Synthesis Time of β -Galactosidase in *Escherichia coli* B/r as a Function of Growth Rate

By DAVID G. DALBOW* and RY YOUNG
 Institute for Molecular Biology, University of Texas, Dallas, P.O. Box 688,
 Richardson, Tex. 75080, U.S.A.

(Received 25 September 1974)

By analysing the kinetics of β -galactosidase accumulation after induction, the synthesis time of β -galactosidase in *Escherichia coli* B/r was found to be 75 s in rapidly growing cells (1.36 and 2.1 doublings/h), and 90 s in slowly growing cells (0.63 doubling/h). These values correspond to peptide-chain-elongation rates of 16 and 13 amino acids/s respectively, in agreement with previous findings, indicating that the peptide-chain growth rate is constant (presumably maximal) in fast-growing bacteria, but decreased in slowly growing bacteria [Forchhammer & Lindahl (1971) *J. Mol. Biol.* 55, 563–568].

The question whether the peptide-chain-elongation rate in bacteria is constant or increases with the bacterial growth rate (cell doublings/h) is important for the problem of translational control: an increase in the peptide-chain-elongation rate means an increase in the synthesis rate of any given protein, without a corresponding increase in the synthesis of mRNA. (This is of particular interest for the control of ribosome synthesis, since both the synthesis rate of rRNA and of mRNA for r-proteins must change in a co-ordinated manner as a function of the bacterial growth rate.) This question is controversial. Engbaek *et al.* (1973) found the peptide-chain growth rate of β -galactosidase in *Salmonella typhimurium* to be independent of the cellular growth rate (about 18 amino acids/s). In contrast, from measurements of the number of active ribosomes and the amount of protein synthesized during growth at different rates, Forchhammer & Lindahl (1971) have calculated that there is a significant difference between the peptide-chain-elongation rate in slow-growing and in fast-growing cells.

By analysing the kinetics of β -galactosidase accumulation after induction, we observed here that the synthesis times of β -galactosidase for *Escherichia coli* B/r growing exponentially at 0.63, 1.36 and 2.1 doublings/h are 90, 75 and 74 s respectively, corresponding to peptide-chain growth rates of 13, 16 and 16 amino acids/s, which is consistent with the findings of Forchhammer & Lindahl (1971).

* Present address: Department of Pathology, Colorado State University, Fort Collins, Colo. 80521, U.S.A.

Fig. 1. Scheme of the early section of the kinetics of β -galactosidase accumulation after the addition of inducer at zero time ($t = 0$)

t_s , synthesis time of β -galactosidase [for exact definition see the Discussion section part (a)]; t_0 , time at the calculated origin of the parabolic section of the kinetics; for a pure parabola $t_0 = t_s$; t_1 , t_2 are two timepoints at which the amounts of enzyme are a_1 and a_2 (over basal value). In order to find t_0 from experimental kinetics, quarter-height analysis is used: a point (●) on the kinetics given by the co-ordinates t_2, a_2 is chosen arbitrarily. A second point (○) given by the co-ordinates t_1, a_1 is then found according to the condition $a_1 = 0.25 a_2$ ('quarter-height'). If the kinetics is parabolic between t_0 and t_2 , then the distance $(t_2 - t_1)$ must be equal to the distance $(t_1 - t_0)$ such that $t_0 = t_1 - (t_2 - t_1)$. t_x = extrapolation of the final linear section of the kinetics to the basal level. It can be shown that the distance $(t_x - t_s)$ is essentially equal to the average functional life of β -galactosidase mRNA.

