Evidently Kirchhoff's Law will not apply in this region, and an experimental indication of this was that meaningful gas temperatures could not be derived from the ratio of the double-beam signals. This small region round the tail of the expansion wave corresponds to the dashed portion of the experimental temperature profile in Fig. 3*a*.

Allowing for the diaphragm opening delay already referred to, the general agreement between theoretical and experimental profiles obtained shows that the picture of the flow pattern derived from theory is broadly correct, though the degree to which the wave is centred has not been investigated; it is hoped to check this in later work in which measurements will be taken at two downstream positions simultaneously. To avoid spurious distortion of the temperature and pressure profiles due to shock attenuation, a larger diameter shock tube would be desirable.

The observed shock attenuation would have led one to expect temperatures in the region of the contact surface above the equilibrium value, based on the shock speed at the second diaphragm position. In fact, however, in all runs, values less than equilibrium were recorded (Fig. 3*a*).

This may indicate that the running time was not quite sufficient for equilibrium to be reached, but may also be a further illustration of an effect peculiar to spectrum-line-reversal measurements using sodium in hot oxygen observed in some earlier work and not yet explained[11,12]. It was found that while changes of temperature were accurately followed, the temperature values were low by constant systematic amounts ranging up to nearly 400° K in some cases. This effect was not present with sodium in nitrogen.

The present work is of interest in relation to some recent spectrum-line-reversal temperature measurements obtained by Hurle, Russo and Hall[13], in a steady nozzle expansion where it was necessary to postulate a relaxation time for de-excitation of nitrogen fifteen times shorter than that derived from vibrational excitation investigations behind strong normal shock waves. From this they suggest that the Landau–Teller description of vibrational de-excitation may be inapplicable in non-equilibrium situations where the difference between vibrational and translational energy is large, and that the shorter relaxation time indicates that the probability of de-excitation may depend on the vibrational as well as on the translational energy of the colliding molecules.

The present results, however, suggest that for the oxygen relaxation observed in these experiments such assumptions are unnecessary. The general agreement seen with Appleton's calculations implies that his description of the relaxation process was adequate; he used a linear rate equation, Camac's experimental data (from shock wave investigations)[14], and Landau–Teller theory.

The consequences of large changes of the effective relaxation time on the temperature profiles of unsteady expansion waves in nitrogen have been looked into theoretically by Woodley[8]. He has found that a substantial increase in the rate of vibrational de-excitation (of the order of that mentioned by Hurle *et al.*) will shift the temperature minimum to earlier times and reduce its value considerably as might be qualitatively expected. This effect was not observed in the present experiments with oxygen, the results of which are consistent with the accepted value for the effective relaxation time within a factor of two. In fact, the experimental observation of temperatures above theoretical (Fig. 3*a*) might, on the contrary, suggest that de-excitation proceeds more slowly than was assumed. According to Appleton's argument, the assumption of frozen dissociation in these experiments is justified, so that the enthalpy of recombination of the oxygen atoms in the wave cannot contribute to the vibrational temperature. A possibility that was considered was that these higher temperatures were due to reflected sodium light entering the photo-multipliers, but this seems unlikely since the aperture of the optical system was small and earlier work[12] involving temperature measurements of the incident shock never at any time revealed a sodium radiation signal preceding the shock.

Extension of this work to reacting gases with the view of measuring the temperature dependence of recombination rates is feasible and might incidentally show whether coupling between the rates of vibrational and chemical relaxation is significant.

The use of unsteady expansion waves for chemical kinetic measurements in shock tubes was suggested by A. R. Fairbairn (1958) in an unpublished Ministry of Aviation note, and the experiment in its present form arose from discussions with D. A. Spence. J. P. Appleton performed the calculations reported in ref. 7 in support of this work, and I am indebted to J. G. Woodley for the further computations in Fig. 3.

