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Rate of Ribonucleic Acid Chain Growth in *Mycobacterium tuberculosis* $H_{37}R_v$

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Two methods were employed to measure the rate of ribonucleic acid (RNA) chain growth in vivo in *Mycobacterium tuberculosis* $H_{37}R_v$ cultures, growing in Sauton medium at 37°C, with a generation time of 10 h. In the first, the bacteria were allowed to assimilate [^{3}H]uracil or [^{3}H]guanine into their RNA for short time periods. The RNA was then extracted and hydrolyzed with alkali, and the radioactivity in the resulting nucleotides and nucleosides was measured. The data obtained by this method allowed the calculation of the individual nucleotide step times during the growth of RNA chains, from which the average rate of RNA chain elongation was estimated to be about 4 nucleotides per s. The second method employed the antibiotic rifampin, which specifically inhibits the initiation of RNA synthesis without interfering with the elongation and completion of nascent RNA chains. Using this method, the transcription time of the 16*S*, 23*S*, and 5*S* ribosomal RNA genes was estimated to be 7.6 min, which corresponds to a ribosomal RNA chain growth rate of 10 nucleotides per s.

Currently, we have been investigating the molecular aspects of the growth of *Mycobacterium tuberculosis* $H_{37}R_v$, the causative organism of tuberculosis in man, with a view to applying the results to combating the disease. One of the difficulties in working with this organism is its extremely slow growth rate: the generation time is 18 to 24 h on stationary cultures and 10 to 12 h on shake cultures. The slow growth of the organism perhaps reflects a retarded rate of macromolecular synthesis and a consequent step-down of its metabolic machinery. We have focused our attention on the synthesis of ribonucleic acid (RNA), chiefly because we have found that the RNA polymerase in this organism is different in its properties from that of a fast-growing organism like *Escherichia coli* (7). The kinetics of RNA synthesis in *M. tuberculosis*, it was hoped, might reflect the slow growth of the organism.

The rate of de novo synthesis of RNA in growing bacteria is a function of two parameters: the number of nascent RNA chains that are growing at any one time, and the rate of growth of nascent chains. We have studied the second of the two parameters in *M. tuberculosis* $H_{37}R_v$, by making use of two different methods. The first was originally used by Manor et al. (8) for studying the rate of RNA chain growth in *E. coli* under different growth conditions. Here, the bacterium is allowed to incorporate a radioactively labeled nucleotide precursor into its RNA chains for a certain period, and the average chain length during the labeling period is ascertained by measuring the ratio of the total radioactivity incorporated into the RNA to that present in the 3′ ends of the growing chains. Such measurements provide a means of calculating the nucleotide step time, which is the time elapsed between the addition of successive nucleotide residues to the growing 3′ ends of the RNA. An overall rate of RNA chain growth can be estimated by using an averaged value of the individual nucleotide step times.

The second method (2) makes use of the drug rifampin, which inhibits RNA chain initiation but not elongation, to estimate the rate of RNA chain growth in *E. coli*. The procedure essentially consists of following the kinetics of labeling total, stable, and unstable RNA under judiciously chosen conditions of rifampin inhibition. The data thus available allow one to arrive at the growth rate of the stable as well as the unstable RNAs.

The methods of Manor et al. (8) and Bremer et al. (2) have suitably been adapted to our organism and experimental conditions. The results of these studies are communicated in this paper.

MATERIALS AND METHODS

Chemicals. [^{3}H]uracil and [2-^{14}C]uracil were purchased from the Department of Atomic Energy, Trombay, Bombay. [8-^{3}H]guanine and [8-^{14}C]guanine were obtained from the Radiochemical Centre, Amersham, England. Unlabeled nucleotide mono-

phosphates and nucleosides were obtained from the California Biochemical Corporation, Los Angeles, Calif. Rifampin was from Sigma Chemical Co., St. Louis, Mo.

Method (i). Only a brief description of the experimental detail is given below. For theoretical calculations and experimental details, the original paper (Manor et al. [8]) should be referred to.

