hold true when stimuli other than endotoxin are used to produce EP. Third, the semiquantitative data obtained by testing EP—or any other substance employed in pyrogen assays—improves the accuracy of these assays and yields more meaningful information for comparison of different pyrogenic substances. Fourth, providing it is clearly defined, the concept of the minimum pyrogenic dose is useful in standardizing the pyrogenic response, and to compare data from different laboratories.

It should be realized that, as a form of bioassay, fever is a relatively gross measurement and depends on many variables inherent in the recipient, including the species of animal employed(5). It seems unlikely that differences between individual recipients or even within the same recipient will ever be eliminated. For this reason alone, attempts to control the procedures employed in this assay are indicated.

Summary. A semiquantitative assay for endogenous pyrogen in serum is described. The test depends on using at least 3-4 recipients at at least 4 ranges of dosage, and the use of the fever index rather than height of the fever curve. This permits construction of a dose response curve which delineates the sensitive range of pyrexia. The minimum pyrogenic dose can then be calculated and should serve as a useful standard for comparison of different pyrogenic substances.

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Received September 2, 1964. P.S.E.B.M., 1964, v117.

Folic Acid Deficiency and Hepatic DNA Synthesis.* (29685)

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Clinical evidence of folate deficiency consisting of a low serum folic acid(1), increased urinary excretion of formiminoglutamic acid (2), and macrocytic anemia(3) are frequently found in cirrhosis. This deficiency appears to result principally from inadequate intake coupled with decreased hepatic storage of this vitamin(4). The demonstration that folic acid is required for cell proliferation(5,6) suggests the regenerative process may contribute to encountered deficits and that folate depletion may interfere with hepatic DNA synthesis and regeneration. The present study was undertaken to assess the influence of liver regeneration on tissue levels of folic acid and folinic acid, and to determine the effect of folic acid depletion on hepatic DNA synthesis.

* Supported by grants from U.S.P.H.S., N.I.H., and National Vitamin Foundation.

Materials and methods. Three hundred weanling Sprague-Dawley rats received a folic acid deficient diet, which was normal in all other basic constituents. At the end of 2 months, by a pre-determined randomized schema, litter mates were each treated with 30 μg folic acid,† 30 μg folinic acid,† 100 μg of vit. B_{12},†, 50 mg uridylic acid,‡ 50 mg thymidilic acid,‡ or 1.0 ml normal saline daily for 10 days. Animals were given 0.2 ml of saline or CCl_4 per 100 g weight orally, and killed by decapitation 12, 24, 48, 72, 96, or 120 hours later. Four hours prior to killing, animals were given 1 μc per g weight of tritiated thymidine (sp. activity 1.9 c per mmole)§(7), intraperitoneally.

† Furnished by Dr. J. M. Ruegsegger, Lederle Laboratories.

‡ Obtained from Calbiochem, New York.

§ Purchased from Schwarz BioResearch, Inc., New York.

At time of killing, the liver was rapidly removed and washed with saline, until free of debris and blood. In selected animals, blood was obtained by aortic puncture for measurement of serum folic acid and folinic acid. A section of liver was removed for light microscopy and autoradiography, and the rest frozen for measurement of tissue protein, folic acid, and folinic acid. For histologic examination, the liver was fixed in 10% formaline, a paraffin block prepared, and routine sections stained with hematoxylin and eosin. Sections of 6 to 8 μ were dipped in NTB_3 Kodak emulsion, allowed to dry, and exposed in a light-tight box at 10°C for 23 days. The autoradiographs were developed in Kodak D-19 developer for 2 minutes, fixed in Kodak fixer, and washed in cool running tap water for 10 minutes. They were counterstained with Harris hematoxylin and mounted in polyvinyl pyrrolidine. The number of labeled nuclei, exclusive of inflammatory cells, was counted on each autoradiograph as an estimate of hepatic DNA synthesis. The total number of liver cells was determined and labeled nuclei expressed per 100,000 liver cells. Frozen specimens of liver were homogenized and lyophilized, and assayed for folic acid activity using *Lactobacillus casei*(8), for folinic acid activity using *Pediococcus cerevisiae*(8), and nitrogen using Coleman Nitrogen Analyzer, Model 29(9).

Results. Twelve hours after receipt of CCl_4, conspicuous centrilobular necrosis was present without change in the number of labeled cells or mitoses. Centrilobular necrosis was more advanced at 24 hours and accompanied by a 10-fold increase in labeled cells(10). At 48 hours, a diminution of the necrotic reaction, a 150- to 200-fold increase in labeled cells, and an 80- to 100-fold increase in mitoses, was noted. The necrotic reaction subsided and the number of labeled cells and mitoses returned to pre-treatment levels by 120 hours. Differential counts of labeled cells showed a 4:1 mesenchymal and ductular cell to liver cell ratio in the normal liver; this ratio increased to 10:1 at 24 hours and became 1:1 at 48 hours.

