transferred to the endoplasmic reticulum which, to many cytologists, is still closely akin to cell membrane.

It would add elegance to the symmetry I have postulated if the whole system of adaptive immunity could be traced back to the morphogenetic necessity that cell to cell contact, as Paul Weiss[60] suggested many years ago, should be controlled by complementary steric relationships between macromolecules. Compatibility and incompatibility between cells are wholly dependent on cell surfaces. If the separating surfaces between a mouse L cell in tissue culture and a fowl erythrocyte can be removed by viral enzymes, the nucleus of the erythrocyte is activated and flourishes in the alien cytoplasm[61]. If, in the primitive cyclostomes, the two complementary functions I have been discussing were needed to deal with a developing crisis, it is from such complementary aspects of cell membrane contacts that they would be sought and the ways by which the necessary diversities were obtained could well have been basically similar for both moieties.

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Effect of Thymine Concentration on the Replication Velocity of DNA in a Thymineless Mutant of *Escherichia coli*

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Changes in thymine concentration in the growth medium affect the chromosome replication time of thymineless cells. This alone may account for changes in cell composition and provide a demonstration of the absence of coupling between replication velocity and growth rate.

THE rate of DNA synthesis by a bacterial culture in a steady state of growth appears to be independent of the velocity of DNA replication (the rate of addition of nucleotides to a growing polynucleotide chain)[1,2]. It is determined solely by the frequency with which successive cycles of chromosome replication are initiated[3,4]. In other words, replication velocity and growth rate appear not to be coupled.

This absence of coupling could have important technical implications in studies with mutant strains of bacteria which require thymine for growth, for this compound is a specific precursor of DNA. It is conceivable that in the

presence of a particular concentration of thymine in the growth medium the internal concentration of thymidine triphosphate (dTTP) might be sufficiently less than that present in a non-mutant strain in the same growth conditions as to cause a reduction in the replication velocity without at the same time causing a detectable difference in the growth rate. If this were the case it would not be valid, as it is with most nutritional mutants, to find the "optimal" amount of thymine to be added to the growth medium by determining the concentration which gives the maximum growth rate and yield, for this could be given by a concentration which was nevertheless "sub-optimal" in the sense that it caused a reduction in the replication velocity. Our attention was drawn to this possibility by data[5] which suggested that thymineless mutants of *E. coli* are able to incorporate thymine into DNA only by maintaining a lower internal dTTP concentration than that in non-mutant strains, and that although this concentration increases as the amount of thymine in the medium is increased it cannot rise to the normal level present in *thy*$^+$ strains whatever thymine concentration is used.

In view of its theoretical as well as technical implications, we have investigated the effect of thymine concentration on the replication velocity of DNA in the strain *E. coli* 15T$^-$, 555–7. This strain was chosen because it has been frequently used for studies on DNA synthesis in bacteria (see ref. 6). The experiments reported here show that the replication velocity in this strain is indeed influenced by the concentration of thymine in the growth medium.

Replication velocity was not measured directly[10]. It was calculated from changes in the composition of cells growing in the presence of different concentrations of thymine. To calculate the replication velocity from cell composition, four assumptions were made. (*a*) Initiation of a cycle of replication occurs when the cell mass per chromosome origin reaches a fixed value[4,11]. (*b*) Completion of a cycle of replication provides a signal for cell division which occurs $C+D$ min after initiation, where C is the time taken for a replication fork to traverse the length of the chromosome, and D is the time between completion of a cycle of replication and the cell division which follows it[1,12]. (*c*) The replication forks traverse the chromosome with uniform velocity at steady states of growth. (*d*) Removal of a required amino-acid from the growth medium permits completion of cycles of replication that were initiated before amino-acid deprivation but inhibits further initiation[13–16].

Applying Powell's[17] function for the age distribution of cells to the age distribution of replication forks[18] in a culture in a steady state of exponential growth, assumptions (*b*) and (*c*) generate the relationship[1]

$$\bar{G} = \frac{\tau}{C \ln 2}[2^{(C+D)/\tau} - 2^{D/\tau}] \qquad (1)$$

where $\bar{G}$ is the average amount of DNA per cell in chromosome equivalents, and τ is the generation time. Similarly, assumptions (*a*), (*b*) and (*c*) generate the following relationship[4,11]

$$\bar{M} = k2^{(C+D)/\tau} \qquad (2)$$

where $\bar{M}$ is the mean cell mass in such a culture, and k is a constant. If these relationships are valid, $\bar{G}$ and $\bar{M}$ will alter in a predictable way with changes in C and τ.

Because we do not know whether C and D are independent variables, measurements of $\bar{G}$ and $\bar{M}$ at different concentrations of thymine can only provide estimates of changes in the value of $(C+D)$. Dividing (1) by (2), however, gives

$$\bar{G}/\bar{M} = \frac{\tau}{kC \ln 2}(1-2^{-C/\tau}) \qquad (3)$$

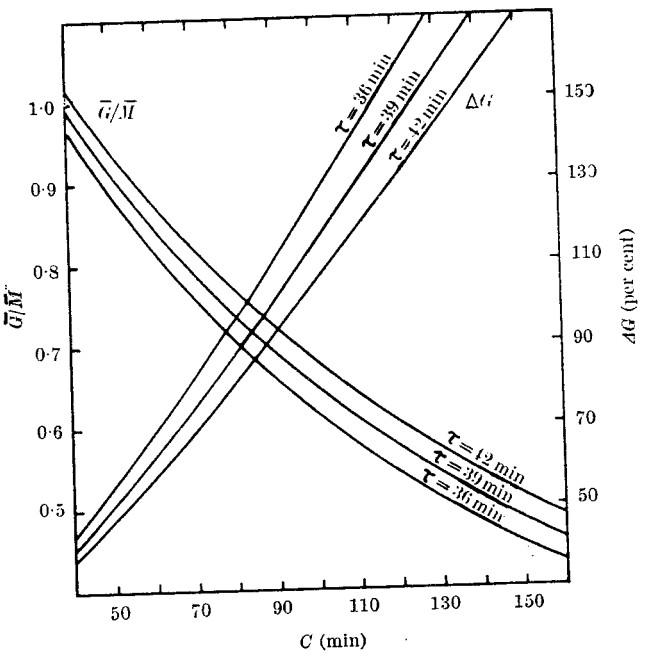

Fig. 1. $\bar{G}/\bar{M}$ and ΔG as functions of C at various growth rates ($1/\tau$), calculated from equations (3) (4) and (5) respectively. $\bar{M}$ was normalized by putting $k = 1/2 \ln 2$ in order to make it equal to $\bar{G}$ (equation (1)) when $C = \tau$.

