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Utilization of Yeast Polyglutamate Folates in Man (38977)

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A number of studies have been published on the oral utilization of yeast as source of folates (1–7). Lucy Wills (1) was the first to demonstrate, some 45 yr ago, the efficacy of Marmite, a special yeast-extract concentrate, in the treatment of "pernicious anaemia of pregnancy" and in "tropical anaemia." Subsequent studies indicated that only a small percentage (5–30) of the folates present in yeast were absorbed from the intestine, as compared to pteroylmonoglutamate (PGA) folate (2–6).

Perry and Chanarin (5) and Chanarin (6) compared the elevation of plasma folates in healthy subjects after oral doses of equimolar amounts of PGA and of pteroylheptaglutamate concentrated from yeast. According to these investigators feeding of 1500 μg of heptaglutamate folate (or about 20 μg/kg) resulted in absorption of 30%, as compared to the absorption of the monoglutamate; moreover, the folate which was found in the plasma after feeding the heptaglutamate was mainly "free folate" as evidenced from the similar activities obtained with *Lactobacillus casei* and *Streptococcus faecalis* bioassays. Pretreatment of the yeast preparation with pancreatic enzyme (conjugase) increased only slightly the serum folate levels after ingestion. A similarly poor utilization of polyglutamates from yeast was obtained by these investigators in a more recent study (7). On the other hand, Swenseid *et al.* (8), Rosenberg *et al.* (9), and recently Tamura and Stokstad (10) reported a much better utilization of yeast folates in man.

These conflicting results prompted us to reinvestigate the availability of yeast folates. The effect of oral administration of small and increasing amounts of polyglutamate folates from yeast on the serum folate activity is reported in this communication. The initial amounts of yeast extract fed to healthy individuals contained very small concentrations of free folate measurable with *L. casei* prior to conjugase treatment.

Materials and methods. Folates from bakers yeast (250 g) were extracted with boiling water (2 liters) for 10 min in the presence of ascorbate. The extract, which was allowed to cool, was stored at 4° for 48 hr, following which it was cleared by centrifugation. The supernatant fluid was concentrated to dryness by lyophilization and redissolved in 125 ml water. The folate content of the yeast extract was determined with *L. casei* without further treatment (free folate) and after treatment with chick pancreas conjugase (total folate, i.e., free folate plus polyglutamate folate).

Nineteen medical students and laboratory personnel participated in the study. Yeast extracts containing polyglutamate folate equivalent to between 100–3000 μg of PGA, and synthetic pteroylmonoglutamate folate from 100 to 300 μg in 50 ml water was given orally to the fasting subjects. The serum folate activity (with *L. casei*) was determined prior to the oral dose and 1, 2, 3, and 5 hr after feeding. The subjects received a very light breakfast 1 hr after folate ingestion; it consisted of sweetened black coffee and two pieces of bread. Preliminary trials have shown that such a meal had no effect on the serum folate level.

Results. The effect of consumption of 100, 200, and 300 μg PGA and of 100, 200, and 300 μg of polyglutamate folate from yeast containing only 10, 20, and 30 μg of free folate, respectively (as assayed with *L. casei* without conjugase treatment) is given in Figs. 1A and 1B. As can be seen, feeding of 100 μg PGA hardly increased the serum folate above the preingestion level; in two out of the four subjects tested almost no elevation of the serum folate activity was noticed while in the other two, small increases (3.0 and 4.3 ng/ml) were found. Feeding of 200 and 300 μg PGA resulted in

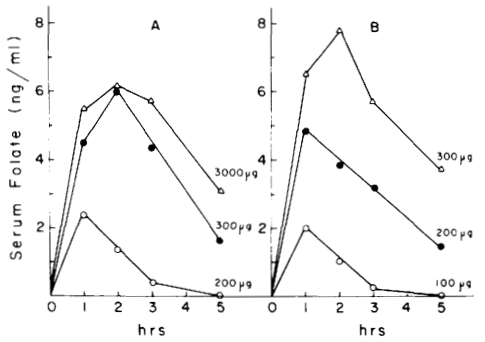

FIG. 1. Effect of folate ingestion on serum folate level. A. Ingestion of polyglutamate folate from yeast. B. Ingestion of synthetic monoglutamate folate (PGA). The net increases in serum folate activity (*Lactobacillus casei* assay) after ingestion of amounts as indicated in the figure. The data represent average values on groups of three subjects, except for the 100 μg PGA (Fig. 1B) given to four.

higher elevations above the zero time values, especially with the latter amount fed (4.9 and 7.8 ng/ml, respectively). When, however, instead of PGA, 300 μg of polyglutamate folate from yeast was administered, of which only 30 μg was directly assayable with *L. casei*, the serum folate level rose to a level equivalent to 300 μg PGA. Smaller quantities ingested in the absence of conjugase resulted in minor elevations or no increase at all. Feeding of higher amounts of polyglutamate folate from yeast (equivalent to 1000 and 3000 μg PGA and containing only 100 and 300 μg free folate, respectively) did not produce higher elevations of serum folate than the administration of 300 μg of PGA. An aliquot of the preparation of yeast folate was incubated with chick pancreas conjugase for 16 hr at 37°, under toluene, and its folate content was estimated with *L. casei*; it was found to contain 300 μg/5 ml folate. Feeding 300 μg of *in vitro* deconjugated yeast-folate increased the serum folate level to the same extent as the preparation which was not deconjugated.

Discussion. Some 75–90% of folate in a mixed diet is present in the form of polyglutamate, i.e., the pteroylglutamate moiety is attached to a γ-polyglutamyl peptide chain of 6 or more residues (11). During absorption the peptide chain is hydrolyzed by folate conjugase (5) and the resulting monoglutamate folate is adsorbed and reaches the circulation (8, 9, 12–14).

The data presented here demonstrate that small amounts of yeast folate fed to healthy subjects are fully utilized. Other investigators, who used much higher amounts of yeast folates in their trials observed a much lesser degree of utilization. Thus, while in our experiments 300 μg of polyglutamate folate was used, Perry and Chanarin (5), as well as others, employed 1000 μg and more. These observations suggest that complete deconjugation in the gut is apparently obtained only when small amounts of yeast folate are ingested. The partial utilization of larger amounts of yeast folate is probably due to the limiting concentration of conjugase present in the gut and/or to the unfavorable conditions for its activity (e.g., unsuitable pH in the gut, etc.). The presence of an inhibitor of conjugase activity contaminating the yeast preparation, as shown by various investigators (7, 8, 13, 15, 16) has also to be considered. The latter assumption is supported by the finding of decreased utilization of higher amounts of ingested polyglutamates possibly due to the introduction of higher amounts of inhibitor of the deconjugation reaction. A very efficient utilization in man of another rich source of folates, e.g., liver folates, as evidenced from the rapid and sustained increases in the serum folate level, was also reported by us (17).

Summary. Ingestion by healthy humans of small amounts of polyglutamate folates from yeast, equivalent to 300 μg of monoglutamate folate and containing 30 μg of "free folate," resulted in an appreciable elevation of the serum folate corresponding to 300 μg of synthetic pteroylmonoglutamate (PGA). Ingestion of higher amounts of polyglutamate folate did not result in higher serum folate elevations than did 300 μg. It is concluded that small amounts of polyglutamate folate from yeast are fully utilized, presumably by deconjugation in the gut prior to absorption. The relative ineffectiveness of larger doses of polyglutamate folates from yeast may be due to limiting conjugase activity in the gut, unfavorable conditions for its activity (such as

unsuitable pH) or to an inhibitor of the enzyme present in impure preparations.

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