Serum Folate and Vitamin B_{12} Levels in Acute and Chronic Renal Disease. Effect of Peritoneal Dialysis

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Summary: Serum folate and vitamin B_{12} levels have been measured in 32 patients with renal failure. The initial mean serum folate level was raised above normal in seven patients with acute renal failure whereas the mean level in eight patients severely ill from chronic renal failure was significantly lower than normal. Serum folate levels fell during peritoneal dialysis and rose between dialyses in all these patients and also in one patient who was dialysed for acute pancreatitis.

The mean serum B_{12} level was raised in patients with both acute and chronic renal failure, but there was no consistent change in serum B_{12} level during dialysis.

Hypersegmented polymorphs were present in the peripheral blood film of most of the patients with acute or chronic renal failure. Their presence bore no relation to the clinical state, blood urea, serum folate, or serum B_{12} level of the patients.

Introduction

With the increasing use of dialysis treatment for both acute and chronic renal failure complications of this therapy are being widely recognized. One such complication is the removal of water-soluble vitamins from the patient's blood by the dialysis. Palmer *et al.* (1966) suggested that this could occur as part of a general "depletion syndrome" in patients having long-term peritoneal dialysis, and Wernicke's encephalopathy has been described in one patient (Clinicopathological Conference, 1966). Lasker *et al.* (1963) demonstrated low plasma levels of folate and nicotinic acid in uraemic patients but failed to show any significant changes in the levels of these substances or in the levels of other vitamins during either peritoneal dialysis or haemodialysis. Nevertheless, Hampers *et al.* (1967) described megaloblastic haemopoiesis in a number of patients undergoing intermittent haemodialysis. Serum folate levels in these and also in other uraemic patients not undergoing dialysis were low. These authors showed that haemodialysis could lower the serum folate in vitro and suggested but did not directly demonstrate that dialysis lowered the serum folate level in vivo. Subsequently Whitehead *et al.* (1968) and Mackenzie *et al.* (1969) reported the effects of haemodialysis on the serum folate level. Mowbray *et al.* (1965) found low serum folate levels and megaloblastic changes in the bone marrow in patients who had received transplant kidneys and azothiaprine therapy and suggested but did not show that removal of folate by dialysis may render the patient unduly sensitive to immunosuppressive drug therapy.

This paper compares the serum folate and vitamin-B_{12} levels in patients with uraemia due to different types of renal disease and shows the effects of peritoneal dialysis on these levels. The results show that peritoneal dialysis rapidly lowers the serum folate level and also suggest that the kidneys normally play an important part in maintaining the serum folate level in man constant.

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Materials and Methods

Thirty-two patients with renal failure were studied and were divided into four groups on the basis of their clinical status.

Group 1 consisted of seven patients with acute renal failure who had no clinical evidence, or histological evidence where renal biopsy was performed, of previous renal disease. All were studied initially during the first week of their illness. Six of these patients were treated by dialysis and one (Case 7, Fig. 2) was treated conservatively. All seven recovered.

Group 2 consisted of 10 patients with acute deterioration of renal function and associated clinical or histological evidence of chronic renal disease. All were dialysed and subsequently required intermittent haemodialysis or renal transplantation.

Group 3 consisted of eight patients with steadily progressive chronic renal failure who were severely ill for many weeks before admission for dialysis and subsequently required intermittent haemodialysis or renal transplantation.

Group 4 consisted of seven patients with chronic renal failure who were clinically well while taking a 20-g. protein diet and who did not require dialysis.

In addition, one patient with normal renal function (Case 33) who had dialysis for acute pancreatitis was studied. None of the 33 patients had received previous dialysis treatment at the time of their initial studies here.

Control Group.—Serum folate levels were also measured in a group of 25 normal healthy adult volunteers.

Serum folate and vitamin-B_{12} levels were measured on admission to hospital in all patients. Repeat measurements were made during and after dialysis in six patients with acute renal failure, in 10 patients with chronic renal failure, in the patient with pancreatitis, and throughout the course of her illness in the one patient with acute renal failure treated conservatively. On admission stained peripheral blood films were examined for the presence of hypersegmented polymorphs—that is, polymorphs with more than five nuclear lobes.

Serum folate levels were measured by the microbiological assay with *Lactobacillus casei* described by Waters and Mollin (1961). Red cell folate levels were measured by the method of Hoffbrand *et al.* (1966). The normal range is from 160 to 640 mμg./ml. packed red cells. Serum vitamin-B_{12} levels were determined by the microbiological assay with *Euglena gracilis* described by Anderson (1964). The normal range is from 160 to 925 $\mu\mu$g./ml. Routine haematological methods were those described by Dacie and Lewis (1963).

