STUDIES ON THE ROLE OF RIBONUCLEIC ACID IN THE GROWTH OF BACTERIA*

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SUMMARY

Measurements have been made of the protein, the RNA, and the DNA composition of cultures of *Aerobacter aerogenes* during balanced growth at different rates. The results indicate that at a given temperature the RNA/protein ratio of a culture is a direct function of the growth rate and that these two variables are linearly related at growth rates greater than 0.6 doublings per h. The RNA of these cultures is approximately 14 % soluble and 86 % ribosomal at all growth rates examined.

In the transition from a high to a lower growth rate the decrease in the rate of synthesis is immediate and marked for RNA and more gradual for protein and for DNA. The rate of RNA synthesis increases to the level characteristic of the new low growth rate only after the RNA/protein ratio has been reduced by preferential protein synthesis to the value normally observed during balanced growth at this new rate. In the transition from a low to a higher growth rate, the response of RNA synthesis is again more rapid than that of protein and DNA. The synthesis of RNA during this transition period proceeds at a rate greater than that finally to be established. The pattern of incorporation of radioactive guanine revealed that the usual proportion of soluble and of sedimentable RNA is made during this period, and the pattern of incorporation of radioactive leucine indicated that the sedimentable RNA consists of complete ribosomes.

The significance of these results in understanding the control of bacterial growth is discussed.

INTRODUCTION

Embarrassingly little is known of the role played by RNA in the growth and metabolism of bacteria. As in higher organisms, two classes of RNA are readily discernible: (1) "particulate RNA", comprising 80–90 % of the total RNA, and consisting of ribonucleoprotein particles (called ribosomes) of sedimentation velocity constants varying between 30 S and 100 S depending on the method of isolation, and (2)

The following abbreviations are used in this paper: RNA, ribonucleic acid; Tris, tris-(hydroxymethyl)aminomethane; DNA, desoxyribonucleic acid; TCA, trichloracetic acid; EDTA, ethylenediamine tetraacetic acid; DNAase, desoxyribonuclease.

* Preliminary reports of parts of this work were presented at the CIBA Foundation Symposium[1], London, 1958, and before the Society of American Bacteriologists, Saint Louis, May, 1959.

"soluble RNA", comprising 10-20% of the total RNA, and consisting of rather smaller molecules (mol. wt. of about 20,000) which are not protein-bound when isolated[2].

Soluble RNA appears to play the same role in bacteria as in higher organisms—that of an acceptor of activated amino acids in an early step of protein synthesis.* Furthermore, work with subcellular fractions of higher organisms[3] provides evidence that the particulate RNA of these systems plays a direct role in one of the final stages of protein synthesis. So far, however, the role of particulate RNA in microorganisms has remained a matter for conjecture.

Since the ribosomal RNA constitutes the bulk of the total RNA, one hint of the role of ribosomal RNA may be found in the observation that the rate of protein synthesis and the total amount of RNA are concomitant variables in most biological systems[4]. The present study was initiated to investigate this variation in a species of bacteria with the hope of elucidating the function of RNA. The procedures used were to measure the amounts of RNA, protein, and DNA in cultures growing at different rates, to determine the kinetics of the synthesis of these macromolecules during alterations in growth rate, and to describe the RNA in these studies in terms of its soluble and particulate components. The results shed light on the way the growth rate of a bacterial culture is controlled during balanced growth ("growth is balanced over a time interval if, during that interval, every extensive property of the growing system increases by the same factor"[5]), and during adjustment to changes in the external medium.

While this work was being completed the similar studies of SCHAECHTER, MAALØE AND KJELDGAARD[6,7] were published. Many of our observations are in close agreement with theirs.

MATERIALS AND METHODS

Organism

Two strains of *Aerobacter aerogenes* were isolated by the penicillin technique for use in these studies: strain 5-P14, which requires arginine and guanine for growth, and strain 32-P14, which requires leucine and guanine as growth factors. Both of these double auxotrophs were derived from the guanine-requiring strain P-14, which had been derived[8] from the wild strain, 1033.

Media

1. *Basal salts solution.* The inorganic salt composition (w/v) used in all the media was: $Na_2HPO_4 \cdot 7H_2O$, 1.34%; KH_2PO_4, 1.36%; $CaCl_2$, 0.0011%; $MgSO_4 \cdot 7H_2O$, 0.0246%; and $(NH_4)_2SO_4$, 0.2%. This solution (pH = 6.5) was prepared fresh at least twice weekly.

2. *Carbon sources.* The particular compound which was to serve as the major source of carbon and energy was prepared in either a 5% or a 10% solution and autoclaved separately. Sufficient amounts were then added to the basal salts solution to give a final concentration of 0.4%. Substances employed in this manner included glucose, *myo*-inositol, glycerol, and L-histidine.

3. *Growth factors.* The usual amounts of growth factors which were employed

* Personal communication from F. GROS AND S. LACKS.

to provide an excess of each component were: guanine, 50 μg/ml (or, alternatively, an equivalent amount of guanosine); L-arginine, 100 μg/ml; and L-leucine, 50 μg/ml. The amino acids were kept in sterile solutions; guanine was kept in dilute KOH and autoclaved with the salt solution.

During short-term incorporation experiments the amount of the particular labelled growth factor was less than the above amounts, and is specified in each experiment.

4. *Supplements.* (a) Purine and pyrimidine ribosides. This supplement was a solution of adenosine, guanosine, uridine, cytidine, and thymidine, each at $6 \cdot 10^{-3}$ *M*, and was used in the amount of 1 ml for every 20 ml of medium.

(b) Amino acid mixture. This solution consisted of a 10^{-2} *M* solution of the L-isomer of each of the following amino acids: arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, tyrosine, valine, alanine, glutamic acid, proline, aspartic acid, serine, and glycine. This mixture was used at a 1:100 dilution. Arginine and leucine were present in the supplement only when not already included in the medium as an essential growth factor.

(c) Vitamin mixture. This supplement was the commercial preparation BME Vitamin Mixture (Microbiological Associates, Inc.), and was used at a 1:100 dilution.

(d) Yeast extract-tryptone. This supplement was added to the medium to give a final concentration of 0.2 % yeast extract (Difco) and 0.2 % Bacto-Tryptone (Difco).

(e) Casamino acids. This commercial preparation (Difco) of hydrolyzed casein was used to give a final concentration of 0.2 %.

Method of cultivation and measurement of growth

For all experiments cells were grown at 35° on a platform shaker with an excursion of 5 cm and a frequency of 90 excursions/min. Cultures were contained either in Erlenmeyer flasks filled to no more that one-tenth their capacity, or in special wide-bottom flasks that could contain up to 500 ml. Using this procedure, and employing cultures that contained no more than 350 μg dry weight of cells per ml, conditions of aerobiosis could easily be maintained.

