Plasma folate conjugase activities and folate concentrations in patients receiving hemodialysis

Emily J. Livant, Tsunenobu Tamura, Kelley E. Johnston, W. Harry Vaughn, Suzanne M. Bergman, Jane Forehand, and Judy Walthaw

Departments of Nutrition Sciences and Medicine, University of Alabama at Birmingham, Birmingham, AL USA

Folate conjugase activities and folate concentrations in blood obtained from patients with chronic renal failure and healthy controls were measured by radioassay using pteroyldiglutamyl-[^{14}C]-glutamic acid as substrate and microbiologic assay, respectively. A total of 32 patients receiving hemodialysis for an average of 5.4 years participated in the study. Folate conjugase activities in posthemodialysis plasma were significantly higher than those in prehemodialysis samples and were similar to those of controls. Plasma folate conjugase was inhibited by the in vitro addition of the heated extract of prehemodialysis plasma. The heated extract of urine from a healthy control also inhibited the enzyme. Furthermore, plasma folate conjugase activities were reduced by the in vitro addition of sulfate, one of the major constituents in urine, although the addition of urea or uric acid had no such effect. Data indicate the presence of an unidentified heat-stable inhibitor(s) that is excreted by the normal kidney, and sulfate may be one of them. During hemodialysis, plasma folate concentrations decreased, while erythrocyte folate concentrations remained unchanged. Five of 32 patients demonstrated plasma folate concentrations lower than normal. These findings suggest that a careful evaluation of folate requirements is necessary in patients maintained with hemodialysis. (J. Nutr. Biochem. 5:504–508, 1994.)

Keywords: folate; folate conjugase; folate conjugase inhibitor; plasma; hemodialysis; chronic renal failure

Introduction

Folate conjugase cleaves glutamyl residues of polyglutamyl folates and is believed to play a vital role in the absorption and metabolism of folates.[1] These include (a) digestion and absorption of dietary folates, which exist mainly as pteroylpolyglutamates; (b) mobilization of stored pteroylpolyglutamates from cells to circulation; and (c) alteration of chain lengths of intracellular folates for the regulation of one-carbon metabolism requiring folate coenzymes.[1,2] Folate conjugase activities have been measured in many human tissues, including plasma.[3–10] The activity of the enzyme in plasma was first identified in 1949 by Wolff et al.[3] Nearly a half century later, however, the exact physiological function of plasma folate conjugase remains to be determined. It may be that the enzyme hydrolyzes pteroylpolyglutamates released by the daily destruction of erythrocytes to monoglutamate, which is the only form identified in human plasma. Although the origin of plasma folate conjugase is unknown, several groups of investigators have determined activities of the enzyme in various disease states.[11–13] However, to our knowledge, there has been no report describing the activities of the enzyme in patients with chronic renal failure. These patients, who are supported by hemodialysis, provide an ideal model for determining whether the kidney is the major source of this plasma enzyme. Therefore, we measured folate conjugase activities in plasma obtained from patients before and after hemodialysis. In addition, we determined the changes in plasma and erythrocyte folate concentrations before and after hemodialysis to estimate the loss of folate during hemodialysis and to evaluate folate nutritional status of patients. We report our findings and discuss the need of folic acid supplementation in patients who are maintained by hemodialysis.

Address reprint requests to Dr. T. Tamura at the Department of Nutrition Sciences, University of Alabama at Birmingham, 218 Webb Bldg., UAB Station, Birmingham, AL 35294-3360 USA.
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Methods and materials

Patients maintained with hemodialysis participated in the study as part of an investigation of the relationship between geophagia and

nutritional status. The procedures were reviewed and approved by the Institutional Review Board, and a consent form was obtained from each of 32 patients (24 females and eight males). Mean age was 54.5 years (SD, 9.2; range 36 to 71). The average duration of hemodialysis was 5.4 years (SD, 3.1; range, 1.5 to 16.0). The patients were maintained by 3.5 to 4.0 hours of hemodialysis three times weekly using a high-flux polysulfone membrane (F50, Fresenius, Concord, CA USA). Dialysis was performed between 6:30 and 10:30 AM or 11:00 AM and 3:30 PM. All patients demonstrated normal liver function tests 1 to 2 months before the study. Of 32 patients, 20 were given a prescription for multivitamin tablets containing folic acid (0.68 to 2.27 μmol per day); however, compliance was not monitored. Controls were 14 healthy volunteers (10 females and four males with a mean age of 36 years ranging from 24 to 54) from the staff of the Department of Nutrition Sciences. Dietary folate intake was not obtained from patients or controls.

