response technique. These relations exist within a single ejaculatory series and also in the dynamics of response associated with the progression from ejaculation to ejaculation (*3*).

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## Sparing of Folinic Acid by Thymidine

*Abstract.* Thymidine, which is inactive when added alone to *Pediococcus cerevisiae* (*Leuconostoc citrovorum*) 8081, was found to increase its growth in the presence of folinic acid. The addition of thymidine increased both the sensitivity of the folinic acid assay and the total growth yield. The mechanism of the synergistic effect of thymidine is briefly discussed.

Folinic acid (leucovorin) is known to meet the requirement of *Pediococcus cerevisiae* (*Leuconostoc citrovorum*) 8081 for the citrovorum factor, whereas the related pteroylglutamic acid is very inefficient (*1*). Although thymidine has been shown to supply the citrovorum factor for this organism, much larger quantities (10,000 times more than leucovorin) were required (*2*).

Wood and Hitchings (*3*) have shown recently that cell-free extracts of *P. cerevisiae* catalyze the transformation of pteroylglutamic acid into the citrovorum factor. They explained that the inefficiency of pteroylglutamic acid as a growth factor, in contrast to folinic acid, is due to its inability to permeate the cell membrane of *P. cerevisiae*.

We began to study the relationship of thymidine to folinic acid to elucidate the role of folic acid derivatives in the metabolism of *P. cerevisiae*. In the initial stage of this work we found that thymidine increased growth in the presence of folinic acid, but had no growth-promoting activity in its absence. The present report is confined to the elucidation of this interesting finding.

Figure 1 shows the effect of the addition of thymidine to graded amounts of folinic acid (*4*). At low concentrations of folinic acid (50 $\mu\mu$g/ml), the addition of thymidine caused a fourfold increase in growth, while at higher concentrations (200 $\mu\mu$g/ml) the increase was twofold; even at the highest concentration used (600 $\mu\mu$g/ml) the addition of thymidine resulted in a heavier growth than when folinic acid alone was added. The effective concentrations of thymidine were between 0.1 to 5 $\mu$g/ml. However, in the absence of folinic acid no visible growth was obtained even with concentrations of thymidine as high as 50 $\mu$g/ml.

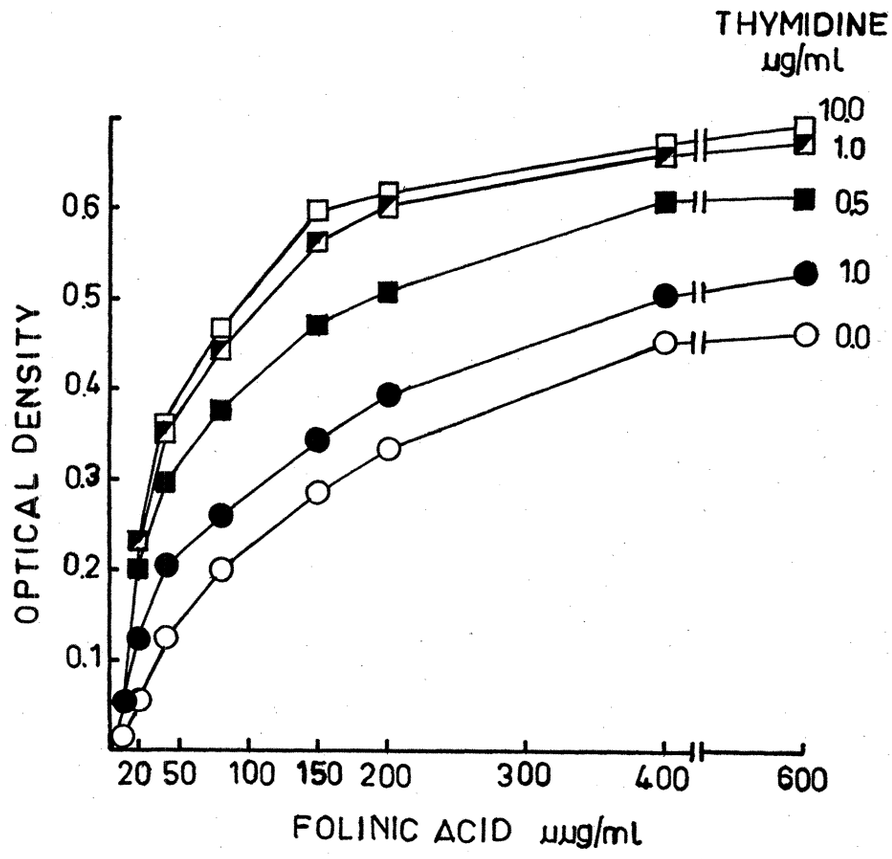


Fig. 1. Effect of thymidine on the growth response of *P. cerevisiae* (*L. citrovorum*) 8081 to graded amounts of folinic acid. Growth, expressed as optical density, was measured in the Coleman Junior spectrophotometer at 650 m$\mu$ after incubation for 48 hr at 37°C.

Thymidine was also found to increase the growth of *P. cerevisiae* in the presence of high concentrations of pteroylglutamic acid. In the presence of thymidine (1 $\mu$g/ml), visible growth occurred at an acid concentration of 0.2 $\mu$g/ml, whereas 10 times as much (2 $\mu$g/ml) was required to obtain similar growth in the absence of thymidine (*5*).

The medium was that of Toennies *et al.* (*6*), and the strain was obtained from the American Type Culture Collection.

The strain that shows the sparing effect of folinic acid by thymidine seems to be different from the original *P. cerevisiae* 8081 (*2*). Apparently, mutants of this organism are obtained rather easily. Nichol (*7*) selected strains that responded to lower concentrations of pteroylglutamic acid and which were found to display an increased sensitivity towards aminopterin.

The mechanism of the thymidine effect is not clearly understood at present. It would appear, however, that in this strain of *P. cerevisiae* folinic acid not only acts as a catalyst for the synthesis of thymidine but also for some other essential metabolite. Thus, the addition of thymidine spares folinic acid for its other functions.

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