The necrotic liver exhibited a 45% decrease in folic acid and 66% decrease in

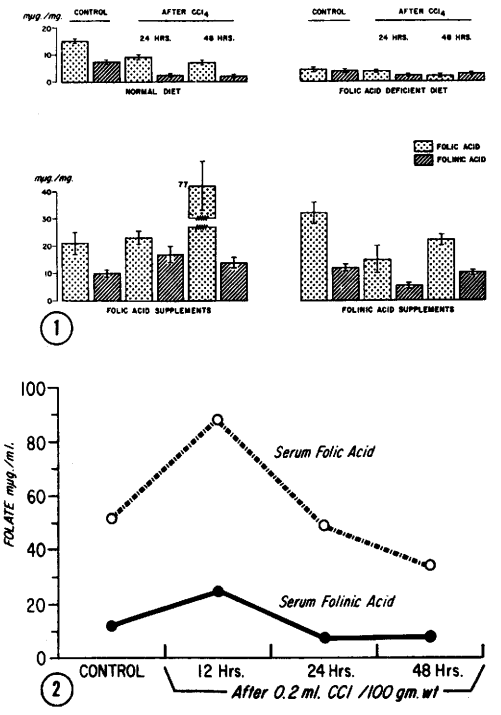

FIG. 1. Effect of experimental (CCl_4) liver injury and dietary deficiency on liver folate.
FIG. 2. Effect of experimental (CCl_4) liver injury on serum folic acid and folinic acid.

folinic acid content when related to dry weight or adjusted for protein content. Folate levels in the liver decreased further during regeneration (Fig. 1). Both folic acid and folinic acid supplements increased tissue folate levels during maximum necrosis and regeneration; in contrast neither vit. B_{12}, uracil, nor thymidine altered folate levels. Reduced hepatic folate during maximum necrosis was preceded by a marked increase in serum levels of both folic acid (60%) and folinic acid (100%), 12 hours earlier (Fig. 2). Further lowering of tissue folate was accompanied by decreased serum folic acid while serum folinic acid remained unchanged.

Folate deficiency induced by combined dietary deficiency and liver injury decreased both the number of labeled cells and mitoses. Mesenchymal, ductular, and liver cell replication were equally reduced. Folate-depleted animals, not given folic acid, folinic acid, or thymidilic acid, had a 50% mortality at 48 hours and negligible survival rate after 72 hours. Neither labeling nor mitotic counts

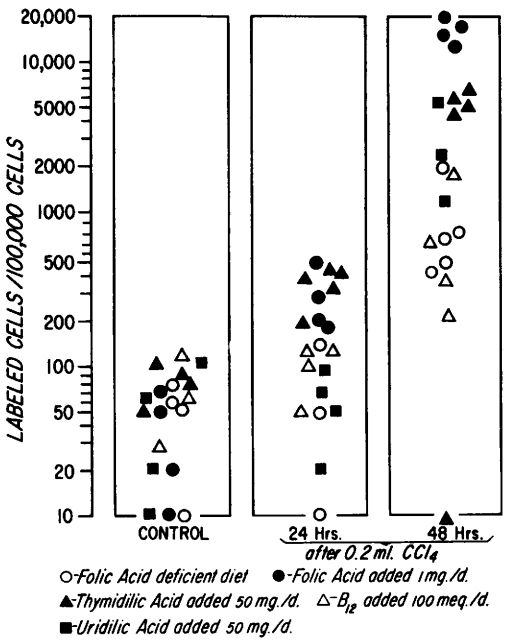

FIG. 3. Effect of dietary-induced folate deficiency on hepatic DNA synthesis after receipt of .2 ml of CCl_4 per 100 g weight.

were influenced by administration of vit. B_{12} or uracil, whereas, thymidine caused a significant increase in both (Fig. 3). Folic acid and folinic acid supplements returned labeling patterns to normal in folate-depleted animals but had no effect on folate-repleted litter mates.

Comment. Previous studies in our laboratory show an increase in hepatic folic acid conjugase and reductase activity during liver regeneration(11). The present data indicate that liver cell necrosis causes release of folate and liver regeneration increases its utilization; these events may produce significant degrees of folate deficiency. Folate depletion produced by liver injury in the setting of dietary deficiency interferes with hepatic DNA synthesis and regeneration, and may perpetuate liver injury. Similar observations have been made in folate-deficient man, using an *in vitro* technique for studies of hepatic DNA synthesis in percutaneous biopsies(12, 13).

A study of the differential effects of vit. B_{12}, uridylic acid, and thymidilic acid on incorporation of tritiated thymidine into DNA confirms the key role of folate in this process. The present study indicates that vit. B_{12}, an activator of serum folic acid coenzymes(14), has no effect on hepatic DNA synthesis with marked reduction in tissue folate. The lack of responsiveness to uridylic acid and the significant increase in labeling with thymidilic acid provides evidence that folate facilitates conversion of uridylic acid to thymidilic acid in mammalian systems(6).

Summary and conclusions. Release of folate from necrotic liver cells and its utilization during regeneration is responsible for reduction in liver content of folic acid and folinic acid in carbon tetrachloride-induced liver injury. Superimposition of hepatic necrosis on dietary-induced folate depletion interferes with DNA synthesis and prevents or delays regeneration. Thymidine facilitates hepatic DNA synthesis in the presence of folate depletion, whereas, uracil and vit. B_{12} have no effect.

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