A few experiments employed a chemostat kindly lent to us by Dr. LUIGI GORINI.

Growth was usually followed by measuring the turbidity of the culture in a Klett-Summerson photoelectric colorimeter (Filter no. 42) which had been calibrated and standardized with exponential-phase cells of this organism dried to constant weight. The measurements were expressed either as dry weight of cells per ml of culture, or simply as Klett units. This method probably introduces an error due to cell-size differences under different conditions, but none of our results are dependent for their validity on exact knowledge of the dry weight of a culture.

Growth rates were expressed as k, the number of doublings per hour.

Fractionation of cells into soluble and particulate components

Cultures were chilled rapidly by pouring them into pre-chilled flasks immersed in an ice-salt bath. They were then centrifuged, usually in plastic bottles, in a refrigerated International centrifuge. The culture fluid was then decanted, the walls of the containers rinsed, and the cells suspended (with or without additional washing) in 11 ml of a buffer (pH 7.4) composed of 0.001 *M* Tris and 0.01 *M* $MgCl_2$; DNAase (Pabst) was added to a concentration of approximately 10 μg/ml. This suspension

was then treated in a Raytheon 10 kc magnetostrictive oscillator for 4 min. Alternatively, the cells were ground with about 3 parts of alumina powder in the cold and extracted with 10 ml of the same buffer. In either case, the resulting extract was centrifuged at 10,000 rev./min in a Servall Superspeed centrifuge for 20 min, and the clear supernatant fluid carefully decanted. This preparation is referred to as an unfractionated, cell-free extract.

Such an extract was then centrifuged in a Spinco Ultracentrifuge (preparative model) at 38,000 rev./min for 90 min. This procedure yielded a crystal-clear supernatant liquid and a compact, gummy pellet. The liquid was carefully decanted and the pellet suspended in the Tris–$MgCl_2$ buffer. For the purpose of these investigations, no further purification steps were deemed necessary.

This procedure is based on that developed by TISSIÈRES AND WATSON[2].

Colorimetric analyses

The colorimetric assays employed were: the orcinol method for RNA[9], the phenol method for protein[10], and the indole method for DNA (CERIOTTO[11] as modified by KECK[12]). To assay these components of whole cultures, 5–10 ml of culture were sampled and brought to 5 % TCA. The samples were stored overnight at 4°, then centrifuged and the pellet washed once with cold 5 % TCA. The washed pellet was resuspended in exactly 5 ml of 5 % TCA, heated at 100° for 30 min, the mixture cooled, centrifuged, and the supernatant fluid carefully decanted. Aliquots of the supernatant fluid were assayed directly for RNA and DNA. The pellet was dissolved in 1 *N* NaOH, diluted 10 fold, and assayed for protein.

The procedure was similar when these assays were performed on cell-free extracts. The nucleic acids and proteins were precipitated and washed with cold 5 % TCA, and extracted with hot 5 % TCA; the hot TCA extract was assayed for ribose and the residual precipitate was dissolved in 1 *N* NaOH and assayed for protein.

Despite the many pitfalls of these colorimetric methods, we have found reasonably good agreement between them and independent methods of analysis (260 mμ absorption for nucleic acid, guanine incorporation for nucleic acid, and amino acid incorporation for protein).

Measurement of [8-^{14}C]guanine incorporation

(a) *Differential incorporation into RNA and DNA*. The culture was chilled and the cells ($\sim$ 75 mg) were harvested and washed free of culture fluid by successive centrifugation. The cells were then extracted with cold 5 % TCA, twice with ethanol–ether (3:1), and finally dissolved in 1.0 ml of 1 *N* KOH. After 1 h of hydrolysis at 37° the mixture was chilled, neutralized with 1.5 *N* HCl, and acidified to 5 % TCA. The precipitate (consisting of protein and DNA) was washed once with cold 5 % TCA and hydrolyzed in 5 % TCA at 100° for 30 min. The mixture was centrifuged and the supernatant fluid concentrated and fractionated on a descending paper chromatogram in isopropanol–HCl–H_2O[13]. The guanine spot was eluted with 0.1 *N* HCl, the concentration determined by u.v. absorption in a Beckman DU spectrophotometer, and an aliquot counted in a gas-flow Geiger counter.

The supernatant liquid containing hydrolyzed RNA was made 0.1 *N* with respect to HCl and twice extracted with 5.0 ml of ether to remove the TCA. The pH was adjusted to 4 and 100 mg of Darco charcoal added. The suspension was

shaken for 30 min, then centrifuged and the charcoal washed once with 2.0 ml of H_2O. Next, 3 ml of 0.01 *M* EDTA (brought to pH 7.5 with NH_4OH) was added and the mixture was shaken and then centrifuged. The charcoal was then treated with ethanol–H_2O (50 %) containing 1 % NH_3 for 20 min with shaking. The eluate, which contained the nucleotides, was then subjected to paper electrophoresis in 0.04 *M* citrate at pH 3.4–3.5 for 2 h with a voltage gradient of 33 V/cm. The guanylic acid spot was eluted, the concentration measured by u.v. absorption in the Beckman DU spectrophotometer and an aliquot counted in a gas-flow Geiger counter.

(*b*) *Incorporation into soluble and particulate RNA*. Following separation of the cell-free extracts into soluble and particulate fractions (as described above), the two fractions were brought to 5 % TCA and centrifuged. The supernatant fluids were discarded and the pellets washed once with cold 5 % TCA. Each pellet was then hydrolyzed for 1 h at 37° in 1 ml of 1 *N* KOH. The hydrolysates were carefully neutralized with perchloric acid, the precipitate $KClO_4$ removed, and then sufficient perchloric acid added to give an additional concentration of 5 %. The precipitate (consisting of protein, and any DNA that has escaped the action of the DNAase in the Tris buffer) was discarded. The supernatant fluid was neutralized with KOH, the precipitated $KClO_4$ removed, and the supernatant concentrated and fractionated by paper chromatography using the isopropanol–HCl–H_2O solvent described previously. The guanylic acid spots were eluted, the concentration of the eluates was measured spectrophotometrically, and their radioactivity was determined by the methods described above.