Venous blood samples from patients were taken at the beginning and at the end of hemodialysis from the arterial line using a plastic syringe and immediately transferred to evacuated tubes containing sodium heparin (Vacutainer, No. 6527, Becton Dickinson, Rutherford, NJ USA). These tubes were kept in the dark at 6° C until plasma separation. Blood samples were taken from controls using the same type of evacuated tubes between 8:00 and 11:00 AM. The patients and controls were not asked to fast before the blood sampling.

Plasma folate conjugase activity was determined using the method originally described by Krumdieck and Baugh[7] using pteroyldiglutamyl-[^{14}C]-glutamic acid (PteGlu$_2$-[^{14}C]-Glu) as substrate (kindly provided by Dr. C.L. Krumdieck, University of Alabama at Birmingham), which was synthesized by solid-phase techniques.[14] The incubation mixture consisted of 174 μmol/L glutamic acid; 46 mmol/L sodium acetate buffer (pH 4.5); and 23.3 μmol/L PteGlu$_2$-[^{14}C]-Glu (specific activity, 0.35 μCi/μmol) in a final volume of 430 μL with 15 to 20 μL of plasma as enzyme source. This mixture was incubated at 37° C for 10 minutes. The reaction was terminated by the addition of 50 μL of 2.4 mol/L trichloroacetic acid and mixed with 400 μL of 10 g/L charcoal suspension in a 50 mmol/L glutamic acid solution. After centrifugation, radioactivity was measured in 500 μL of supernatant to estimate the quantity of liberated [^{14}C]-glutamic acid. Enzyme activity was expressed as μmol of glutamic acid hydrolyzed per liter of plasma per hour.

The effect of the in vitro addition of prehemodialysis and posthemodialysis plasma and urine from a healthy individual on plasma folate conjugase was tested. These plasma and the urinary samples were heated at 100° C for 10 minutes and centrifuged. The supernatants (20 to 50 μL) of these were added to the incubation mixture to test the effect on the enzyme activity. The effect of urea, uric acid, and potassium sulfate on folate conjugase activity was determined separately using various concentrations with the ranges of 3 to 23 mmol/L, 17 to 122 μmol/L, and 5 to 36 mmol/L, respectively. These concentrations were selected based on the normal constituents in human urine.[15] The effect of the in vitro addition of [6R,S]-5-methyltetrahydrofolate (Sigma Chemical, St. Louis, MO USA) was also investigated at concentrations ranging between 60 and 470 nmol/L, which far exceeded the normal plasma folate concentrations.[16] Plasma and erythrocyte folate concentrations were determined using *Lactobacillus casei* microbiologic assay.[17]

Paired Student's *t* test, and Mann-Whitney nonparametric and linear regression analyses were carried out using GB-STAT (Dynamic Microsystems, Silver Springs, MD USA) and a *P* value less than 0.05 was considered significant.

Results

Plasma folate conjugase activities increased after hemodialysis in all but one patient (*Figure 1*). Mean activity in post-