Hence change in $\bar{G}/\bar{M}$ will be a function solely of C and τ and independent of possible changes in D (Fig. 1).

Estimates of the relative values of $(C+D)$ and C, obtained from measurements of $\bar{M}$ and $\bar{G}/\bar{M}$ respectively, at different thymine concentrations would be invalid if the mass per chromosome origin at the time of initiation also changed as a function of the thymine concentration. It is therefore necessary to get another estimate of C which is independent of the time of initiation of chromosome replication. If assumption (*d*) is valid, the increment in DNA (ΔG) after removal of a required amino-acid is given by[18]

$$\Delta G = \left(\frac{2^n \cdot n \cdot \ln 2}{2^n - 1} - 1\right) 100 \qquad (4)$$

and because it can be shown (refs. 16, 19 and calculated in collaboration with J. Collins) that

$$n = C/\tau \qquad (5)$$

then, at a given growth rate, C uniquely defines ΔG Measurements of the increment in DNA following amino-acid deprivation in cultures which have been growing in the same growth conditions but supplied with different thymine concentrations can therefore provide independent estimates of C in absolute terms.

At a given instant of time the rate of incorporation of ^{14}C-thymine into DNA by a growing culture will be a function of the number of replication forks in the culture and the replication velocity. If the thymine concentration (but not the specific activity) is suddenly increased and results in an increase in the replication velocity, this will be reflected in an increase in the rate of incorporation of label, the increase being proportional to the increase in velocity. This technique can therefore provide a third estimate of C as a function of thymine concentration which is independent of those described previously.

A fourth estimate of C can be obtained by shifting a culture in a steady state of exponential growth (that is, with a constant $\bar{G}/\bar{M}$) on one thymine concentration to a higher concentration and determining the time taken for $\bar{G}/\bar{M}$ to attain the new steady state value appropriate to the higher thymine concentration. It can be shown that the time taken will be equal to C at the higher thymine concentration.

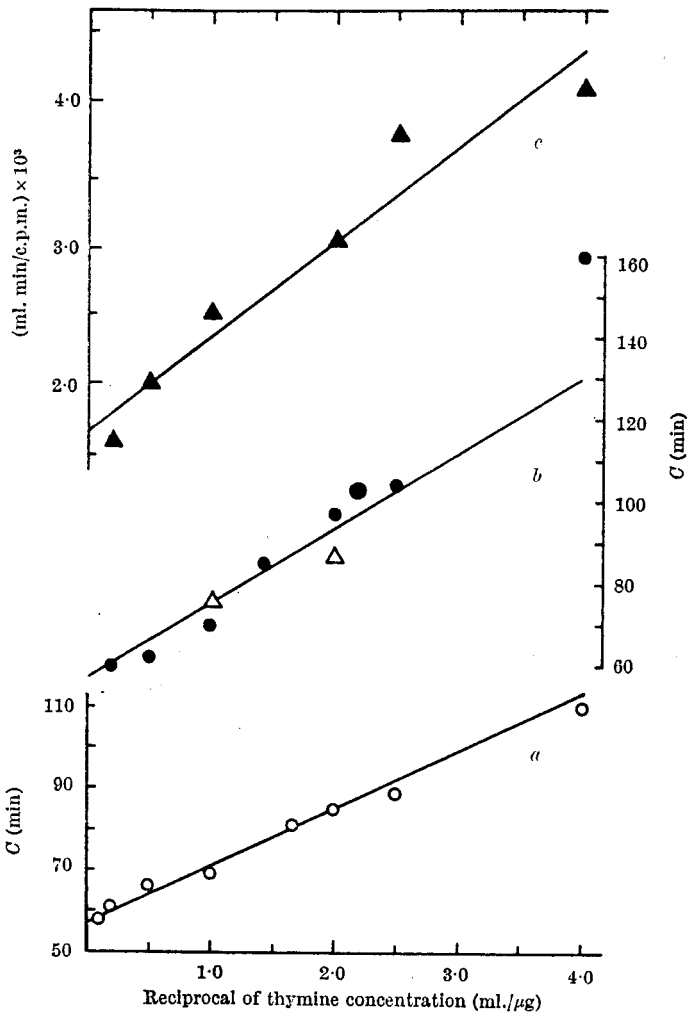

Fig. 2. Relative values of C as a function of the reciprocal of the thymine concentration obtained in three different ways: (*a*) from ΔG using the data presented in Table 3; (*b*) from $\bar{G}/\bar{M}$ using the data (●) presented in Table 2, and data (△) from a second experiment; (*c*) from the instantaneous rate of incorporation of ^{14}C-thymine after a step-up. The following experimental procedure was used to obtain curve (*c*): cells were grown in 'M9' supplemented with 0·25 μg/ml. thymine. When the culture reached $A_{450\,nm} = 0{\cdot}3$ it was filtered, washed with an equal volume of pre-warmed 'M9' and resuspended in a small volume of 'M9'. The washed culture was distributed almost simultaneously into six pairs of tubes containing 'M9' supplemented with six different concentrations of ^{14}C-thymine at exactly the same specific activity. An equal volume of cold trichloroacetic acid (10 per cent) was added to one member of each pair after 5 min incubation at 37° C and to the other after 8 min. Incorporation measured as described under Table 1. Values of c.p.m./min were derived by subtracting each result for the 5 min tube from that for the corresponding 8 min tube, and dividing by 3. These values are a function of the replication velocity (V). The relationship between V, C and L (chromosome length) is as follows: $V = L/C$. If we define the length of the chromosome as unity, then $V = 1/C$. The reciprocal of the rate of incorporation will therefore be proportional to C.

