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The Hematologic Effect of Folinic Acid (Citrovorum Factor) in Persons with Pernicious Anemia[1]

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A factor in refined liver extract necessary for the growth of *Leuconostoc citrovorum* has been isolated by Sauberlich and Baumann (*1*). The growth-promoting properties of this factor cannot be replaced by vitamin B_{12} or thymidine, but growth of the organism will occur in the absence of this factor if very large amounts of folic acid are provided. Subsequently, Sauberlich (*2*) demonstrated that "citrovorum factor" overcomes the inhibitory effect of aminopterin on *Leuconostoc citrovorum*. The excretion of the "citrovorum factor" in the urine of rats or human beings is enhanced by the administration of folic acid (*3*). Nichols and Welch (*4*) have shown that "citrovorum-factor" activity of liver slices from normal and folic-acid–deficient rats is increased by incubation with folic acid, and that ascorbic acid enhances this effect. These data indicate that folic acid is a precursor of the "citrovorum factor" and suggest that ascorbic acid plays a part in the conversion.

Bond, Bardos, Sibley, and Shive (*5*) isolated a substance called "folinic acid" which overcomes the inhibition of methyl folic acid on the growth of *Lactobacillus casei* more effectively than folic acid. This substance is similar to the "citrovorum factor" in promoting the growth of *Leuconostoc citrovorum*. The same workers (*6*) have described a method for the synthesis of a substance with properties similar to "citrovorum factor" and "folinic acid," and it is probable that these two substances are identical.

May *et al.* (*7*) have reported that crystalline folinic acid is more effective than folic acid in relieving the megaloblastic anemia of monkeys deficient in folic and ascorbic acids. This observation, together with the work of Nichols and Welch mentioned above (*4*), suggests that folinic acid may be a biologically important intermediate in the metabolism of folic acid.

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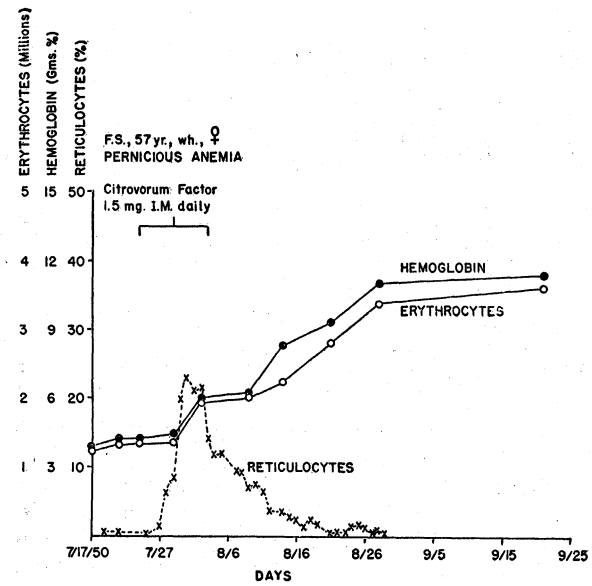

FIG. 1. Hematologic response to citrovorum factor (folinic acid) in patient with pernicious anemia in relapse.

There is evidence that folic acid may be converted to a metabolically active form in persons with pernicious anemia in relapse before it exerts its hematopoietic effect (*8–10*). Such a substance should induce a hematologic effect in doses much smaller than those usually required with folic acid. Since "folinic acid" has the potentiality of being this active form of folic acid, its hematopoietic effect has been tested in persons with pernicious anemia in relapse.

Three such subjects have received intramuscular injections of this substance for 10 consecutive days; the daily dose in 2 of the subjects was 3 mg and in the other, 1.5 mg. In all 3 patients hematologic responses occurred. Erythrocyte and hemoglobin rises were as good as would be expected with similar amounts of folic acid. Reticulocytes increased in all three instances, with peaks of 9%, 13.7%, and 23.1% on the sixth or seventh days of treatment. The megaloblastic bone marrow was converted rapidly to a normoblastic type, as happens after oral or parenteral treatment with folic acid. One of the hematologic responses is recorded in Fig. 1.

Folinic acid was no more effective than folic acid, however. One of the subjects who responded to the daily administration of 3 mg had previously failed to respond to 0.6 mg of the substance daily for 10 days. Another subject with pernicious anemia, previously responsive to refined liver extract, vitamin B_{12}, and folic acid, had relapsed hematologically while receiving 20 mg of folic acid daily. He was given 3 mg of "citrovorum factor" daily for 10 days. There was no reticulocytosis, and erythrocytes and hemoglobin failed to rise following this therapy. Subsequently, he was given vitamin B_{12}, 15 μg daily for 3 weeks and then 20 μg weekly. There was a desultory hematologic response similar to that previously described in similar subjects (*9*).

