vision(6), calls a "short latent period" response, the prompt 2- to 3-fold increase in mitotic rate typically seen in tissues with normally high mitotic index upon exposure to various stimuli which speed up energy metabolism.

No attempt has been made, as yet, to identify the agent in serum responsible for its demonstrated marrow-stimulating activity. Perhaps an adrenal hormone, released as part of the body's response to "stress" of acute infection, constitutes the circulating marrow stimulant. It has been demonstrated, for instance, that cortisone may have a myelopoietic effect on bone marrow(7).

However, most earlier studies have indicated that substances responsible for leukocytosis accompanying acute inflammation are produced at the inflammatory site itself(1,8). In our study, this substance might be a product of either the infecting bacteria, disintegrated leukocytes, or the inflamed tissues. Failure to demonstrate a direct marrow-stimulating activity of the exudate itself does not rule out any of these possibilities. The contaminated purulent exudates in our experiments might contain a high enough concentration of substances toxic to marrow cultures to obscure the effect of any marrow-stimulating factor; or it is possible that, *in vivo,* such a locally-produced substance might act on marrow only *indirectly,* through the endocrine system. Such an indirect action of the exudate would, of course, not be demonstrable in our tissue culture system.

Summary. Serum from acutely infected rats produced a 2- to 3-fold increase in mitotic index of rat marrow cells in short-term tissue cultures. These results suggest that a serum-borne marrow-stimulating substance may play a part in producing the characteristic peripheral granulocytosis which normally accompanies acute infection.

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Histidine Metabolic Loading Test to Distinguish Folic Acid Deficiency From Vit. B_{12} in Megaloblastic Anemias.*† (24936)

A. LEONARD LUHBY, JACK M. COOPERMAN AND DAVID N. TELLER
Dept. of Pediatrics, N. Y. Medical College, N. Y. City

In the past, differentiation of clinical deficiency of folic acid from that of Vit. B_{12} in megaloblastic anemias has depended upon extended therapeutic trials with Vit. B_{12} and folic acid. Failure of hematologic response to B_{12}, but a typical reticulocyte and red blood cell rise following minute doses of folic acid indicated folic acid deficiency(1). Formiminoglutamic acid (FIGLU) has been shown to accumulate in urine of patients with folic deficiency but not in uncomplicated Addisonian pernicious anemia or normal subjects

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(2,3). Because many patients with megaloblastic anemia and suspected folic acid deficiency did not excrete significant quantities of urinary FIGLU, it was reasoned that differences in FIGLU excretion by folic acid deficient individuals may vary with dietary intake of protein containing histidine, the amino acid from which FIGLU normally arises. A histidine metabolic loading procedure was thus devised to equalize the quantity of histidine presented for metabolism in all individuals. This uniformly permitted differentiation of clinical folic acid deficiency from that of Vit. B_{12}. Details of this test are reported herein.

Materials and methods. Patients with various types of megaloblastic anemia were studied. Diagnosis of megaloblastic anemia was established in each case by bone marrow megaloblastic erythroid and myeloid pattern, peripheral blood macrocytic anemia, hypersegmented macropolymorphonuclear leucocytes and, often, thrombocytopenia. In non-pernicious megaloblastic anemia, gastric acidity was usually normal, and evidence of combined system (CNS) disease was always absent. Serum Vit. B_{12} level and gastrointestinal absorption of radioactive Vit. B_{12} was normal except in macrocytic anemia associated with sprue or malabsorption syndromes, in which they were decreased. Megaloblastic anemia of Addisonian pernicious anemia type in relapse was characterized by histamine refractory gastric achlorhydria, low serum Vit. B_{12} level and markedly decreased gastrointestinal absorption of radioactive Vit. B_{12}, which improved following administration of Vit. B_{12} with intrinsic factor concentrate. Whenever possible, patients with non-pernicious megaloblastic anemia were given therapeutic trials with 10-25 γ parenteral Vit. B_{12} followed by 1-1.5 mg parenteral folic acid. They invariably made a typical response to folic acid but not to Vit. B_{12}(1). Pernicious anemia in relapse did not respond to 1-1.5 mg folic acid, but responded dramatically to 10 γ parenteral Vit. B_{12}. Control subjects included children of different ages, adults and pregnant women at various stages of gestation. A metabolic load of L-histidine monohydrochloride, 15 g daily, was given in 3 divided doses one-half hour before meals in apple juice or water for 2 to 3 days. Urine was collected under acid as previously described(3). Urinary FIGLU was measured by modification of enzyme technic of Tabor & Wyngarden(4).

Results. After 48-72 hours on this metabolic loading procedure, patients with non-pernicious megaloblastic anemias listed in Table I had urinary FIGLU excretion rang-

TABLE I. Urinary FIGLU Excretion following 48-72 Hr Histidine Metabolic Loading.