In the experiments which follow, substantial agreement in our estimates of C in medium containing different thymine concentrations was obtained using each of the methods described above.

Measurement of $\bar{M}$, $\bar{G}$ and $\bar{G}/\bar{M}$

It was first necessary to determine over what range of thymine concentrations growth of strain 555–7 is normal. Our criterion of "normal" was that absorbance, colony forming units and particle number should increase exponentially at the same rate. To a first approximation, growth was normal by this criterion in 'M9' minimal medium supplemented with thymine at concentrations of 0·25 μg/ml. or more. This appears to be in agreement with similar measurements on the related strain *E. coli* 15 TAU studied by Maaløe and Rasmussen[20], who stated that "at 0·2 μg/ml. the growth rate of the TAU strain is just perceptibly reduced". We have found, however, that in our strain and other thymineless strains of *E. coli* there is a subtle aberration in cell division which occurs in all cultures, irrespective of the concentration of thymine in the medium, which causes the curves of particle number and colony forming units on the one hand and absorbance on the other to have slightly different slopes. This effect can also be seen in growth curves of strain 555–7 reported by Lark *et al.*[15], and of *E. coli* B/r *thy*⁻ reported by Pierucci and Helmstetter[21]. We shall present a full analysis of this phenomenon separately. In the present context it is sufficient to point out that it prevents a meaningful estimate of $\bar{M}$ or $\bar{G}$ from being obtained, for, as can be seen from the data in Table 1, their values change progressively with time. The DNA/mass ratio, on the other hand, does reach a steady state value (Table 1; Fig. 6) and it is therefore possible to compare its value in cultures growing on different concentrations of thymine.

Table 1. DNA PER UNIT MASS OF A CULTURE IN SUCCESSIVE GENERATIONS

Sampling time (min)	A_{450nm} × 10³	Particles/ml. × 10⁷	A_{450nm} / 10⁹ particles	c.p.m./ml.	c.p.m. / 10⁵ particles	c.p.m. / A_{450nm} × 10⁻³
0	55	1·70	3·24	1,075	6·32	19·55
90	130	3·80	4·42	2,764	7·27	21·26
130	150	3·65	4·11	3,267	8·95	21·78
165	140	3·45	4·06	3,243	9·40	23·16
200	145	3·15	4·60	3,229	10·25	22·27
230	130	2·55	5·10	2,844	11·15	21·88
270	140	2·55	5·50	2,945	11·55	21·04
305	140	2·25	6·22	2,736	12·16	19·54
340	120	1·80	6·67	2,398	13·32	19·98
380	115	1·55	7·42	2,447	15·79	21·28
425	120	1·55	7·74	2,456	15·85	20·47
460	135	1·65	8·18	2,939	17·81	21·77
480	70	0·70	10·00	1,563	22·33	22·33

A single colony was suspended in 'M9' synthetic medium[8] supplemented with L-arginine L-methionine, L-tryptophan (50 μg/ml. of each) and ^{14}C-thymine (0·4 μg/ml.; 0·05 μCi/μg) and incubated at 37° C with vigorous shaking. Sampling commenced after 3–4 cell doublings and the culture was diluted by half with fresh prewarmed medium after each sample was taken. Samples (2 ml.) were taken into 0·5 ml. formaldehyde (1·2 per cent). Absorbance was measured at 450 nm and particle number with a Coulter electronic particle counter Model B. Samples (1 ml.) were simultaneously taken into 1 ml. of cold trichloroacetic acid (10 per cent), filtered and washed six times with hot (95° C) water. The filters were then dried and counted in a liquid scintillation counter.

To calculate the DNA/mass ratio we have assumed that absorbance at 450 nm is proportional to mass, for this has been shown to be so over a wide range of growth rates and cultural conditions in the closely related species *Salmonella typhimurium*[22]. DNA was determined by using ^{14}C-thymine in the growth medium and measuring the amount of label present in the acid-insoluble fraction, because we were primarily interested in the relative amounts of DNA per unit mass rather than absolute amounts. This technique would be invalid if strain 555–7 were "leaky" and consequently incorporated a significant amount of endogenously synthesized ^{12}C-thymine into its DNA, the proportion varying with the concentration of labelled thymine present in the growth medium. We therefore determined the leakiness of this strain by growing it in 'M9' synthetic medium containing ^{14}C-glucose and ^{12}C. thymine, and measuring the specific activity of the DNA thymine using a modification of the procedure described by Cohen and Barner[7]. When 0·5 and 5·0 μg/ml. thymine were used in the growth medium, at least 95 and 97 per cent respectively of the thymine in DNA was derived from that in the growth medium. These values are in close agreement with other estimates obtained for this strain and others carrying the same *thy*⁻ mutation[7–9]. This degree of leakiness will introduce a negligible error into our data.