Theory

The induction kinetics (amount of enzyme per sample volume of culture as a function of time after induction) of the enzyme β -galactosidase shows three phases (Fig. 1). (1) Initially the amount of enzyme remains constant at the basal value for a period of time corresponding to the synthesis time (essentially time for initiation, elongation and termination of β -galactosidase chains, but see the Discussion section for more exact definition). (2) There follows a period of transition which begins with a parabolic [actually nearly parabolic; see the Discussion section part (b)] increase, reflecting the initial increase in the number of both β -galactosidase mRNA molecules and of translating ribosomes per β -galactosidase mRNA molecule; the kinetics are then gradually changing into the final linear phase; this transition also reflects the decay of β -galactosidase mRNA. (3) The final phase is reached when the amount of β -galactosidase mRNA per cell is constant (equilibrium between synthesis and breakdown) such that the rate of enzyme synthesis per cell is constant in time, and the amount of enzyme per volume of culture increases exponentially, in proportion to the increase in cell number.

To determine the β -galactosidase-synthesis time, it is obviously necessary to find the beginning of the second phase, i.e. the origin of the parabola, from the parabolic shape of the induction kinetics immediately after the amount of enzyme has begun to increase. This is done as follows: at an arbitrarily

chosen early time (t_2) an amount of enzyme (a_2) has accumulated (over basal value). An earlier time (t_1) at which an amount corresponding to $1/4 a_2$ has accumulated is then found from the induction kinetics. The time difference ($t_2 - t_1$) is equal to time between zero and t_1 ; i.e. the synthesis time $t_0 = t_1 - (t_2 - t_1)$ (Fig. 1). If this operation is repeated for various pairs of t_2 and a_2 , identical values of t_0 should be obtained if the kinetics were truly parabolic. Actually small deviations for t_0 are observed, in the order of a few seconds, which are expected, since the kinetics are not expected to be exactly parabolic [see the Discussion section part (b)]. This type of analysis will be designated 'quarter-height analysis'.

The final (exponential) phase of the induction kinetics can be made to appear linear by using an expanded time-scale (McCarthy & Britten, 1962) which corrects for the growth of the culture. This has the advantage that the final kinetics can be extrapolated back to the basal value, where it defined an extrapolation time t_x (Fig. 1). This time is related to the parameters which determine the early phases of the induction kinetics, i.e. it can be shown that t_x is the sum of the β -galactosidase synthesis time (t_s) plus the average functional life (τ) of β -galactosidase mRNA plus the average life (θ) of the *lac* operator-repressor-inducer complex (see the Discussion section). With the bacterial strain and under the conditions used here, t_x was found to be 150 ± 10 s, independent of the bacterial growth rate (between 0.6 and 2 doublings/h; Dalbow & Bremer, 1975).

Fig. 2. Accumulation of β -galactosidase activity (E_{420}) during the first 3 min after induction in cultures growing in succinate (a; 0.63 doublings/h), glucose-minimal medium (b; 1.3 doublings/h) and glucose-amino acids medium (c; 2.2 doublings/h)

Thin horizontal lines represent the basal value of enzyme in the uninduced culture (background owing to spontaneous substrate hydrolysis is already subtracted). The origin of the parabolic section for each kinetics was determined to be at 89, 74 and 73 s respectively (Table 1). See the Materials and Methods section for a description of the assay conditions.

The implications of this finding for the fine analysis of the induction kinetics and more precise determination of the β -galactosidase synthesis time are discussed below.

Table 1. *Quarter-height analyses of the enzyme-induction kinetics shown in Fig. 2*

t_2 , Time-point being subjected to quarter-height analysis; a_2 , height of curve (above basal value) at the given time (amount of induced enzyme at t_2); $a_2/4 = a_1 = 0.25 a_2$ ('quarter height'); t_1 , time-point at which curve is equal to quarter-height value (midpoint between t_0 and t_2); t_0 , origin of parabolic section of kinetics, calculated from the relationship $t_0 = t_1 - (t_2 - t_1)$ (see the Theory section). The basal value was subtracted from all E_{420} values (for the three media, the subtracted values are 0.021, 0.014 and 0.012 respectively). In regions where the data scatter, the best smooth curve was used.