Measurement of L-[^{14}C]*leucine incorporation into proteins*

When incorporation of L-leucine into soluble and particulate protein was to be measured, cell-free extracts of the cultures were prepared and fractionated by the procedures described above. Each fraction was then acidified with 5 % TCA containing 2 mg of unlabelled L-leucine/ml and heated at 90° for 20 min. The residual precipitates were washed once with the same reagent and then dissolved in 1 ml of 1 *N* NaOH containing 2 mg of L-leucine/ml. This mixture was acidified by the addition of 1 ml of 20 % TCA and the resulting precipitate extracted twice (with warming) by 5-ml portions of each of the following: acetone, alcohol–ether (3:1), and finally, ether. The air-dried proteins were then dissolved in 1 *N* NH_4OH and aliquots taken for estimation of protein by dry weight and by colorimetric assay, and for measurement of radioactivity. The amount of protein plated on planchets for counting never exceeded 100 $\mu g/cm^2$, and corrections for self-absorption were unnecessary.

This method is a modification of that of MANDELSTAM[14].

Chemicals

L-[^{14}C]leucine (uniformly labelled), 5.7 $\mu C/\mu mole$, was obtained from the Nuclear Chicago Corporation (Chicago). [8-^{14}C]Guanine, 1.23 $\mu C/\mu mole$ was obtained from Isotope Specialities Company ,Inc. (Burbank, California).

All purine and pyrimidine bases and ribosides, and all amino acids were obtained from the California Corporation for Biochemical Research (Los Angeles) and were of a purity equivalent to their A Grade.

All other organic and inorganic chemicals were of reagent grade.

RESULTS

Variation of the RNA content of A. aerogenes

Many microorganisms exhibit a marked fluctuation in their RNA content depending on the conditions of cultivation. To determine the behavior of *A. aerogenes*, strains 5-P14 and 32-P14, measurements were made of the RNA, DNA, and protein of cultures of these strains grown in different ways.

Initially, a stationary phase culture was defined simply as one which had been incubated aerobically for 18 h in the minimal medium described above, with glucose as the major source of carbon and energy. The first line of Table I presents the composition of such a culture assayed by the methods already described. These values should be compared to those in the second line which were obtained when the measurements were made on cells taken from the exponential phase of growth in a medium of the same composition. The most striking difference between the two cultures is in their content of RNA. Whether the RNA level is expressed in relation to mass of protoplasm (measured turbidimetrically), protein, or DNA, the RNA of exponential phase cultures is three to four times that of stationary phase cultures.

TABLE I

THE COMPOSITION OF *Aerobacter aerogenes*, STRAIN 5-P14, DURING THE STATIONARY PHASE AND DURING EXPONENTIAL GROWTH ON DIFFERENT SOURCES OF CARBON AND ENERGY

Carbon source	*Growth rate constant (k)*	*Composition of culture of standard turbidity* (μg/ml)*		
		Protein	*DNA*	*RNA*
Glucose	(stationary)	55	6.0	8.5
Glucose	0.97	51	4.5	27.5
Glycerol	0.72	53	4.2	21.0
Inositol	0.50	60	5.0	18.0
Histidine	0.29	47	4.8	14.0

These results were obtained using the colorimetric techniques described under MATERIALS AND METHODS. See text for details of the experimental procedure. Each figure represents the average of three or more determinations.

* A Klett reading of 150.

Measurements were next made to ascertain whether exponential phase cultures possess the same high RNA content regardless of the method of cultivation. Accordingly, samples were taken from cultures in the exponential phase of growth with glycerol, *myo*-inositol, or L-histidine serving as the major source of carbon and energy and were analyzed for RNA, DNA, and protein. The results of these analyses are presented in Table I also. It may be seen that, again, there is little variation in the ratio of DNA to protein among these cultures. The RNA content, however, shows considerable variation under the different conditions, though it is always significantly above the level of the stationary-phase culture.

The results summarized in Table I provoke two questions: (1) how is the RNA content of growing cultures of bacteria reduced when they enter the maximum stationary phase, and (2) how is it related to the chemical composition of the medium? Experiments relevant to these questions are described in the next two sections.

Change in RNA levels of cultures entering the stationary phase

In answering the first question, involving cells which are not growing exponentially, it seemed desirable to specify the condition restricting growth. For this reason it was decided to investigate the effect of different nutritional limitations on the composition of strain 5-P14. Experiments were performed in which exhaustion from the medium of one of the following components resulted in a transition from exponential growth to a stationary condition: (1) the essential amino acid, L-arginine, (2) the essential purine, guanine, and (3) the major source of carbon and energy, glucose.

All three of the experiments were performed in the same way. Exponential phase cells were inoculated (to a final concentration of about 40 μg dry weight per ml) into media containing the usual excess amounts of all components but one. The particular limiting factor was added in an amount calculated to support growth only to about 200 μg dry weight of cells per ml. Growth was followed turbidimetrically, and samples were taken at various times to be analyzed for RNA, DNA, and protein by the usual methods. In Fig. 1 the results are plotted from the time when the first deceleration of growth (as measured turbidimetrically) was noted. The values are expressed in terms of the levels of all components being 1.00 at this (somewhat arbitrary) time.

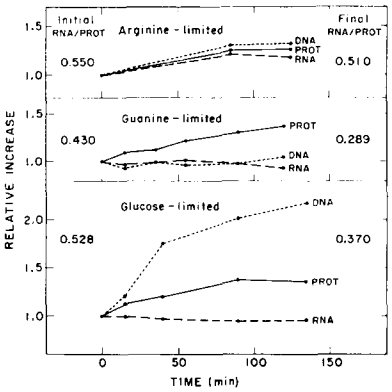

Fig. 1. The relative increases of DNA, RNA, and protein in cultures of *A. aerogenes*, strain 5-P14, entering the maximum stationary phase because of limiting amounts of L-arginine, guanine, or glucose. The excess and the limiting concentrations, respectively, of these medium constituents were: L-arginine, 100 μg/ml and 10 μg/ml; guanine, 50 μg/ml and 5 μg/ml; glucose, 2000 μg/ml and 250 μg/ml. RNA, DNA, and protein were measured by colorimetric methods, and the increases in these components, relative to their values when growth ceased to be exponential, are plotted against time. The numbers to the left of the curves are the RNA/protein ratios at the time when growth ceased to be exponential; the numbers to the right are the RNA/protein ratios of the final samples.

It is apparent that the kinetics characteristic of decelerating growth depend partially on the nature of the limiting factor. (1) When arginine became limiting there was a very rapid and simultaneous deceleration in the rate of net synthesis of all three of these major cell components; the RNA/protein ratio 125 min after the arginine had become limiting had changed very little (0.55 to 0.51). (2) In the culture limited by the guanine concentration, RNA and DNA ceased increasing at the time that growth (measured turbidimetrically) slowed down, but the synthesis of protein continued at a slow rate throughout the 125-min period studied, dropping the RNA/protein ratio from 0.43 to 0.29. (3) In the culture in which glucose was limiting, there was an immediate cessation of net RNA synthesis a full 40 min before the synthesis of DNA was affected. The protein of this culture continued to increase throughout the experiment at a slow but easily measured rate, reducing the RNA/protein ratio from 0.53 to 0.37.