The data from a series of experiments like that described under Table 1 but using different concentrations of thymine in the growth medium are shown in Table 2. They show that there is a progressive decrease in the DNA/mass ratio with decreasing thymine concentrations. On the assumption that the change in this ratio is due to a progressive increase in C as the thymine concentration is reduced, we have calculated the corresponding values of

Table 2. DNA PER UNIT MASS AS A FUNCTION OF THYMINE CONCENTRATION

Thymine concentration (μg/ml.)	$\frac{\text{c.p.m.}}{A_{450nm}} \times 10^{-3}$	$\frac{(\text{c.p.m.}/A_{450nm})}{(\text{c.p.m.}/A_{450nm})_{5\cdot0}}$	$\frac{\text{c.p.m.}/A_{450nm}}{(\text{c.p.m.}/A_{450nm})_{5\cdot0}} \times 0{\cdot}85$	C ($\tau = 39$ min)
0·25	19·5	0·540	0·459	160
0·4	26·8	0·744	0·632	104
0·5	28·0	0·778	0·661	97
0·7	30·2	0·839	0·713	85
1·0	33·7	0·933	0·793	70
2·0	35·6	0·986	0·838	62
5·0	36·0	1·000		

Parallel cultures were grown in 'M9' containing the indicated concentrations of thymine. Specific activity was identical in each case (0·05 μCi/μg). Cultures were sampled as described under Table 1. The values for c.p.m./A_{450nm} are averages of at least 6 samples taken in successive generations. C was calculated in each case assuming a value of 60 min for 5·0 μg/ml. thymine (see text). This value of C gives $\bar{G}/\bar{M} = 0{\cdot}85$ when $\tau = 39$ min (Fig. 1).

C for each DNA/mass ratio using equation (3) and normalizing the ratio given by the culture growing on 5·0 μg/ml. thymine to 60 min. $C = 60$ min for 5·0 μg/ml. thymine was chosen, for this was the value obtained from measurement of ΔG (next section). A graph of C as a function of the reciprocal of the thymine concentration is given in Fig. 2*b*.

Measurement of ΔG

In principle, measurement of the increment in DNA after amino-acid deprivation can provide an estimate of the average number of forks per chromosome in the culture and therefore of C. One problem associated with this method is that the capacity to replicate DNA declines during amino-acid starvation[23], and therefore not all chromosomes necessarily complete replication[16]. The experiment shown in Fig. 3 also provides evidence supporting this. A culture was grown for five to seven generations in ^{14}C-thymine at a concentration of 0·5 μg/ml. and the required amino-acids were removed by filtration. The subsequent increment of DNA was determined by further incubation in medium containing ^{14}C-thymine at different concentrations but with the same specific activity. Notice that the rate of synthesis of DNA increases with increasing thymine concentration, as expected if there is a progressive decrease in C. The final yield also increases and the curves suggest that after about 70 min replication ceases whether or not all replication forks have reached the chromosome terminus. Notice, however, that there is no significant difference in the increment of DNA in the presence of thymine concentrations greater than 1·0 μg/ml.

ΔG was now measured in a series of cultures previously grown on different thymine concentrations. In each case it was determined in a set of cultures containing various concentrations of thymine during the amino-acid starvation. The results are shown in Table 3. There is a progressive increase in ΔG as the thymine concentration in the previous growth medium is reduced. In Fig. 2*a* the values of ΔG have been transformed into values for C, using equations (4) and (5), and are plotted against the reciprocal of the thymine concentration. Notice that the slope of this line calculated from values of ΔG is very similar to that calculated from values of $\bar{G}/M$. This suggests that ΔG is not seriously influenced by failure of some chromosomes to complete replication during amino-acid starvation or by a significant number of new acts of initiation after removal of the required amino-acids, and also that $\bar{G}/M$ is not seriously influenced by differences in the timing of initiation during growth on different concentrations of thymine.

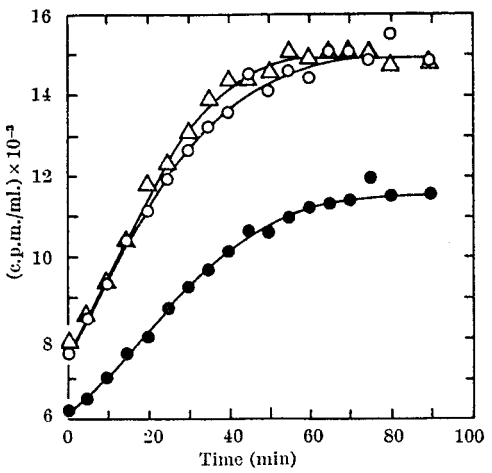

Fig. 3. Increment in DNA during amino-acid starvation. Cells were grown in 'M9' supplemented with 0·5 μg/ml. ^{14}C-thymine (0·05 Ci/μg) for 5–7 generations. When the culture reached $A_{450nm} \simeq 0{\cdot}4$ it was filtered, washed with approximately the same volume of prewarmed 'M9' without amino-acids or thymine and resuspended in a small volume of the same medium. This procedure took usually less than 3 min. Portions were withdrawn at time zero into the same medium supplemented with ^{14}C-thymine at various concentrations. Data obtained for the following concentrations are shown: ●, 0·5 μg/ml.; ○, 1·0 μg/ml.; △, 9 μg/ml. The amount of ^{14}C incorporated was determined as described under Table 1

Table 3. ΔG AS A FUNCTION OF THYMINE CONCENTRATION BEFORE AND AFTER AMINO-ACID DEPRIVATION

Pre-step-up thymine concentration (μg/ml.)	ΔG for the indicated post-shift thymine concentrations (per cent) 1·0	2·0	5·0	9·0	$\overline{\Delta G}$ (per cent)	C (τ = 39 min)
0·25	136	115	131	—	127	110
0·4	102	97	97	—	99	89
0·5	94	93	95	90	93	85
0·6	93	87	84	—	88	81
1·0	73	73	71	74	73	69
2·0	77	67	64	74	70	66
5·0	—	71	62	58	64	61
10·0	—	—	62	60	61	58

The data shown for the pre-step-up concentration of 0·5 μg/ml. thymine are taken from curves obtained in the experiment shown in Fig. 3. The data shown for the other pre-step-up thymine concentrations were obtained from similar experiments. $\overline{\Delta G}$ is the average of all ΔG values shown in each line. Values of C were calculated from the corresponding values of $\overline{\Delta G}$ using equations (4) and (5).