(i) Growth conditions. The culture media (L-asparagine, 0.4%; citric acid, 0.2%; $MgSO_4$, 0.05%; K_2HPO_4, 0.05%; ferric ammonium citrate, 0.005%; glycerol, 3.5% [vol/vol] [pH 7.2] [11]) were inoculated with a fresh stationary-phase culture of *M. tuberculosis* $H_{37}R_v$ (NCTC 7416), so as to give a 500-fold dilution, and grown on a gyratory shaker at 37°C. Growth was monitored by measuring the turbidity in a Klett-Summerson colorimeter, using the green (no. 54) filter. The doubling time of the bacteria under the growth conditions employed was found to be 10 to 12 h.

(ii) Rapid labeling and sampling experiment. When the culture attained a turbidity of 190 Klett units, 25-ml portions were transferred to four flasks kept at 37°C on a magnetic stirrer. Then 1 μCi of either [2-^{14}C]uracil (49 mCi/mmol) or [8-^{14}C]guanine (55 mCi/mmol) was added to each flask, and the cultures were grown with rapid stirring for 10 h. Rapid labeling of the cultures with either [^{3}H]uracil (6.1 Ci/mmol) or [8-^{3}H]guanine (6.8 Ci/mmol) was initiated by injection of 250 μCi of the labeled compound into each of the flasks. Uptake of the radioactive label was terminated in the flasks at 20-s intervals, starting at 60 s after the addition of the ^{3}H compound, by injection of 15 ml of stopping solution (75 ml of ethanol, 21 ml of 0.1 M sodium acetate [pH 5.1], 2 ml of redistilled phenol, and 2 ml of 0.1 M ethylenediaminetetraacetic acid [EDTA]). The contents of the flasks were centrifuged in a Sorvall RC-2B refrigerated centrifuge for 20 min at 10,000 rpm, and the pellet obtained was washed several times with the washing solution (80 ml of ethanol and 20 ml of 0.1 M sodium acetate [pH 5.1]). Bacterial lysis, nucleic acid extraction, alkaline hydrolysis of RNA, and chromatography were carried out exactly as described by Manor et al. (8), except that the concentration of egg white lysozyme used during processing was four times that used by them.

(iii) Scintillation counting of radioactivity. The uridine 5'-monophosphate (UMP), guanosine 5'-monophosphate (GMP), cytosine 5'-monophosphate (CMP), uridine (Urd), cytidine (Cyd), and guanosine (Guo) spots of the chromatogram were cut out and counted in a liquid scintillation spectrometer (Beckman LS-100) in 10 ml of the scintillation fluid (500 mg of PPO [2,5-diphenyloxazole] in 50 ml of toluene plus 50 ml of Cellosolve). Since the uptake experiments had been designed to produce a high ratio of ^{3}H/^{14}C counts, the counting channels were so chosen that 40% of the ^{14}C counts were also recorded in the ^{3}H channel, but that none of the ^{3}H counts was recorded in the ^{14}C channel. Radioactivity in the nucleosides was counted for 20 min and that in the nucleotides was counted for 10 min.

(iv) Chemical analysis of the bacterial content of RNA and DNA. The bacteria were treated with cold perchloric acid (1.5% at 0°C), and the precipitated material was collected by centrifugation. The nucleic acids were extracted from the precipitate with hot perchloric acid (3.5% at 70°C for 45 min). The amounts of deoxyribonucleic acid (DNA) and RNA in the extract were determined by the diphenylamine reaction according to Burton (3) and the orcinol reaction according to Ceriotti (4), respectively.

Method (ii). *M. tuberculosis* cells were made permeable to rifampin by a short treatment with EDTA as described below (from Bremer et al. [2]). At a turbidity of approximately 190 Klett units, a portion of the bacterial culture was centrifuged (7,000 × *g* at 0°C) for 10 min. The supernatant medium was discarded and the bacteria were resuspended in sodium phosphate-EDTA buffer (0.1 M phosphate-0.001 M EDTA, pH 6.8) in one-tenth of the original volume and held at 37°C for 2 min with aeration. EDTA treatment was terminated by dilution to the original volume with medium prewarmed to 37°C. In most experiments, this culture was further diluted as indicated in the legends for figures.