Culture medium	t_2 (s)	a_2 (E_{460})	$a_2/4$ (E_{420})	t_1 (s)	t_0 (s)
Succinate	180	0.178	0.045	132	84
	170	0.145	0.036	128	86
	160	0.118	0.029	124	88
	150	0.088	0.022	119	88
	140	0.063	0.016	115	90
	130	0.039	0.010	109	88
Glucose	180	0.178	0.045	124	68
	170	0.153	0.038	120	70
	160	0.124	0.031	115	70
	150	0.100	0.025	112	74
	140	0.077	0.019	107	74
Glucose-amino acids	180	0.220	0.055	124	68
	170	0.188	0.047	120	70
	160	0.148	0.037	115	70
	150	0.119	0.030	111	72
	140	0.102	0.026	107	74
	130	0.071	0.018	101	72

Materials and Methods

Bacteria and growth conditions

Cultures of *Escherichia coli* B/r (A.T.C.C. 12407) were grown at 37°C in minimal C medium (Helms-tetter, 1967), supplemented with 0.2% succinate, glucose or glucose and a mixture of 20 L-amino acids (20 µg/ml each). Experimental cultures were inoculated by at least a 500-fold dilution of a fresh stationary-phase culture in the same medium. Growth was followed by measuring the turbidity at 460 nm (1 cm light-path). The doubling times for the various media were 90–95, 43–46 and 27–30 min respectively.

Induction and assay of β -galactosidase

Cultures, on reaching a turbidity (E_{460}) of 0.1, were treated with cyclic AMP (Sigma Chemical Co., St. Louis, Mo., U.S.A.) to a final concentration of 0.01 M, to decrease catabolite repression. Then 5 min later synthesis of β -galactosidase was induced by the addition of the inducer isopropyl- β -D-thiogalactopyranoside (Sigma), to a final concentration of 0.01 M. These concentrations of cyclic AMP and inducer have been shown to produce maximum induction of β -galactosidase (Dalbow & Bremer, 1975). After induction, 180 µl portions ($\pm 1\%$) of the culture were taken at 2 s intervals ($\pm 0.2\%$), by using an apparatus which operates with the rotating stop-cock principle (Lonberg-Holm, 1959), modified to provide vigorous aeration at 37°C.

β -Galactosidase activity was assayed as described by Dalbow & Bremer (1975) except that the incubation at 30°C was allowed to continued for 24 h.

Substrate hydrolysis was found to be proportional to both assay time and concentration of induced culture under these conditions up to an E_{420} of about 0.3. To avoid correction of data for non-linearity of the assay, cultures were induced at an E_{460} of 0.1;

Table 2. *Summary of β -galactosidase-induction kinetics*

Basal value is normalized to $E_{460} = 0.1$ at induction. The calculated synthesis time is equal to origin of parabolic kinetics, calculated from quarter-height analysis (Table 1, for $t_2 = 130$ –150 s). Peptide-chain-elongation rate is based on 1190 amino acid residues in β -galactosidase (Craven *et al.*, 1968).

Culture medium	μ (doublings/h)	Basal value (E_{420})	Calculated synthesis time (s)	Peptide-chain- elongation rate (amino acids/s)
Glucose-amino acids*	2.2	0.012	72–74	16.3–16.1
Glucose-amino acids	2.07	0.014	71–75	16.8–15.4
Glucose*	1.3	0.014	70–74	15.6–14.7
Glucose	1.4	0.015	75–81	16.9–16.1
Succinate*	0.63	0.021	88–90	13.5–13.2
Succinate	0.62	0.022	86–94	13.8–12.7

* Kinetics shown in Fig. 2.

thus E_{420} values of less than 0.25 obtained at the maximum sampling time of 3 min after induction.