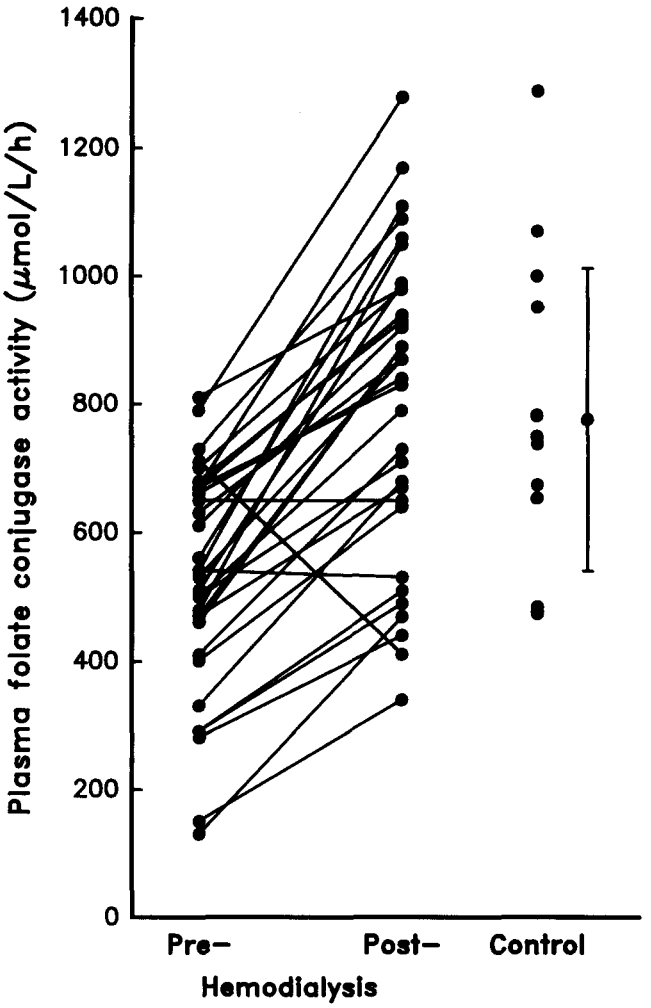

Figure 1 Plasma folate conjugase activities in prehemodialysis and posthemodialysis plasma samples and those in plasma from healthy controls. The vertical line represents the SD of the activities of 14 controls

hemodialysis plasma (805 μmol/L/hour; SD, 241; range 340 to 1,280) was significantly higher than that in prehemodialysis samples (526 μmol/L; SD, 175; range 130 to 810, $P < 0.0001$). As shown in *Figure 1*, folate conjugase activities in posthemodialysis plasma samples from patients were similar to those in plasma from controls (777 μmol/L; SD, 237; range 473 to 1,290, $P > 0.60$).

Folate conjugase activity in plasma obtained from a healthy control was inhibited by the in vitro addition of the heated extract of prehemodialysis plasma. Inhibition of 60% was observed when two volumes of the heated extract was added to one volume of control plasma in the assay mixture, while the heated extract of posthemodialysis plasma had no such effect. In addition, the heated extract of urine from a healthy individual inhibited plasma folate conjugase. Furthermore, the in vitro addition of potassium sulfate inhibited plasma folate conjugase by 62% at the concentration of sulfate of 36 mmol/L, while urea or uric acid did not affect the enzyme activity. No effect of the in vitro addition of 5-methyltetrahydrofolate on plasma folate conjugase activity was observed.

Plasma folate concentrations decreased after hemodialysis in 31 of 32 patients (*Figure 2*). The mean plasma folate concentration before hemodialysis was 56.5 nmol/L (SD, 82.7) and decreased to 30.9 nmol/L (SD, 37.7) after hemodialysis ($P < 0.02$). The decline in plasma folate concentrations during hemodialysis among individuals showed a significant correlation with the folate concentrations in prehemodialysis plasma ($r = 0.93$, $P < 0.0001$). On the other hand, erythrocyte folate concentrations of posthemodialysis samples (1,723 nmol/L; SD, 1,623; range 350–7,638) were similar to those in prehemodialysis samples (1,741 nmol/L; SD, 1,709; range 220–7,928). There was a significant negative correlation between plasma folate concentrations and folate conjugase activities in prehemodialysis or posthemodialysis samples ($r = -0.58$, $P < 0.0005$; $r = -0.56$, $P < 0.002$, respectively). A similar negative correlation was observed for plasma from controls ($r = -0.57$, $P < 0.004$).