These results indicate that the low RNA/protein ratios of stationary phase cultures can be due, at least in part, to a preferential increase in the protein of the culture after RNA increase has ceased.

RNA content of exponential-phase cells

Experiments were next undertaken to determine the effect of the chemical composition of the medium on the RNA level of exponential-phase cultures.

Cultures of strain 5-P14 were grown under each of ten different conditions for a minimum of three generations, and then samples were taken and analyzed for RNA, DNA, and protein. The methods of cultivation differed from each other in one or more of the following ways: (a) the nature of the major source of carbon and energy, (b) the presence or absence of an exogenous supply of amino acids, and (c) the rate of supply of the growth-essential amino acid, L-arginine. The latter parameter was varied by means of a chemostat.

Each method of cultivation resulted in a different growth rate and in a different RNA/protein ratio of the culture. As shown by Fig. 2, the data indicate a direct relation between the RNA content of a culture and its growth rate. Since all of the cultures were in balanced growth, this relation is also true between the RNA

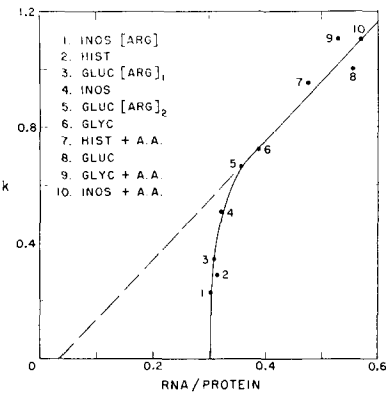

Fig. 2. The relation between growth rate and RNA content of *A. aerogenes*, strain 5-P14. The growth rate is expressed as the value of the growth rate constant, k (doublings/h), and the RNA content is expressed as the RNA/protein ratio based on colorimetric measurements. Each of the numbered points in the figure represents the average of at least two experiments, analyzed in duplicate. The cells were grown in the various media for at least three generations before any samples were taken. Except where indicated otherwise, all of the media contained basal salts supplemented with the usual excess amounts of guanine and L-arginine. The major carbon and energy sources, supplied at 0.2% final concentration, were: (1) *myo*-inositol, with L-arginine supply limited by the chemostat; (2) L-histidine; (3) glucose, with L-arginine supply limited by the chemostat; (4) *myo*-inositol; (5) glucose, with the supply of L-arginine by the chemostat greater than in (3) but still limiting; (6) glycerol; (7) L-histidine plus hydrolyzed casein; (8) glucose; (9) glycerol plus hydrolyzed casein; (10) *myo*-inositol plus hydrolyzed casein.

content and the rate of protein synthesis. Starting at the highest growth rate (k = 1.10) and proceeding to slower rates, there is a proportional decrease in RNA content for every decrease in growth rate until at a growth rate of k = 0.6 and below, a minimum level of RNA is approached. The smooth curve described by the data indicates that the composition of the medium determines the level of RNA only insofar as it determines the growth rate of the cells.

Having established the steady-state relationship between growth rate and RNA content, it became important to know the manner in which an exponentially growing culture adjusts its RNA content when undergoing an increase or a decrease in its growth rate. Studies were first made of the kinetics of RNA synthesis during a decrease in growth rate caused by a change in the major source of carbon and energy.

RNA synthesis during diauxic growth

Cells of this organism grow very well when *myo*-inositol is the sole source of carbon and energy, metabolizing this substance by a series of inducible enzymes[15]. The synthesis of these enzymes is prevented during growth on glucose, even when the inducer, *myo*-inositol, is present[16]. This repression of enzyme formation gives

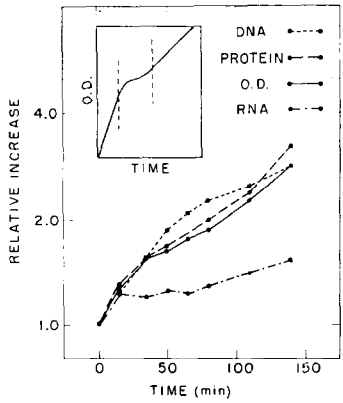

Fig. 3. The relative increases of DNA, RNA, protein, and O.D. in a culture of *A. aerogenes*, strain 5-P14, during a diauxic lag. The cells were grown in 250 ml of the basal salts solution supplemented with guanine (50 μg/ml) and L-arginine (100 μg/ml), and containing glucose (600 μg/ml) and *myo*-inositol (2000 μg/ml) as major sources of carbon and energy. The inset to the figure describes the diauxic growth curve of this culture, as measured by its O.D. During the period included by the dashed lines in the inset, when the culture was adjusting from growth on glucose to growth on *myo*-inositol, samples were taken and assayed for RNA, DNA, and protein by the methods previously described. The results are plotted in the main part of the figure as relative increases in these components as a function of time. The increases are relative to the individual values at 0 time, shortly prior to the beginning of the diauxic lag.

rise to the familiar diauxic type of growth curve when the culture is supplied with both glucose (in limiting amounts) and *myo*-inositol. As the inset of Fig. 3 shows, there is a period of exponential growth (during which it can be demonstrated that the cells are growing at the expense of the glucose exclusively) followed by a lag period which lasts while the cells synthesize the appropriate inducible enzymes. Finally there is a phase of exponential growth on the *myo*-inositol.

During the period included by the dashed lines in the inset, when a culture of strain 5-P14 was adjusting from growth on glucose ($k = 0.93$) to growth on *myo*-inositol ($k = 0.50$), samples were taken and analyzed for RNA, DNA, and protein by the usual colorimetric methods. The increase in each component, relative to the value at 0 time (shortly prior to the beginning of the diauxic lag), is plotted in the main part of the figure. It can be seen that DNA accumulation continues at its original rate during the first part of the lag period, and then decreases to the new rate characteristic of growth on *myo*-inositol. Protein continues to accumulate throughout the lag period, though at a slightly lower rate than DNA. On the other hand, very little net increase in RNA could be detected throughout the period of diauxic lag; RNA began to increase more rapidly again only after the transition from glucose metabolism to *myo*-inositol metabolism had been accomplished.

The failure to observe any net increase in RNA during the diauxic lag could be the result of two processes: (1) a cessation of RNA synthesis, or (2) a breakdown of RNA masking a continued synthesis. To distinguish between these alternatives the experiment was repeated, and a small amount of [8-^{14}C]guanine (5.2 μmoles) of specific activity (1.23 μC/μmole) was added to the guanine-containing culture (20 μg/ml) at the beginning of the lag phase; the cells were harvested at the end of the lag phase (1 h) and their nucleic acids were fractionated by the methods described above. The molar radioactivity of the RNA-guanine and of the DNA-guanine was determined and compared with the molar radioactivity of the guanine present in the growth medium. The results (Table II) indicate that much less guanine was incorporated into RNA than into DNA. Therefore, despite an incorporation corresponding to a synthesis of 11%, the marked decrease in accumulation of RNA during the diauxic lag seems to be caused mainly by a decreased rate of synthesis.

