A STUDY OF THE RIBONUCLEIC ACID OF NORMAL AND CHLOROMYCETIN-INHIBITED BACTERIA BY ZONE ELECTROPHORESIS*

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Relatively little is known of the chemical state of ribonucleic acid within bacterial cells[1] although such information is of considerable biochemical interest. That much of the bacterial ribonucleic acid (RNA) is in the form of nucleoproteins was indicated by studies with the ultracentrifuge in which extracts of bacteria of a number of species were shown to have most of their RNA in the form of a few relatively monodisperse collections of large particles containing both RNA and protein[2]. Electrophoresis in the Tiselius apparatus of extracts of various cells shows little RNA in the free form[3,4]. The situation with respect to bacterial desoxyribonucleic acid (DNA) is less clear, and both free and bound DNA have been demonstrated in bacterial extracts[5].

In order to examine further the question of the nature of the bacterial nucleic acids, extracts obtained from bacteria in various phases of growth, as well as from cells with altered protein metabolism, were subjected to zone electrophoresis. Protein metabolism was affected by growth of the cells in the presence of chloromycetin, which totally inhibits net protein synthesis[6,7].

MATERIALS AND METHODS

E. coli strain B was grown on a salts plus glycerol medium[8] with aeration at 37° C. When ^{32}P incorporation was desired a low-phosphate medium was used[9]. *B. megatherium* was grown under the same conditions in 2% peptone. The bacteria were grown to a density of about $6 \cdot 10^8$/ml, were harvested and washed once with 0.001 *M* PO_4, pH 7.0, and an extract was prepared in 0.001 *M* phosphate to give a nucleic acid content of about 1.5 mg/ml.

Extracts were made in several ways. Bacteria were exposed for 5 minutes in the Raytheon 9KC Sonic Oscillator, or centrifuged cells were ground with 2.5 times their wet weight of alumina[10], or were shaken for 10 minutes with 0.6 g of 0.1 to 0.15 mm glass beads per ml cell suspension in a MICKLE disintegrator[11]. In addition, *B. megatherium* was lysed by exposure to 0.5 mg/ml lysozyme for 15 minutes at 25° C.

Starch electrophoresis was performed as described by PAIGEN[12]. Usually the trough contained 40 g washed starch mixed with 50 ml pH 7.6 tris(hydroxymethyl)aminomethane buffer (tris), ionic strength 0.015. A transverse hole was cut in the starch about 4 cm from the cathode end and this was filled with 1.5 ml of extract mixed with 1.2 g starch. The runs were made at 4° C, with a potential gradient of 5.5 volts/cm for approximately 15 hours. At the completion of the run the starch was blotted by applying a strip of Whatman 3 mm paper edgewise. Approximate positions of nucleic acids and proteins were determined by observation of the strip under ultraviolet light and staining with aqueous bromphenol blue-$HgCl_2$[13], respectively. The starch was then cut into 60

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References p. 172/173.

5 mm sections, each section was eluted by mixing with 5 ml H_2O and after allowing the starch to settle the eluates were used for subsequent analyses. Similar analyses were also carried out on aliquots of the bacterial cells and the extracts.

Total nucleic acids (NA) were estimated approximately by readings of optical density at 260 mμ in the Beckman spectrophotometer. (One μg NA/ml was assumed to have an optical density of 0.03.) The amounts of protein, RNA, and DNA were estimated by the Folin[14], orcinol[15], and indole[16] methods, respectively. For these determinations starch was removed from the eluates by centrifugation since it was found to interfere. For the determination of radioactive phosphorus incorporated into nucleic acids, samples were precipitated with 5 % trichloroacetic acid (TCA) after addition of 1 mg ovalbumin. The precipitates were then washed and extracted[15] and radioactivity was determined by counting dry samples using an end window Geiger-Müller counting tube. The enzyme β-galactosidase was determined by a modification of the procedure using *o*-nitrophenol-β-D-galactoside[8], and D-serine deaminase was determined by pyruvate production with pyridoxal phosphate added as cofactor[17].

Analyses for bases of RNA were made chromatographically, essentially by the method of HERSHEY *et al.*[18]. Hydrolysis of RNA was accomplished by heating for 1 hour at 100° in 1 *M* HCl in sealed tubes, and the free bases were separated by ascending chromatography on Whatman No. 1 paper using as eluent 170 ml *iso*propanol, 41 ml conc. HCl and water to make 250 ml. Chromatography in the second dimension was not required.