(i) Labeling of RNA and DNA. After termination of EDTA treatment, a 10-ml portion of the bacterial culture (suitably diluted; see legend to Fig. 1) was added to a test tube containing [^{3}H]uracil (6.1 Ci/mmol; final concentration, 50 pmol/ml) and rifampin (100 μg/ml) and incubated at 37°C. One-milliliter samples were removed at various times, and nucleic acids from aliquots of 0.5 ml each were precipitated with 2 ml of cold 5% trichloroacetic acid. The incorporation of label into RNA and total nucleic acid was determined (see below). In other experiments [^{3}H]uracil (50 pmol/ml) was added to 10 ml of a suitably diluted culture (see legend, Fig. 2) and, at

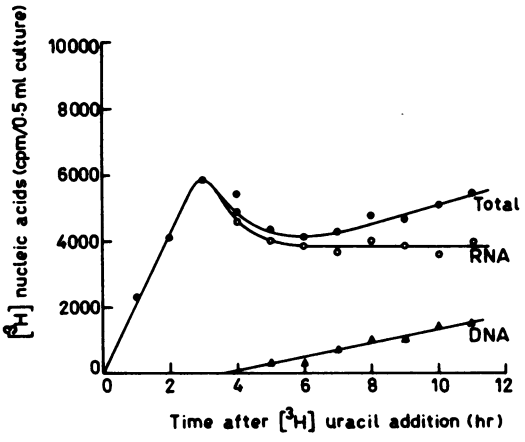

FIG. 1. *Kinetics of labeling nucleic acid in the presence of rifampin. The culture, at a turbidity of 60 Klett units, was added to an incubation tube containing rifampin and radioactive uracil. One-milliliter samples were removed, and aliquots of 0.5 ml each were precipitated with trichloroacetic acid. Incorporation of label into RNA (⊙), DNA (▲), and total nucleic acid (●) was determined. The RNA remaining after 6 h represents stable RNA labeled in the presence of rifampin.*

various times after incubation at 37°C, 0.5-ml aliquots were removed and added to a tube containing rifampin (100 μg/ml). These tubes were further incubated for 5 h, the reaction was terminated by addition of 2 ml of 5% trichloroacetic acid, and the radioactivity in RNA was determined.

To measure the radioactivity in total nucleic acids (RNA plus DNA), the acid-precipitated cells were filtered through membrane filters (0.45-μm pore size, Millipore Corp.). After they were washed several times with 5% trichloroacetic acid, they were dried and counted in a liquid scintillation spectrometer (Beckman LS-100) in toluene-based scintillation fluid containing 4 g of PPO and 50 mg of POPOP [1,4-bis-(5-phenyloxazolyl)-benzene] per 1,000 ml of toluene. For determination of label in RNA, the acid-precipitated cells were centrifuged and washed with 5% trichloroacetic acid. After drying, the precipitate was suspended in 1 ml of 0.2 N NaOH and left for 18 h at 25°C for complete hydrolysis of RNA. The alkali-resistant material was precipitated at 0°C by 1 ml of 0.5 N perchloric acid. The soluble nucleotides resulting from the hydrolysis were separated from the insoluble material by filtration through membrane filters (Millipore Corp.). They were washed several times with 0.5 N perchloric acid and dried, and the radioactivity was measured. The difference between the radioactivity retained by the alkali-treated cells and that retained by untreated cells gives a measure of the incorporation of radioactivity into RNA.

The method of Bremer et al. (2), with the experimental procedures already described, was also used to measure the rate of RNA chain growth in *E. coli* (ATCC 11246) growing at 37°C in Sauton medium (11) in which glycerol was replaced by 0.2% glucose.