Five of the 32 patients had prehemodialysis plasma folate concentrations less than normal (< 11.0 nmol/L), and two had less than 7.0 nmol/L, indicating that these patients were at "early negative folate balance" as defined by Herbert.[16] Furthermore, four patients had erythrocyte folate concentrations less than normal (< 454 nmol/L).

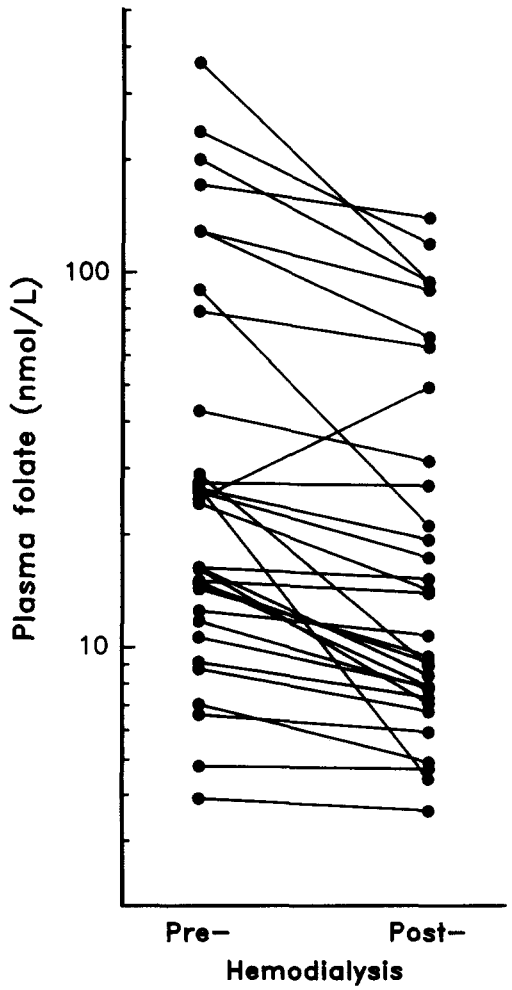

Figure 2 Changes in plasma folate concentrations during hemodialysis.

Discussion

We found that plasma folate conjugase activity was normal in patients with end-stage renal disease following hemodialysis. This observation indicates that the main source of this enzyme in plasma is not the kidney. Lavoie et al.[18] suggested that human serum folate conjugase does not originate in the jejunum, based on the different heat stabilities of the enzymes from these two tissues. It may be reasonable to speculate that the liver, where the majority of folates are stored, is the main source of plasma folate conjugase. Increased plasma folate conjugase activity has been described in patients with infectious mononucleosis, which commonly causes liver cell damage, suggesting a possible hepatic origin for the enzyme.[19] Lakshmaiah and Ramasastri[20] suggested that the liver is the origin of this enzyme in the rat; however, the source of human plasma folate conjugase remains to be investigated.

The activities of plasma folate conjugase in posthemodialysis samples were 53% higher than those in prehemodialysis plasma (*Figure 1*). This finding suggests that an inhibitor(s) of plasma folate conjugase accumulated in the circulation between hemodialyses and was removed by hemodialysis. The physiological significance of the finding is not known; however, it is possible that similar changes occur not only in circulation, but also in extracellular as well as intracellular spaces, which may affect folate metabolism in the patients with chronic renal failure.

