

Folic acid clearances and basal serum folate levels in patients with thyroid disease¹

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SYNOPSIS The plasma clearance of intravenously administered folic acid was abnormally rapid in all of five patients studied with untreated hyperthyroidism; in three, the basal serum *L. casei* folate level was subnormal. Four of these patients and another patient were studied at various intervals after partial or complete correction of the hyperthyroid state. Partial correction resulted in a continued fast clearance in one patient, a return to borderline-normal values in the second, and a normal clearance in the third. Complete euthyroidism was achieved in two patients; their clearance values had returned to borderline-normal at this time. In two patients studied after partial correction, the serum *L. casei* folate levels had become normal whereas the clearance values remained abnormally rapid.

These findings suggest that hyperthyroidism in man is associated with depletion of folate stores and subclinical deficiency of this vitamin. This is attributed to an increased demand for folic acid in the hypermetabolic state.

Decreased hepatic stores of folic acid have been observed in experimental animals made hyperthyroid by the administration of thyroid hormone (Noronha and Sreenivasan, 1959). No studies of folic acid metabolism in humans with thyrotoxicosis, however, have been reported.

The anaemia occasionally seen in thyrotoxicosis is usually associated with a normoblastic bone marrow and has been attributed to superimposed iron deficiency (Biström, 1946; Axelrod and Berman, 1951; Wallerstein and Castle, 1962). Clinical folic acid deficiency as manifested by megaloblastic anaemia has not been recognized in patients with hyperthyroidism. However, more than 85 cases of coincidental thyrotoxicosis and megaloblastic anaemia attributed to pernicious anaemia and responding to liver extract or vitamin B₁₂ have been reported (Boenheim, Schwimmer, and McGavack, 1945; McNichol, 1961). Since the diagnosis of pernicious anaemia was not confirmed by serum vitamin B₁₂ assays or Schilling tests, it is possible that some of these patients may have had folic acid deficiency.

In recent years, two tests of folic acid deficiency, the plasma clearance of intravenously administered folic acid and the basal serum *L. casei* folate level, have been shown to correlate well with clinically

occurring folic acid deficiency states in man (Chanarin, Mollin, and Anderson, 1958; Metz, Stevens, Krawitz, and Brandt, 1961; Sheehy, Santini, Guerra, Angel, and Plough, 1963; Herbert, Baker, Frank, Pasher, Sobotka, and Wasserman, 1960; Cooper and Lowenstein, 1961; Waters and Mollin, 1961). Preliminary observations suggest that both tests may become abnormal in subclinical deficiency states before the development of overt haematological abnormalities (Metz, Stevens, Krawitz, and Brandt, 1961; Herbert, 1962b; Herbert, 1962c; Klipstein, 1963; Klipstein and Lindenbaum, 1963). The present study was undertaken to ascertain the presence of possible subclinical folic acid deficiency in patients with thyroid disease.

MATERIALS AND METHODS

Two groups of subjects were studied: 1 Five patients with untreated thyrotoxicosis (cases 1-5), and four of these were restudied after varying degrees of improvement had occurred following therapy. Case 6, the sixth patient with hyperthyroidism, was initially studied after having received three months' treatment. 2 Six patients with untreated hypothyroidism (cases 7-12). The clinical diagnosis in each case was confirmed by studies of thyroid function (Table I).

Basal serum folate levels were obtained in the fasting state and assayed with *L. casei* using a modification of the method introduced by Baker, Herbert, Frank,

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Pasher, Hutner, Wasserman, and Sobotka (1959). In this laboratory, values of $<5\text{m}\mu\text{g./ml.}$ are considered indicative of folic acid deficiency; values of 5 to 7 $\text{m}\mu\text{g./ml.}$, are diagnostically indeterminate; and values of 7 to 18 $\text{m}\mu\text{g./ml.}$ are normal. Folic acid clearance studies were performed by the technique described by Chanarin *et al.* (1958). An intravenous dose of 15 $\mu\text{g.}$ folic acid per kilogram body weight was administered and serum samples, obtained three and 15 minutes after the injection, were assayed with *Streptococcus faecalis*. Normal subjects have been found to have serum folic acid concentrations of 75-186 $\text{m}\mu\text{g./ml.}$ at three minutes and 21-80 $\text{m}\mu\text{g./ml.}$ at 15 minutes (Chanarin *et al.*, 1958). (The organism *S. faecalis* has been found reliable for assay of administered folic acid in folic acid clearance and absorption studies.) In contrast, for determination of basal serum folate activity, assay with *L. casei* is preferred, since the growth of *L. casei* is supported by 5-methyl tetrahydrofolic acid, recently shown to be the major form of folic acid activity in human serum (Herbert *et al.*, 1962). Serum vitamin B_{12} concentrations were assayed with *Lactobacillus leichmannii* (Spray, 1955); normal values in this laboratory range from 150 to 900 $\mu\mu\text{g./ml.}$