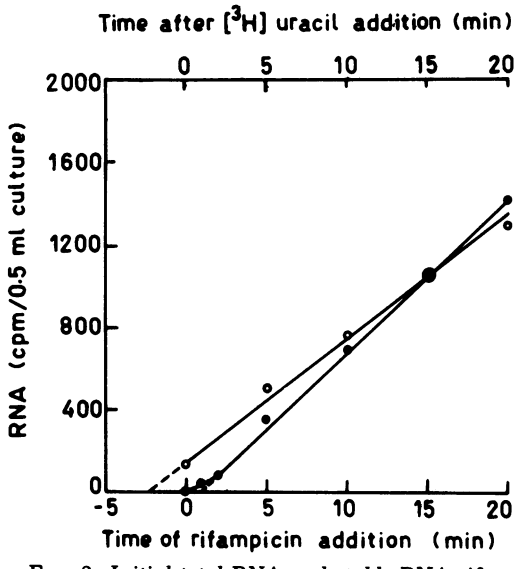

FIG. 2. *Initial total RNA and stable RNA-rifampin kinetics. The culture at a turbidity of 40 Klett units was added to [3H]uracil at zero time. Samples (0.5 ml) were taken and either precipitated directly for determination of the total RNA-labeling kinetics (●) or added to rifampin for further incubation before precipitation and determination of stable RNA rifampin kinetics (⊙). $L_s^{ru}(t)$ kinetics extrapolate to the time axis at −2.5 min. In the labeling kinetics of total RNA, the labeling lag determined by extrapolation to the time axis is 1 min.*

RESULTS

Method (i). (i) Measurement of nucleotide step times. Table 1 represents the radioactivity recovered in UMP and Urd after alkali hydrolysis of the RNA synthesized by pulsing the bacterial culture with [3H]uracil for various times. The radioactivity associated with Urd (E_u) arises from the 3′ end of the RNA chains during alkali hydrolysis, whereas that associated with UMP arises from the internal UMP residues of the RNA. The radioactivities in UMP as well as Urd were added together to get the total incorporation of UMP into the RNA (τ_u). The ratio E_u/τ_u gives the apparent reciprocal chain length of the RNA synthesized after addition of [3H]uracil to the culture. The culture had been prelabeled with [2-14C]uracil for 10 h before addition of [3H]uracil, and the 14C radioactivity associated with UMP after alkaline hydrolysis of RNA was also measured. The ratio of 3H/14C in UMP was determined (I_u) and is a reliable index of total 3H incorporated per unit mass of

TABLE 1. *Step time (τ_u) of uridylic acid in Sauton medium at 37°C*

Labeling time (s)	[3H]UMP/[14C]UMP (I_u)	[3H]UMP (cpm)[a]	[3H]Urd (cpm) E_u[a]	τ_u (cpm)	E_u/τ_u	τ_u (ms)	
						Apparent[b]	Corrected[c]
60	24.05	11,500	400	11,900	0.03364		
80	116.15	25,970	530	26,500	0.02000	277.4	
100	560.00	45,398	682	46,080	0.01480	286.4	262.3
120	1,380.00	69,438	832	70,260	0.01184	295.4	260.7

[a] The counts per minute are corrected for differences in the counting efficiencies of nucleotides and nucleosides.

[b] Calculated by use of equation (4′) (reference 8).

[c] Calculated by use of equation (4) (reference 8).

bacteria, independent of any variable losses of these nucleotides during the extraction and isolation procedures.

On the basis of these data estimates of the UMP step time (τ_u) have been made using the appropriate equations (4 and 4' [8]). This gives an average step time of 262 ms for UMP. Similarly, step times for CMP(τ_c) and GMP(τ_g) have been calculated to be 330 ms and 152 ms, respectively (Tables 2 and 3). The step time for adenosine 5'-monophosphate (AMP) could not be calculated by this method and hence is assumed to be the same as that for GMP (8).

(ii) Determination of RNA/DNA ratios in *M. tuberculosis* and *E. coli*. The RNA/DNA ratio per gram of mid-log-phase culture was 10-fold lower for *M. tuberculosis* as compared with that for *E. coli* (data not shown).

Method (ii).(i) Labeling of nucleic acids in the presence of rifampin. If [^{3}H]uracil and rifampin are added coincidentally to *M. tuberculosis*, the kinetic pattern of total nucleic acid labeling can be resolved into two superimposing kinetics of DNA and of RNA labeling (Fig. 1). The radioactivity in DNA increases continuously, since DNA synthesis is not primarily affected by the drug. The kinetics of RNA labeling show a maximum after about 3 h, representing label in stable and unstable RNA. After 3 h, the label in RNA decreases to a constant level, reflecting the decay of unstable RNA and the cessation of RNA synthesis (1, 10). The final level represents stable RNA molecules which, nascent at the time of addition of [^{3}H]uracil and rifampin, were completed.

