

TABLE 2. Relative rates of rRNA synthesis measured by pulse-labeling^a

Pyrimidine source (concn) (μg/ml)	Doubling time (min)	$\frac{(^3\text{H}/^{32}\text{P})_{\text{rRNA}}}{(^3\text{H}/^{32}\text{P})_{\text{total RNA}}}$ at:	
		1.5 min	30 min
Uracil (20)	43	0.24	0.51
Orotate (50)	79	0.16	0.51

^a Strain NF321 was grown in the minimal medium containing 0.3 mM inorganic phosphate supplemented with thiamine (1 μg/ml), leucine (20 μg/ml), glucose (1 mg/ml), adenine (5 μg/ml), and the pyrimidine source as indicated. The cultures were background labeled with ³²P (5 μCi/ml) for 2 generations and pulse-labeled (time = 0) with 200 μCi (i.e., 4,400 Ci/mol, final specific activity) of [³H]adenine per ml. (Adenine was included in the growth medium prior to the pulse, because addition of even very small concentrations of adenine dramatically reduces the pool of 5-phosphoribosyl-1-pyrophosphate [1]. This, in turn, disturbs the orotate phosphoribosyltransferase reaction [44] upon which our experiments are based. The inclusion of adenine made it difficult for us to make very short pulses [the 1.5-min pulses represented only 630 hybridized ³H cpm in the presence of uracil and 270 hybridized ³H cpm in the presence of orotate], but on the other hand, we have not perturbed the steady states.) The ratio of ³H to ³²P was determined in the total RNA and in RNA hybridized to λ *rnnX* DNA. The observation that at 30 min only 51% of the RNA hybridized to the λ *rnnX* DNA indicates that tRNA and mRNA do not hybridize to the probe and that the hybridization reactions were not complete.