Results

The induction kinetics of *E. coli* B/r cultures growing with doubling times of 95, 46 and 27 min respectively are shown in Fig. 2; Table 1 contains the quarter-height analyses of the curves in Fig. 2. Table 2 contains a summary of these analyses plus analyses done on additional kinetics performed in an identical fashion. Thus the origin of the parabolic induction kinetics appears to be at 74 ± 2 s after induction for cells grown on glucose and 75 ± 4 s for cells grown on glucose and amino acids. Taking this time as equal to the translation time for β -galactosidase (135 000 daltons = 1190 'average' amino acids), one obtains an average peptide-chain-elongation rate of 16 amino acids/s for bacteria grown in these two media. However, the origin of the parabolic phase of the induction kinetics for bacteria grown in succinate-minimal medium is found at 90 ± 3 s after induction, giving 13 amino acids/s for the average peptide-chain-elongation rate. Although precise statistical analysis is not meaningful for three sets of two measurements each, there is no overlap between the range of parabolic origins found for the fast-growing cells ($\mu = 2.1$ – 2.2 doublings/h) and that found for the slow-growing cells ($\mu = 0.6$ doubling/h). As expected, in all the kinetics there is a detectable departure from parabolic shape at about 2 min after induction.

Discussion

(a) Components of the synthesis time

We define the time-interval between the addition of the *lac* inducer and the origin of the parabolic increase in β -galactosidase activity as the synthesis time for the complete β -galactosidase molecule. Included in this time-interval are a series of necessary events: (1) mixing of inducer into the culture volume; (2) penetration of inducer into bacterial cells and binding to repressor; (3) dissociation of the *lac* repressor from operator; (4) initiation of transcription at the *lac* promoter; (5) synthesis of the ribosome-binding mRNA sequence and initiation of translation; (6) translation of the complete *z*-gene (by the first ribosome) and release of the finished *z*-gene product after termination of translation; (7) maturation, folding and assembly of complete active tetrameric enzyme.

Evaluation of the contribution of each of these processes to the total synthesis time follows:

(1) *Mixing of inducer.* We have measured the absorbance of samples taken from liquid volumes injected with an absorbing substance and aerated vigorously in our apparatus and concluded that the

time required for mixing to homogeneity is less than 3 s; even before 3 s, the inducer is at inducing concentrations throughout most of the culture, since we used an inducer concentration 10 times higher than necessary for maximum induction. In any case, mixing time will be the same for all growth rates.

(2) *Penetration of inducer.* Coffman *et al.* (1971) argued that, since passive penetration of inducer into the cell should be dependent on the extracellular inducer concentration and since they observed no change in total lag time before the first induced enzymes appear over a wide range of inducer concentrations, the penetration time is insignificant in comparison with the β -galactosidase-synthesis time. However, their data are not precise enough to justify this conclusion [see part (c) of this Discussion section]. By measuring the capacity of β -galactosidase synthesis after the simultaneous addition of various concentrations of inducer and rifampicin Hirsh & Schleif (1973) show conclusively that the penetration time of the inducer is less than 1 s (for cells grown in a glycerol-minimal medium, with a doubling time of about 1 h). For the purposes of this analysis it is assumed that the penetration time of inducer is insignificant for all growth rates, which is reasonable since the penetration is passive and the cell membrane does not change radically over the growth-rate range considered.

(3) *Dissociation of the repressor from the operator.* Once the inducer binds to the repressor-operator complex, de-repression (removal of the repressor-inducer complex from the *lac* operator) probably occurs within seconds; S. Bourgeois (personal communication) has shown that the average life of the inducer-repressor-operator complex (θ) is less than 5 s *in vitro* at an ionic strength of 0.05 M. The de-repression should be even faster under ionic conditions *in vivo*, since it has been shown that the un-induced repressor-operator dissociation is much more rapid at higher ionic strengths (Riggs *et al.*, 1970).

(4) *Initiation of transcription.* It has been estimated that even in maximally induced cells there are only about two RNA chain initiations/min at each *lac* promoter (Hirsh & Schleif, 1973; Bremer & Dalbow, 1975). Therefore there is an approx. 15 s average lag after de-repression before the first transcription occurs. It might seem that this delay causes a gradual increase (over a period of 15–30 s) in the rate of initiation by the polymerase. However, closer examination reveals that, in a population of cells, the rate of RNA chain initiation increases immediately after de-repression in a stepwise manner to the final value (without any lag). This is because the rate is proportional to the probability of initiation at a promoter per unit time; this probability presumably has only two values, one for the repressed state, the other for the de-repressed state.