Dialysis was performed through a Trocath with Dialaflex solutions, 2 litres per exchange. The fluid was left in the peritoneal cavity for from 10 to 20 minutes and the procedure repeated throughout the 24 hours until the plasma urea fell to about 100 mg./100 ml., after which dialysis was performed for 12 hours a day. Patients with acute renal failure were dialysed daily until the diuretic phase was established. Those with chronic renal failure were initially dialysed daily, and later were treated by intermittent peritoneal dialysis for 36 to 48 hours a week, with a low-protein diet between dialyses.

All patients on admission were given a 20-g. protein, low-sodium, high-calorie diet. This has an estimated folate content

of 150–300 μg./day after cooking. A freer diet was allowed once the plasma urea had fallen, but restrictions were imposed again in chronic patients between intermittent dialyses.

Cases 1 to 5, 7, and 8 received ampicillin in a dose of 1 g./day by intramuscular injection for most of their illness and Case 7 also had 1 g. of cloxacillin intramuscularly daily.

Results

Initial Serum Folate Levels.—The serum folate levels of the 25 normal control subjects ranged from 3·0 to 16·6 mμg./ml. (mean 7·7 mμg./ml.) (Fig. 1). Of the seven patients in group 1, five had normal levels and two had levels above the upper limit of the normal control range (Fig. 1). The mean serum folate level of these seven patients, 12·0 mμg./ml., was significantly greater than normal (P=0·02). Eight of the 10 patients in group 2 and five of the seven patients in group 4 had normal serum folate levels and the mean levels in each of these groups, 6·1 and 5·6 mμg./ml. respectively, were only slightly and not significantly subnormal. On the other hand, subnormal serum folate levels occurred in as many as six of the eight patients with chronic renal failure in group 3, and the mean serum folate level in this group, 1·9 mμg./ml., was significantly less than normal (P<0·001). There was no overall correlation among the 25 patients with chronic renal failure between serum folate, degree of anaemia, or height of the blood urea.

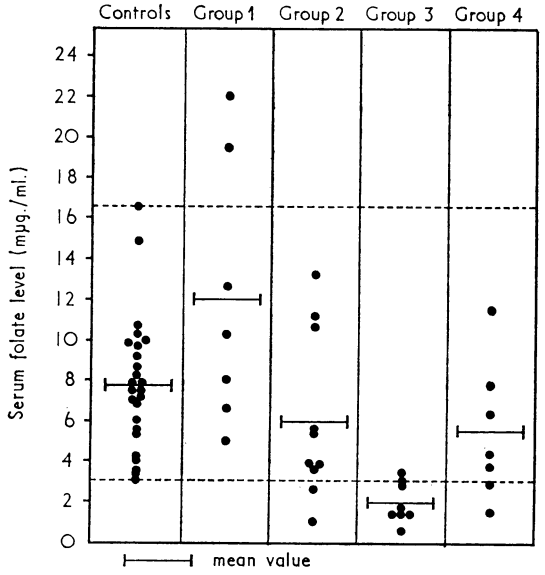

FIG. 1.—Serum folate levels of 25 control subjects and 32 patients with renal failure, divided into four clinical groups (see text).

Red Cell Folate Levels.—These were measured in only eight patients. They were normal in one patient in group 1, 192 mμg./ml. (5·0[1]); in three patients in group 2, 339 mμg./ml. (5·4), 600 mμg./ml. (3·9), and 183 mμg./ml. (5·6); and in two patients in group 4, 314 mμg./ml. (6·4) and 380 mμg./ml. (4·2). On the other hand, red cell folate levels were subnormal in both patients tested in group 3—71 mμg./ml. (1·6) and 88 mμg./ml. (2·9).

Follow-up Serum Folate Levels.—Figs. 2 and 3 illustrate the changes in serum folate level that occurred in three representative patients with acute renal failure who were dialysed and in one patient with acute renal failure treated conservatively (Fig. 2), and in four patients with chronic renal failure who were dialysed (Fig. 3). The Table summarizes pre- and post-dialysis findings in all 17 patients who received dialysis. The serum folate rose with the blood urea before dialysis began in the two patients in whom repeat estimations were made (Fig. 2).

