normally high Rayleigh jet. It is possible that the unusual breakup of the crown (Fig. 4) with depths less than about 5 mm occurs when the reflected energy of the vortex ring reaches the surface during the period while the liquid from the crown is flowing downward. This interpretation would also account for the sharp reduction in the height of the jet with depths less than about 5 mm.

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Radioisotope Uptake as a Measure of Synthesis of Messenger RNA

Abstract. *Exogenously supplied radioactive uracil (or guanine) enters the intracellular pools of RNA precursors in* Escherichia coli *only as nucleotides are removed from these pools by net synthesis of RNA. Consequently uptake of uracil over a short period does not measure the sum of the synthesis of all forms of RNA, unstable and stable, as is often supposed. Uptake of uracil during changing conditions of growth may be influenced by changes in types of RNA's being made; under such conditions that no stable RNA is being made, the synthesis of unstable forms may be greatly underestimated.*

Radioactive uracil, as well as the other nucleic acid bases, is very widely used for measurement of the rate of biosynthesis of RNA. Interpretation of such experiments, however, is complicated by the existence of an unstable fraction of the RNA, the messenger (mRNA), which, at least in bacteria, is undergoing rapid turnover (*1, 2*) (Fig. 1). Over long periods (relative to the half-life of the messenger) the mRNA becomes completely labeled, and uptake data primarily indicate the synthesis of the stable forms of RNA, since the mRNA amounts to only a few percent of the total RNA accumulating in the cell. On the other hand, it is often stated that the amount of radioactive uracil incorporated into RNA, in a pulse of labeling, is proportional to the sum of the rates of synthesis of mRNA and stable RNA. I now examine this point in particular, and the view that messenger synthesis in general can be measured by isotope uptake.

An assumption made in use of the pulse-labeling technique is that the radioactive precursor freely and rapidly enters the cells' internal precursor pools (*3*). In some of the earliest studies of the turnover of mRNA in bacteria, Gros *et al.* showed that, after addition of radioactive guanine to a culture of *Escherichia coli*, the specific activity of the intracellular guanine nucleotides rose only slowly (*2*); they explained this result in terms of a continuing dilution of this pool by the breakdown of mRNA. An additional assumption, implicit in their interpretation, is that the entry of guanine from the medium into the cell is limited in some fashion. This assumption was borne out in an extensive series of studies of the transport of nucleic acid precursors into cells and their incorporation into RNA, in which workers at Carnegie Institution found that the rate of entry of uracil or guanine into cells did not exceed the rate of their utilization in synthesis of RNA (*4*); that is, that uptakes of uracil and guanine were somehow linked to their metabolism.

The experiments now described extend these last observations and point out their implications in terms of the interpretation of isotope-uptake experiments. They show that the rate of incorporation of uracil and guanine by *E. coli*, even over relatively short periods, is clearly limited at some step in the conversion of the base in the medium to a nucleotide in the cell. This limitation is such that the amount of radioactive base entering the cell does not exceed the amount being removed in the net synthesis of RNA; the consequence is that, even over short periods of labeling, isotope incorporation largely reflects the rate of synthesis of the stable forms of RNA. And by extension, under such growth conditions that there is no formation of stable RNA, synthesis of mRNA may go undetected or be greatly underestimated.

In a labeling experiment several factors may contribute to the rate at which the pools of nucleotides become radioactive: First, the synthesis of stable forms of RNA leads to a continuing flow of nucleotides through the pools; if the exogenously supplied base is used preferentially and *de novo* synthesis of the related nucleotides is inhibited, the pools will rapidly become radioactive (*5*). Second, one can envision that the enzymes responsible for the uptake reactions (1 and 2 in Fig. 1), and the interconversions of the various nucleotide derivatives within the pool, might catalyze an exchange reaction; if this were so, the nonradioactive nucleotides in the pools, and those formed by the degradation of mRNA, would be dispersed into the larger amount of radioactive precursors in the medium. Finally, if the addition of a radioactive base to the medium causes very great expansion of the pools of nucleotides in the cell, the nonradioactive molecules will be simply diluted out.