Thus the transcription-initiation rate does not contribute at all to the total synthesis time; regardless of the magnitude of the initiation rate, it is only the time lag (θ) before that initiation rate is established [see part (3) of this section] that contributes to the total synthesis time.

(5) *Initiation of translation.* The first ribosome-initiation site on the new messenger must occur within approx. 40 nucleotides from the transcription-initiation site (Maizels, 1973). According to present estimates of the mRNA-chain-elongation rate (Bremer & Yuan, 1968; Rose *et al.*, 1970), the appearance of the newly synthesized ribosome initiation sequence should thus occur within 1 s after the initiation of transcription.

Once the mRNA sequence specific for ribosome initiation is synthesized, the initiation rate for ribosomes per ribosome binding site is instantly constant [by the same argument of part (4)], unless there are conformational differences between nascent untranslated mRNA and steady-state mRNA. [If *lac* mRNA is translated as efficiently as average mRNA, it is translated about once every 4–8 s, depending on the bacterial growth rate (Dennis & Bremer, 1974).]

(6) *Translation and release from the ribosome.* The translation time for β -galactosidase chains of 1190 amino acid residues can be estimated to be between 60 and 120 s, since all previous estimates of the peptide-chain-elongation rate are between 10 and 20 amino acid residues/s [see section c(2) below].

We are not aware of any direct data on the time required for polypeptide-chain termination and release. If a messenger is translated every 4–8 s, then a termination and release time longer than this value will cause a stacking of ribosomes at the termination region, which was not observed in the electron micrographs of active genes in *E. coli* (Miller *et al.*, 1973).

(7) *Maturation and assembly.* In principle, there may be two classes of maturation events required to convert finished polypeptides into active enzyme: (i) those which can occur after the sampling process (e.g. after chilling or toluene treatment), but before the assay; and (ii) those which require metabolic activity but no protein synthesis (e.g. which can occur in the presence of chloramphenicol but not after toluene treatment). Events of the first type do not influence the induction kinetics and, in fact, are not measurable unless very fast assay methods are used. The events of the second type, however, will influence the induction kinetics, e.g. the kinetics will be different if such events are allowed to occur (by incubation with chloramphenicol) or not (by immediate toluene treatment).

Kepes (1963) and Kaempfer & Magasanik (1967) observed that the amount of β -galactosidase increased somewhat after addition of chloramphenicol to the induced culture in comparison with samples treated

immediately with toluene suggesting that a maturation process of the second class does occur. However, Alpers & Tomkins (1965) did not find such an increase in activity in the presence of chloramphenicol; further, Berg & Zabin (1964) were unable to detect any immunologically cross-reactive material that was not functional enzyme. Also, Adamson *et al.* (1970), who attempted to elucidate whether or not tetramer assembly was a 'rate-limiting factor on induction' by measuring the induction lag under conditions of very low intracellular concentrations of polypeptide, found no evidence for an extended assembly time. (Judging from their data, however, one cannot rule out an assembly time of less than 5 min.)

This controversy suggests that the presence of the 'maturation' effect depends on some undetermined conditions used to sample, kill and assay the induced culture. Therefore we attempted to detect a maturation effect under our conditions by sampling an induced culture into either toluene or chloramphenicol. The results (see Fig. 3) show that there is no significant difference between the samples taken into chloramphenicol and those taken into toluene; thus the

Fig. 3. Effect of incubation of samples with chloramphenicol on the induction kinetics of β -galactosidase

Cultures were grown in glycerol-amino acids medium (1.6 doublings/h) and induced at $E_{460} = 0.6$ (●). Samples were treated with toluene at the times indicated and assayed as in the experiment of Fig. 2, except that incubation with substrate lasted 30 min (○). Samples were first incubated for 15 min with 200 μ g of chloramphenicol/ml, beginning at the times indicated, and then treated with toluene and assayed as above.

maturation effect found by Kepes (1963) and Kaempfer & Magasanik (1967) to be in class (ii) above appears to be in class (i) in our hands (or is not required at all). Hence, under our conditions, the sampling into toluene provides a precise assay for the amount of finished polypeptide, measured as functional enzyme.

