8.1		6.5					31.7
15–20	0			49.0			3.9	28.1
20–25			10.8					
25–30	5.9	12.8		19.2	15.0	8.1	5.1	16.2
30–35		4.3		16.9		5.5		
35–40		6.7		13.1	25.3			7.0
40–45	5.5	3.9		11.4			6.0	
45–50			6.8					
50–55		5.1			8.7			3.8

* Twice weekly 5–7 hr dialysis with a twin-coil hemodialyzer. Plasma samples were tested only when the pateint's clinical condition was stable and neither blood transfusions nor potentially neurotoxic drugs had been given for a least 1 wk before sampling.

‡ Data are expressed as in Table I.

§ The values after 25 wk were obtained following kidney transplantation and discontinuation of dialysis.

by more than 4.5%, and had no effect on the observed TKA inhibition by any sample of uremic plasma or CSF at all or by more than 6.2%. Neither prolongation of the preincubation period to 180 min and of the incubation time to 240 min in the presence of TPP, nor replacement of Krebs-Ringer phosphate buffer by tris(hydroxymethyl) aminomethane (Tris) buffer with or without TPP abolished the TKA inhibition.

DISCUSSION

The present studies describe an assay to measure nervous tissue transketolase adapted for testing the specific inhibitory effects of uremic material. It is evident that the observed TKA inhibition is not due to interference of the employed uremic samples with the action of other enzymes which also affect formation and conversion of S7P and/or with the spectrophotometric determination of sedoheptulose. Lack of the cofactor thiamine pyrophosphate as a cause for the low TKA values is also excluded, which is in agreement with earlier results obtained from uremic erythrocytes showing that TPP was not able to restore suppressed TKA (26). Significant inhibition of TKA was shown by plasma and spinal fluid from chronic uremic patients. No attempts were made to compare the inhibitory effects of plasma and CSF since the relatively few available spinal fluid samples had been secured at different times in relation to plasma collection and length of dialytic treatment. The mode of equilibration of the inhibiting substance(s) between plasma and CSF is impossible to determine at the moment; however, it seems reasonable to conclude that the TKA inhibiting factor(s) crosses the blood–brain barrier.

Dialyzability of the inhibitor(s) is clearly demonstrated when plasma from patients on hemodialysis loses its TKA suppressing properties and inhibitory substance(s) of low molecular weight can be recovered in the dialysates. Elimination of neurotoxic factors by dialysis has been suggested by the observations of several dialysis centers as well as by our own experience that the neurologic deficits of uremic patients are amenable to vigorous dialysis (1–3, 37–39). The finding that the uremic patients' neuropathy and TKA inhibition values of their plasma show a reasonable correlation and a similar response to effective long-term dialytic treatment could reflect a causal relation of these two expressions of the uremic state.

Previously we had noted in undialyzed chronic uremic patients for whom length of renal disease was exactly known that the increasing duration and severity of advanced renal insufficiency was accompanied by gradually rising inhibition effects of their plasma on TKA. On the other hand, eight patients with acute renal failure of various etiologies (GFR <2 ml/min, serum creatinine >18 mg%) showed neither inhibition of TKA by their plasma nor electrophysiologic or clinical evidence of uremic neuropathy although they had various degrees of encephalopathy.[14] These findings clearly indicate that

[14] Unpublished observations.

uremic peripheral neuropathy as well as TKA inhibition by uremic plasma does not only depend on the degree but also on the duration of the underlying renal insufficiency.

Contrary to the peripheral neurologic defects, signs and symptoms of uremic encephalopathy are often subject to rapid changes. The clinical expression of the studied uremic patients' encephalopathy as well as their EEG recordings was affected by many variables such as electrolyte imbalance, variations in plasma osmolality and/or in pH, changes of blood pressure, and blood perfusion of the brain. Similar observations have also been described by Kiley (4) and other workers (2, 6). These concurrent influences were not uniformly present in all examined cases so we are hesitant to make interpretations, even though our findings indicate that patients with clinical evidence of moderate and severe encephalopathy due to chronic uremia have the highest degrees of TKA inhibition.