Table 1. Guanosine triphosphate pool of *Escherichia coli* after addition of guanine. *E. coli* B/1 was grown to an O.D.$_{540}$ of 0.4 in a tris-buffered medium containing $6 \times 10^{-4}M$ phosphate, and supplemented with 0.4 percent glucose, thiamine-HCl at 0.05 mg/liter, adenine at 10 mg/liter, and $H_3{}^{32}PO_4$ at 10 mc/liter. Portions of this medium were analyzed for inorganic phosphate and radioactivity for direct determination of the phosphate specific activity. To a series of culture flasks, guanine was added as indicated, and 2 minutes later, 1-ml samples were removed into 0.5 ml of $0.75M$ perchloric acid; to each sample was added 1.5 μmole of each of guanosine monophosphate, diphosphate, and triphosphate, and 0.05 μc of ^{3}H-guanosine triphosphate. The cells were removed by centrifugation, and the supernatants were desalted by absorption to and elution from charcoal (*12*). The guanosine triphosphate was then isolated by electrophoresis in *p*H 3.5 ammonium formate ($0.07M$) and $10^{-3}M$ EDTA, and after elution a second electrophoresis in *p*H 7.5 phosphate buffer ($0.02M$) and $3 \times 10^{-3}M$ EDTA. The guanosine triphosphate (GTP) spots were counted directly in a mixture of toluene, 2,5-diphenyloxazole, and *p*-bis-[2-(5-phenyloxazole)]-benzene in a liquid-scintillation counter. The recovery of GTP was calculated on the basis of recovery of the ^{3}H-GTP added. An O.D.$_{540}$ of 1.0 corresponds to a dry weight of 0.34 mg/ml. The doubling time was 49 minutes.

Guanine conc. (M)	GTP (mμmole/mg, dry wt)
0	3.7 ± 0.1*
0.33×10^{-5}	4.7
1.0×10^{-5}	4.3
0.33×10^{-4}	4.7
1.0×10^{-4}	5.6
0.33×10^{-3}	4.6

* Four determinations.

The possibility of exchange of uracil in the medium with the uracil derivatives contained in the internal pools was examined by experiment (Fig. 2). Exponentially growing cells of *E. coli* B were given uracil-^{14}C at $10^{-7}M$, a quantity small enough to be utilized in less than 1 minute (*4*). After 2 minutes, a chase of nonlabeled uracil at $10^{-5}M$ was added. Samples were removed periodically for measurement of the total amount of uracil-^{14}C in the cells, the amount in the cells' nucleic acids, and the amount remaining in the culture medium; the quantity in the intracellular pools was taken to be the difference between the total intracellular amount and that in the nucleic acids. It can be seen that at the time of addition of cold uracil almost all the radioactive uracil originally added had been removed from the medium, being distributed about 40 : 60 between the intracellular pool and the nucleic acids. Although the nucleic acids were not further fractionated in this experiment, one would expect a considerable portion, perhaps one-third or more, of its activity to be in the mRNA fraction (*2*).

During the following minutes, counts leave the soluble fraction and appear in the insoluble fraction; after 20 minutes the pool is unlabeled, indicating that the transfer, not only from the soluble pools, but also from the mRNA fraction, is now essentially complete. The increase in counts in the medium in this period is 1 percent of the initial total counts in the cells, or approximately 2.5 percent of the counts initially in the soluble pools. A duplicate experiment gave the same results except that the counts in the medium were unchanged during the course of the experiment (9 minutes). These findings indicate no exchange of the uracil in the nucleotide pools with that in the medium. Experiments with guanine have yielded similar results.

If addition of uracil to the growth medium causes great expansion of the internal pools, this expansion should affect the kinetics by which an added tracer quantity of radioactive uracil flows through the pools and into RNA (*4*); this possibility has been tested in two types of experiments.

Figure 3 compares the incorporation of uracil-^{14}C with that of a tracer amount of uracil-^{3}H added 90 seconds later. To cells growing exponentially in minimal medium, uracil-^{14}C was added to a concentration of $7 \times 10^{-6}M$.

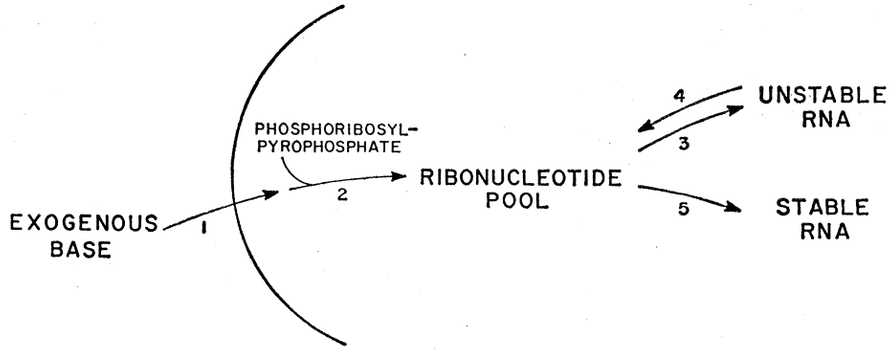

Fig. 1. Interrelation of precursors, nucleotide pools, and synthesis of RNA (*17*).