RNA prepared by a mild treatment from yeast was obtained from Dr. F. W. ALLEN[19] and from tobacco mosaic virus from Dr. H. FRAENKEL-CONRAT. High molecular weight DNA prepared from thymus by the method of SIGNER AND SCHWANDER[20] was obtained from Mr. D. E. HOARD. Chloromycetin was a gift from Parke, Davis and Company.

EXPERIMENTS ON NORMAL CELLS

Results of analysis of a starch electrophoresis run on an extract of alumina-ground cells of *E. coli* in the phase of logarithmic growth are shown in Fig. 1a. Amounts of substances are plotted against distance traveled toward the anode from the origin. Protein was found to be distributed over a wide region extending from behind the origin to the nucleic acid front, being maximal at the main nucleic acid band. The curve for RNA shows one major band. In front of this was a secondary nucleic acid peak which contained a mixture of RNA and DNA, with the DNA somewhat ahead. However, as later experiments showed, the position of DNA was largely dependent on the method of breaking the bacteria. Essentially all of the ultraviolet-absorbing material was insoluble in 5% TCA. Such an experiment demonstrates the relative simplicity of the nucleic acid pattern, with only two major bands present, and the RNA accompanied by protein in both cases.

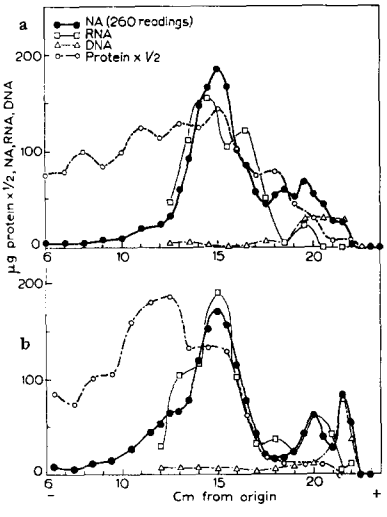

Fig. 1a. Starch electrophoresis patterns of an extract prepared by alumina grinding of *E. coli* B. Electrophoresis was performed in pH 7.6 tris buffer, ionic strength 0.015 at 4°. The μg of materials found at various positions on the trough are shown on the ordinate. The amount of protein is divided by two in order to show it readily on the figure. Fig. 1b. Starch electrophoresis patterns after sonic treatment of the extract used to obtain Fig. 1a.

Three major questions arise. First, to what extent does the degradation of cellular materials during extraction and electrophoresis determine the observed pattern? Second, what are some of the chemical and physical properties of the nucleic acids in two bands? In particular there is the question of whether the nucleic acids are

References p. 172/173.

free or bound to protein, either loosely or in the form of a nucleoprotein with strong specific linkages. Third, does a precursor–product relationship exist between the two RNA-containing bands?

Validity of the pattern

Autolysis, or other degradative changes occurring during electrophoresis did not seem to be a serious factor in determining the pattern. Storage of the extracts for 7 hours at 0° C prior to electrophoresis did not alter the patterns obtained. Material isolated from each of the two nucleic acid bands and resubjected to electrophoresis yielded single bands with no change in mobility. In addition, examination in the ultracentrifuge (described in detail in the following section) showed that the principal components present in fresh extracts were also present following electrophoresis.

In contrast to the stability and reproducibility of the pattern obtained with a single extract considerable variation was found between extracts prepared by different procedures. The more vigorous methods of cell disruption caused an increase in the quantity of material present in the forward RNA band, and a sharpening of the DNA band accompanied by a rise in mobility. These effects are illustrated in Fig. 1b which gives the results obtained when the extract of alumina-ground cells used to obtain Fig. 1a was subjected to sonic treatment. Since identical results were obtained by sonic treatment of cells, the changes observed were not because of any difference in the nature of the materials extracted by the two procedures. Rather they were artifacts produced by exposure to sonic oscillation. There was no detectable adsorption by alumina of the materials present in sonic extracts. When the cells were broken by grinding with alumina the results were dependent upon the ratio of alumina to cells as well as the individual grinding technique employed. Treatment of the cells in a Mickle disintegrator for 10 minutes produced extracts similar to those obtained by grinding with 3 g of alumina per g of wet cells.

