Oral Folic Acid Tolerance Test in Normal Human Subjects and Patients with Pernicious Anemia.* (20011)

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In pernicious anemia, vit. B_{12} appears to produce a complete hematologic and neurologic remission(1). Pernicious anemia seems to represent a conditioned deficiency of vit. B_{12}. Folic acid will also produce a hematologic response in most cases, but patients may experience hematologic or neurologic relapse during treatment with folic acid alone(2). In attempting to explain the action of folic acid in pernicious anemia, one must consider the possibility that a deficiency of this vitamin exists in some cases. Such a deficiency could arise from an insufficient intake, insufficient synthesis by the intestinal flora, absorption or destruction by the intestinal flora, impaired splitting of dietary folic acid conjugates, impaired intestinal absorption, inadequate utilization or rapid destruction in the body, and increased excretion.

These experiments are an attempt to measure the intestinal absorption of folic acid. The balance technic is not applicable to this problem because of the variants introduced by the intestinal flora; accordingly the approach adopted has been to determine the effect of oral dosing upon blood levels; *i.e.*, that of tolerance curves.

Material and methods. Seventeen healthy white adult scientists were used as "normal" subjects. Fourteen were men and 4 women. They ranged in age from 19 to 53 years. Ten patients with well-established pernicious anemia were studied. Five were male and 5 female, and one of the males was a Negro. They ranged in age from 46 to 78 years. Six of the patients had been maintained in remission by intramuscular liver extract‡ for several years and one by vit. B_{12}§ for several years. Three patients were in the early stages of a hematologic response to B_{12} and were anemic when the test was performed. *Folic acid*‖ was given one to 2 hours after breakfast as a freshly prepared aqueous suspension. Venous blood was collected prior to and at intervals after ingestion. The serum was separated and frozen within one to 2 hours after venipuncture. For assay, the serum was diluted with water and assayed microbiologically(3) with *Streptococcus fecalis* ATCC 8043. The assays were read titrimetrically after incubating 72 hours and steaming 10 minutes, because it was found that autoclaving and the quaternary amine used for a germicide caused turbidity of the serum. Recoveries using 3 dilutions of one or 2 serum samples were included with each assay, and no assay was accepted unless these recoveries were between 90 and 115% and the standard curve approached a straight line when plotted on log-log paper. Twenty-four hour urine specimens were collected under toluene from certain of the normal subjects beginning at the time of folic acid ingestion. An aliquot was frozen and later assayed in a fashion similar to that used for serum, except that incubation was continued for only 18 to 24 hours and the assays read turbidimetrically.

Results. The serum level of folic acid prior to folic acid ingestion was 4 mγ (millimicrograms) per cc or less in every subject but one, who 48 hours after omitting a daily dose of 5 mg of folic acid, had a level of 6 mγ per cc. These levels were found to be uniformly low whether or not the subject had eaten breakfast.

Table I shows the highest serum levels

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‡ Liver extract was generously supplied by The Wilson Laboratories, Division of Wilson and Co., Inc.

§ Crystalline vit. B_{12} was made available through the courtesy of Merck and Co., Inc.

‖ Folic acid was supplied through the generosity of the Lederle Laboratories Division of the American Cyanamid Co.

TABLE I. *Maximum Values of Serum Folic Acid Reached Following Folic Acid Ingestion.*

Subject (healthy)	.5 mg dose			1 mg dose			2 mg dose		
	Max, mγ/cc	Time of peak hr after ingestion	Activity 24-hr urinary excr., γ	Max, mγ/cc	Time of peak hr after ingestion	Activity 24-hr urinary excr., γ	Max, mγ/cc	Time of peak hr after ingestion	Activity 24-hr urinary excr., γ
C, Sr.				0	—	—			
W	1	1	6	2	4	—	40	1	140
McL	2	1		2	4	56	27	4	
C	—	—	21	4	1	36	40	2	465
D				4	1	46			
DC				7	2	—			
CD				7	4	26	10	4	114
B				10	6*	34	46	1	235
O	2	1	8	18	4	36	31	2	240
E	1	1	7	20	4	23			
F				36	6*	46			
N	1	4	11						
P	5	4	5				28	4	220
Mc							18	6*	200
DB							19	4	180
U							25	6*	165
GB							33	4	165
Subject (pernicious anemia)									
K				2	2*	—			
E				4	1	—			
T				4	1	—	4	1	180
C				8	2	—			
B				10	1	—			
G				12	1	—			
M				14	1	—			
V				16	1	—			
P				24	1	—			
F							19	1	455