In Case 7 it rose steadily from 4·2 to 10·5 mμg./ml. until a spontaneous diuresis was established, when it fell once more to 6·8 mμg./ml. (Fig. 2). The most striking finding, however, was that in all but one of the 17 patients dialysed the serum folate fell during dialysis (see Table), the fall occurring after as little as one day of dialysis and being most rapid in the first few days of dialysis (Figs. 2 and 3). The lower the initial serum folate level, the lower the level reached at the end of dialysis. In some patients the serum folate stabilized during dialysis but in others it continued to fall until dialysis was discontinued. After dialysis there was a rise in serum folate which could be detected after 24 hours. However, in all three patients with acute renal failure, and in two of three patients with chronic renal failure studied, the serum folate level had failed to reach the pre-dialysis level after from 1 to 20 days of follow-up.

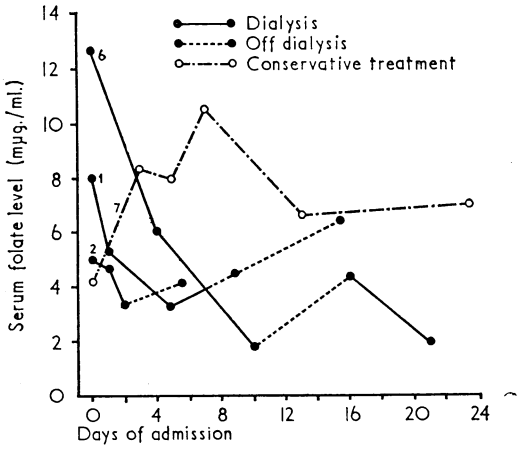

FIG. 2

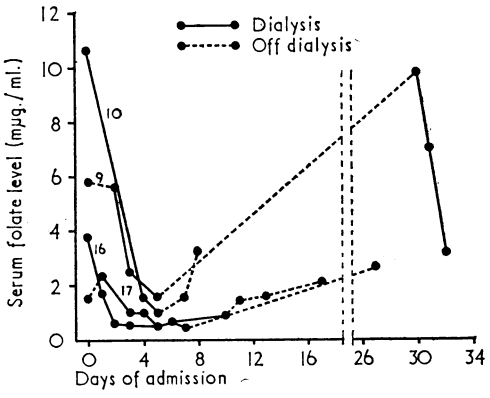

FIG. 3

FIG. 2.—Effect of peritoneal dialysis on serum folate levels of three patients with acute renal failure (Cases 1, 2, and 6). The changes in serum folate level that occurred in the patient with acute renal failure treated conservatively (Case 7) are also shown. Diuresis in this patient was established on the seventh day after admission to hospital. FIG. 3.—Effect of peritoneal dialysis on serum folate levels of four patients with chronic renal failure (Cases 9, 10, 16, and 17).

Serum Vitamin-B_{12} Levels.—Initial serum vitamin-B_{12} levels were above the upper limit of normal in three of the seven patients in group 1, and in the other four in this group they were all in the upper half of the normal range, with a mean for the whole group of 1,230 μμg./ml., significantly greater

[1] Figures in parentheses are the patients' serum folate levels in mμg./ml.

Clinical and Biochemical Findings of 17 Patients who were Dialysed. The Blood Urea, Serum Folate, and Serum-B_{12} Levels are those Immediately Before Dialysis and at the End of Dialysis. Cases 6, 9, and 15 were Studied During Two Dialyses.

Case No.	Age	Sex	Diagnosis	Days Dialysis	Plasma Urea (mg./100 ml.)		Serum Folate (mμg./ml.)		Serum B_{12} (μμg./ml.)	
					Before	After	Before	After	Before	After
			Group 1							
1	34	F	Abortion; tubular necrosis	9	335	97	8·0	4·6	800	1,700
2	22	F	Abortion; tubular necrosis	2	260	185	5·0	3·4	1,200	1,360
3	21	F	Abortion; tubular necrosis	7	330	130	16·0	6·0	2,185	—
4	34	F	Abortion; tubular necrosis	2	410	216	6·6	1·7	610	785
5	53	M	Tubular necrosis ? cause	15	340	84	22·0	7·9	920	860
6	34	F	Malignant hypertension. Acute renal failure	10	610	96	12·7	1·8	670	215
				5	160	40	4·4	2·0	1,480	1,960
			Group 2							
8	29	M	Ch. pyelonephritis. Diet 9/12	16	380	61	13·2	3·0	465	—
9	35	F	Chronic pyelonephritis. On diet 4 months	3	385	92	5·6	1·5	800	240
				3	210	90	9·8	3·4	655	832
10	25	M	Chronic glomerulonephritis	7	530	140	10·7	1·6	735	770
11	21	F	Chronic glomerulonephritis	23	410	164	3·9	1·2	385	240
12	26	M	Chronic glomerulonephritis	6	365	94	2·0	0·8	465	530
13	41	F	Chronic glomerulonephritis	5	285	75	3·6	4·6	720	695
14	38	F	Chronic glomerulonephritis	5	370	108	11·2	3·8	1,600	640
15	38	M	Chronic glomerulonephritis	15	430	120	5·4	1·5	815	290
				5	380	73	7·4	2·5	800	535
16	18	F	Chronic glomerulonephritis	6	310	118	3·8	0·4	345	305
17	37	M	Chronic glomerulonephritis	3	390	164	1·0	0·5	570	480
33	56	M	Acute pancreatitis. Normal renal function	2	40	—	6·5	2·6	600	510