The values of ΔG provide us with an absolute measure of C, unlike our measurements of the DNA/mass ratio, and the extrapolation in Fig. 2*a* to infinite thymine concentration gives an estimate of 57 min.

Thymine Incorporation Rates after a Step-up

To measure changes in velocity by pulse-labelling a culture in a steady state of exponential growth with ^{14}C-thymine at different concentrations, it was first necessary to determine the time taken for the labelled thymine to replace the unlabelled pool of thymine derivatives present before addition of label, and for the internal concentration of dTTP to equilibrate to its new value. A culture was therefore pre-grown on 0·5 μg/ml. thymine and the rate of incorporation of label followed after addition of ^{14}C-thymine to give final concentrations of 1·0 and 5·0 μg/ml. (Fig. 4). The data indicate that a linear rate of incorporation is achieved after about 4 and 2 min, respectively. Notice that the rate of incorporation in 5·0 μg/ml. thymine is more than twice that on 1·0 μg/ml. thymine. We have not used these differences in rate as measures of the relative replication velocity in the two cultures, for their specific activities were not carefully controlled.

To measure the replication velocity over a broader range of concentrations, the experiment was repeated using six different thymine concentrations with identical

specific activities. The results are plotted graphically in Fig. 2c. An apparently linear relationship between the reciprocal of the rate of incorporation and the reciprocal of the thymine concentration was obtained. If we assume that addition of increasing concentrations of thymine to a culture does not change the frequency of initiation, then the reciprocal of the rate of incorporation will be directly proportional to the replication time C. The slope of the line obtained is very similar to those calculated from $\bar{G}/\bar{M}$ and ΔG.

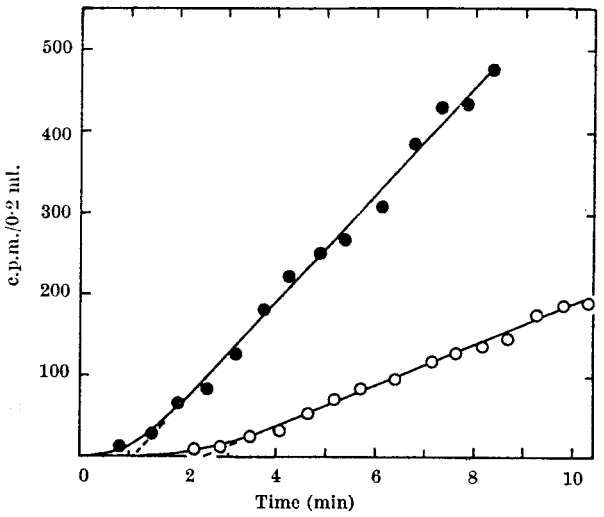

Fig. 4. Incorporation of ^{14}C-thymine after a step-up. Cells were grown in 'M9' supplemented with 0·4 μg/ml. thymine. When culture reached $A_{450nm} \cong 0{\cdot}3$ 10 ml. samples were taken (time zero) into two pre-warmed flasks, containing ^{14}C-thymine such that the resulting thymine concentration was either 1·0 μg/ml. or 5·0 μg/ml. The final specific activities were only approximately the same. Samples (0·2 ml.) were taken as frequently as possible into 0·5 ml. cold trichloroacetic acid (10 per cent). Incorporation measured as described under Table 1.

The experimental results shown in Fig. 5 demonstrate the fourth method of measuring C as a function of the thymine concentration. In this experiment a uniformly labelled culture growing on 0·5 μg/ml. thymine was stepped up to 5·0 μg/ml. thymine at the same specific activity. Notice that an immediate increase in the rate of incorporation was observed without any detectable change in the rate of increase in cell mass. Subsequently the rate of incorporation drops back to the pre-shift rate. This is precisely the effect predicted if an increase in thymine concentration leads to an increase in replication velocity but does not change the frequency of initiation. The rate of incorporation should fall back to the previous rate when the distribution of replication forks equilibrates to the new value appropriate to the new replication velocity. The time taken should be equal to the new replication time C. To facilitate estimation of this time the data have been transformed (Fig. 6) to show the DNA/mass ratio as a function of time. The new equilibrium is reached between 50 and 60 min after the shift, which is consistent with our previous estimate (from ΔG) of C in the presence of 5·0 μg/ml. thymine of 60 min. We have not made an estimate of C over a whole range of concentrations using this technique.

Significance of Changes in Cell Composition

None of the methods we have used by itself demonstrates unequivocally that the changes in cell composition which are observed when cultures are grown in the presence of different thymine concentrations arise solely from changes in replication velocity. The simplest alternative possibility would be that, contrary to our assumption (*a*), the mass/chromosome origin ratio at initiation is affected by the thymine concentration. Qualitatively, all the changes we have observed, except that in ΔG, would be accounted for if this ratio increases with decreasing