(ii) Stable RNA-rifampin kinetics. The kinetics of incorporation of [^{3}H]uracil into total RNA (in the absence of rifampin), and into stable RNA after addition of rifampin, were again determined in the experiments illustrated in Fig. 2. Here, only the final plateau level of incorporation after 5 h, representing stable RNA, was determined. Again these plateau values were plotted against the time of rifampin addition to give the stable RNA-rifampin kinetics [$L_s^{rif}(t)$, reference 2]. The zero-time point of these kinetics corresponds to the amount of label incorporated into stable RNA after coincident addition of [^{3}H]uracil and rifampin.

(iii) Determination of ribosomal RNA chain growth rate. According to equation (8) [see Theoretical Analysis and Fig. 3(b) in reference 2], the shift, t_s, needed to construct the kinetics of labeling stable RNA, is equal to the sum of the extrapolation of the $L_s^{rif}(t)$ kinetics to the time axis, t_x, and the initial lag, t_{lag}, in the kinetics of labeling total RNA. The initial section of rifampin kinetics extrapolates to −2.5 min on the time axis (Fig. 2). Thus, t_x = 2.5 min. The lag in the initial incorporation (t_{lag}) is obtained from the kinetics of labeling total RNA as 1 min, and the shift, t_s, is therefore equal to 2.5 + 1 = 3.5 min.

The transcription time of the ribosomal RNA (rRNA) transcriptional unit, t_u, is obtained according to equation (9) (reference 2), from the extrapolation value, t_x, the incorporation lag, t_{lag}, and lag in the action of rifampin, t_r. Since t_r is about 3 to 5 s (unpublished data) we have omitted it from the equation, so that

$$t_u = \frac{t_x + t_{lag}}{0.46} \quad (1)$$

TABLE 2. *Step time of cytidylic acid (τ_c) in Sauton medium at 37°C*

Labeling time (s)	[^{3}H]CMP/[^{14}C]CMP (I_c)	[^{3}H]CMP (cpm)[a]	[^{3}H]Cyd E_c (cpm)	τ_c	E_c/τ_c	τ_c (ms)	
						Apparent[b]	Corrected[c]
60	2.34	460	30	490	0.06123		
80	35.00	1,874	62	1,936	0.03202	344.5	
100	140.44	4,041	75	4,116	0.01822	361.4	322.2
120	285.3	8,801	81	8,882	0.00912	385.3	336.9

[a,b,c] See Table 1 for explanation of footnote symbols.

TABLE 3. *Step time of guanylic acid (τ_g) in Sauton medium at 37°C*

Labeling time (s)	[^{3}H]GMP/[^{14}C]GMP (I_g)	[^{3}H]GMP (cpm)[a]	[^{3}H]Guo E_g (cpm)[a]	τ_g	E_g/τ_g	τ_g	
						Apparent[b]	Corrected[c]
60	6.68	96,768	802	97,570	0.00822		
80	15.00	226,660	940	227,600	0.00413	152.5	
100	22.82	385,884	1,116	387,100	0.00300	169.7	148.9
120	30.00	607,568	1,132	608,700	0.00186	177.6	152.4

[a,b,c] See Table 1 for explanation of footnote symbols.

Using t_x = 2.5 min and t_{lag} = 1 min,

$$t_u = \frac{2.5 + 1}{0.46} = 7.608 \text{ min}$$

Assuming the length of the rRNA transcriptional unit, which corresponds to 4,620 nucleotides (sum of 16*S*, 23*S*, and 5*S* RNA [5, 6]), to be the same for *M. tuberculosis* and *E. coli*, the chain growth rate of rRNA, c_r, is calculated for the quotient:

$$c_r = \frac{4{,}620}{t_u} = \frac{4{,}620}{7.608} \quad (2)$$
$$= 10.1 \text{ nucleotides per s}$$