The heated extract of either prehemodialysis plasma or urine from a healthy individual reduced the activity of plasma folate conjugase, suggesting that a readily dialyzable heat-stable compound(s), which is normally cleared by the kidney, inhibits plasma folate conjugase. Therefore, we tested the effect of the in vitro addition of urea, uric acid, and sulfate, the major compounds found in urine.[15] Sulfate (36 mmol/L) inhibited plasma folate conjugase by approximately 60%, but no inhibition was observed by urea or uric acid. The inhibition of folate conjugase by sulfate has also been demonstrated in bovine hepatic folate conjugase by Silink et al.[21] Plasma sulfate concentrations in patients with chronic renal failure are reported to be approximately 2.6 mmol/L.[22,23] Therefore, it is unlikely that sulfate alone is responsible for the inhibition of folate conjugase observed in this study. Although we have not identified the compounds in prehemodialysis plasma samples that inhibited folate conjugase, it is likely that the accumulation of sulfate and some other compounds between hemodialyses reduced the enzyme activity. These may include various inorganic compounds (chloride, phosphate, potassium, etc.), as well as a number of organic substances.[24] It has been demonstrated that certain peas and beans contain a water-soluble, heat-stable folate conjugase inhibitor that can be absorbed from the small intestine.[25] Conceivably, such a compound could be present in the plasma of patients with chronic renal failure. The in vitro addition of 5-methyltetrahydrofolate had no effect on the enzyme activity, although we found a significant negative correlation between folate concentrations and the enzyme activities in prehemodialysis and posthemodialysis plasma and control plasma samples.

Plasma folate concentrations declined during hemodialysis (*Figure 2*). Our findings are consistent with the observa-

tions by several investigators, indicating that circulating folate leaches out during hemodialysis.[26–29] However, our results differ from the findings of Ramirez et al.[30] and Ono and Hisasue.[31] Ramirez et al.[30] determined plasma folate concentrations before and after hemodialysis in five patients and showed a slight decline in two patients and a significant increase in three patients. Ono and Hisasue[31] reported that plasma folate concentrations declined only slightly after hemodialysis among the patients who had not been receiving folic acid supplementation for at least 6 months.

The magnitude of decline in plasma folate concentrations during hemodialysis was positively correlated with folate concentrations in prehemodialysis samples. This is consistent with the report by Whitehead et al.[26] We calculated the apparent average daily loss of folate by hemodialysis in our patients based on the following formula:

$$(56.5 - 30.9)\ \text{nmol/L} \times 3^{a} \times 2.5\ \text{L}^{b} \div 7\ \text{days}^{c} = 27.4\ \text{nmol/day},$$

where "a" is the number of hemodialyses per week, "b" is the approximate plasma volume in circulation, and "c" is days per week. Therefore, an average of approximately 27.4 nmol per day of folate is apparently lost during a single hemodialysis. However, this apparent loss may be a gross underestimation because our calculation did not account for the equilibration of plasma folate with extracellular fluids and cells, particularly among the patients who had low folate concentrations in prehemodialysis plasma samples. Nevertheless, the amount of folate lost during hemodialysis is far less than the U.S. Recommended Dietary Allowances.[32]

Several investigators recommended folic acid supplementation for patients receiving hemodialysis.[26,27,33–35] However, for the past two decades, supplementation of folic acid for patients maintained with hemodialysis has been considered unnecessary as long as usual dietary intake is maintained.[28,29,36–39] The recent introduction of erythropoietin administration as a routine part of hemodialysis raises new concerns as to whether folic acid supplementation is essential to maintain erythrocyte formation stimulated by this treatment. To date, only one study addressing this question has been published.[31] Also, high-flux dialyzers, introduced in the mid 1980s, may have increased the clearance of plasma folate. In our study, more than 10% of the patients had inadequate folate nutriture as judged by blood folate concentrations. Therefore, we recommend that the need for folic acid supplementation be reevaluated among patients undergoing hemodialysis.

References

1 McGuire, J.J. and Coward, J.K. (1984). Pteroglypolyglutamates: Biosynthesis, degradation, and function. In *Folates and Pterins, volume 1,* (R.L. Blakley and S.J. Benkovic, eds.), p. 135–190, John Wiley, New York, NY USA

2 Krumdieck, C.L. and Eto, I. (1986). Folates in tissues and cells. Support for a "two-tier" hypothesis of regulation of one-carbon metabolism. In *Chemistry and Biology of Pteridines,* (B.A. Cooper and M.V. Whitehead, eds.), p. 447–466, Walter de Gruyter, Berlin, Germany

3 Wolff, R., Drouet, L., and Karlin, R. (1949). Occurrence of vitamin B_c conjugase in human plasma. *Science* **109,** 612–613