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RHYTHMIC ALTERNATIONS IN THE RATE OF SYNTHESIS AND THE COMPOSITION OF RAPIDLY LABELLED RIBONUCLEIC ACID DURING THE SYNCHRONOUS GROWTH OF BACTERIA

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WE have observed that a remarkable wave pattern of rate of synthesis and of composition results when the ribonucleic acid newly formed in consecutive segments of the generation period of a synchronously growing bacterial culture is examined. A donor strain of *Escherichia coli K*-12 (*HfrH*) was used in these experiments.

Recent observations on *Hfr* strains of *E. coli*[1] and on certain strains of *Bacillus subtilis*[2], and also some autoradiographic evidence[3], which suggested a sequential, unidirectional replication of bacterial chromosomes, starting from a single growing point, determined the choice of organism for the work recorded here. If the

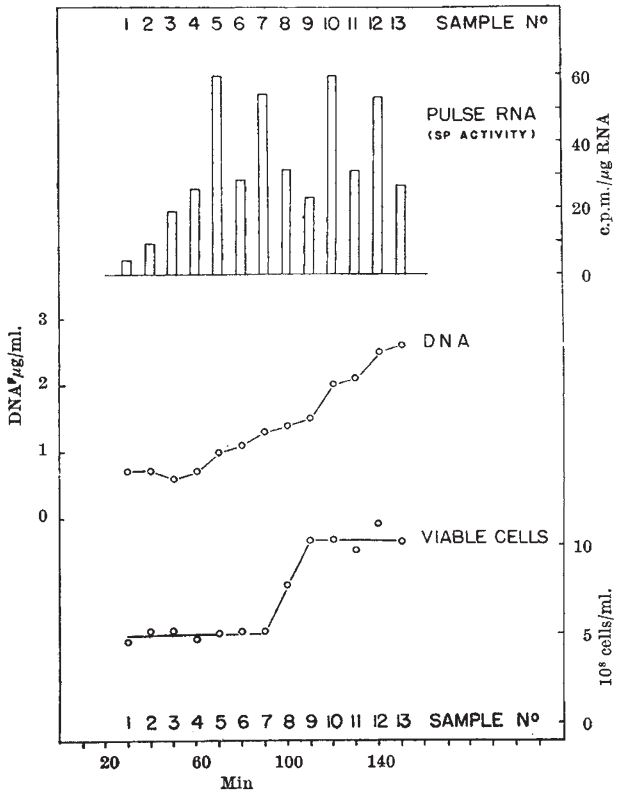

Fig. 1. Synchronous growth of *E. coli HfrH* (m^-, λ, 424), Exp. 1. The samples, taken at intervals of ten minutes, are numbered as in Table 1. The diagrams represent, in upward order: (*a*) viable counts, determined by plating the cells in appropriate dilutions on plates of nutrient agar; (*b*) synthesis of DNA; (*c*) specific activity of pulse-labelled RNA

function of the DNA component of a chromosome is taken to consist in its ability to conserve genetic information (through the duplication of DNA) and to convey it (perhaps through the production of specific RNA molecules), and if the first of these processes advances along the chromosome in an orderly and successive manner, it is not unreasonable to assume that the second does so too. In this event, the rate of synthesis, and the nucleotide composition, of different species of RNA, produced in different intervals within a growth cycle, could well vary. Furthermore, the synthesis of ribosomal and transfer RNA could be expected to occur in a periodic fashion either before or after the replication of those sites on the genome that may determine these RNA species. In order to explore these possibilities, a synchronously dividing population of *E. coli* was subjected to a pulse of ^{32}P-phosphate at regular intervals; the rate of synthesis and the composition of the newly formed RNA were then determined.