was abnormally rapid in all five of the patients studied with untreated thyrotoxicosis (Fig. 1). Basal serum *L. casei* folate levels were assayed in four of these patients and were subnormal in three; however, in no instance were the values depressed to the very low range ($<3\text{m}\mu\text{g./ml.}$) usually associated with megaloblastic anaemias due to folic acid deficiency. Case 6 was studied only after the hyperthyroid state had been partially corrected following three months of therapy. The folic acid clearance and basal serum *L. casei* folate were both normal in this patient.

Four patients (cases 1-4) were studied again after periods of two to four months' therapy had resulted in partial or complete correction of hyperthyroidism. Cases 1 and 4 had achieved euthyroidism. The plasma folic acid clearances were now borderline normal in both subjects and the basal serum *L. casei* folate concentrations, which had previously been subnormal in case 1, were now normal (Fig. 2). Cases 2 and 3 remained hyperthyroid despite four and two and a half months of treatment, respectively. The folic acid clearance remained abnormally rapid in case 2 and was borderline normal in case 3. The previously subnormal serum *L. casei* folate level had returned to within the normal range in case 2.

RESULTS

HYPERTHYROIDISM The results for both groups are set out in Table I. The plasma clearance of folic acid

TABLE I

STUDIES OF THYROID FUNCTION AND FOLIC ACID METABOLISM

Case	Degree of Symptoms (0 to +++)	Pulse Rate (per min.)	Weight Changes (lb.)	Hb (g.%)	Protein-bound Iodine ¹ ($\mu\text{g.}\%$)	Radioactive Iodine Uptake ² (% uptake)	Serum B_{12} ($\mu\mu\text{g./ml.}$)	Serum <i>L. casei</i> Folate ($\text{m}\mu\text{g./ml.}$)	Folic Acid Clearance S. faecalis Serum Levels ($\text{m}\mu\text{g./ml.}$)		Comments
									3 Minutes	15 Minutes	
Thyrotoxicosis											
1a	++++	100	↓ 50	14.0	19		510	4.0	39	3	Untreated
1b	+++	88	None	14.7			550	8.4	63	16	
c	+	80	↑ 17	14.5	5.4		800	9.2	78	18	After Tapazole for 2 mth., thyroidectomy
2a	++	68	↓ 18	14.5	20	37	300	4.4	58	15	Two mth. after thyroidectomy
2b	0	64	↑ 11				380	7.1	45	10	
2c	0	60	↑ 6	14.7	8.4		455	8.4	52	11	
3a	++	110	↓ 20	11.9	10.9	80	380	8.0	76	10	Untreated
3b	+	92	↑ 1		10.1			9.4	48	18	
4a	+++	108	↓ 23	11.2	11.3	69			70	12	Untreated
4b	+	92	↑ 8	13.7	5.5		650	6.0	77	21	
5	+++	80	↓ 10	15.9	15.9	56	525	4.0	50	5	Restudied 4 mth. after therapeutic dose radioactive iodine
6	+	104	↑ 17	13.2	11.2	54	575	7.0	70	30	Untreated
Myxoedema											
7	++++	58	↑ 20	12.5	1.1		55	10.3	134	58	Following 3 mth. treatment with Tapazole; 2 mth. after therapeutic I ¹³¹
8	++++	64	↑ 30	12.0	1.2		450	8.0	200	57	
9	+++	58	↑ 12	12.5	2.3	13	450	14.4	50	35	
10	+++	64	↑ 4	12.2	2.0	5	410	8.0	46	43	
11	+++	60	↑ 10	10.6	1.3	9	725	12.0	80	20	
12	+++	72	↑ 5	12.7	1.0	5	310	13.1	135	18	

¹Protein-bound iodine, normal range is 4.0-8.0 $\mu\text{g.}\%$
²Radioactive iodine uptake, normal range is 15-45% uptake in 24 hours.