In summary, the best interpretation of available data indicates that the synthesis time of the complete β -galactosidase molecule is rather precisely determined by the translation time of β -galactosidase monomers.

(b) Validity of the method

Theoretically, the induction kinetics of β -galactosidase would be parabolic when the functional life of β -galactosidase mRNA (τ) was infinity and the average life (θ) of the *lac* repressor-operator complex in the presence of the inducer was zero. Applying quarter-height analysis to such a theoretical parabola (Table 3, first column: $\tau = \infty$, $\theta = 0$) gives the same origin of the parabola (at $t = 0$) if the time t_2 (see the Theory section) is decreased in successive steps between 108 and 60s after the origin. Assuming the origin to be between 70 and 90s after induction, the

range of 108–60s after origin corresponds to 180–130s after induction, i.e. we are considering the range for t_2 which has been used for quarter-height analysis of the observed data (Table 1).

The next two columns in Table 3 ($\tau = 75$ s, $\theta = 0$; $\tau = 90$ s, $\theta = 0$) show the influence of a finite τ value on the quarter-height analysis. Two effects are seen: first, the time of the origin is always underestimated; secondly, the estimate increases (and approaches the correct value) with decreasing values of t_2 . Since for the evaluation of the observed data, the exact time of the origin is not known, one can only observe the second effect: in these theoretical examples, a +7s increase in the calculated origin (i.e. from -11 to -4 or -10 to -3s respectively for t_2 values between 108 and 60s).

As mentioned in the Theory section, the functional life (τ) of β -galactosidase mRNA is related to the intercept (t_s) at 150s of the final section of the induction kinetics extrapolated backwards to the basal value:

$$\tau = t_s - (t_s + \theta)$$

such that (for $\theta = 0$) $\tau = 150 - t_s$. If the synthesis time t_s is assumed to be between 60 and 90s, one obtains approximate values for τ between 75 and 90s, i.e. the range considered in Table 3.

In the last columns of Table 3 the influence of θ on the quarter-height analysis is explored. Since θ is in the order of a few seconds [see section a(3) above] two small values (7.5 and 15s) were considered. By comparing the last three columns of Table 3 ($\tau =$ constant at 90s, θ increasing from 0 to 15s) one can see that already small values of θ produce an increase in the estimated (non-observable) time of the origin and a decrease in the (observable) drift of the calculated origin with decreasing values of t_2 (in the t_2 range considered; see above). Thus the observed drift of 4–5s (average from Table 1) is consistent with a θ value of somewhat less than 7s. Also, small θ values fortuitously compensate for the error in the estimate of the origin (t_s) produced by τ alone.

Considering these effects, we conclude that our values for the synthesis time are theoretically accurate within 5%.

(c) Peptide-chain-elongation rate

The peptide-chain-elongation rates calculated here for three different growth media give 16, 16 and 13 amino acids/s for cells growing with 2.1, 1.3 and 0.63 doublings/h (Table 2), similar to but somewhat lower than some previous measurements (Forchhammer & Lindahl, 1971: 20, 21 and 16 amino acids/s for cells with 2.1, 1.5 and 0.87 doublings/h) and also similar to more recent values obtained from evaluation of the radioactive-labelling kinetics of nascent peptide chains (R. Young & D. G. Dalbow, unpublished work: 17, 17 and 12 amino acids/s). This

Table 3. Application of quarter-height analysis to five theoretical (one truly parabolic and four non-parabolic) induction kinetics (see Fig. 4), showing the influence of a finite functional life (τ) of β -galactosidase mRNA and of a delay (θ) in the dissociation of the repressor from the operator

The Table shows the time of the calculated origin (by using quarter-height analysis) in relation to the true origin of the curves (for these theoretical examples, the true origin is known; for observed data the origin is not known). Negative and positive values refer to the number of seconds before or after the true origin respectively, and therefore would correspond to under- or over-estimates of the translation time of β -galactosidase if the translation time were to be estimated from the theoretical curves of Fig. 4. Each set of kinetics is characterized by two parameters, τ and θ (see also legend to Fig. 4); if $\tau = \infty$ and $\theta = 0$, the kinetics is a true parabola. t_2 is the time-point subjected to quarter-height analysis (see Table 1 for details) in seconds after induction; thus a t_2 value of 60s here corresponds to a t_2 value of 135–150s in Table 1.

