EVALUATION OF ASSESSMENT OF FOLIC-ACID DEFICIENCY BY SERUM FOLIC-ACID ACTIVITY MEASURED WITH L. CASEI*

BERNARD A. COOPER, M.D., F.R.C.P.[C]†
and LOUIS LOWENSTEIN, M.D., F.A.C.P.,
Montreal

THE FOLIC-ACID activity of human serum may be assayed microbiologically using *L. casei*. This technique, adapted by Baker *et al.*[1] from that described by Usdin, Phillips and Toennies[2] and by Jukes,[3] has been reported to provide a reliable and convenient means of recognizing folic-acid deficiency in man.[1, 4] Because of conflicting evaluations of this technique which have been reported recently,[1, 4, 5] we have attempted to assess the reliability of this assay in detecting clinical folic-acid deficiency.

MATERIALS AND METHODS

Lactobacillus casei (A.T.C.C. No. 7469) was subcultured at weekly intervals in the maintenance medium recommended by Baker *et al.*[1]

The assay procedure was carried out exactly as described by Herbert,‡[4] with the following exceptions:

1. The assay was carried out in 20-ml. screw-capped culture tubes.
2. The serum-buffer was incubated for 90 minutes, as recommended by Baker *et al.*[1]
3. The inoculum was prepared by adding 1 ml. of a one-week culture of the organism to 10 ml. of maintenance medium, which then was incubated for six hours. (One ml. of this culture in 10 ml. of basal medium constituted the inoculum.)
4. Volatile preservative was added to the medium to a concentration of 1%. No preservative was added to the buffer. Prior to use, the medium was filtered through Whatman's No. 2 filter paper, to aid in complete removal of the preservative.
5. After incubation, the turbidity of the culture tubes was determined in a Coleman Model-14 spectrophotometer.

Crystalline pteroylglutamic acid was diluted as recommended by Baker *et al.*,[1] and kept frozen until use.

Blood was obtained in sterile syringes. After coagulation the blood was centrifuged at 1000 G., and the serum was collected and stored at –20° C. until assayed. All glassware, other than syringes, was cleaned in sulfuric acid chromate solution and then autoclaved.

*From the Hematology Service, Department of Medicine, Royal Victoria Hospital, and the McGill University Clinic, Montreal.
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†Medical Research Associate, Medical Research Council of Canada.
‡The reagents used in the medium were obtained from Fisher Scientific of Canada, Limited, or from California Corporation for Biochemical Research.

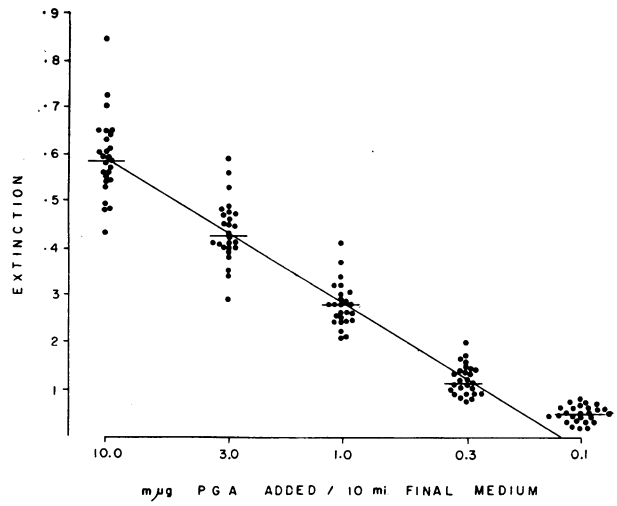

Fig. 1.—Growth response of *L. casei* to pteroylglutamic acid in assay medium.
Growth response (optical density) of *L. casei* in the assay medium containing different quantities of added pteroylglutamic acid. These represent duplicate standard curves of alternate routine assays over five months.

Serum vitamin-B_{12} level was determined in all patients by the technique of Mollin and Ross,[6] using *Euglena gracilis* var. *bacillaris*.

The diagnosis of pernicious anemia was confirmed by the Schilling test, using 0.5 μg. of Co^{58}-B_{12}, and 48-hour urine collection with two flushing injections, of 1000 μg., 24 hours apart, as described previously.[7]

RESULTS

As shown in Fig. 1, the growth response of the *L. casei* organism in the assay medium was proportional to the concentration of folic acid added, above 250 mμg. of folic acid per 10 ml. of medium. Since, in this procedure, the test serum is diluted 1/10, this would indicate that the lowest concentration of folic-acid activity which can be determined quantitatively in serum by this technique is 2.5 mμg./ml.