Ninety seconds later, uracil-^{3}H was added at high specific activity so as not to increase the concentration of uracil in the medium appreciably (10 percent). The difference in the uptake curves of the two isotopically labeled uracils is slight. Had the first addition of uracil-^{14}C caused great expansion of the internal pools, there would have been a relative delay in the uptake of the uracil-^{3}H.

The data given in Fig. 2, describing the pool-exchange experiment, also show that exogenous uracil, over a 100-fold range of concentration, does not appreciably affect the size of the nucleotide pools. The Fig. 2 insert shows that the rate at which a prelabeled pool is chased out is the same whether the concentration of the uracil chase is 0, 10^{-5}, or $10^{-4}M$.

The fact that the concentration of guanine in the growth medium does not substantially expand the cell's guanine nucleotide pools has been established by direct measurement of the

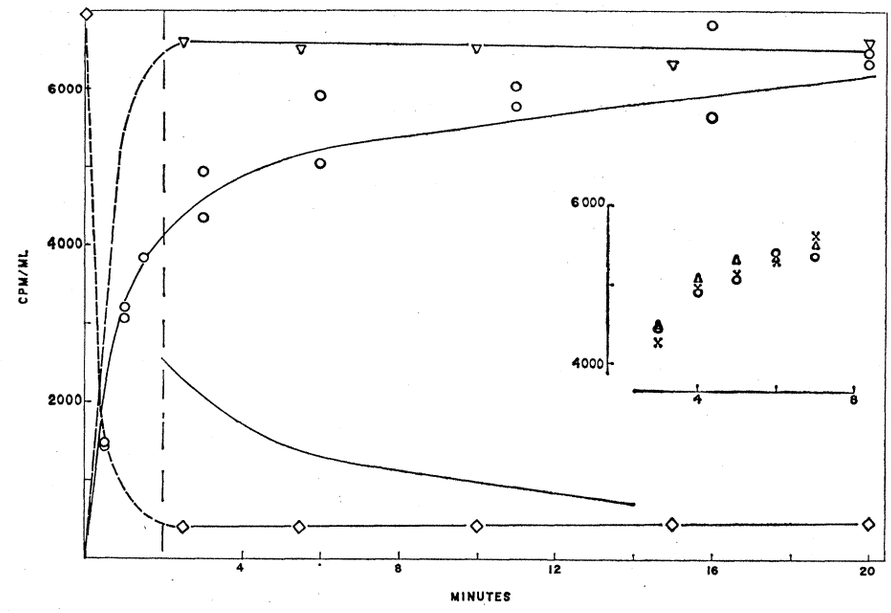

Fig. 2. Cultures of *E. coli* B/1 (2×10^{8} cells per milliliter) in mineral-salt medium, supplemented with glucose at 4 mg/ml and arginine at 20 μg/ml, were made $10^{-7}M$ in uracil-^{14}C (30 mc/mmole), and 2 minutes later uracil-^{12}C was added as explained. Samples for acid-insoluble counts were taken, made 5 percent in trichloroacetic acid, collected and washed on membrane filters, and counted (*17*). Total radioactivity in the cells and radioactivity in the medium was determined by taking 1-ml samples and immediately filtering them through membrane filters (*4*). The filters were sucked dry and counted, without further washing, to determine total cellular counts; a portion of the filtrate was counted for determination of radioactivity in the medium. Acid-insoluble counts: ○, $10^{-5}M$ uracil chase; △, $10^{-4}M$ uracil chase; X, without chase. Total-cell count: ▽, $10^{-5}M$ uracil. Count in medium: ◇, $10^{-5}M$ chase. The unbroken line shows the difference between the total and the acid-insoluble curves. The insert shows the incorporation into acid-insoluble material, while the concentration of the chase was varied, in a separate but otherwise identical experiment.