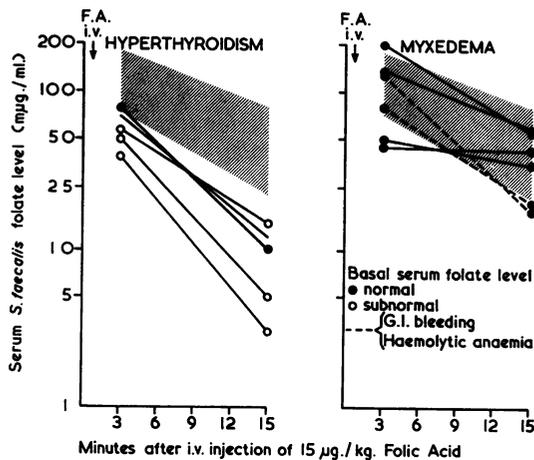


FIG. 1.

Serum vitamin B₁₂ concentrations were within the normal range in all patients in this group. Two patients were mildly anaemic. This was shown to be on the basis of iron deficiency in both instances and had been corrected by replacement therapy with iron in case 4 at the time of the study. None of the peripheral smears of the patients in this group showed macrocytosis or hypersegmentation of the neutrophils.

HYPOTHYROIDISM Basal serum *L. casei* folate concentrations were normal in all six patients with untreated hypothyroidism (cases 7-12). The plasma clearance of folic acid (Fig. 1) was within the normal range in four subjects (cases 7-10) and borderline normal in two patients (cases 11 and 12). Case 11 had an overt acquired haemolytic anaemia with a reticulocytosis of 6.3% at the time of study and case 12 was shown to have occult gastrointestinal bleeding.

Serum vitamin B₁₂ concentrations were within the normal range in all subjects in this group, with the exception of case 7. The subnormal serum vitamin B₁₂ level of 55 µg./ml. in this patient was shown by absorption studies with Co⁶⁰-labelled vitamin B₁₂ to be on the basis of latent pernicious anaemia. Macrocytosis was observed in the peripheral smear of this patient but in no other subject in this group. Case 11 was the only patient with anaemia in this group. This patient had an acquired haemolytic anaemia secondary to sulphone therapy which responded to withdrawal of the offending drug.

DISCUSSION

Abnormally rapid plasma clearances of folic acid

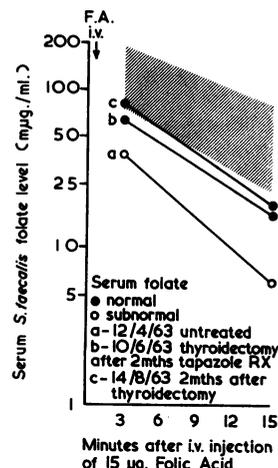


FIG. 2.

and subnormal concentrations of serum *L. casei* folate have been reported in states in which clinical folic acid deficiency has been recognized: megaloblastic anaemias associated with dietary deficiency of folic acid (Herbert, 1962a; Zalusky and Herbert, 1961), malabsorption (Chanarin *et al.*, 1958; Klipstein, 1963), liver disease (Herbert, Zalusky, and Davidson, 1963; Klipstein and Lindenbaum, 1964), diphenylhydantoin therapy (Klipstein, 1964), neoplastic diseases (Chanarin and Bennett, 1962; Rama Rao, Lagerlöf, Einhorn, and Reizenstein, 1963), pregnancy (Chanarin, MacGibbon, O'Sullivan, and Mollin, 1959b; Lowenstein, Hsieh, Brunton, De Leeuw, and Cooper, 1962) and haemolytic anaemias with secondary megaloblastic change (Chanarin *et al.*, 1959a; Lindenbaum and Klipstein, 1963). Abnormal values for both these determinations have been observed in patients considered to have folic acid deficiency before the appearance of haematological abnormalities (Metz *et al.*, 1961; Herbert 1962b,c; Klipstein, 1963; Klipstein and Lindenbaum, 1964; Hansen and V. Klewesahl-Palm, 1963). The available evidence suggests, therefore, that the folic acid clearance and serum *L. casei* folate concentration provide sensitive indications of the adequacy of tissue stores of folic acid derivatives. The finding of abnormally rapid folic acid clearances in all five patients with untreated thyrotoxicosis and low basal serum *L. casei* folate levels in three of these patients is suggestive of depletion of folate stores with subclinical folic acid deficiency and is in accord with the laboratory observation of low hepatic stores of folic acid in hyperthyroid rats (Noronha and Sreenivasan, 1959).