The organism examined was an *HfrH* (λ, 424) strain requiring methionine obtained from Dr. T. Nagata, who had shown that the genome of this strain replicates in a sequential, polarized fashion from the *F* locus forward[1]. The bacteria were grown in a *tris*-salts medium[4] to which were added, per litre of medium, 87·1 mg dihydrogen potassium phosphate, 300 mg vitamin-free casamino acids, 50 mg L-methionine, and 2 g glucose. The cells were synchronized by fractional filtration[5], and the filtrate comprising the small cell fraction was incubated in fresh, previously warmed, medium at 37°. Samples were withdrawn from the master culture every 10 min for the following operations: (*a*) 0·1 ml. for viable counts; (*b*) to 1 ml. of chilled 50 per cent trichloroacetic acid solution 9 ml. of the culture were added, and DNA was determined in the precipitate by the diphenylamine reaction[6] (in Exp. 2, RNA was also determined by means of the orcinol reaction); (*c*) a sample of 100 ml. of the culture was exposed to 2-mc. carrier-free ^{32}P-orthophosphate for 80 sec when, in order to terminate the pulse, the cells were poured on crushed ice. The bacteria were then washed to remove extraneous radiophosphate and 1 g (wet weight) of unlabelled *HfrH* cells grown in the same medium was added as carrier. After the extraction of RNA by a modification of several known procedures utilizing 'Duponol' and phenol, determination of radioactivity, and hydrolysis with alkali in the usual manner the (2′ + 3′)-nucleotides were separated by two-dimensional chromatography[7] and estimated spectrophotometrically and by measurement of their radioactivity. The figures for newly formed RNA were expressed as c.p.m./μg of RNA ; the estimates of the RNA content of the several preparations were again derived from spectrophotometry. Correction was made for non-nucleotidic ^{32}P-compounds that accompany the RNA specimens in varying amounts (20–30 per cent of the radiophosphate incorporated in a non-dialysable form) through the summation of counts recorded for the purified mononucleotide components.

Figs. 1 and 2 depict, in two independent experiments, the synchronism of cell division in the bacterial culture; the increase in the level of DNA during growth; and the patterns of synthesis of new RNA revealed by the application of a series of short pulses of ^{32}P-phosphate to consecutive segments of the generation period. In addition, the rise of the levels of RNA in the master culture of Exp. 2 and the computed total counts of RNA present in the

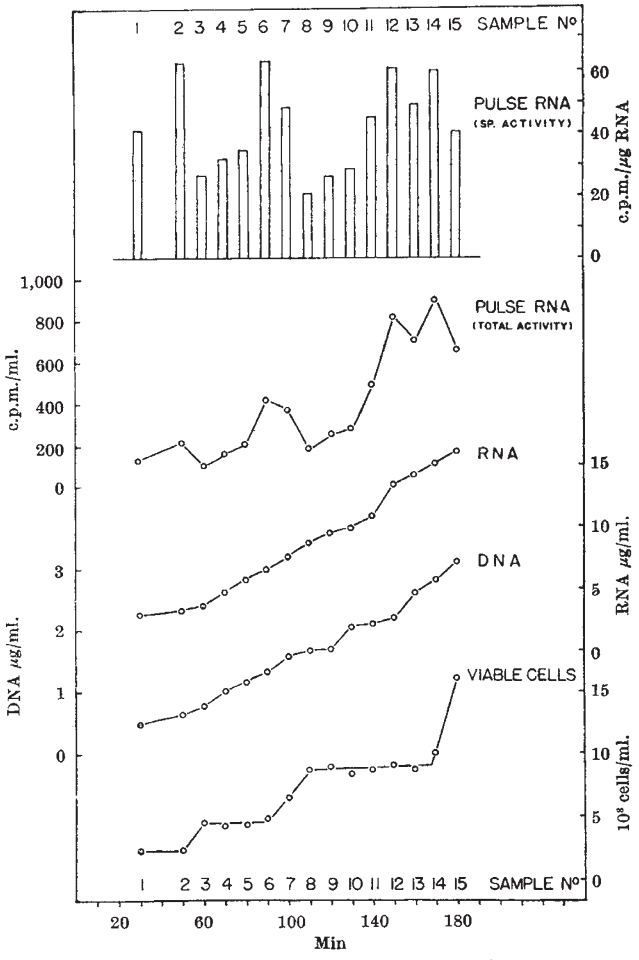

Fig. 2. Synchronous growth of *E. coli HfrH* (m^-, λ, 424), Exp. 2. The samples are numbered as in Table 2. The diagrams represent, in upward order: (*a*) viable counts; (*b*) synthesis of DNA; (*c*) synthesis of total RNA in the master culture; (*d*) total radioactivity of pulse-labelled RNA per ml. culture, computed from the values of specific activity and from the estimation of RNA content (orcinol) of the several samples; (*e*) specific activity of pulse-labelled RNA