Direct instillation of "citrovorum factor" into the bone marrow cavity was performed in 3 persons with pernicious anemia in relapse; the amounts used were 0.06 mg, 1.5 mg, and 3 mg, respectively. In no instance was there evidence on Wright-Giemsa–stained marrow smears of the erythrocyte maturation effect that was observed locally after marrow instillation of vitamin B_{12} (*8*) but which did not occur after the instillation of 1 or 2 mg of folic acid into the marrow. In this respect, also, folinic acid is similar to folic acid.

This study demonstrates that "folinic acid" or "citrovorum factor" is a potent hematopoietic agent in pernicious anemia in relapse, but is no more effective than a similar dose of folic acid. The failure of the substance to produce a local erythrocyte maturation effect on instillation into the marrow cavity suggests that "citrovorum factor" or "folinic acid," like folic acid (*10*), must be altered elsewhere in the body before becoming active in hematopoiesis.

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Otitis Media and Audiogenic Seizures in Mice[1]

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Infection of the middle ear in rats has been shown to be a factor in the occurrence of audiogenic seizures (*1–3*). The original report by Patton (*2*) stressing the complications that might thus arise in using the incidence of seizures in rats as an index of nutritional deficiency has, however, been misinterpreted by some workers. They seem to believe that Patton proved that purulent otitis media is the only and sufficient cause of audiogenic seizures. Patton, however, stated that his observations "do not define the role of middle ear disease in the etiology of sound induced seizures," and that "the infection has not complicated the severe sound induced seizures associated with specific deficiencies, e.g., magnesium. . . ." Sound-induced seizures in rats are thus possible without concomitant otitis media (*1–3*). As Pilgrim and Patton (*3*) state, "The precise relationships between convulsions and the infection have not yet been elucidated."

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Laboratory mice exhibit audiogenic seizures similar to those of rats, and mice may be better than rats as test animals for certain purposes (*4*). Before mice are widely used for tests of auditory reactions, however, it seems advisable to determine the relationship, if any, between otitis media and seizures in these animals.

Mice of three strains were used in this study: dba Subline 1, C-57 black, Subline 6, and mongrel albino, so-called Swiss. The first two were obtained originally from the market stock of The Jackson Memorial Laboratory, Bar Harbor, Maine, the third from an animal dealer. All individuals used in our experiments were reared in our laboratory.

Seizures were induced by subjecting mice imprisoned in a small wire-mesh cage to a sound field at 10 kc frequency and 110 db average sound pressure. The animals were tested daily from 15 to 50 days of age. Mice of all three strains are susceptible during some part of this period. Details of the apparatus and procedures have been published elsewhere (*5*). The occurrence of otitis media was determined by autopsy carried out under a binocular dissecting microscope. The bulla and tympanum were exposed and penetrated, and the middle ear was carefully examined for inflammation and pus.

The mice autopsied were of the following classes: (1) animals which either had no seizures during the test period or had seizures during the early part of the period (20–30 days of age) but stopped having seizures at least 10 days before the examination; and (2) animals which died as a result of clonic-tonic seizures. The second group certainly includes the most susceptible mice in the colony. In the first group (controls) were 70 albinos, 10 dba's, and 10 C-57's; in the second group were 131 albinos, 53 dba's, and 16 C-57's. Approximately half the mice in each group were males and half females, and they varied in age from 18 to 50 days, most of the animals that died being 20–30 days old.

No case of otitis media was found in the control group, and only one case of the disease was found in animals dying in seizure, a unilateral infection in a dba. One other case appeared during the study. An albino which had only 2 seizures, at 20 and 21 days of age, developed, at 41 days of age, definite symptoms of middle ear infection and labyrinthitis, holding the head to the side and swinging in a circle when held by the tail. Dissection of the middle ear, when the mouse was 42 days old, confirmed the diagnosis. Since this animal had a low seizure record, it seemed advisable to examine animals with similar records but without clinical symptoms of the disease. Twenty albino mice with similar records were examined, and no case of otitis media was found.

It is obvious that the incidence of otitis media in our colony of laboratory mice is very low. This accords well with the report of Caussé (*6*) that otitis media is found in at most 1% of white mice. The low incidence found here is matched by that discovered quite independently for dba's and C-57's by Miller and