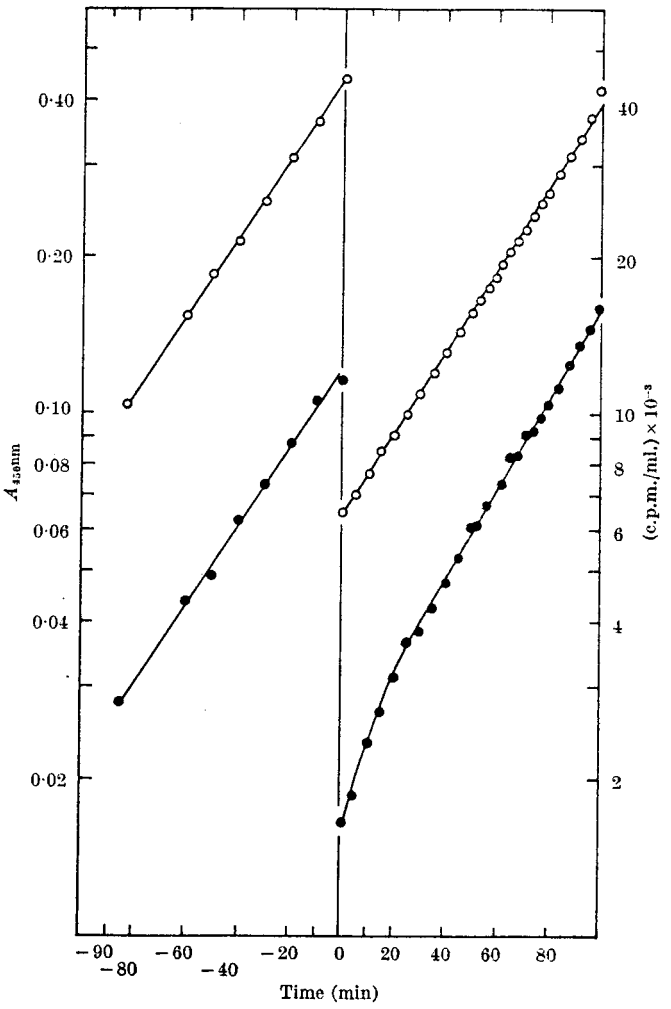

Fig. 5. Increase of A_{450nm} (○) and of DNA (●) of a culture before and after a step-up. Cells were grown for 3–4 generations in 'M9' supplemented with 0·4 μg/ml. ^{14}C-thymine (0·05 μCi/μg). A_{450nm} and c.p.m./ml. were measured (as described in legend to Table 1) thereafter. When the culture reached $A_{450} = 0{\cdot}4$ it was diluted seven-fold into fresh prewarmed 'M9' supplemented with ^{14}C-thymine to give a final concentration of 5·0 μg/ml. (same specific activity). This was defined as time 0.

thymine concentrations. If this were the explanation, however, it would be necessary to invoke an additional effect to account for the changes found in ΔG. Because all the changes observed are consistent, both qualitatively and quantitatively, with an effect on C alone and because there are other reasons[5] for believing that the internal dTTP concentration is sensitive to the external thymine

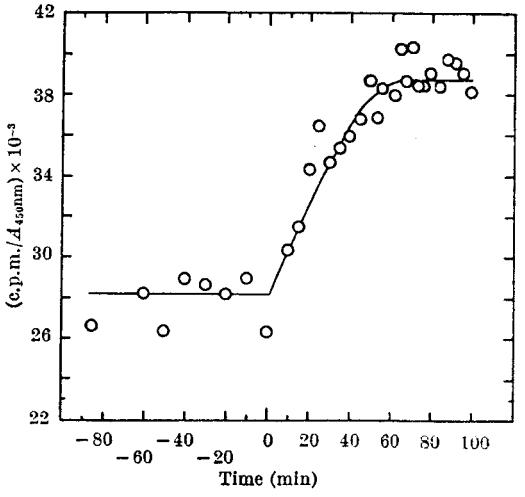

Fig. 6. Change of DNA/mass ratio in a culture due to a step-up. The data in Fig. 5 are replotted to show the change in c.p.m./A_{450nm} as a function of time after a step-up.

concentration, thus providing a straightforward explanation for an associated variation in replication velocity, we favour this hypothesis.

There is at least one alternative way by which a reduction in the internal concentration of dTTP might reduce the replication velocity. This is by causing an increase in the internal pool of dATP and a consequent inhibition of polynucleotide ligase (work of J. R. Lehman[24]) which seems to be essential for normal DNA synthesis[25]. Reduction of the dTTP pool is known to be associated with an increase in the dATP pool[26].

There is evidence that the phenomenon occurs in other strains. Friesen and Maaløe[9] performed an experiment similar to that shown in our Fig. 2*c* using a number of unrelated strains of *E. coli* and obtained qualitatively similar results in each case. "Leakiness" of the *thy*⁻ mutations as an explanation of their data was ruled out for each strain, but no explanation of the phenomenon was offered.

Between thymine concentrations of 0·25 and 10·0 μg/ml. the replication time differs by a factor of 2–3 without significantly affecting the growth rate or cell viability. Our data therefore provide striking support for the hypothesis that the replication velocity and the growth rate are not coupled. Our values of C in 555–7 extrapolate to 57 min at infinite thymine concentrations. This value is higher than that found in *E. coli* B/r[1], but we cannot yet evaluate the significance of this difference. The two strains may have different replication times; there may be a systematic error in our measurements of C, which are based primarily on ΔG; or it is possible that in *E. coli* 15, C is 41 min as in *E. coli* B/r but that in its thymineless derivative 555–7, its value is higher irrespective of the thymine concentration in the growth medium.

The amount of DNA per cell (G) has also been reported to be greater in 555–7 than in B/r[1], but we do not think this comparison is meaningful owing to the progressive increase in $\bar{G}$ which occurs during exponential growth of *thy*⁻ strains (see Table 1).

Finally, it is clear that in future studies with thymineless strains of bacteria, the thymine concentration in the growth medium will need to be more carefully controlled and explicitly stated than has often been the case in the past. Concentrations between 1·0 and 10 μg/ml. have usually been used in growth studies with 555–7 and, as we have shown, significant changes in cell composition occur over this range. In addition, data obtained from *thy*⁻ and *thy*⁺ strains can no longer be assumed to be comparable without independent evidence that in the growth conditions used the composition of cells of each strain is identical.

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Towards a Four-dimensional Analysis of Meteorological Data

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Rapid advances in the gathering of meteorological data and the design of atmospheric models are making the conventional two-dimensional analysis inadequate. This article describes some progress towards a four-dimensional analysis system.