* Last sample drawn.

measured in each test following the ingestion of 0.5 to 2.0 mg of folic acid. Twelve of the healthy subjects attained a maximum within 4 hours and 4 showed a higher level at 6 hours. An illustrative course of blood serum levels following the ingestion of 1.0 mg by one healthy subject is given in Fig. 1.

One mg appeared to be the critical dose at which some of the normal subjects showed an increase in serum level sufficient to be regarded as evidence of absorption. It was, therefore, chosen as a standard dose for the patients with pernicious anemia. Six of the healthy subjects whose serum level did not rise above 10 mγ per cc were given 5 mg folic acid daily for one week and the 1 mg tolerance test repeated 48 hours after the last dose. Table II demonstrates that each subject showed a somewhat higher peak in the second test, but the increase was not striking.

The 24-hour urinary excretion of folic acid by several of the normal subjects following varying doses of folic acid are also presented in Table I. Difficulties were encountered in assaying these urines. Recoveries from individual samples varied from 50 to 200%, using both titrimetric and turbidimetric assays. Therefore, the urinary excretion is expressed as "folic acid activity" in recognition of this limitation.

Nine of the patients with pernicious anemia were given 1 mg of folic acid and blood samples collected before, and 1, 2, and 3 hours after ingestion. Two of the patients were given 2 mg and blood samples collected before, and 2, 4, and 6 hours after ingestion. The peak values reached did not differ significantly from those of the normal subjects, but the peaks appear to occur earlier than in the normal subjects (Table I).

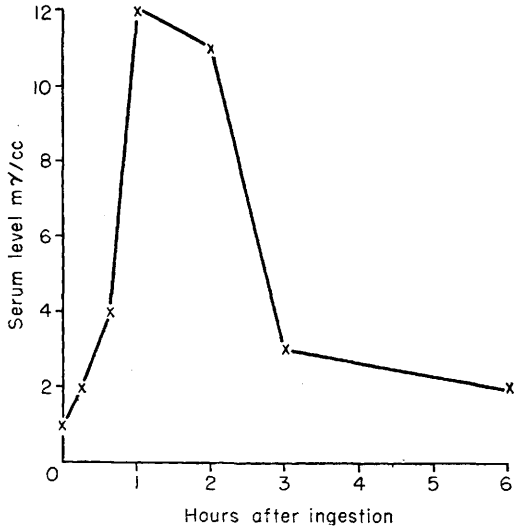

FIG. 1. Blood Serum levels of folic acid following ingestion of 1 mg of folic acid. The subject had received 5 mg of folic acid daily for 7 days ending 48 hr prior to this test. Male, 31 years old.

Discussion. The reproducible results and good recoveries obtained with the serum assays indicate a degree of accuracy that permits measurement of the slight increases in serum levels encountered in this study. It seems reasonable to assume that the increases measured represent free folic acid in the serum because they occurred following folic acid ingestion, because *S. fecalis* is unresponsive to folic acid conjugates(4), and because the serum was frozen shortly after collection, to prevent the release of bound folic acid by serum enzymes.

This study confirms previous reports(5) that the normal diet does not cause a significant rise in serum levels of free folic acid and justifies the use in such studies of the postprandial state. Furthermore, these data fail to indicate that a meal previous to ingestion causes delay in absorption.