Table 1. DISTRIBUTION OF RADIOACTIVITY AMONG NUCLEOTIDE CONSTITUENTS OF RNA, IN RELATION TO DNA REPLICATION, IN SUCCESSIVE STAGES OF SYNCHRONOUS GROWTH OF *E. coli HfrH* (EXP. 1)*

Sample No.	Min after filtration	DNA: increase as % of initial in each generation	RNA: total c.p.m. recovered (×10³)	Nucleotide constituents of RNA†						
				% of total radioactivity				Molar ratios		
				A	G	C	U	(A+G)/(C+U)	(A+U)/(G+C)	(A+C)/(G+U)
1	30	0	52·5							
2	40	0	84·2	24·0	27·7	25·6	22·7	1·07	0·88	0·98
3	50	0	164·2	24·4	25·3	26·3	24·0	0·99	0·94	1·03
4	60	12	190·8	23·4	26·1	25·8	24·7	0·98	0·93	0·97
5	70	44	423·4	*23·3*	*32·2*	*23·5*	*21·0*	1·25	0·80	0·88
6	80	52	292·5	22·9	29·4	25·1	22·6	1·10	0·83	0·92
7	90	80	423·5	*22·3*	*31·8*	*25·5*	*20·4*	1·18	0·75	0·92
8	100	100	218·0	24·4	28·3	23·3	24·0	1·11	0·94	0·91
Division										
9	110	8	122·4	23·1	26·5	26·0	24·4	0·98	0·90	0·96
10	120	46	506·6	*24·4*	*32·1*	*23·5*	*20·0*	1·30	0·80	0·92
11	130	52	259·0	22·9	29·7	24·7	22·7	1·11	0·84	0·91
12	140	78	443·2	*22·8*	*32·4*	*25·3*	*19·5*	1·23	0·73	0·93
13	150	90	256·3	24·8	25·7	25·0	24·5	1·02	0·97	0·99

* Compare Fig. 1 for other results of this experiment.
† About 1 mg of RNA was hydrolysed with 0·2 ml. of 0·5 N aqueous potassium hydroxide for 20 h at 37°. After separation by two-dimensional paper chromatography, the radioactivity of each (2′+3′)-nucleotide was measured in a gas flow counter. The figures express the distribution of phosphorus-32 incorporated into each nucleotide as per cent of total radioactivity, that is, of the summation of all counts given by the sample. The standard deviation ranged from 0·25 to 0·94. In sample No. 1 the counts were too low for calculation. The figures in italics denote the samples for which bursts of RNA synthesis were recorded (see Fig. 1). The three columns of molar ratios list the ratio of purines to pyrimidines, the dissymmetry ratio, and the ratio of 6-amino to 6-keto nucleotides, respectively. Compare Table 3 for the composition of bacterial DNA and RNA. Abbreviations: A, adenylic acid; G, guanylic acid; C, cytidylic acid; U, uridylic acid.

