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Conversion of Diopterin and Teropterin into Folic and Folinic Acid Activity by the Rat Liver.* (28514)

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The synthetic folic acid conjugates pteroyl-alpha-glutamylglutamic acid (Diopterin) and pteroyl-gamma-glutamyl-gamma-glutamylglutamic acid (Teropterin), respectively, have been effective in treating the megaloblastic anemia of sprue(1) and pernicious anemia (2). Such hemopoietic effects probably result from conversion of these conjugates into folic acids active for man(2). Although Diopterin (1) and Teropterin(3) are partially excreted in the urine as free folic acid, neither the sites of conversion nor the exact nature of the resulting products are known.

The present studies were therefore carried out to determine the liver's ability to transform Diopterin and Teropterin into products having folic and folinic acid activity and to see if differences in structure of the two molecules cause one to be more effectively converted than the other. For comparison purposes, the reduction of pteroylglutamic acid (PGA) to folinic acid was studied according to the same protocol.

Materials and methods. Three groups of Sprague-Dawley rats, each containing 3 animals, were maintained on Purina Laboratory Chow. The livers of all rats were removed and perfused in an apparatus adapted from the one described by Miller(4). One liver from each group was perfused from a reservoir of 200 ml heparinized blood containing a starting concentration of either Diopterin (6.4 μg per 100 ml) Teropterin (7.3 μg per 100 ml), or PGA (5.0 μg per 100 ml). Blood flow was maintained at a constant rate of 60 drops per minute. The minimum bile flow was 0.2 ml per hour. Blood samples were collected, 15, 45, 75, and 105 minutes from the beginning of perfusion from a filter proximal to the liver inflow cannula. At the end of each perfusion the liver was placed in formalin; and histologic sections, stained with hematoxylin and eosin, were prepared. Blood was diluted 1:1 with 0.05 M sodium phosphate buffer. The blood-buffer solution was autoclaved for 10 minutes at 118°C and then centrifuged for 5 minutes at 3000 rpm to remove coagulum. Clear supernatant was removed and used for folic acid activity assay with *Lactobacillus casei* and *Streptococcus faecalis* and for folinic acid activity assay with *Pediococcus cerevisiae*(5). Conversion of Diopterin to folic acid activity was expressed as per cent increase of *L. casei* activity in perfusing blood. For Teropterin this same calculation was made using *S. faecalis* activity, since this conjugate is 60 to 80% as active when assayed with *L. casei* but only 2 to 6% as active when assayed with *S. faecalis* as is PGA itself(6). Conversion of PGA, Diopterin, and Teropterin to folinic acid activity was quantified as per cent increase of *P. cerevisiae* activity in perfusing blood. Increase of activity in each case was expressed as percentage of starting concentration. Diopterin and Teropterin were assayed *in vitro* in the concentrations employed in these experiments using both *L. casei* and *S. faecalis*. Diopterin supported growth of neither organism. Teropterin permitted growth of *L. casei*, as expected, but did not permit growth of

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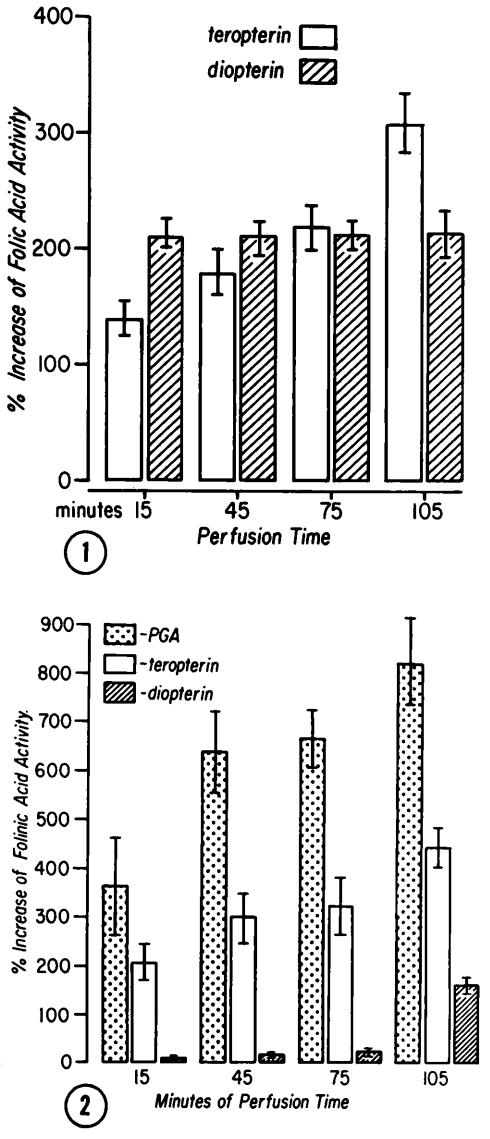

FIG. 1. Per cent increase of folic acid activity in perfusates containing starting concentrations of either Teropterin (7.3 μg/100 ml) or Diopterin (6.4 μg/100 ml).