than the normal mean, 472 μμg./ml., of Anderson (1964) ($P<0·01$). Only one patient in group 2 and one in group 4 of the 25 patients with chronic renal disease had a serum-B_{12} level greater than normal, and the mean serum-B_{12} level in the patients in groups 2, 3, and 4 of 696, 525, and 671 μμg./ml. respectively were all above the normal mean. For the patients in group 2, the difference was significant ($P<0·05$). As with the serum folate level, there was no correlation among all 25 patients with chronic renal failure between serum-B_{12} level and length of history, level of blood urea, or degree of anaemia. In contrast to the finding with the serum folate level, there was no consistent change in serum-B_{12} level before or during dialysis (see Table).

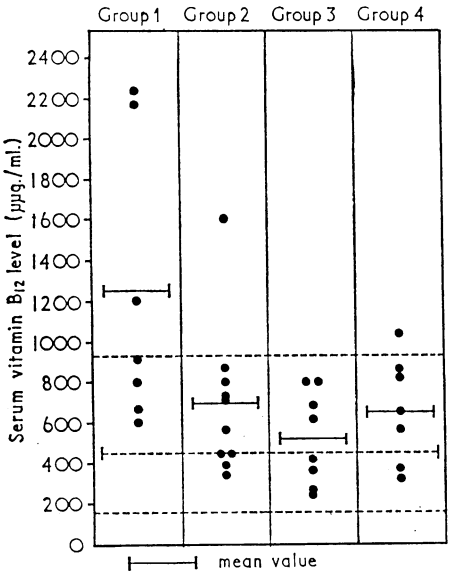

FIG. 4.—Serum B_{12} levels of the 32 patients with renal failure, divided into four clinical groups. The upper, middle, and lower horizontal broken lines represent the upper limit, mean, and lower limit of normal serum B_{12} levels (Anderson, 1964).

Hypersegmented Polymorphs.—These occurred in the peripheral blood films of three of the seven patients in group 1, 8 of the 10 in group 2, six of the eight in group 3, and five of the seven in group 4. There was no correlation between the presence of hypersegmented polymorphs and serum folate level, since hypersegmented polymorphs were present in the films of 8 of the 10 patients with subnormal serum folate levels (80%) and of 14 of the 22 patients with normal serum folate levels (64%). There was also no correlation between the presence of hypersegmented polymorphs and the length of history, blood urea, degree of anaemia, or serum-B_{12} level.

Discussion

The low initial serum and red cell folate levels in our patients with chronic renal failure who were severely ill (group 3) are similar to those found by Lasker *et al.* (1963) and Hampers *et al.* (1967) in patients with chronic renal failure. The explanation is probably a prolonged period of inadequate dietary intake of folate combined with losses of the vitamin due to vomiting. In patients with chronic renal failure who were less severely ill (group 4) or had been severely ill for only a matter of days (group 2) serum and red cell folate levels were almost invariably normal.

Serum folate levels in acute renal failure have not previously been reported. These were normal or above normal and the mean level was significantly raised. The explanation for these high levels is uncertain. Goresky *et al.* (1963) showed that, at least in the dog, plasma folate enters the glomerular filtrate and is reabsorbed by an active process by the renal tubules. Under normal conditions, however, some folate escapes absorption and enters the urine, the amount depending to a large extent on the dietary intake (Register and Sarett, 1951). In patients with acute renal failure folate enters the plasma from the diet and, possibly in excessive amounts, from breaking down tissues, but cannot be excreted in the urine. In addition, bone marrow cell turnover may be depressed in uraemia, and thus the main pathway of folate utilization is diminished. In the face of these factors causing accumulation of folate in plasma, the liver, the principal site of folate storage, seems unable to remove folate rapidly enough from plasma to prevent the plasma level rising before kidney function and consequent folate excretion is re-established.