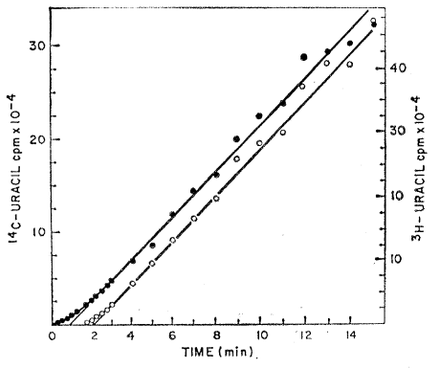

Fig. 3. Cells growing as for Fig. 2. At zero time uracil-^{14}C (15 mc/mmole) was added to a concentration of 0.1 μc/ml. At 90 seconds uracil-^{3}H (2200 mc/mmole) was added to a concentration of 1.0 μc/ml. Samples (1.0 ml) were taken into 0.1 ml of 50-percent trichloroacetic acid; they were collected and washed with 5-percent trichloroacetic acid and uracil at 100 μg/ml and then with H_2O, on membrane filters, and, after drying, were counted in a mixture of toluene, 2,5-diphenyloxazole, and *p*-bis-[2-(5-phenyloxazole)]-benzene, in a Beckman LS-2 liquid-scintillation counter. The abscissa is plotted proportional to the increase in cell mass, with sampling times indicated (*4*).

size of the pool of guanosine triphosphate, the major guanine component. Table 1 shows that, in raising the guanine concentration of the culture from 0 to $0.33 \times 10^{-5}M$, the guanosine triphosphate pool increases by only 25 percent, while further increase up to $0.33 \times 10^{-3}M$ leads to no further expansion.

These experiments show that entry of a radioactive base into the intracellular nucleotide pools is not facilitated by exchange reactions, and only slightly so by pool expansion (*6*). One possible explanation for these results is that one of the steps in the transport of the base into the cell, or the reaction in which the base reacts with phosphoribosyl pyrophosphate to form the nucleoside monophosphate, may be subject to negative feedback control. Berlin and Stadtman have observed that the enzyme responsible for the formation of guanosine monophosphate from guanine is subject to inhibition by guanine nucleotides (*7*). Further evidence regarding the conversion of guanine to guanosine monophosphate as a site of control comes from studies of yeast cells (*8*); here it was observed that, whereas addition of guanine to the growth medium very markedly expanded the intracellular pool of free guanine (*9*), it had no effect on the pool of guanine nucleotides. From this and the general similarity in the kinetics of utilization of guanine and uracil, one would predict that the formation of uridine monophosphate should also prove subject to feedback inhibition. One may add that the nucleotide pyrophosphorylases for adenine, hypoxanthine, and thymine, and the kinases for deoxythymidine and deoxycytosine, have been shown to be subject to inhibition by the appropriate nucleotides (*7, 10*). Finally, these same reactions, being essentially irreversible, could also explain the apparent nonexchangeability of the pool nucleotides with the free bases in the medium (*11*).

The consequence of the limitation of the uptake of a base from the medium is that uptake will not be greater than the net rate of utilization of nucleotides for the synthesis of RNA—this net rate would be equal to the synthesis of the stable forms of RNA plus a small contribution by the increase in the amount of mRNA in the culture with cell growth. Normally incorporation of a labeled precursor into a macromolecule would be expected to show a lag during which the pool of intracellular intermediates became labeled, especially if the pool labeling were not speeded by exchange or expansion; this then would be expected of incorporation of uracil or guanine into RNA. However, this is not so: the uptake of the RNA bases proceeds with relatively little lag or phase of acceleration (see Fig. 3) (*4*). This phenomenon can be largely explained by the effect of turnover of messenger, which rapidly sweeps precursors from the pool into RNA, independently of the rate at which the precursors are entering the cell (*12, 13*). This fact explains an apparent anomaly in that, after a short period of labeling, most of the isotope incorporated is found in the mRNA, while the amount incorporated is limited by the rate of formation of stable forms (*14*).

A logical extension of these statements, that uptake of uracil measures only the rate of formation of stable forms of RNA, would be that uptake of uracil would cease where there was no net synthesis of RNA, even in the presence of rapid turnover of RNA. This possibility could partly explain the large and abrupt reduction of uptake of isotope by cells of *E. coli* after infection with T-even phage; here it is known that the synthesis of host ribosomal- and transfer-RNA stops, while one infers that synthesis of mRNA continues from the fact of the uninterrupted rate of protein synthesis (*15*). In fact it has been shown (*16*) that, during amino acid starvation of a stringent strain of *E. coli*, neither uracil nor bromouracil enters the nucleotide pools, and I have shown that under similar conditions, during both amino acid starvation and amino acid shift down, guanine does not enter the guanosine triphosphate pool (*1*). Therefore under these conditions isotope uptake alone cannot be used for determination of the extent of synthesis of mRNA.

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