Preferential RNA synthesis during increases in growth rate

It can be seen by reference to Fig. 2 that an increase in the rate of growth from

TABLE II

THE INCORPORATION OF [8-^{14}C]GUANINE INTO THE NUCLEIC ACIDS OF *Aerobacter aerogenes*, STRAIN 5-P14, DURING A DIAUXIC LAG

	RNA guanine	*DNA guanine*
Radioactivity of the isolated guanine relative to that in the medium (97,000 counts/min/μmole)	0.103	0.330
Increase calculated from the radioactivity	11 %	49 %
Increase calculated from colorimetric measurements	7 %	40 %

The analytical techniques are described under MATERIALS AND METHODS. The experimental procedure is described in the text.

$k = 0.2$ to $k = 1.2$ would entail an increase in the RNA/protein ratio from about 0.3 to about 0.6. It would be of interest to know whether the increased RNA/protein ratio is a prerequisite for the increase in growth rate or whether it is simply a consequence of the increased growth rate. One approach would be to determine whether one detectably preceded the other. Fortunately, it proved relatively simple to demonstrate a temporal difference between an increase in the level of RNA in a culture and an increase in the rate of protein synthesis.

The appropriate experiment was first performed by the addition of a mixture of yeast extract and tryptone (see MATERIALS AND METHODS for the composition) to a culture of strain 5-P14 growing on L-histidine as the major source of carbon and energy. Aliquots were taken at various times before and after the supplementation and analyzed for RNA, DNA, and protein by the usual colorimetric methods.

The results are shown in Fig. 4, where relative increases in RNA, protein, and DNA (with all values assumed to be 1.00 at the time of addition of the supplements) are plotted on a logarithmic scale against time. The samples taken at 0 and at 50 min show that during the growth on L-histidine with a $k = 0.28$ there was balanced synthesis of RNA, DNA, and protein. At 50 min the supplement was added, and almost immediately there was a great increase of synthesis of RNA. This rapid synthesis (at a rate temporarily greater than the rate which was to be established) continued for about 25 min, during which time the rate of synthesis of DNA and of

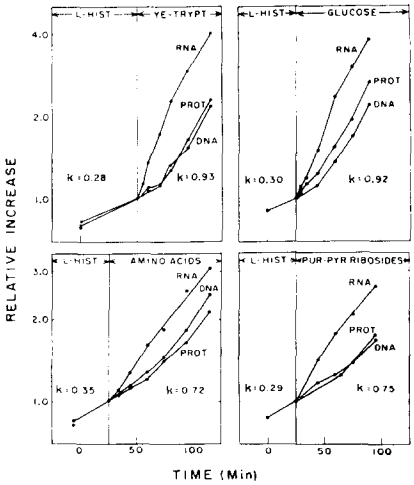

Fig. 4. The relative increases of DNA, RNA, and protein in cultures of *A. aerogenes*, strain 5-P14, during accelerating growth following the addition of (a) yeast extract–tryptone, (b) glucose, (c) a mixture of amino acids, or (d) a mixture of purine and pyrimidine ribosides to an L-histidine medium. See the text for details of the experimental procedure, and MATERIALS AND METHODS for the composition of the media and the supplements.

protein increased only slightly. By this time the preferential RNA synthesis had raised the RNA content of the culture to the level characteristic of the new growth rate, and the rate of synthesis of DNA and of protein then quickly reached the new value, and the synthesis of all three macromolecules proceeded at the same differential rate ($k = 0.93$).

The results of this experiment make it unlikely that high RNA content is simply a consequence of fast growth rather than a necessary antecedent. But if this conclusion is correct, then one should be able to vary the nature of the supplement without changing the results of the experiment. Fig. 4 also shows the results which were obtained when (a) glucose, (b) a mixture of amino acids, and (c) a mixture of purine and pyrimidine ribosides were added to cells of strain 5-P14 growing on L-histidine. In all cases the attainment of the new high growth rate was preceded by a rapid, preferential synthesis of RNA. It was inconsequential whether the supplement consisted mainly of precursors of nucleic acid or of protein.

It now seemed of interest to investigate the nature of the RNA which is synthesized under these conditions.

Particulate and soluble components of A. aerogenes

The bulk of the RNA in bacterial cells exists in the form of ribonucleoprotein particles (ribosomes) of high molecular weight, and with sedimentation coefficients ranging from 30 S to 100 S, depending on the particular conditions employed to prepare them. The remainder of the RNA consists of low molecular weight units which are not sedimented with the ribosomes and which are soluble down to a pH of 5. This fraction has been termed the "soluble RNA" and has been implicated in the incorporation of amino acids into proteins.

Using the procedure of TISSIÈRES AND WATSON (see MATERIALS AND METHODS) to fractionate the RNA of cultures of our strains of *A. aerogenes*, it was determined that about 15 % of the total RNA is not sedimented by a force of 100,000 × *g* acting for 120 min, while the remainder forms a compact and easily isolated pellet.

Such a fractionation was performed on two separate cultures of strain 5-P14: one growing on L-histidine and one on glucose. Aliquots (5 ml) were taken periodically from each culture for RNA and protein determinations and the two exponential-phase cultures (each of 250-ml volume and with a bacterial density of 250 μg dry weight per ml) were chilled rapidly, harvested by centrifugation, and washed once with the Tris–Mg buffer. Each pellet was ground with alumina and the resulting extracts were fractionated into soluble and particulate components. Each fraction was then assayed colorimetrically for RNA and for protein. (See MATERIALS AND METHODS.)

The results of these analyses were combined with the values for the total RNA and total protein of the respective cultures, and the soluble and particulate constituents of the original cultures were calculated. The results are given in Table III and may be summarized as follows:

(1) Rapidly growing cells have both more soluble and more particulate RNA; the ratio of soluble to particulate RNA seems to be independent of the growth rate.

(2) The ratio of the protein to the RNA of the particulate fraction seems to differ significantly between cultures growing at different rates. The range of values, however, agrees very well with that found by other investigators[2].