SINCE the advent of electronic computers, meteorologists have had the problem of interpolating data values at the points of a regularly spaced grid on the x,y map. Each set of gridpoint values provides an analysis or representation of the actual state of the atmosphere. These analyses have an obvious importance in their own right, but they also give at each gridpoint the initial values for the numerical forecasting programme which computes future flow patterns by integrating approximate forms of the mathematical equations in a simple model of the atmosphere.

The raw data have traditionally been observations from irregularly distributed locations on the x,y map (the weather stations) at globally fixed times, known as the synoptic hours. In the vertical, data for these locations and times are obtained from radiosonde soundings of pressure altitude, temperature, humidity, and wind for certain standard pressure levels such as 1,000, 850, 700, 500 up to 100 mbar and above. The values of pressure height at these standard levels are obtained by linear log p interpolation from temperature readings taken at levels at which the lapse rate of temperature undergoes a significant

antibiotic usage may be selecting gonococci with both greater antibiotic resistance and, indirectly, greater sensitivity.

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Control of microbial surface-growth by density

IN synchronous cultures of bacteria, the rate of cell elongation[1] or of envelope synthesis[2] seems to increase abruptly at a particular age. These and other findings suggest[3–5] that bacterial envelope is formed at a constant rate, possibly by enzymes organised in zones, and that some event in the cell cycle leads to a discrete increase in the amount of enzymes or number of zones concerned. (The actual event involved has been variously identified with initiation of chromosome replication[4], with termination[1,5] and with the attainment of a critical cell length[6].) Growth zones are known to occur in the filamentous fungi[7]. The rate at which these zones are able to add new envelope is limited, and since mass increases exponentially[8], hyphal density eventually begins to rise; when it reaches a critical value, new zones are formed. Thus density (or indeed the concentration of any product synthesised exponentially[9]) could control the rate of surface growth, and we suggest that such is the case in bacteria.

We postulate a constant rate of surface-area synthesis β that doubles once during the bacterial cycle, d min before division[10]. For a cylinder with open ends, the cell volume $V(a)$ at age a is given by

$$V(a) = 2^{m-1} R\beta\tau\,[1+(a+d)/\tau-m] \text{ for } a \leqslant (m+1)\tau-d$$
$$= 2^{m} R\beta\tau\,[(a+d)/\tau-m] \quad \text{for } a \geqslant (m+1)\tau-d \qquad (1)$$

where τ is the mean doubling time of the cell, R is the radius, and $m \equiv [d/\tau]$. If we define ρ_d as the density of the cell at age $\tau-d$, then

$$\rho_d \equiv (M_d/V_d) = (M_i/R\beta\tau)2^{(C+D-d)/\tau} \qquad (2)$$

where M_d and V_d are the mass and volume at age $\tau-d$. Here C and D are the times taken to replicate the chromosome and to complete division processes, respectively[11], and M_i is the (constant) ratio of cell mass to chromosome origins at the time of initiation of replication[12].

There is reliable experimental evidence that R does not vary very much over the cell cycle during steady-state growth[13]. If we take it to be completely independent of a, then

$$\bar{V} = (M_i/2\rho_d \ln 2)2^{(C+D)/\tau} \qquad (3)$$

where $\bar{V}$ has been defined in the usual way as

$$\bar{V} = \int_0^\tau V(a)\nu(a)da \Big/ \int_0^\tau \nu(a)da,$$

and $\nu(a)$ is the frequency function of age.

Because ρ_d is considered to be the critical density that leads to the addition of envelope growth-zones, one would expect it to be independent of τ; $\bar{V}$ should thus vary with τ according to the exponential term. We have tested this prediction against the dimensions of *Escherichia coli* B/r strain H266 growing with a variety of doubling times (Table 1). Non-linear regression analysis to fit equation (3) to average cell volume gave excellent results ($P < 10^{-6}$ from a two-tailed F-test of explained variance). The value obtained for $C+D$ (78.7±7.6 min) is so close to accepted values[14] as to preclude the possibility that ρ_d is exponentially dependent on the growth rate to any great extent. It should be stressed that equation (3) has been derived without any assumptions concerning the dependence of β, R or d on τ. Furthermore, the proportionality of $\bar{V}$ to $2^{(C+D)/\tau}$ found here may also hold true for other Gram-negative bacilliform bacteria. This follows from the similar dependence of the average mass of *Salmonella typhimurium*[12,15] on $(C+D)/\tau$ and the independence[16] of the average cell density ρ.

In addition to a maximum at $\tau-d$, cell density goes through a minimum during each cycle. This minimum precedes $\tau-d$ by a fixed fraction of τ, $2-1/1n2$ or about 56%, and will thus occur after, at or before cell division according as $d/\tau <, =$ or $> m-1+1/1n2$; in all cases, the ratio ρ_{min}/ρ_d is constant and equal to $\frac{1}{2}e\ln 2 \approx 0.94$. (By comparison, ρ/ρ_d, which is also independent of τ, is $2(1n2)^2 \approx 0.96$.)

Evidence[17] for just such a variation in ρ over the cell cycle has recently been reported for exponentially growing *E. coli* K12, with measured values of 0.95 for ρ_{min}/ρ_d and 0.97 for ρ/ρ_d; ρ/ρ_{min} was precisely as predicted, 1.020. The occurrence of the maximal density at cell birth and division is consistent with a d of around 44 min; the least-squares estimate provided by the surface-area model (Rosenberger *et al.*, submitted for publication) is 49±4 min. The location of the minimal density, near the middle of the cell cycle, is also as expected.