Table 2. DISTRIBUTION OF RADIOACTIVITY AMONG NUCLEOTIDE CONSTITUENTS OF RNA, IN RELATION TO DNA REPLICATION, IN SUCCESSIVE STAGES OF SYNCHRONOUS GROWTH OF *E. coli HfrH* (EXP. 2)*

Sample No.	Min after filtration	DNA: increase as % of initial in each generation	RNA: total c.p.m. recovered (×10³)	Nucleotide constituents of RNA						
				% of total radioactivity				Molar ratios		
				A	G	C	U	(A+G)/(C+U)	(A+U)/(G+C)	(A+C)/(G+U)
1	30	63	152·4	26·1	27·3	24·5	22·1	1·15	0·93	1·02
2	50	84	276·3	*26·7*	*31·3*	*23·2*	*18·8*	1·38	0·83	1·00
3	60	100	118·8	26·2	26·5	23·7	23·6	1·11	0·99	1·00
Division										
4	70	31	143·0	25·2	26·1	26·3	22·4	1·05	0·91	1·06
5	80	54	139·6	23·1	28·7	25·2	23·0	1·07	0·86	0·93
6	90	75	302·4	*26·2*	*29·4*	*22·7*	*21·7*	1·25	0·92	0·96
7	100	100	160·7	25·6	27·6	25·1	21·7	1·14	0·90	1·03
Division										
8	110	9	149·2	23·0	25·9	26·5	24·6	0·96	0·91	0·98
9	120	10	134·6	25·2	26·3	25·1	23·4	1·05	0·95	1·01
10	130	34	99·4	24·7	26·7	26·2	22·4	1·06	0·89	1·04
11	140	37	132·0	24·4	27·0	25·5	23·1	1·06	0·90	1·00
12	150	42	261·9	*24·6*	*30·1*	*24·0*	*21·3*	1·21	0·85	0·95
13	160	69	242·6	25·2	25·8	25·5	23·5	1·04	0·95	1·03
14	170	82	282·0	*24·4*	*30·7*	*23·4*	*21·4*	1·23	0·85	0·92
15	180	100	195·8	24·1	26·7	25·0	24·2	1·03	0·93	0·96
Division										

* See Fig. 2 for additional results and Table 1 for explanations.

15 pulsed samples are shown in Fig. 2. As concerns the synthesis of DNA, it exhibits a definite retardation at the outset and then proceeds in what would appear to be a stepwise manner (usually two steps in a growth cycle) interrupted by periods of almost no synthesis. It would have been possible to fit a straight line to most of the points indicating the DNA levels; but we believe that our observations represent meaningful variations.

During the initial lag of DNA formation new RNA molecules are apparently produced, in accordance with previous observations[8,9]. RNA is synthesized continually; but when the rates of synthesis are probed into at different points of the growth cycle, rhythmic fluctuations are seen. These are shown graphically in Figs. 1 and 2 in the form of the specific activity of the RNA. At the onset of the growth cycle the increase in the rates of synthesis of RNA, as measured by the uptake of phosphorus-32, was nearly linear; thereafter, the specific activities of pulse-labelled RNA alternated between high and low levels, two bursts being discernible during an average generation time of 70 min (Fig. 1). In another experiment, in which the generation time was somewhat shorter, only one peak was noticed in the first complete generation period under observation (sample No. 6 in Fig. 2). These bursts of RNA synthesis seem to be regularly spaced with respect to DNA replication, occurring generally in the strain under investigation here when DNA had increased by 40–45 per cent and 75–85 per cent. (Compare samples No. 5, 7, 10, 12 in Fig. 1 and No. 2, 6, 12 and 14 in Fig. 2.)

These alternations of the rates of RNA synthesis were found to be associated with remarkable changes in the apparent composition of the newly made RNA molecules. This information will be found in Tables 1 and 2 which list, in addition to figures on the percentage increase in DNA and the total counts recovered in the isolated RNA specimens, the distribution of radioactivity among the (2′ + 3′)-nucleotide components of each RNA sample. These values, it will be remembered, describe the RNA made in the course of a short period of exposure to phosphorus-32, by synchronously multiplying cells at different stages of their cellular history. For purposes of comparison it may be useful to consider these figures in conjunction with those listed in Table 3, in which data on the composition of the DNA and of various RNA fractions of the bacterial strain are presented. It will be noticed that the DNA contains, of course, equal molar quantities of purine and of pyrimidine nucleotides, whereas in the ribosomal and also in the total RNA the molar concentration of purine nucleotides surpasses that of pyrimidine nucleotides by 25 per cent or more.