Normally the value for t_x has to be corrected for changes in the specific activities of the uracil pool during the labeling period. The specific activity of the precursor pool can be obtained from the relation

$$S(t) = \frac{l_s(t)}{r_s(0)} \cdot e^{-\ln 2 \cdot t/D} \quad (3)$$

where $l_s(t)$ is the labeling rate of stable RNA at time t, $r_s(0)$ is the initial rate of stable RNA synthesis, and D is the doubling time of the bacterium. The values of $l_s(t)$ have been obtained at various values for t as the mean slopes of the stable RNA kinetics (inset Fig. 3). It was found that the increase in the labeling rate of the stable RNA between 3.8 min (0.5 t_u) and 7.6 min (t_u) was only 1.11-fold. The exact specific activities of the precursor pools could not be determined because the value of $r_s(0)$ is not known. However, the ratio of the specific activities at the two time intervals could be calculated by using equation 3. It was found that there was only 1.09-fold increase in the specific activity of the precursor pool from 3.8 to 7.6 min. Hence, the observed increment of radioactivity in the stable RNA after rifampin addition (ΔL_s^{rif}) is (0.75 × 1 + 0.25 × 1.09) = 1.02 times higher than would be expected. Hence, the observed zero point of the rifampin kinetics $L_s^{rif}(0)$ is 1.02 times too high and must be decreased by a correction factor $\epsilon_0 = (1 - 1/1.02) L_s^{rif}(0) = 0.02 L_s^{rif}(0) = 0.02 \times 1.4 \times 10^2 = 2.8$. However, this correction does not appreciably alter the value of $t_x = -2.5$ min. (For details regarding the corrections, the reader is referred to section E of Theoretical Analysis and Experimental Results, reference 2.)

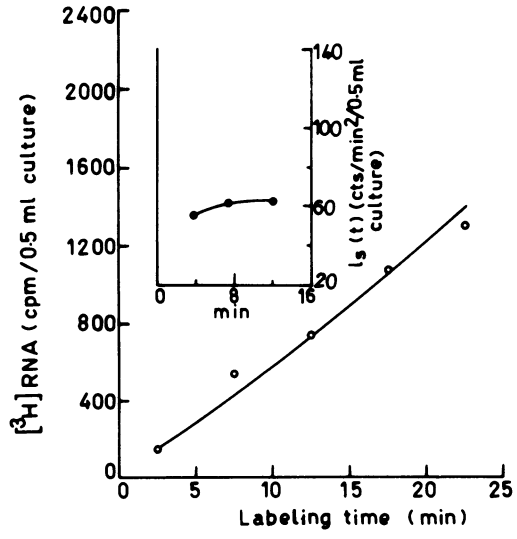

FIG. 3. *Kinetics of labeling stable RNA. The kinetics of labeling of stable RNA* (⊙) *were obtained by shifting the rifampin kinetics* $L_s^{rif}(t)$ *from Fig. 2, 2.5 min in the positive direction of the time axis. The inset shows the labeling rate of stable RNA* (●) *obtained from the slope of the stable RNA curve for intervals between 2.5 and 5 min, 5 and 10 min, and 10 and 15 min.*

In experiments with *E. coli* as well, the rates of RNA chain growth have been calculated by the same procedure. We have obtained a value of 50 s for t_u, which corresponds to an rRNA transcription time of 92 nucleotides per s. These values are in good agreement with those reported by Bremer et al. (2).

DISCUSSION

The two methods employed by us for calculating the growth rate of RNA chains in *M. tuberculosis* $H_{37}R_v$ are adaptations of those already employed for obtaining RNA elongation rates in *E. coli* (2, 8). The basic theoretical principles underlying the two approaches are different. The first method arrives at the step time of addition of a nucleotide to the growing end of the RNA by measuring the chain length of RNA synthesized during a short time in the presence of the radioactive precursor nucleotide. On the other hand, the second one follows the kinetics of RNA labeling with the radioactive precursor in the presence of rifampin, which is an inhibitor of initiation of RNA synthesis but not of elongation of the growing RNA chains.