4 Klipstein, F.A. (1967). Intestinal folate conjugate activity in tropical sprue. *Am. J. Clin. Nutr.* **20,** 1004–1009

5 Rosenberg, I.H., Streiff, R.R., Godwin, H.A., Castle, W.B. (1969). Absorption of polyglutamic folate: Participation of deconjugating enzymes of the intestinal mucosa. *New Engl. J. Med.* **280,** 985–988

6 Hoffbrand, A.V. and Peters, T.J. (1970). Recent advances in knowledge of clinical and biochemical aspects of folate. *Schweiz. Med. Wschr.* **100,** 1954–1960

7 Krumdieck, C.L. and Baugh, C.M. (1970). Radioactive assay of folic acid polyglutamate conjugase(s). *Anal. Biochem.* **35,** 123–129

8 Landon, M.J. (1972). Placental γ-glutamyl carboxypeptidase. *Int. J. Biochem.* **3,** 387–388

9 Wang, T.T.Y., Chandler, C.J., and Halsted, C.H. (1986). Intracellular pteroylpolyglutamate hydrolase from human jejunal mucosa. Isolation and characterization. *J. Biol. Chem.* **261,** 13551–13555

10 Chandler, C.J., Wang, T.T.Y., and Halsted, C.H. (1986). Pteroylpolyglutamate hydrolase from human jejunal brush borders. Purification and characterization. *J. Biol. Chem.* **261,** 928–933

11 Santini, R., Pérez-Santiago, E., Walker, L., and Butterworth, C.E. Jr. (1966). Folic acid conjugase in normal human plasma and in the plasma of patients with tropical sprue. *Am. J. Clin. Nutr.* **19,** 342–344

12 Magnus, E.M. (1977). Plasma conjugase activity in health and disease. In *Folic acid. Biochemistry and Physiology in Relation to the Human Nutrition Requirement,* (Food and Nutrition Board, National Research Council), pp. 147–151, Washington, D.C.

13 Baggott, J.E., Heimburger, D.C., Krumdieck, C.L., and Butterworth, C.E., Jr. (1987). Folate conjugase activity in the plasma and tumors of breast-cancer patients. *Am. J. Clin. Nutr.* **46,** 295–301

14 Krumdieck, C.L. and Baugh, C.M. (1969). The solid-phase synthesis of polyglutamates of folic acid. *Biochemistry* **8,** 1568–1572

15 Hawk, P.B., Oser, B.L., and Summerson, W.H. (1954). Urine: Physiological constituents. In *Practical Physiological Chemistry,* p. 788–822, McGraw-Hill, New York, NY USA

16 Herbert, V. (1990). Development of human folate deficiency. In *Folic Acid Metabolism in Health and Disease,* (M.F. Picciano, E.L.R. Stokstad, and J.F. Gregory III, eds.), p. 195–210, John Wiley, New York, NY USA

17 Tamura, T. (1990). Microbiological assay of folates. In *Folic Acid Metabolism in Health and Disease,* (M.F. Picciano, E.L.R. Stokstad, and J.F. Gregory, III, eds.), p. 121–137, John Wiley, New York, NY USA

18 Lavoie, A., Tripp, E., and Hoffbrand, A.V. (1975). Sephadex-gel filtration and heat stability of human jejunal and serum pteroylpolyglutamate hydrolase (folate conjugase). Evidence for two different forms. *Biochem. Med.* **13,** 1–6

19 Lawrence, M.S., Baugh, C.M., Krumdieck, C.L., Scott, C.W., Jr., and Butterworth, C.E., Jr. (1971). Elevated serum pteroyl-polyglutamyl hydrolase (PPGH, folic acid "conjugase") in infectious mononucleosis and certain forms of acute leukemia. *Clin. Res.* **19,** 727

20 Lakshmaiah, N. and Ramasastri, B.V. (1975). Folic acid conjugase from plasma. II. Studies on the source of the enzyme in blood. *Int. J. Vit. Nutr. Res.* **45,** 194–200