If, with this in mind, the values compiled in Tables 1 and 2 are scrutinized, two principal groups of composition will emerge. One group, with a ratio of purines to pyrimidines of 1·2 or higher, comprises all those RNA specimens the synthesis of which coincided with the peaks of incor-

Table 3. COMPOSITION OF DNA AND RNA OF *E. coli HfrH**

Nucleic acid	Fraction	Moles per 100 g-atoms of nucleic acid phosphorus					Molar ratios		
		A	G	C	U	T	A + G/C + U(T)	A + U(T)/G + C	A + C/G + U(T)
DNA	Total	24·6	25·5	25·6		24·3	1·00	0·96	1·01
RNA	Total (Exp. 1)	23·5	31·9	24·5	20·1		1·24	0·77	0·92
	Total (Exp. 2)	23·5	31·8	25·6	19·1		1·24	0·74	0·96
	Ribosomal, 16S	25·7	31·4	21·5	21·4		1·33	0·89	0·89
	Ribosomal, 23S	26·0	31·6	21·8	20·6		1·36	0·87	0·92
	Transfer, 4S	19·6	31·8	30·2	18·4		1·06	0·61	0·99

* T denotes thymidylic acid; other abbreviations as in Table 1. The figures for total RNA represent the averages of the spectrophotometric analyses of the nucleotide composition of the 13 RNA samples collected in Exp. 1 (Table 1) and of the 15 RNA samples of Exp. 2 (Table 2). The specimens of ribosomal and transfer RNA were separated by means of a linear gradient of sodium chloride on a column of methylated serum albumin-kieselguhr (ref. 10).

poration of phosphorus-32, that is, samples No. 5, 7, 10, 12 in Table 1 and Fig. 1; samples No. 2, 6, 12, 14 in Table 2 and Fig. 2. These specimens are altogether quite similar in composition—if one were to judge from the results of the distribution of phosphorus-32 in their constituent nucleotides—to that of total or ribosomal RNA. The base-pairing characteristic of DNA is entirely absent. Guanylic acid is the preponderant nucleotide, with the ratio of guanylic to cytidylic acids 1·3 or higher in nearly all instances.

The other group is less homogeneous in appearance, though many specimens share the property of exhibiting molar equivalence with respect to at least one of the two base-pairs: the ratio of adenylic to uridylic acids is near one in samples No. 6, 8, 11 in Table 1 and in samples No. 5 and 15 in Table 2. Similarly, guanylic and cytidylic acids are nearly equimolecular in samples No. 4 and 9 in Table 1, and in samples No. 4, 8, 10 and 13 in Table 2. In a recent paper from this laboratory it was pointed out that the occurrence of only partial base-pairing seems to hold true of most messenger-RNA preparations described in the literature[11].

There are several samples of pulse-labelled RNA in our list that could be regarded as quite similar to *E. coli* DNA in base proportions, particularly samples No. 3 and 13 in Table 1; the examples in Table 2 are less convincing, but samples No. 8, 13, and 15 could be cited. If another characteristic of DNA is adduced, namely, the equimolarity of purines and pyrimidines, samples No. 3, 4, 9, 13 in Table 1, and No. 8, 13, 15 in Table 2 may be said to resemble DNA in this respect. One receives the impression that, on the whole, the pulse-labelled RNA specimens produced at the beginning or at the end of a growth cycle, when DNA synthesis has not yet begun or has tapered off, are more similar to DNA in composition than those made at other periods.

There are other possible correlations, though the experimental material is not large enough for certainty. When DNA has increased by about 10 per cent, the distribution of the label in the RNA made at this stage is very similar in both experiments (compare samples No. 4 and 9 in Table 1, and No. 9 in Table 2). Another RNA composition appears to occur when DNA has increased by about one-third (No. 4 and 10 in Table 2); and yet another, with a high content of guanylic acid, when about one-half of the DNA has been replicated (No. 6 and 11 in Table 1 and No. 5 in Table 2). The ratio of 6-amino to 6-keto nucleotides[12] was near one in many of the specimens, with a mean of 0·97 for all 27 specimens.