Kinetics t_2 (s)	Calculated origin t_0 (before or after true origin)				
	$\tau = \infty$	$\tau = 75$ s	$\tau = 90$ s	$\tau = 90$ s	$\tau = 90$ s
	$\theta = 0$	$\theta = 0$	$\theta = 0$	$\theta = 7.5$ s	$\theta = 15$ s
108	0	-11	-10	-2	+4
96	0	-9	-8	0	+5
84	0	-7	-6	+2	+6
72	0	-5	-5	+2	+7
60	0	-4	-3	+3	+7
48	0	-3	-2	+4	+7

Fig. 4. Influence of a finite lifetime (average = τ) of β -galactosidase mRNA and of a delay (average = θ) in the dissociation of the repressor from the operator on the early induction kinetics; theoretical examples

Abscissa: time (t) after appearance of first finished enzyme (i.e. t_s of Fig. 1 is here set at zero); ordinate: amount of enzyme (E) in arbitrary units, normalized such that all curves intersect at 72s (1.44 ordinate units): —, $\tau = \infty, \theta = 0$ (true parabola); Δ , $\tau = 75s, \theta = 0$; $\circ$, $\tau = 90s, \theta = 0$; $\bullet$, $\tau = 90s, \theta = 7.5s$; $\bullet$, $\tau = 90s, \theta = 15s$. All five curves shown can be described by the following equation (R. Young & H. Bremer, unpublished work):

$$E = [t - \frac{\tau^2}{\tau - \theta} (1 - e^{-t/\tau}) + \frac{\theta^2}{\tau - \theta} (1 - e^{-t/\theta})]$$

This equation reduces to $E = t^2/2$ for $\tau \rightarrow \infty$ and $\theta \rightarrow 0$. The constant which makes both sides of the equation dimensionally consistent has been omitted because of the normalization involved. Koch (Appendix to Coffman *et al.*, 1971) presented an equation which can be shown to be identical with one such simpler form where $\theta = 0$.

agreement supports the conclusion [section (a) above] that the synthesis time for β -galactosidase is essentially equal to the translation time.

In contrast, Engbaek *et al.* (1973) concluded that the β -galactosidase-synthesis time was growth-rate-independent in the 0.5–1.9 doublings/h range studied. Given the relative insensitivity of their method (they find a 15 s variation in the synthesis time to be within their experimental error), their results are not inconsistent with those reported here.

Coffman *et al.* (1971) also came to the conclusion that the synthesis time for fast- and slow-growing cells was constant (90–95s), but their methods of evaluation are subject to question. For cells growing in succinate medium (1.03 doublings/h) they used a linear least-squares fit to the (non-linear) induction curve; for a glucose-grown culture (1.2 doublings/h), they fit the data to a second-order polynomial, which

requires that the origin of the polynomial be already known. A rough application of quarter-height analysis to their data on the glucose induction kinetics, although difficult because of the paucity of points, yields a synthesis time of about 65–75s, where they find about 98s. Their linear extrapolation of the succinate-culture induction kinetics yields 95s for the synthesis time; the agreement with our findings must be fortuitous, considering their arbitrary extrapolation and the difference between their succinate doubling times (58 min) and ours (95 min).

West & Stein (1973) found that somewhere between 108 and 150s the amount of β -galactosidase enzyme was distinctly higher than the basal value and assumed this time (about 130s) to be the synthesis time. This method is absolutely dependent on the sensitivity of the assay, such that each increment in assay sensitivity results in a decrease of the apparent synthesis time.