	No. of patients	Urinary FIGLU γ/ml	Urinary FIGLU mg/24 hr
Non-pernicious megaloblastic anemia:			
Nutritional macrocytic anemia	12	125– 750	250– 550
Macrocytic anemia of pregnancy	14	90– 875	225– 600
Macrocytic anemia associated with sprue or malabsorption syndromes	10	100– 920	185– 675
Megaloblastic anemia of infancy	3	125–1909	625–2047
Macrocytic anemia associated with liver disease	6	300–1052	300–1850
Addisonian pernicious anemia in relapse:	18	2–30	1.5–35
Non-megaloblastic anemia:			
Iron deficiency anemia	7	1.0–20	1.7–24
" " " of pregnancy	9	1.2–23	1.9–28
Acute leukemia	8	.5–15	.3–16
Chronic "	2	.3, 20	.6, 25
Aplastic anemia	2	.1, 7.1	.9, 8.2
Hodgkins disease	1	13.4	13.3
Chronic hemolytic anemia	3	3.1– 7.2	5.5–11
Anemia associated with gastrointestinal malignancy	6	1.1–28	1.5–30
Control subjects:			
Normal children	8	.1–10	1.9– 7.2
" adults	25	.1–28	.5–30
" pregnant women	10	.5–25	1.2–26

ing from 90 to 1909 γ/ml; final 24-hour output varied from 185 to 2047 mg. In sharp contrast, patients with uncomplicated Addisonian pernicious anemia in relapse, following identical metabolic loading, excreted only 2-30 γ FIGLU/ml urine; final 24-hour urinary FIGLU output ranged from 1.5 to 35 mg. In fact, most such patients rarely achieved FIGLU urinary concentration at 72 hours of 18 γ/ml and 24-hour excretion rarely over 15 mg, values well within the normal range.

Patients with non-megaloblastic anemias and other disorders listed in Table I, when subjected to histidine metabolic loading procedure outlined, showed urinary FIGLU concentration ranging from 0.1 to 28 γ/ml. The final 24-hour output varied from 0.6 to 30 mg. Control subjects, as well as pregnant women at various stages of gestation without apparent disease or hematologic abnormality, after the histidine metabolic loading described, had urinary FIGLU concentration ranging from 0.1 to 28 γ/ml and 24-hour output ranging from 0.5 to 30 mg. Most normal subjects had urinary FIGLU concentration of 0.3 to 5 γ/ml; a 24-hour output of 1-4 mg.

Discussion. It is apparent from our data that individuals with non-pernicious megaloblastic anemia due to folic acid deficiency, as judged from hematologic response to minute doses of folic acid, but refractory to parenteral Vit. B_{12}, excrete from 3 to over 1000-fold the quantity of FIGLU excreted by patients with Addisonian pernicious anemia, in which deficiency is that of Vit. B_{12}. Subjects with other anemias and disease in which no therapeutic benefit to folic acid could be demonstrated, and hence in whom no folic acid deficiency existed, also excreted decidedly normal amounts of FIGLU after this standardized histidine metabolic load.

Increased urinary FIGLU excretion after histidine loading, as described herein, reflects biochemical insufficiency of folic acid cofactors(5,6,7) in such patients. The test thus offers a biochemical index for diagnosis and assessment of clinical folic acid deficiency and a means of differentiating megaloblastic anemias due to folic acid deficiency from those due to Vit. B_{12} deficiency, a problem of considerable clinical importance for which no direct method of this short duration was previously available.

The 15 g histidine monohydrochloride metabolic load was arrived at after many trials with various other dosages. At this level, no patient with uncomplicated pernicious anemia in relapse tested to date showed above normal excretion. All patients with other types of megaloblastic anemia tested showed an excretion value sufficiently above normal to be unequivocal. In fact, certain individuals with minimal megaloblastic changes, in which folic acid deficiency could at best be classified as "borderline" or "subclinical" were distinctly diagnosable. Normal individuals as well as those with megaloblastic anemia due to uncomplicated pernicious anemia could not be induced to excrete a urinary concentration of FIGLU above 30 γ/ml until loading doses of 36 to 45 g per day were administered. Salts of histidine or an equivalent amount of free base are equally effective.

Summary. 1) A new procedure for diagnosis of clinical folic acid deficiency is described which distinguishes it from Vit. B_{12} deficiency. This permits differentiation of Addisonian pernicious anemia in relapse from megaloblastic anemia due to folic acid deficiency. 2) The procedure is based upon oral administration of 15 g of L-histidine monohydrochloride for 48-72 hours and measurement of urinary concentration or 24-hour excretion of formiminoglutamic acid, which accumulates in folic acid deficiency. Under these conditions, urine FIGLU levels do not exceed 30 γ/ml in non-folic acid deficient individuals; are 3 to 1000 fold greater in folic acid deficiency.

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