TABLE III

PARTICULATE AND SOLUBLE COMPONENTS OF *Aerobacter aerogenes*, STRAIN 5-P14, GROWING AT DIFFERENT RATES

Cell constituent	*Fraction**	*Major carbon source in growth medium*	
		Glucose (k 0.90)	*L-histidine (k 0.31)*
RNA	soluble, μg/ml	3.6	2.2
	particulate, μg/ml	22.8	12.8
	soluble/particulate	0.16	0.17
Protein	soluble, μg/ml	43.3	43.2
	particulate, μg/ml	11.7	7.8
	soluble/particulate	3.7	5.5
Ribosomes	total, μg/ml**	34.5	20.6
	protein/RNA***	0.55	0.66

* All values are expressed as μg/ml of a culture of standard turbidity (Klett reading of 150). See text for experimental details.
** Calculated as the sum of the particulate protein and the particulate RNA.
*** Calculated as the ratio of the particulate protein to the particulate RNA.

With this information in hand, it was now possible to return to the original problem: the nature of the RNA made preferentially during accelerating growth.

Nature of the RNA made during accelerating growth

The incorporation of [8-^{14}C]guanine into the RNA of a culture of strain 5-P14 during accelerating growth was compared with the incorporation into a control culture. Acceleration was achieved by the addition of a rich supplement (amino acids, purine and pyrimidine ribosides minus guanosine, vitamins, and glucose) to a culture growing relatively slowly on *myo*-inositol (with arginine (100 μg/ml) and guanine (20 μg/ml) present as required growth factors). At the time of supplementation, a small amount of [8-^{14}C]guanine (4.56 μmoles) of high specific activity (1.23 μC/μmole) was added to the culture, and an identical amount added to a control culture which received no supplement.

After 20 min of continued incubation the two cultures were chilled and harvested by centrifugation. Cell-free extracts were prepared and fractionated in the usual manner. Colorimetric analyses for RNA were performed on the soluble and the particulate fractions of the two extracts, as well as on samples of the two cultures taken at 0 time and at 20 min. The results of these measurements are shown in Part A of Table IV, and in Part C these results have been used to calculate the increase in soluble and in particulate RNA in the two cultures.

The specific activity of the guanine in the soluble and in the particulate fractions was measured by the usual methods, with the results that are presented in Part B of Table IV. Calculation of the increase in the two fractions of both cultures are given in Part C.

The colorimetric and the isotope incorporation data are in excellent agreement. They show a 20–25 % increase in both the soluble and the particulate RNA in the control culture, and a 40–45 % increase in both fractions of RNA in the experimental culture.

TABLE IV

THE NATURE OF THE RNA SYNTHESIZED BY *Aerobacter aerogenes*, STRAIN 5-P14, DURING ACCELERATING GROWTH

A. Colorimetric assay of the total RNA and of the ratio of soluble to particulate RNA during accelerating growth.

Time min	*Ribonucleic acid of control culture*		*Ribonucleic acid of experimental culture*	
	total RNA μg/ml	*sol./part. RNA*	*total RNA (μg/ml)*	*sol./part. RNA*
0	42.8	0.15*	42.8	0.15*
20	52.0	0.17	60.5	0.15

* Separate experiment.

B. Incorporation of [8-^{14}C]guanine into the soluble and the particulate fractions of RNA during accelerating growth.

Flask	*Specific activity (counts/min/μmole)*		
	guanine in medium	*guanine in soluble RNA*	*guanine in particulate RNA*
Control	53,000	10,800	9,300
Experimental	53,000	16,100	15,600

C. Calculated syntheses of soluble and of particulate RNA.

Culture	*Component*	*Increase calculated from colorimetric measurements (data of Part A.)*	*Increase calculated from radioactivity (data of Part B.)*
Control	soluble RNA	21 %	25 %
	particulate RNA	21 %	23 %
Experimental	soluble RNA	41 %	43 %
	particulate RNA	41 %	42 %

Protein synthesis during accelerating growth

Since the RNA made during accelerating growth proved to be, like that made during balanced growth, mostly (85 %) particle-bound, it became of interest to know whether the protein moiety of the particles is also synthesized preferentially during accelerating growth.

Two 300-ml exponential-phase cultures of strain 32-P14 were prepared with L-histidine as the major carbon and energy source and with the required growth factors, guanine (50 μg/ml) and L-leucine (10 μg/ml). At 0 time a small amount (1.2 μmoles) of uniformly-labelled L-[^{14}C]leucine of high specific activity (5.7 μC/μmole) was added to each of the two cultures. One of the cultures received a simultaneous supplement of amino acids, purine and pyrimidine ribosides, vitamins, and glucose, while the other received no supplement. Small samples of both cultures were taken for colorimetric analyses at 0 time and 25 min later, when both cultures were harvested and cell-free extracts prepared and fractionated as before. This time, however, the protein of the two fractions was isolated (see MATERIALS AND METHODS)

and the specific activities of the whole proteins were determined as counts per minute per milligram of dried protein. From these activities it was possible to calculate the specific activities of the L-leucine in the proteins (Table V) for the following reasons: (1) strain 32-P14 must use the L-leucine presented to it for protein synthesis as it is incapable of manufacturing any, (2) L-leucine added to the medium appears in protein exclusively as leucine, and (3) the average leucine content of the soluble and particle-bound proteins of this organism has been measured and found to be 8.5 % (w/w) at two different growth rates[14].

TABLE V

THE SYNTHESIS OF SOLUBLE AND OF PARTICULATE PROTEIN BY *Aerobacter aerogenes*, STRAIN 32-P14, DURING ACCELERATING GROWTH

Culture	*Time*	*Colorimetric assay*				*Incorporation of [^{14}C]leucine*			
		Total RNA		*Total protein*		*Specific activity of bacterial protein* (counts/min/μg leucine)*		*Increase in protein based on specific activity*	
		μg/ml	*increase*	*μg/ml*	*increase*	*Soluble*	*Particulate*	*Soluble*	*Particulate*
Control	0 min	22.6		86		0	0		
	25 min	25.9	14.6 %	99	15.1 %	197	212	21 %	23 %
Experimental	0 min	24.8		86		0	0		
	25 min	46.9	89 %	111	29 %	201	432	22 %	62 %

* The specific activity of the [^{14}C]leucine in the medium of both cultures was 1130 counts/min/μg.

Colorimetric analyses of the aliquots taken at the time of supplementation and at the time of harvesting showed that the control (unsupplemented) culture increased about 15 % in total RNA and in total protein. Leucine incorporation indicated a somewhat higher rate of synthesis (about 25 %) for both the soluble and particulate protein fractions. (This discrepancy is assumed to be an analytical artifact.) The experimental flask exhibited a dramatic 89 % increase in total RNA and a 29 % increase in total protein, measured colorimetrically. Comparing the specific activities of the soluble and particulate protein fractions in this culture, it can be seen that there has been a preferential synthesis of particle-bound protein; while the soluble fraction increased no more than did that of the control culture (22 %), the particle-bound fraction increased almost three times as much (62 %) as did that of the control. Therefore, we may conclude that the rapid synthesis of particulate RNA during accelerating growth is accompanied by a preferential synthesis of particulate protein. This experiment has been repeated several times, and has been performed using different carbon and energy sources and different labelled amino acids, always with similar results.