FIG. 2. Per cent increase of folinic acid activity in perfusates containing starting concentrations of either PGA (5 μg/100 ml), Teropterin (7.3 μg/100 ml), or Diopterin (6.4 μg/100 ml).

S. faecalis. The possibility is therefore excluded that either conjugate was significantly contaminated with pteroylglutamic acid.

Results. In Teropterin-containing perfusates folic acid *(S. faecalis)* activity reached a maximum level, 310% (SD ± 50) of initial concentration, at 105 minutes. Dioptern perfusates attained maximum folic acid *(L. casei)* activity, 212% (SD ± 26), in 15 minutes; subsequent small increases observed were not significant (Fig. 1).

PGA, Teropterin, and Diopterin each caused a progressive rise in folinic acid *(P. cerevisiae)* activity to 105 minutes; increases being respectively 830% (SD ± 182), 449% (SD ± 79), and 165% (SD ± 32) of initial concentration (Fig. 2).

Histologic sections of all perfused livers were entirely normal by light microscopy.

Discussion. These studies delineate the liver as a site for conversion of Diopterin and Teropterin into products having folic and folinic acid activity. They do not exclude the possibility that such conversion of these conjugated folic acids occurs in other tissues as well. Of the two conjugates Teropterin appears to be the better precursor of folic acid and is obviously the more effective antecedent of folinic acid. Neither has as great folinic acid conversion potential as PGA (Fig. 2).

It has been shown that N^5-methyl tetrahydrofolate(7) and the apparently identical "prefolic A"(8) support the growth of *L. casei* but not of *S. faecalis*. If this coenzyme form of folic acid was elaborated during the course of Teropterin perfusions its presence would not have been detected. It is therefore possible that folic acid activity derived from Teropterin may have been even somewhat higher in relation to folic acid activity derived from Diopterin than was demonstrated in these experiments.

It seems probable that Teropterin's molecular structure makes it a better substrate than Diopterin for liver folic acid conjugases and reductases. One may assume that because Teropterin is conjugated in the gamma position, unlike Diopterin which is conjugated in the alpha position, the liver enzymes handle it with greater facility.

Summary. Isolated rat livers were perfused with blood containing equimolar concentrations of PGA, Diopterin, and Teropterin. Both conjugates gave rise to folic and folinic acid activity. Teropterin, a gamma-conjugate, was a more effective precursor for folinic acid than Diopterin, an alpha-conjugate.

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Liver Nonprotein Sulfhydryl in Vitamin B_6-Deficient Rats: Effects of Age, Feeding, and Fasting.* (28515)

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Hsu *et al*(1,2) reported that liver and erythrocyte glutathione increased in Vit. B_6-deficient rats. In contrast, Williams *et al*(3) found that Vit. B_6-deficient rats fed an amino acid diet showed a small but significant decrease in liver nonprotein sulfhydryl (NPSH), which is principally glutathione(4). Possible reasons for the apparent differences in the reported effect of Vit. B_6 deficiency on liver glutathione are the age of the rats, composition of the diet, and type of control used for comparison. The Vit. B_6-deficient rats of Williams *et al* were 6-7 weeks old, were fed an amino acid diet containing 1.5% nitrogen, 0.56% methionine, and 0.22% cystine, compared with pair-fed controls, and not fasted before the analyses. The deficient rats of Hsu *et al*(1) were fed a casein-sucrose diet, presumably were compared with *ad libitum*-fed controls, and may have been fasted(5).

Since the metabolism of pair-fed controls may vary considerably from that of *ad libitum*-fed animals(6,7), it was possible that the NPSH and glutathione content of the pair-fed controls might be greater than that of the *ad libitum*-fed controls. Consequently, the concentration of these compounds in the liver in Vit. B_6-deficient rats might appear reduced if compared with the pair-fed controls but elevated if compared with the *ad libitum*-fed controls.

The following experiments were done (a) to compare the liver NPSH content of Vit. B_6-deficient rats with those of both pair-fed and *ad libitum*-fed controls and (b) to determine whether the age of the deficient rats, duration of the deficiency, and fasting significantly changed liver NPSH concentration.

Methods and materials. Animals and diets. Male rats of the Long-Evans strain raised in our laboratory were used in Experiments 1-3. Both male and female rats were used in Experiment 4. Liver NPSH values were similar for both sexes. The rats were weaned at 3 weeks of age (45-55 g), caged individually in galvanized screen-wire cages, and fed a casein-sucrose diet†, except for the group in

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† Composition, g/100 g diet: "vitamin-free" casein (Nutritional Biochemicals Corp., Cleveland), 20.0; cottonseed oil, 5.0; salts (U.S.P. 14, Nutritional Biochemicals Corp., Cleveland), 4.0 or 3.5 (*Fed. Proc.*, 1963, v22, 261); choline bitartrate, 0.18; powdered sucrose to 100. Two ml of a 20% ethanol solution of B vitamins and menadione were fed 3 times