The increase in material in the forward RNA band, and the sharpening and increase in mobility of the DNA band were particularly noticeable when cells or previously prepared alumina extracts were subjected to sonic treatment. The changes observed in the DNA band are probably the result of cleavage of the molecules upon exposure to sonic oscillation[2]. A similar effect was observed with thymus DNA which in the native state gave a broad, slow moving zone, and which was converted by sonic treatment to a material giving a very narrow rapidly moving band (Fig. 2a). It seems likely that this change is a consequence of the mechanical holding back of the large native molecules of DNA, but not the broken pieces, by the starch[21], since sonic treatment was not found to change the mobility of DNA in free electrophoresis[22].

Sonic treatment for 1.5, 5 or 15 minutes yielded essentially the same pattern, indicating that the increase in the RNA component was at the expense of a limited fraction of the major band.

Since the use of more vigorous methods for cell breakage increased the size of the faster moving RNA band, it appeared possible that this component might be entirely a product of the mechanical rupture of the cells. In order to check this possibility an extract of cells of *B. megatherium* broken by treatment with lysozyme (0.5 mg/ml for

References p. 172/173.

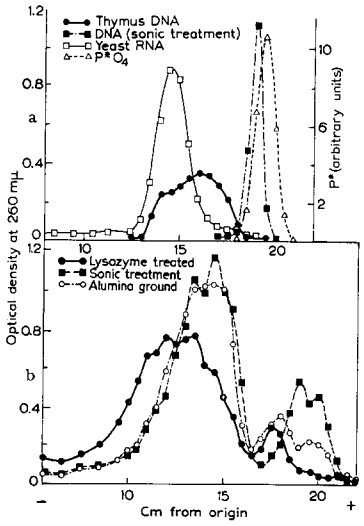

Fig. 2a. Starch electrophoresis patterns of thymus DNA, sonically treated thymus DNA, mixed sonically treated and undegraded RNA, and $^{32}PO_4$.

Fig. 2b. Starch electrophoresis pattern of nucleic acid of *B. megatherium* extracts prepared by three methods.

15 minutes at 25°C) was compared with extracts of the same culture prepared by alumina grinding and by sonic treatment (Fig. 2b). The extract produced by lysozyme treatment showed a somewhat broader major band than the others, which might be attributed to the much higher viscosity of this extract. In addition it showed a definite fast moving RNA-protein band. Grinding with alumina produced another, more rapidly moving component which was essentially all DNA. Sonic treatment of the bacteria increased both fast moving bands and also increased the mobility of the more rapid RNA band somewhat. Less protein was found ahead of the major band in this last case than in the first two. It was therefore concluded that these bacterial cells also contain a stable major RNA component plus a minor RNA component having a relatively higher mobility. Harsh methods of extraction appear to split the linkage between this latter RNA component and protein, and to effect an increase in the mobility of DNA.

Physical and chemical characteristics

Material from a fraction near the apex of the major nucleic acid band was eluted with tris buffer and was observed in a Spinco analytical ultracentrifuge equipped with ultraviolet absorption optics. The major portion of the nucleic acid in this fraction was found to be roughly equally divided between materials with sedimentation coefficients of approximately 32 and 54 *S*, in fair agreement with the sedimentation behavior of the particles found in crude extracts (29 and 40 *S*)[2]. Both components had a high extinction at 265 mμ. Thus the principal RNA electrophoretic band appears to be composed of the same material as the two major rapidly moving peaks previously observed in the ultracentrifuge, as would be anticipated since a majority of the bacterial RNA was reported to be in these components[2].

The more rapidly moving electrophoretic band, however, appeared to be composed of relatively low molecular weight material. Measurement was made of the percentage of the RNA of each fraction that failed to sediment in an angle centrifuge at 100,000 *g* after 160 minutes at 0° C. The fractions were prepared from cells broken by grinding with alumina. While only 15% of the RNA of the major band remained in the supernatant, over 70% of the RNA of the more rapidly moving band failed to sediment. The material in the minor, fast moving band was also examined in the analytical ultracentrifuge using a synthetic boundary cell and ultraviolet absorption optics[23]. No sedimentation of the boundary was observed after 30 minutes at 259,000 *g*, indicating an average molecular weight of less than 5,000.

Chemically the two bands consist of nucleic acid and protein, with ratios of 1.6 mg protein per mg nucleic acid for the major band and 1.0 mg per mg for the minor, faster band. Determinations were also made of the base ratios of the RNA present. Values for the base composition of the major band were consistent in three separate

References p. 172/173.

experiments, but the values for the minor band varied considerably, apparently depending on which portion of the band was assayed (Table I). It would appear, therefore, that the latter band consists of a heterogeneous material of relatively small size which is easily separable from the variable amounts of protein to which it is bound.