A direct estimation of transketolase in uremic nervous tissue was hindered by the difficulty in obtaining a large number of well-preserved brain tissue samples from uremic patients. In the three studied uremic brains, TKA was significantly lower than in the nonuremic brain tissue regardless of the time interval which had elapsed between death of the patient and performance of the assay. These observations, albeit limited in number, would support the assumption of suppressed TKA in uremic nervous tissue which is based on the noted in vitro inhibition of normal tissue TKA in the presence of uremic material. Here, it is of interest that in many of the described uremic subjects, TKA of erythrocytes were also tested and found to be below normal but rose following dialytic therapy (26).

Depressed erythrocyte TKA is a finding which patients with chronic uremia have in common with thiamine-deficient subjects. In various animal species as well as in man, cerebral and spinal cord transketolase, like other enzymes of the pentose phosphate pathway, have been found predominantly in myelin-rich areas of the nervous system (e.g., subcortical white matter, brain stem, spinal cord) whereas two other thiamine-dependent enzymes, pyruvate decarboxylase and alpha ketoglutarate decarboxylase, were shown to have higher activity in the neurosomal masses of the nervous tissue (15–21, 40). Dreyfus has indicated that transketolase activity measured in nervous tissue may reflect the amount and integrity of myelin supporting structures. It has been proposed that depression of nervous tissue TKA contributes to the characteristic demyelinative changes of progressive thiamine deficiency by interference with the normal functions of the pentose phosphate pathway in myelin metabolism (18–20, 22, 41). McCandless, Schenker, and Cook observed that diminished transketolase activity may not be the sole factor responsible for the first signs of encephalopathy in acutely thiamine-deficient rats (21). Such an early symptomatology however, is rarely associated with conspicuous axon degeneration, demyelination, and glia proliferation in affected areas of the peripheral and central nervous tissue as seen with prolonged depletion of vitamin B_1 (40, 42, 43).

The electron microscopy studies of Prineas (44) revealed in rats after 5–7 wk of thiamine deficiency that distal axonal degeneration in small myelinated intramuscular nerves was more extensive than changes of the accompanying myelin sheaths. Although in this study, the unmedullated nerve fibers were not affected, the author considered that the observed lesions were primarily due to the impairment of axonal transport systems.

Simultaneous evaluations of ultrastructural and related biochemical parameters of athiaminotic or of uremic neuropathy are not available at the present time. It is therefore impossible to decide whether the functional significance of transketolase with its cofactor TPP is limited to myelin metabolism or whether this enzyme plays an additional or even critical role in axonal or neuronal metabolism of the medullated structures.

Nevertheless, our findings of specific inhibition of nervous tissue transketolase activity under uremic conditions suggest that the pathogenesis of the demyelinative changes underlying uremic neuropathy may be analogous to the development of neurologic defects in thiamine deficiency. One may thus infer that in patients with advanced chronic renal failure, toxic factors accumulate and inhibit nervous tissue transketolase activity; this biochemical defect—uncorrectable by thiamine—interferes with the metabolism of medullated nerve fibers and contributes to their degenerative changes, and thus, over a prolonged period of time, to overt uremic neuropathy. Since the inhibiting material is dialyzable, reversal of the metabolic defect is possible, permitting the slow regeneration of impaired myelinated structures and stabilization, or even improvement, of the neurologic defects in patients on chronic hemodialysis.

Although thiamine deficiency per se seems excluded as a causative factor in uremic neuropathy and in the inhibition of nervous tissue transketolase, measurement of other thiamine-related enzymes, e.g., pyruvate decarboxylase and alpha ketoglutarate decarboxylase, may provide additional information as to biochemical correlates of functional and morphological defects in uremia. At present the determination of nervous tissue transketolase inhibition, may serve as an indicator for uremic neuropathy, a guide for effectiveness of chronic hemodialysis, and as an experimental model for the identification of dialyzable uremic toxins.

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