21 Silink, M., Reddel, R., Bethel, M., and Rowe, P.B. (1975). γ-Glutamyl hydrolase (conjugase). Purification and properties of the bovine hepatic enzyme. *J. Biol. Chem.* **250,** 5982–5994

22 Freeman, R.M. and Richards, C.J. (1979). Studies on sulfate in end-stage renal disease. *Kidney Intern.* **15,** 167–175

23 Michalk, D., Klare, B., Manz, F., and Schärer, K. (1981). Plasma inorganic sulfate in children with chronic renal failure. *Clin. Nephrol.* **16,** 8–12

24 Bergström, J. and Fürst, P. (1983). Uraemic toxins. In *Replacement of Renal Function by Dialysis. A Textbook of Dialysis,* (W. Drukker, F.M. Parsons, and J.F. Maher, eds.), p. 354–390, Martinus Nijhoff, Boston, MA USA

25 Butterworth, C.E., Jr., Newman, A.J., and Krumdieck, C.L. (1974). Tropical sprue: A consideration of possible etiologic mechanisms with emphasis on pteroylpolyglutamate metabolism. *Trans. Am. Clin. Climatol. Assoc.* **86,** 11–22

26 Whitehead, V.M., Comty, C.H., Posen, G.A., and Kaye, M. (1968). Homeostasis of folic acid in patients undergoing maintenance hemodialysis. *New Engl. J. Med.* **279,** 970–974

27 Siddiqui, J., Freeburger, R., and Freeman, R.M. (1970). Folic acid, hypersegmented polymorphonuclear leukocytes and the uremic syndrome. *Am. J. Clin. Nutr.* **23,** 11–16

28 Andersen, K.E.H. (1977). Folic acid status of patients with chronic renal failure maintained by dialysis. *Clin. Nephrol.* **8,** 510–513

29 Cunningham, J., Sharman, V.L., Goodwin, F.J., and Marsh, E.P. (1981). Do patients receiving haemodialysis need folic acid supplements? *Br. Med. J.* **282,** 1582

30 Ramirez, G., Chen, M., Boyce, H.W.,Jr., Fuller, S.M., Butcher, D.E., Brueggemeyer, C.D., and Newton, J.L. (1986). The plasma and red cell vitamin B levels of chronic hemodialysis patients: A longitudinal study. *Nephron* **42,** 41–46

31 Ono, K. and Hisasue, Y. (1992). Is folate supplementation necessary in hemodialysis patients on erythropoietin therapy? *Clin. Nephrol.* **38,** 290–292

32 National Research Council. (1989). *Recommended Dietary Allowance. 10th ed.* National Academy Press, Washington DC USA

33 Hampers, C.L., Streiff, R., Nathan, D.G., Snyder, D., and Merrill, J.P. (1967). Megaloblastic hematopoiesis in uremia and in patients on long-term hemodialysis. *New Engl. J. Med.* **276,** 551–554

34 Stein, G., Sperschneider, H., and Koppe, S. (1985). Vitamin levels in chronic renal failure and need for supplementation. *Blood Purif.* **3,** 52–62

35 Kopple, J.D. and Swendseid, M.E. (1975). Vitamin nutrition in patients undergoing maintenance hemodialysis. *Kidney Intern.* **7,** S-79–84 (suppl)

36 Lash, J. and Smith, E.C. (1990). Is folate supplementation necessary in dialysis patients? *Int. J. Artif. Organs* **13,** 785–786

37 Sharman, V.L., Cunningham, J., Goodwin, F.J., Marsh, F.P., Chaput De Saintonge, D.M., and Evans, S.W.J. (1982). Do patients receiving regular haemodialysis need folic acid supplements? *Brit. J. Med.* **285,** 96–97

38 Swainson, C.P. and Winney, R.J. (1983). Do dialysis patients need extra folate? *Lancet i,* **239**

39 Hutchins, L. and Lipschitz, D. (1985). Iron and folate metabolism in renal failure. *Semin. Nephrol.* **5,** 142–146