In Table I, the serum folic-acid activity determined by this technique is compared to the clinical assessment of deficiency in 100 patients. Folic-acid deficiency was recognized by the presence of a megaloblastic bone marrow, with a history of deficient diet, and subsequent response of the bone marrow to hospital diet or to the intravenous injection of a small dose of folic acid. The

TABLE I.—CORRELATION OF SERUM FOLIC-ACID ACTIVITY AND CLINICAL DEFICIENCY

Serum folic-acid activity (mμg./ml.)	*Numbers of patients*		
	Normoblastic	*Megaloblastic Folic-acid deficiency*	*Pernicious anemia*
≥6.0	43	—	9*
4.5 - 5.9	19†	—	6
3.0 - 3.9	6	—	—
<3.0	3	12	2

*One patient with nutritional vitamin-B_{12} deficiency included.
†One patient with rare macrogranulocyte in bone marrow (see text).

single, small, therapeutic dose used was 15 μg. of folic acid per kg. of body weight, and in the six folic-acid-deficient patients so studied it induced conversion of the bone marrow from megaloblastic to normoblastic within 72 hours. In three patients with vitamin-B_{12} deficiency and normal serum folic-acid levels, this dose of folic acid did not alter the megaloblastic appearance of the bone marrow.

As shown in Table I, the serum folic-acid activity of all patients with clinically recognized folic-acid deficiency was below 3.0 mμg./ml. Three patients with normoblastic bone marrow showed apparently deficient levels. Two of these had disseminated malignant neoplasm, and their folic-acid intake had been poor. The third patient was admitted with a history of gross malnutrition but with normoblastic bone marrow.

Only one patient was found to have bone-marrow changes suggestive of megaloblastic anemia, without abnormally low serum levels of vitamin B_{12} or of folic-acid activity. This patient was admitted to hospital with marked iron deficiency (serum iron 7 μg. per 100 ml. of blood, unsaturated iron-binding capacity 412 μg. %) secondary to acute and chronic gastrointestinal hemorrhage. The hemoglobin value was 7.3 g. % and the reticulocyte count 6.4%. Serum vitamin-B_{12} level was 548 μμg./ml., and serum folic-acid activity 5.1 mμg./ml. Bone marrow showed marked erythroid hyperplasia which was normoblastic in appearance, but rare giant macrogranulocytes were found. The patient gave no history of deficient dietary intake, and made a complete recovery on iron therapy alone.

Two of the patients with pernicious anemia were found to have low serum folic-acid levels. Both were elderly women with complaints of vomiting and anorexia for one to three months, and one complained of diarrhea also. One of these patients was treated initially with vitamin B_{12}, with clinical improvement but with suboptimal reticulocytosis and a slow recovery of hemoglobin level. The other responded partially to hospital diet, but residual megaloblastic changes in the bone marrow did not disappear until she received injections of cyanocobalamin.

DISCUSSION

It would appear that the technique described for the microbiologic assessment of folic-acid deficiency, using *L. casei*, correlates well with clinical deficiency. We have found this assay to be reliable for measuring folic-acid activity above 2.5 mμg./ml. of serum. The relatively poor growth curves with added folic acid reported by Cooperman, Luhby and Avery[5] need not be interpreted as indicating that the assay is insensitive to folic-acid activity.

The clear separation between deficient and non-deficient patients by the level of 3.0 mμg./ml. would justify the use of this value to differentiate between patients with and those without clinical folic-acid deficiency.

All patients with serum values below 3.0 mμg./ml. almost certainly had folic-acid deficiency; it may be that some of those with slightly higher values but without megaloblastic marrow were similarly deficient. A number of patients in the latter group had metastatic carcinoma or gross malnutrition, but they could not be differentiated from normal subjects by single determinations of serum folic-acid activity. Serial determinations might have demonstrated a developing deficiency. Thus, with the data available, the lower limit of the normal range of serum folic-acid activity cannot be defined precisely.

Recent work from Israel[8] suggests that, using *L. casei*, the assay of folic-acid activity in whole blood may be somewhat superior to the serum assay as a measure of folic-acid deficiency. If this work is confirmed, the whole-blood method may permit a sharper distinction of the lower limit of the normal range than does the serum method.

SUMMARY

Serum folic-acid activity, determined with *L. casei*, differentiates between normal subjects and patients with clinical folic-acid deficiency. All patients with megaloblastic anemia due to folic-acid deficiency were found to have serum values below 3.0 mμg. per ml. by this technique.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

Sir James Paget, in his presidential address before the Pathological Society of London in 1887, says, "Surely, it would be hard to name a discovery in biology which more deserves the name of scientific than does Jenner's discovery of vaccination; and yet it was made in the plainest, practical manner while he was a country practitioner. But, observe, Jenner was a thorough naturalist, trained by John Hunter; and I suspect that all the best advances in clinical pathology, the best not only in their utility, but in their fitness for adjustment among the largest principles of our science, have been made by practitioners who were either by nature or by cultivation men of scientific mind. And it is as sure as anything of the kind can be that similar studies by men of similar mind will still attain as good results."—A. Primrose: Address in Surgery, *Canad. M. A. J.*, 1: 610, 1911.