The present results clearly indicate that during peritoneal dialysis serum folate levels fall rapidly even in patients with initially subnormal levels, and Whitehead *et al.* (1968) suggested that haemodialysis also lowers the serum folate level. Folate is only loosely and non-specifically attached to protein in serum and presumably easily enters dialysis fluid. The slow rise of serum folate after dialysis suggests that the liver is unable to replenish plasma folate rapidly when this is acutely lowered. Indeed, it may be that the plasma folate is replenished after dialysis from the diet and not from liver stores at all.

Raised serum-B_{12} levels are well known to occur in renal failure (Matthews and Beckett, 1962). These authors discussed

several possible explanations, including decreased renal excretion of B_{12}, liberation of B_{12} from damaged kidney tissue, or a rise in the serum-B_{12}-binding α-globulin in parallel with the rise in the serum levels of other α-globulins in renal disease. A rise in the serum-B_{12}-binding α-globulin has been demonstrated in uraemia (Herbert, 1967). This protein may be derived from breaking down tissues (white cells are normally an important source of the α-globulin-binding protein) (Simons and Weber, 1966), but the exact source of the raised B_{12}-binding protein in uraemia remains uncertain. Dialysis had no immediate effect in vivo on the serum-B_{12} level comparable with its effect on the serum folate level. This is not surprising, since, unlike folate, B_{12} is firmly bound to protein in serum and cannot be readily dialysed in vitro.

The presence of hypersegmented polymorphs in the blood films of uraemic patients with normal serum folate and B_{12} levels supports the view that factors other than the folate or B_{12} deficiency produces this picture. Hampers *et al.* (1967) failed to detect a change in the white cell morphology when they gave folic acid in large doses to such patients. The explanation for the hypersegmented polymorphs in renal failure therefore remains obscure.

We wish to thank Dr. Oliver Wrong for permission to study patients under his care; the many house-physicians and members of the nursing staff who were involved in the clinical care of these patients; and Mr. J. Morgan, who performed the microbiological assays. In addition, we are grateful to Miss R. Harrison and the dietetic department, who provided the special diets and estimated their protein and folate content.

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Relapses and Remissions in Brain Stem Tumours

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Summary: Three cases of brain stem astrocytoma and one of a para-stem vascular tumour are described, each showing a history of relapses and remissions. The diagnoses were confirmed by necropsy, and it is suggested that the relapsing course may have been due to oedema and necrosis in both the tumour and the surrounding brain tissue. Features indicating repeated recurrence of a lesion at the same site in the brain stem justify full neuroradiological investigation before a diagnosis such as multiple sclerosis can be accepted.

Introduction

Though brain stem gliomas are more common in children than in adults, all age groups may be affected. They usually present as a progressive disorder, consisting of oculomotor palsies, defects of conjugate ocular deviation, and other scattered cranial nerve palsies. Pyramidal or cerebellar defects may appear early or may be long delayed, but signs of raised intracranial pressure are uncommon until the later stages. It is not widely appreciated, however, that these tumours may show a relapsing and remittent course, the remission sometimes being so complete and over such a long period that a diagnosis of multiple sclerosis may have been made, and regarded as correct until the final deterioration with headache, vomiting, coma, and respiratory failure brings the true diagnosis to light. Ford (1966) mentioned two children with brain stem glioma who had complete and spontaneous recovery and Sachs (1949) described a case in which the diplopia cleared up for several days before recurring. Apart from these reports, no other record of remission has been found in the literature.

The following four cases illustrate the course that some brain stem and para-brain-stem tumours take, and are recorded in the hope that they will emphasize the importance of keeping this diagnosis high among the differential possibilities when signs and symptoms continuously and repeatedly point to a lesion in this one part of the nervous system.

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Case 1

A woman aged 21 gave a five-month history of right-sided dysaesthesia followed by diplopia and right internal strabismus. This recovered completely, but was followed by a left internal strabismus. She had had left-sided ataxia for eight weeks, left deafness and tinnitus for three weeks, early morning headache with occasional vomiting for four weeks, and amenorrhoea for three months.