This demonstration that the protein moiety of the ribosomes is made concomitantly with the RNA during accelerating growth (Table V) shows, in fact, that an 89 % increase in RNA was accompanied by only a 62 % increase in ribosomal protein. A partial explanation of this difference stems from the fact that the protein/RNA ratio of the ribosomal fraction prepared from rapidly growing cells is consistently 10–20 % lower than that of the same fraction of slowly growing cells (*cf.* Table IV). This difference would almost account for the discrepancy (25 %) observed in the incorporation studies. Since no attempt was made to purify the ribosomal prepara-

tions beyond that described in MATERIALS AND METHODS, it is possible that the variation of the protein/RNA ratio of these preparations is due to small amounts of contaminating non-ribosomal protein. Other explanations are conceivable, but further information would be required to discuss them profitably.

DISCUSSION

It is by now a well established fact that growing bacterial cultures are richer in RNA than are non-growing cultures. WADE AND MORGAN[18], for example, found that the level of RNA in "dividing cells" was from 1.3 to 16.5 times higher than in "resting cells" of the dozen bacterial species they examined. Our strain of *A. aerogenes* exhibits a three-fold variation in its RNA level depending on the physiological condition of the culture. Such variation in a major constituent of the cell is of particular interest since the physiological role of this component is not fully understood.

In view of the reported stability of RNA during growth[19,20], it seemed of interest to learn how the RNA content is reduced when cultures enter the stationary phase. It was realized, however, that the stationary phase does not represent a unique physiological state. Cultures may have ceased increasing for any one (or more) of a number of reasons: exhaustion of an organic or an inorganic growth factor, exhaustion of the major carbon and energy source(s), limitation of oxygen, or accumulation of toxic metabolic products. We have studied three conditions restricting growth: exhaustion of a required amino acid, of a required purine, and of the major carbon and energy source. When growth became limited by the exhaustion either of the purine or of the major carbon and energy source, the net synthesis of RNA was reduced much more drastically than was the net synthesis of protein or of DNA. Thus, the RNA/protein ratio became gradually reduced during the transition from exponential growth to a stationary phase by means of a continued synthesis of protein after RNA accumulation had ceased. When an amino acid became limiting, however, there was an abrupt and virtually simultaneous reduction of the synthesis of RNA, protein, and DNA.

The findings with carbon source or purine limitation are similar to those of SCHMIDT *et al.*[21] who observed that phosphate exhaustion in a yeast culture results in a continued, slow synthesis of protein after RNA synthesis has ceased. These results, together with our own, make it clear that cultures can undergo a considerable reduction in RNA content simply by a continued synthesis of other cellular components (*e.g.*, protein and DNA) after RNA accumulation has halted.

The RNA/protein ratio of stationary phase cells predicted by Fig. 2 is about 0.30, while that observed in an overnight culture (Table I) is about 0.16. This discrepancy is probably due to the fact that when cells are incubated for long periods of time (10 h or more) under non-growing conditions there is an actual destruction of some RNA[21–23]. In general, however, one observes little or no net destruction of RNA for several hours after a culture has ceased exponential growth due to the exhaustion of some constituent of the medium.

The next series of experiments showed that there is not a unique RNA level which is characteristic of a "growing" cell as compared to that of a "resting" cell, but rather that the RNA content of growing cultures can vary over a wide spectrum of values. This variation is shown by our work, as well as that of many others[6,7,18,24–27]

to be associated with variations in the growth rate of the cultures. Different authors have described the relationship between RNA content and growth rate in microorganisms in quantitatively different terms, but in general the following conclusion can be (and has been) drawn: at a given temperature the RNA content of a culture is a direct function of the growth rate and is affected by the composition of the medium only in so far as the latter influences the growth rate. From our information about *A. aerogenes* we may draw the additional conclusions: (1) in rapidly growing cultures ($k > 0.60$) the relation between growth rate and RNA/protein ratio is approximately linear, and (2) the amount of soluble RNA (that which is not sedimented in 120 min by 100,000 $\times$ g) seems to be a constant proportion (about 14 %) of the total amount of RNA in a culture, regardless of the growth rate.

These measurements of the RNA, DNA, and protein content of cultures in balanced growth at different rates were followed by studies of the transition of cultures from one rate of balanced growth to another. When cells are caused (by changing their carbon source) to slow down their rate of growth, the transition is characterized by a marked decrease or complete cessation of RNA accumulation, followed by a more gradual slowing down of protein and of DNA synthesis (Fig. 3). RNA formation resumes only after the RNA/protein ratio has been reduced to that characteristic of the new low growth rate. The synthesis of RNA appears, therefore, to be much more sensitive to the decreased supply of energy and of carbon compounds than is that of the other two macromolecules studied.

When, by the addition of various enrichments to the medium, a culture is caused to accelerate its growth rate, the transition is characterized by a rapid acceleration of RNA synthesis to a rate temporarily greater than that to be finally attained in the new environment (Fig. 4). The pattern of incorporation of radioactive guanine during this period revealed that the usual proportion of soluble and of sedimentable RNA was being made. The pattern of incorporation of radioactive leucine indicated that the sedimentable RNA synthesized during this period consisted of complete ribosomes. After this rapid, preferential synthesis of RNA has raised the level of RNA in the cells to that characteristic of the new high growth rate, the synthesis of protein and that of DNA are accelerated to the new rate and balanced growth ensues. Thus, quite analogous to the results obtained during a period of decreasing growth rate, the synthesis of RNA is the reaction most immediately sensitive to a sudden enrichment of the medium. This conclusion is strengthened by the finding that the exact biochemical nature of the enrichment used to accelerate growth is of no importance in determining the response of the cells; the same rapid increase in RNA formation preferential to that of protein occurs whether the supplement consists of amino acids or of purine and pyrimidine ribosides.

Two implications of our findings are of general significance and deserve comment. The first concerns the role of RNA in the biosynthesis of protein, and the second the metabolic control of the biosynthesis of RNA.

It has been postulated that the ribosomal RNA plays the role of a passive template in protein synthesis[28]. According to this concept, the rate of protein synthesis would be determined by the RNA content of the cell and by the concentration of the activated amino acids which are to be polymerized. Were these latter present in sufficient concentration to saturate the template, the rate of protein synthesis would be directly proportional to the RNA content of the cellular mass. This theoret-

ical picture accurately describes the situation in cultures of *A. aerogenes* growing at rates greater than $k = 0.6$, where the rate of protein synthesis is directly proportional to the RNA content.