The application of a pulse to a bacterial culture or to an organism raises many problems of interpretation with some of which a recent paper from this laboratory[11] has attempted to deal. The use of a narrowly spaced series of pulses administered to a synchronized cell population no doubt offers many advantages; but it remains that this grid, be it ever so fine, is imposed on, or made to slice through, the infinitely complex pulsations of a growing cell. We have probed along the time axis of a dynamic system—a synchronously dividing bacterial culture—and have obtained a set of values. How should they be interpreted and what is their bearing on present-day hypotheses concerning the transfer of biological information?

At the outset, one would wish to ask whether the analytical methods used in this article, as in all others dealing with this subject, in order to reveal the composition of the pulse-labelled RNA specimens, are really adequate; that is, whether regardless of the presumably varying specific activities of the nucleoside 5′-phosphates serving as the precursors of the polymers, the transfer of the phosphate groups to the (2′ + 3′)-positions, as it takes place in the course of the cleavage of RNA by alkali, does give rise to a randomization. This has been questioned repeatedly[11,13]. It may, however, be conceded that the technique is least objectionable when applied, as here, to the comparison of a series of samples produced under conditions having, ostensibly, only one variable, namely, time. Another point that should be emphasized is that, had a longer interval between samples than 10 min been chosen, a much simpler, but doubtlessly erroneous, picture could have emerged: the rapidly labelled RNA might have been in all cases only of the ribosomal type or only of the DNA type or mixed. It is the last-mentioned type that has, in fact, been encountered in investigations with non-synchronous bacterial populations[11,14–17]. If, on the other hand, an even finer net of pulse intervals could have been imposed—but this would be very difficult technically—it is quite conceivable that at one time or another a pulse-labelled RNA species of the composition of transfer RNA (compare Table 3) would have been found. It is quite clear that the examination of the composition or of other chemical and physical properties of an RNA specimen cannot be sufficient for a decision whether any of the many varieties of rapidly formed RNA observed in the course of a growth cycle conform with the concept[18] of messenger-RNA. There is, however, little question that the observation of a characteristic wave pattern in the synthesis and composition of RNA—a pattern apparently related to the extent of replication of DNA—is of some interest.

The alternations observed in this work possibly reflect a progressive transcription of the nucleotide sequence of the bacterial DNA in the form of a series of different pulse-labelled RNA molecules. Whether these, in turn, mirror the composition of individual cistrons in the DNA remains undetermined. The finding that the RNA newly made in the various cultures at identical levels of DNA replication is very similar in composition, but different from RNA produced at other levels of DNA increase, is consistent with the concept of an orderly transcription of the genome. The observation that the pulse-labelled specimens of RNA produced in different phases of synchronism vary in their composition is also in agreement with several previous investigations in which entirely different techniques (elution patterns[19], hybrid formation[20]) were used. Recent attempts to demonstrate the stepwise production of certain enzymes in synchronously dividing cells should also be mentioned in this context[21,22].

The work recorded here was carried out with one donor strain of *E. coli* (*HfrH*) with its given polarity of chromosome replication[1]. The outstanding shifts in the nucleotide distribution in the newly made RNA towards that

expected for total or ribosomal RNA appeared referable to the periods when approximately 40 and 80 per cent of the DNA had been replicated. Does this now mean that at these times those nucleotide tracts on the DNA chain become available that are responsible for the formation of ribosomal RNA, and is this spacing characteristic of the particular strain of *E. coli* ? These and related questions, for example about the intervals during which the DNA-like RNA is produced, cannot yet be answered. But if the proposition is correct that different types of RNA are synthesized sequentially in a synchronized population, other donor strains of *E. coli Hfr* may exhibit another characteristic spacing. This problem is being investigated at present with strains having different chromosomal polarities.