In summary, our results indicate that the peptide-chain-elongation rate is about 16 amino acids/s for fast-growing cells but is decreased to about 13 amino acids/s for slowly growing cells. These values may actually slightly underestimate the true peptide-chain-elongation rates, since they are based on the proposition that the synthesis time equals the translation time for β -galactosidase. Also, these values may not be equal to the average peptide-chain-elongation rate (for bulk protein synthesis), if β -galactosidase specifically has an unusual codon composition or if the ribosome movement is limited by the RNA polymerase generating the nascent messenger, but we conclude that the growth-rate dependence of the peptide-chain-elongation rate is real.

We thank Professor Hans Bremer in whose laboratory this work was done and without whose assistance and guidance this paper could not have been written. We also thank Joyce Lee for her inexhaustible patience and accuracy in preparing this manuscript and Ruth Ricamore for her excellent graphics. The work of Ekkehard Kuner on the sampling apparatus was absolutely essential for the method. This work was performed under N.I.H. grant GM 15142 and the UTD grant GM 15142 and the UTD Research Fund; in addition, R. Y. was supported by an NSF Graduate Fellowship, 1971–74. Work described in this paper was carried out by R. Y. in partial fulfilment of the requirements for the degree of Ph.D. in Molecular Biology at the University of Texas at Dallas.

References

- Adamson, L., Gross, C. & Novick, A. (1970) in *The Lactose Operon* (Beckwith, J. R. & Zipser, D., eds.), pp. 317–323, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Alpers, D. H. & Tomkins, G. M. (1965) *Proc. Natl. Acad. Sci. U.S.A.* **53**, 797–802

- Berg, A. & Zabin, I. (1964) *J. Mol. Biol.* **10**, 289-294
- Bremer, H. & Dalbow, D. G. (1975) *Biochem. J.* **150**, 9-12
- Bremer, H. & Yuan, D. (1968) *J. Mol. Biol.* **38**, 163-180
- Coffman, R. L., Norris, T. E. & Koch, A. L. (1971) *J. Mol. Biol.* **60**, 1-19
- Craven, G. R., Steers, E. & Anfinsen, C. B. (1968) *J. Biol. Chem.* **240**, 2468-2477
- Dalbow, D. G. & Bremer, H. (1975) *Biochem. J.* **150**, 1-8
- Dennis, P. P. & Bremer, H. (1974) *J. Bacteriol.* **119**, 270-281
- Engbaek, F., Kjeldgaard, N. O. & Maaløe, O. (1973) *J. Mol. Biol.* **75**, 109-118
- Forchhammer, J. & Lindahl, L. (1971) *J. Mol. Biol.* **55**, 563-568
- Helmstetter, C. E. (1967) *J. Mol. Biol.* **31**, 519-540
- Hirsh, J. & Schleif, R. (1973) *J. Mol. Biol.* **80**, 433-444
- Kaempfer, R. O. R. & Magasanik, B. (1967) *J. Mol. Biol.* **27**, 475-494
- Kepes, A. (1963) *Biochim. Biophys. Acta* **76**, 293-309
- Kepes, A. & Beguin, S. (1966) *Biochim. Biophys. Acta* **123**, 546-460
- Lonberg-Holm, K. K. (1959) *Biochim. Biophys. Acta* **5**, 464-472
- Maizels, N. M. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3585-3589
- McCarthy, B. J. & Britten, R. J. (1962) *Biophys. J.* **2**, 35-47
- Miller, O. L., Hamkala, B. A. & Thomas, C. A. (1973) *Science* **169**, 392-395
- Riggs, A. D., Bourgeois, S. & Cohn, M. (1970) *J. Mol. Biol.* **53**, 401-418
- Rose, J. K., Mosteller, R. O. & Yanofsky, C. (1970) *J. Mol. Biol.* **51**, 541-550
- West, I. C. & Stein, W. D. (1973) *Biochim. Biophys. Acta* **308**, 161-167