Multiple vitamin deficiencies have been repeatedly observed in the experimental animal rendered

FIG. 1. Serial studies of the effect of therapy on serum folate values and folic acid clearances in a thyrotoxic patient.

FIG. 2. Folic acid clearance studies during treatment of hyperthyroidism. In both diagrams the shaded area represents the normal range for the folic acid clearance.

hyperthyroid by the administration of large doses of thyroid hormone. Increased requirements for vitamin A, thiamine, pyridoxine, pantothenic acid, vitamin B₁₂, and vitamin C, with concomitant depletion of body stores of these vitamins, have been demonstrated in a variety of laboratory animals (Drill, 1962). The most likely interpretation of the findings in the present report is that they are a reflection of an increased metabolic demand for folic acid associated with a generalized increase in coenzyme requirements associated with the hyperthyroid state. The hyperactive bone marrow typically seen in thyrotoxicosis (Axelrod and Berman, 1951) may also contribute to such an increased demand and utilization of folate. The mechanism for the subclinical folate deficiency in thyrotoxicosis may thus be analogous to that in patients with severe haemolytic anaemia. A more than four- to eight-fold increase in requirement for folic acid has recently been demonstrated in a patient with sickle cell anaemia (Lindenbaum and Klipstein, 1963).

The finding of persistently rapid folic acid clearances after partial correction of the hyperthyroid state appear to indicate a continued increased demand for folic acid even in the mildly hyperthyroid individual. The return of the serum folate levels to normal while the plasma clearances were still abnormally rapid in two patients, as well as the initial finding of a rapid plasma clearance associated with a normal serum folate level in another patient, suggests that the serum folate level is a less sensitive method than the clearance for detecting minimal depletion of folate stores. A similar experience with these two determinations has been obtained in patients with liver disease (Klipstein and Lindenbaum, 1964).

No evidence of folic acid deficiency was observed in the patients studied with myxoedema. Serum *L. casei* folate levels were normal in all six subjects as were folic acid clearances in the four uncomplicated cases. The borderline clearance values found in two subjects, one of whom had acquired haemolytic anaemia and the other gastrointestinal bleeding, may well be attributed to these complications since both have been associated with rapid folic acid clearances, possibly secondary to increased folate demands of the hyperactive marrow (Chanarin *et al.*, 1959a and b; Chanarin *et al.*, 1958; Sheehy *et al.*, 1963; Klipstein and Lindenbaum, 1964).

While experience with megaloblastic anaemias to date has shown a high correlation between folic acid deficiency states and the tests employed in this study, caution must be exercised in the final interpretation of these tests of folic acid deficiency. It is possible that factors other than depletion of body stores of folate derivatives may contribute to

abnormally rapid folic acid clearances or low serum folates. Circulatory factors may play an important role in the clearance tests. In a thyrotoxic patient with an increased cardiac output, it is conceivable that increased perfusion of the tissues during the 15 minutes after the intravenous administration of folic acid could result in increased extraction of the vitamin, leading to a rapid clearance in the absence of depletion of tissue stores. However, the finding of persistently rapid folic acid clearances even after the pulse rate had returned to normal in a partially treated patient (case 2, Table I) would argue against the contribution of circulatory factors at that time.

The possible role of the state of activity of the reticuloendothelial system also merits further consideration. Generalized hyperplasia of lymphoid tissues, and in a minority of cases, even splenomegaly, are recognized features of thyrotoxicosis (Baldrige and Peterson, 1927). The role played by the various organs of the reticuloendothelial system in the immediate extraction of folic acid from the plasma after an intravenous injection is unknown at present. Whether hyperactivity of this system could result in a rapid clearance in the absence of depletion of tissue stores is, in our present state of knowledge, merely speculative. However, in conditions such as the anaemia of infection or that associated with rheumatoid arthritis, there may be a rapid clearance of intravenously-administered iron and a depression of the serum iron level, in the absence of depletion of body iron stores (Cartwright and Wintrobe, 1952).

At the present time, the definite interpretation of the finding of abnormally rapid folic acid clearances and subnormal basal *L. casei* folate levels awaits correlation of assay of biopsy material for folate stores performed in conjunction with the plasma studies.

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