The appearance of free folic acid in the serum as early as one hour after ingestion indicates that this vitamin is rapidly absorbed. The failure of some of the normal subjects to show an increased serum level following ingestion of 1 mg of folic acid may signify delayed absorption, more rapid conversion to "bound" folic acid in the blood, rapid uptake by the tissues due to tissue desaturation, or an increased rate of excretion. The 24-hour excretions did not differ between those subjects whose serum level rose and those showing no increase. The small increase in the maximum level attained when the tolerance test was repeated following a period of 5 mg of folic acid per day suggests that unsaturation of the tissue is not the factor determining the height of the tolerance curves in these subjects. Therefore, the observed differences in maximum levels of folic acid in the serum following a standardized dose seem to reflect primarily variations in the rate of absorption.

The urinary excretions do not vary importantly from those found in other studies (6), and fail to account for most of the ingested dose. Activators and inhibitors present in some urines cause inaccuracies in the assay.

The data of this study indicate that there is no abnormality in the intestinal absorption of folic acid by patients with pernicious anemia. The fact that oral and parenteral folic acid are equally effective in treating pernicious anemia(7) supports this conclusion, as does the finding of Castle(8) that intrinsic factor did not enhance the hematopoietic effectiveness of oral folic acid in pernicious anemia.

The fact that peak levels were reached later and high levels maintained longer in the normal subjects than in the pernicious anemia patients suggests that the latter group removed free folic acid from the blood more rapidly. This could be due to a relative deficiency of folic acid and tissue desaturation (or more avid binding of folic acid in the

TABLE II. Change in the 1 mg Tolerance Test Produced by the Ingestion of 5 mg of Folic Acid Daily for 1 Week.

Subject	Peak level of serum folic acid reached after ingestion of 1 mg of folic acid	
	Original test, mγ/cc	Peak after 5 mg folic acid daily for 1 wk, mγ/cc
C	4	9
W	2.5	12
B	10	13
D	4	6
MeC	2	5
CD	7	8

blood). Such a theory is reinforced by the observation that 2 of the normal subjects given 5 mg of folic acid daily for a week showed more prolonged high levels and a later peak in the 1 mg tolerance test after this "saturating dose" than before.

From these limited observations, the oral folic acid tolerance test appears to be a useful tool in the study of folic acid metabolism. Additional studies of the level and duration of serum folic acid increases should provide evidence concerning the rate of folic acid absorption as well as the rate of its removal from the blood in suspected deficiency states.

Summary. An oral folic acid tolerance test is described and its limitations discussed. Evidence is presented that the test is useful in assessing the absorption of folic acid. No defect in the gastrointestinal absorption of folic acid in patients with pernicious anemia has been demonstrated.

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Thyroid Function in Hypophysectomized Mice Bearing Intraocular Pituitary Implants. (20012)

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There are conflicting reports(1-8) on the degree of secretory activity of the mammalian pituitary transplanted from its normal site. Most investigations were conducted with animals of unspecified genetic constitution. Since genetic heterogeneity may have affected the viability of the transplants, it was felt that a reinvestigation of the problem utilizing a genetically homogeneous group of animals might be of value. In the present experiments, intraocular pituitary transplants maintained a level of thyroidal radioiodine metabolism much higher than that of hypophysectomized controls but had no significant effect on body weight or on the weight of the thyroid, adrenals, ovaries, or uterus.

Materials and methods. BALB/c x C_3H mice were used in all experiments. As F_1 derivatives of 2 closely inbred lines, transplantation among them produces no incompatibility reaction and implants persist indefinitely. Two-month-old females were used as hosts except in Exp. VII, where 4-week-old females were implanted. In one experiment, not included in Table I, males were used as hosts. There was no indication of a sex difference in the response. Donors were embryos at the 14th or 15th day of gestation (Exp. I, II, VI, VII) or 1-2 day old newborn mice (Exp. III, IV, V) irrespective of sex. A single pituitary was transplanted into each animal except in Exp. IV, in which 4 pituitaries were implanted, 2 into each eye. The pituitaries were placed into the anterior chamber by a method previously described(9). Exp. VIII was designed to test the potency of adult pituitaries. Here 3-month-old females were donors and 1/3 pituitary was placed in each