By using the method developed by Manor et al. (8) we have calculated the step times for the addition of UMP, CMP, and GMP to the growing RNA chains to be 262, 330, and 152 ms, respectively. The method does not permit an accurate estimate of the step time for AMP, since the cellular abundance of adenosine at the 3′ end of transfer RNAs makes the solution for the step time of AMP inaccurate. Hence, it has been assumed that τ_a is equal to τ_g. Assuming that the mole fractions of all four

bases are the same in RNA and using an averaged step time τ = 224 ms, the average growth rate of the RNA chains in *M. tuberculosis* growing in Sauton medium at 37°C was calculated as 4 nucleotides per s. By using a different approach (2) we found the rate of growth of rRNA chains to be 10 nucleotides per s. If one assumes that, as in *E. coli*, the rate of chain growth of unstable RNA in *M. tuberculosis* is only half that of the stable RNA and that, at any instant of time, 71% of the RNA synthesized is of the stable type (2), one can deduce that the average growth rate of RNA chains in this organism would be 8 to 9 nucleotides per s, which is twofold higher than the value we have obtained by the method of Manor et al. (8). However, the method of Manor et al. might slightly underestimate the elongation rate because of the complicated correction for the change in specific radioactivity of the precursor pool and the termination of RNA chains during the labeling period. It has been found in the more recent estimates of RNA chain growth in *E. coli* (2) that the value is 80 to 100 nucleotides per s as opposed to 43 nucleotides per s given by Manor et al. It seems, therefore, that an RNA growth rate of 4 to 10 nucleotides per s in *M. tuberculosis*, growing at 37°C, is a reasonably accurate estimate. Bremer et al. (2) have also estimated the elongation rate of mRNA's in *E. coli* and found it to be lower than that of rRNA. However, since this calculation requires a knowledge of the replication time of DNA, the time elapsed between the synthesis of DNA and actual cell division, the number of genomes per cell, etc., and since we have little knowledge of these parameters in *M. tuberculosis*, we were unable to undertake the estimation of messenger RNA growth rate in this organism. It must be mentioned that the method by Manor et al. (8) assumes an equal mole fraction of all four bases in the RNA for calculating the growth rate of the RNA chains, and we have also used this assumption in our calculations. Since mycobacterial DNA is known to have a high guanine plus cytosine content, one might be tempted to question the validity of such an assumption. Nevertheless, it has been found by Midgley and McCarthy (9) that the RNAs of several bacteria with DNAs of widely varying guanine plus cytosine contents still have equimolar distribution of the four bases.

Comparison of our results with those from *E. coli* showed that the rate of RNA chain growth in *M. tuberculosis* is 10 times lower than that in *E. coli*. It has been reported by Manor et al. (8) that *E. coli* growing in different media with different growth rates exhibit variations in their rate of RNA chain growth. According to their results, nucleotide step times did grow longer with bacterial generation time, upon passage from glucose-Casamino Acids to succinate or proline media. However, the increases in step time alone were not enough to account completely for the observed decreases in the net rate of bacterial RNA synthesis. Thus, it was concluded that the bacteria growing in a richer medium achieved their higher rate of RNA synthesis, allowing faster growth, mainly by providing greater numbers of nascent RNA molecules, although a small contribution is also made by a reduction in nucleotide step time. Examination of the RNA contents per DNA in *E. coli* and *M. tuberculosis* has shown that the latter contains a 10-fold lower amount of RNA per genome (assuming that the size of the genome is the same in both organisms). Since *M. tuberculosis* undergoes 20-fold fewer divisions per hour than *E. coli*, it follows that the net rate of RNA synthesis per DNA template is 20 $\times$ 10 = 200-fold lower in this organism than in *E. coli*. Thus a 10-fold decrease in the RNA chain elongation rate does not wholly explain the rather large difference in the net RNA synthesis rates between these organisms. It would therefore appear that such marked changes in the rates of RNA synthesis must reflect the initiation frequency of RNA molecules, as well as the number of RNA polymerase molecules actively engaged in RNA synthesis at a specified time.

It is also interesting to speculate on the possibility that *M. tuberculosis* may have more than one RNA polymerase with varying degrees of affinity for the nucleoside triphosphate precursors and which may perhaps be involved in the synthesis of specific classes of RNA. It might then turn out that the different polymerases would engender different nucleotide step-times, and the relative proportions of these enzymes would determine the overall rate of RNA synthesis. Such a possibility does seem interesting in view of our preliminary results suggesting a heterogeneity of RNA polymerase in *M. tuberculosis* (7). Currently, we are engaged in detailed studies on the separation and characterization of the different RNA polymerases from this organism.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Detailed studies on the separation and characterization of two RNA polymerases in *M. tuberculo-*

sis will appear in the December issue of the *Journal of the Indian Institute of Science*.

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