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ION-EXCHANGE CHROMATOGRAPHY OF AMINO-ACIDS: IMPROVEMENTS IN THE SINGLE COLUMN SYSTEM

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PIEZ and Morris[1] have described a system for the quantitative analyses of amino-acids using a single column of ion-exchange resin, and instruments based on this principle are now commercially available (Phoenix Precision Instrument Co., Philadelphia, Penn.; Technicon Instrument Co., Ltd., Hanworth Lane, Chertsey, Surrey). With these the complete analysis and regeneration cycle take about 24 h. We wish to describe two sets of modifications in experimental conditions, the first giving a complete chromatogram in 15 h, the second in 5·5 h with no loss of resolution for the protein amino-acids.

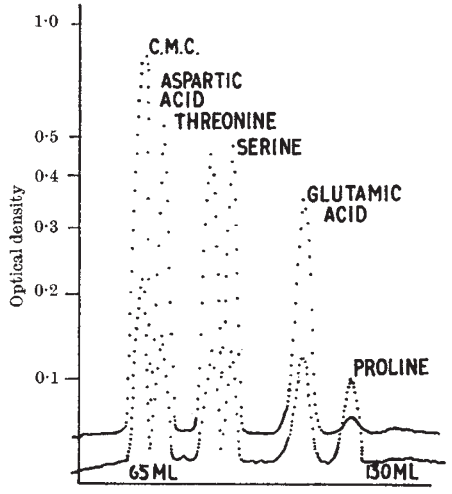

Fig. 1. Part of 15-h chromatogram. 0·2 μmoles each amino-acid, 0·5 μmoles *S*-carboxymethyl-cysteine (CMC)

(1) 15-*h chromatogram.* This may be effected as follows: (i) The flow of buffer to the column is increased to 47 ml./h. (ii) After regeneration with 0·2 N sodium hydroxide the column is equilibrated with 0·05 M sodium citrate (0·2 N with respect to Na^+) *p*H 2·9 in 10 per cent aqueous methanol. Methanol is required to maintain the resolution of threonine and serine and its use gives better separation of these amino-acids than is normally achieved with the 24 h chromatogram. 10 per cent methanol in the corresponding Varigrad buffer further improves threonine–serine resolution, but proline then tends to run into glutamic acid and the separation of cystine and methionine is impaired. (iii) Two additional chambers containing *p*H 5·0 sodium citrate are incorporated in the Varigrad to provide a safety margin for the overnight run. The other conditions are as for the 24-h chromatogram.

Temperature programming can also be used to improve threonine–serine resolution, but we have found this less convenient. Fig. 1 shows part of a typical chromatogram from carboxymethyl-cysteine to proline indicating the resolution obtained. The reproducibility of the system is unaffected by these changes.

(2) 5·5-*h chromatogram.* In investigations of protein structure, the rate of quantitative amino-acid analysis is often a limiting factor. Recently, details of a method

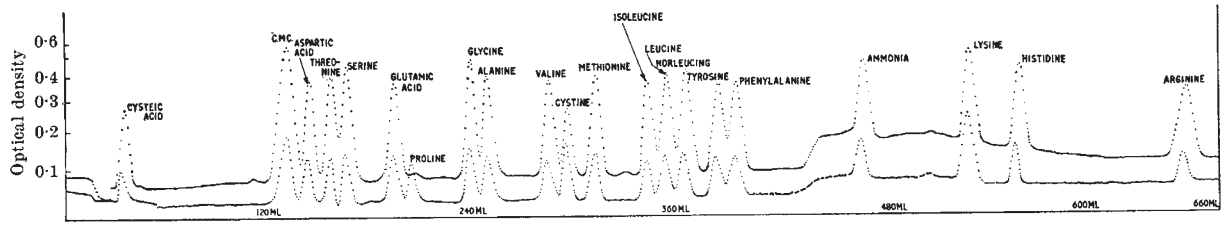

Fig. 2. Typical 5·5-h chromatogram (loading and abbreviation as for Fig. 1)