The time before division at which the rate of envelope synthesis doubles, d, will depend on R and β and the way they vary with τ. The radius of *E. coli* B/r H266 decreases with τ (Table 1): over 90% of the variance ($P < 10^{-6}$) in $R(\tau)$ can be accounted for by the expression $R(\tau) = R_\infty 2^{\gamma/\tau}$, where $R_\infty = 0.185$ μm and $\gamma = 33.6$ min. In order to define the relationship of d to growth rate, it is thus only necessary to determine how β varies with τ. In the absence of definitive data, two simple relationships are considered[10] (see also Rosenberger *et al.*, submitted for publication): no dependence between β and τ, and inverse proportionality. The former predicts d to be a weak function of τ; the latter to be a constant, $C + D - \gamma$, about 45 min. In either case, and depending on the growth rate, d could coincide with either the initiation or the termination of chromosome replication. This may explain the general lack of agreement in the literature regarding the particular event in the cell cycle considered to be associated with the rate change in envelope synthesis[1,5,6].

The dimensions predicted by models postulating linear and exponential rates of envelope synthesis are very similar[18]. We would thus like to point out that exponential surface extension between discrete changes in the number of zones can also explain the experimental results, as follows. If the rate of surface growth from a zone increases in proportion to cell mass until the zone reaches its maximum capacity, as is the case in fungi[7], then cell density will be constant except for the period when all existing zones are acting at full capacity and new ones have not yet become operative; provided this period is short compared to τ, $\bar{V}$ will still be proportional to $2^{(C+D)/\tau}$.

While there seem to be no recognised molecular mechanisms through which changes in density are able to derepress enzyme synthesis, changes in solute concentrations can do so.

Table 1 Mean length $\bar{L}$ and radius R of *E. coli* B/r at various doubling times τ

Medium containing	τ (min)	$\bar{L}$ (μm)	R (μm)
Alanine	160	2.39	0.217
	160	2.20	0.212
	160	2.19	0.212
	124	2.72	0.238
Succinate	105	2.27	0.250
Alanine + proline	72	2.64	0.229
	72	2.80	0.288
Glycerol	60	2.53	0.232
Glucose	45	2.62	0.308
	45	2.66	0.300
	45	2.68	0.312
Glucose + casamino acids (Difco)	32	3.14	0.321
	31	3.35	0.446
	31	3.08	0.406
	31	3.07	0.417
	31	3.22	0.429
Glucose + casein Hydrolysate (Sigma)	24	3.58	0.479
	24	3.29	0.466

E. coli B/r strain H266 was grown in minimal salts medium supplemented with the carbon source indicated[10]. Cells, fixed in OsO_4 and agar-filtered, were measured from projections of electron micrographs at a final magnification of 12,000.

If the limiting precursor of an envelope component were synthesised with the same kinetics as is mass, its concentration too would reach a maximum at $\tau - d$; for a relatively large flux and low average concentration, such an increase could be quite substantial.

One should perhaps consider another possibility: that cell radius varies during the cell cycle in such a way as to maintain ρ constant. Of course, if the cell wall were completely free to adjust to changes in hydrostatic pressure, R rather than ρ would reach a maximum at $\tau - d$. Here too, the maximum would be only 4% above the average value and 6% above the minimum—variations that are probably beyond experimental resolution[13]. But cell wall, once formed, is able to withstand considerable internal pressures and yields very little (if at all) to forces of expansion. Mitchell[19] measured the phosphate-impermeable volume of stationary *E. coli* as a function of external NaCl concentration in conditions that were later found to cause a substantial cleavage of the peptides crosslinking murein chains[20]. Nevertheless, less than half the increase in internal osmotic pressure was compensated for by a change in cell volume, the remainder being balanced by the hydrostatic pressure exerted by the cell wall[21]. In principle, in the case of growing cells, new cell wall could attain the necessary magnitude as it is being synthesised in order to maintain constant density despite the rigidity of formed wall. If we assume, as a conservative upper limit, that formed wall can adjust sufficiently to compensate for half the change in volume required to keep ρ constant, then one can show (and verify easily) that in order for new wall to compensate for the other half, the radius must increase exponentially along the length of the cell from a value of $R_0 (= 2M_0 \ln 2/\beta\rho\tau$, where M_0 is the mass at birth) at the location corresponding to cell birth to a maximum of $2R_0 2^{-d/\tau}$ at doubling. There it drops abruptly to half its size and then resumes its exponential climb back to R_0. Such behaviour is clearly at variance with observation, and we must reject the possibility of constant ρ and adjustable R.

The applicability of our model will depend in part on the extent to which it can explain the differences in shape observed in closely related strains of *E. coli* growing in a variety of conditions[5,6,10,14,15,22], including those produced by specific mutations[5,23,24]. In this connection, the bizarre alterations of shape found in *E. coli* 15T⁻ growing rapidly in low thymine concentrations[25], may be of particular interest.

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Suppression of fever at term of pregnancy

THE newborn human is often unable to develop a fever following exposure to various infectious agents[1–3] and newborn lambs are similar, in that they do not develop a fever in response to a bacterial endotoxin challenge in the first few days of life[4]. Lack of the febrile response is thought to be due to the need for a process of 'sensitisation' of the lambs, or to the immaturity of some aspect of the fever-production process[5,6]. Newborn guinea pigs and rabbits also, do not become febrile or show a diminished response to a challenge with endotoxin during early postnatal life[7,8]. We have examined the response of the pregnant ewe to pyrogens at and near the time of birth, for although they become febrile during pregnancy[9], their response to endotoxin at term has not been studied. We report here that the febrile response induced by a bacterial endotoxin or by endogenous pyrogen is suppressed in the ewe for a period extending from 2 to 5 d prepartum to several hours postpartum.

Suffolk, Dorset and cross-bred ewes were studied at various times ranging from 8 d prepartum to 60 h postpartum. The ewes were injected by way of the jugular vein with 30 μg of bacterial pyrogen derived from *Salmonella abortus equi* (SAEP) in a 3-ml volume of sterile physiological saline. This quantity of SAEP gave a fever of 1.3 ± 0.15 °C in non-pregnant adult sheep. The SAEP was administered to each ewe at 3-d intervals, in order to avoid the production of refractoriness to the pyrogen. Temperatures were measured continuously by a thermistor probe inserted about