On the other hand, if the concentration of the activated amino acids were to be below that needed to saturate the template, then an increase in the supply of these precursors would result in an increased rate of protein synthesis without a concomitant increase in the RNA content of the cellular mass. This picture accurately describes the situation in cultures of *A. aerogenes* growing at rates less than $k = 0.6$, where the rate of protein synthesis is independent of the RNA content of the bacteria.

Protein synthesis continues long after the external level of a required purine or of the major carbon and energy source has been reduced to a value insufficient to permit RNA accumulation. This observation is quite compatible with the concept that the role of ribosomal RNA in protein synthesis is a passive one.

The most significant observation in this regard, however, concerns the transition from a slow to a rapid growth rate. The fact that the rate of protein synthesis does not attain its new high value until after the RNA level of the protoplasm has been increased to that characteristic of the new growth rate strengthens the argument for a passive role of ribosomal RNA in protein synthesis. Were it the rate of RNA synthesis *per se* which determines the rate of protein synthesis, then one would have expected as rapid an acceleration in the latter as in the former.

Turning now to the question of the control of RNA synthesis, we would like to suggest that the rate of RNA synthesis is more sensitive to the composition of the medium than is the rate of protein synthesis. It would appear that some metabolite whose intracellular concentration is raised by enrichment of the medium preferentially stimulates RNA synthesis. From the present experiments it is impossible to learn the nature of such a metabolite, for every compound which stimulates growth when added externally causes this marked stimulation of RNA synthesis, and all of them, of course, are interconvertible by the cell. One guess as to its nature, however, stems from the fact that the synthesis of RNA in the presence of chloramphenicol requires the availability to the cell of all of the amino acids[29,30]: activated amino acids or amino acid–nucleotide complexes might be the limiting factor in RNA synthesis.

Now if we assume that RNA synthesis is particularly sensitive to the level of some (unknown) constituent whose concentration depends on the metabolic activity of the cell, what would be the consequence of changes in the composition of the medium? A sudden drop in the available supply of carbon and of energy would temporarily diminish RNA synthesis to a greater extent that it would protein synthesis. Conversely, a sudden increase in the available supply of carbon and energy would stimulate RNA formation preferentially.

An important subject for further investigation is the discovery of the particular compound which is rate-limiting for RNA synthesis. But it is already possible with the present results to assess the significance of the control of protein and RNA synthesis to the economy of the cell. The fluctuations of RNA content in growing cells permit the cells to grow at a faster rate in any particular chemical environment than would be possible with an inflexible system. Thus, the cell growing on *myo*-inositol, which is not metabolized rapidly enough to give the maximal growth rate, uses a smaller proportion of its resources to make RNA than that used by a cell

growing on glucose; hence, a larger portion of the energy and building blocks produced by the degradation of *myo*-inositol is available for the synthesis of the other components of the bacterial cell. With an inflexible system in which the protein-forming machinery were always present in maximal amount, the cell would grow with maximal efficiency only at its maximal growth rate.

The existence of this adjustment mechanism in bacterial metabolism is far from surprising: studies on the control of synthesis of individual enzymes in bacteria have shown these organisms to be quite economical in the handling of their resources. The same economy seems to apply in part at least to the production of RNA.

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REFERENCES

1 B. MAGASANIK, A. K. MAGASANIK AND F. C. NEIDHARDT, in G. E. W. WOLSTENHOLME AND C. M. O'CONNER, *Regulation of Cell Metabolism* (CIBA Foundation Symposium), Little, Brown and Company, Boston, 1959.
2 A. TISSIÈRES AND J. D. WATSON, *Nature*, 182 (1958) 778.
3 R. LOFTFIELD, *Progress in Biophysics and Biophysical Chemistry*, Vol. XIII, Pergamon Press, London, 1958.
4 J. BRACHET, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. II, Academic Press, Inc., New York, 1955.
5 A. CAMPBELL, *Bacteriol. Rev.*, 21 (1957) 263.
6 M. SCHAECHTER, O. MAALØE AND N. O. KJELDGAARD, *J. Gen. Microbiol.*, 19 (1958) 592.
7 N. O. KJELDGAARD, O. MAALØE AND M. SCHAECHTER, *J. Gen. Microbiol.*, 19 (1958) 607.
8 D. USHIBA AND B. MAGASANIK, *Proc. Soc. Exptl. Biol. Med.*, 80 (1953) 626.
9 W. C. SCHNEIDER, *J. Biol. Chem.*, 161 (1945) 293.
10 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
11 G. CERIOTTI, *J. Biol. Chem.*, 198 (1952) 297.
12 K. KECK, *Arch. Biochem. Biophys.*, 63 (1956) 446.
13 G. R. WYATT, *Biochem. J.*, 48 (1951) 584.
14 J. MANDELSTAM, *Biochem. J.*, 69 (1958) 110.
15 B. MAGASANIK, *J. Biol. Chem.*, 205 (1953) 1019.
16 F. C. NEIDHARDT AND B. MAGASANIK, *Biochim. Biophys. Acta*, 21 (1956) 324.
17 F. C. NEIDHARDT, unpublished experiments.
18 H. E. WADE AND D. M. MORGAN, *Biochem. J.*, 65 (1957) 321.
19 A. D. HERSHEY, *J. Gen. Physiol.*, 38 (1954) 145.
20 A. L. KOCH AND H. R. LEVY, *J. Biol. Chem.*, 217 (1955) 947.
21 G. SCHMIDT, K. SERAIDARIAN, L. M. GREENBAUM, M. D. HICKEY AND S. J. THANNHAUSER, *Biochim. Biophys. Acta*, 20 (1956) 135.
22 J. T. HOLDEN, *Biochim. Biophys. Acta*, 29 (1958) 667.
23 J. MENDELSOHN AND A. TISSIÈRES, *Biochim. Biophys. Acta*, 35 (1959) 248.
24 P. C. CALDWELL AND C. HINSHELWOOD, *J. Chem. Soc.* (1959) 3156.
25 W. H. PRICE, *J. Gen. Physiol.*, 35 (1952) 741.
26 H. E. WADE, *J. Gen. Physiol.*, 7 (1952) 24.
27 R. JEENER, *Arch. Biochem. Biophys.*, 43 (1953) 381.
28 F. H. C. CRICK, in *Biological Replication of Macromolecules*, Symposium XII of the Society for Experimental Biology, Academic Press, Inc., New York, 1958.
29 A. B. PARDEE AND L. S. PRESTIDGE, *J. Bacteriol.*, 71 (1956) 677.
30 F. GROS AND F. GROS, *Biochim. Biophys. Acta*, 22 (1956) 200.