TABLE I

BASES IN RNA BANDS OF *E. coli*

Expt.	*Band*	*Guanine*	*Adenine*	*Cytosine*	*Uracil*
I	Major	0.32	0.26	0.22	0.20
II	Major	0.33	0.26	0.21	0.20
III	Major	0.33	0.24	0.22	0.21
I	Minor	0.31	0.34	0.28	0.18
II	Minor	0.35	0.22	0.26	0.17
IIIA	Minor	0.34	0.22	0.27	0.17
IIIB	Minor	0.38	0.38	0.13	0.11

IIIA is from the slower moving half of the minor band; IIIB is from more rapidly moving half. The numbers represent the ratio of the amount of each base in moles to the sum of the moles of all four bases.

Nature of the NA to protein linkage

Of some importance is the question whether the bacterial nucleic acids exist free, or whether they are bound to protein, either as defined nucleoproteins, or in the form of protein nucleates[1]. It appears from the above results that there are essentially no nucleic acids free of protein in extracts prepared in a mild way. It is not evident whether the nucleic acids are bound to this protein by a specific linkage (nucleoproteins)[24,25,26] or are in strong but non-specific salt linkages (protein nucleates)[27]. In order to estimate the firmness of binding, the ability of added high molecular weight RNA to displace the bacterial RNA from its linkage with protein was tested.

As a necessary preliminary some experiments with free RNA were performed in order to provide a comparison with the nucleic acids present in extracts. RNA isolated from yeast or tobacco mosaic virus gave one band considerably sharper than the RNA bands observed with bacterial extracts. Sonic treatment (5 minutes) did not alter the shape of the isolated RNA bands or their rate of motion on electrophoresis, nor did mixtures of the original and sonically treated RNA show two bands (Fig. 2a). Radioactive orthophosphate gave a similarly sharp band (90% of the applied material was found in 1.5 cm).

To determine whether bacterial nucleic acids could be displaced from protein by added RNA, it was necessary to distinguish between RNA of bacterial origin and added RNA. This was accomplished by labeling the bacterial RNA with ^{32}P by permitting growth to occur in a medium containing radioactive phosphate ($2 \cdot 10^4$ counts ^{32}P/ml). An extract of radioactive *E. coli* was made by alumina grinding, and a portion of the extract was incubated with yeast RNA for 10 minutes at 25°C before electrophoresis. Free yeast RNA, extract alone, and yeast RNA plus extract were run at the same time in three troughs in the starch electrophoresis apparatus. Measurements were made of optical density and radioactive nucleic acids in the eluted samples (Fig. 3a, 3b). It is seen that the distribution of radioactivity was not influenced by the addition of RNA, and that the specific activity of the major band was unchanged. The minor band was

References p. 172/173.

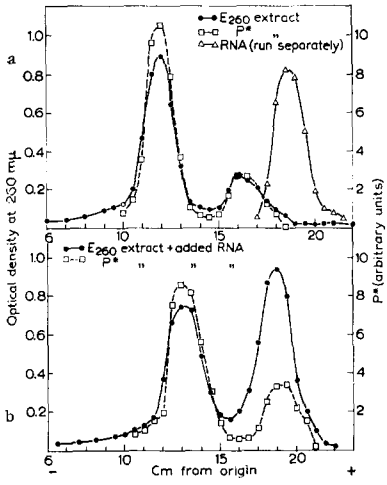

Fig. 3a. Starch electrophoresis patterns of nucleic acids and radioactivity of alumina-ground *E. coli* B. Also shown is a pattern of yeast RNA, run separately.

Fig. 3b. Starch electrophoresis pattern of a mixture of the two materials used to obtain Fig. 3a.

found with the added RNA, as shown by the P* determinations. At most, 5% of the radioactivity shifted from the major band to the minor one. The added RNA appeared as a band in the same position as the original faster moving extract band, a position somewhat behind that where free RNA was found when run alone, but where free bacterial RNA was found after sonic treatment. Protein did not move into the area occupied by the added RNA. Over 90% of the radioactivity applied to the starch was recovered.

A similar experiment was performed with radioactive *B. megatherium*, using an extract prepared by lysozyme treatment. Part of the extract was incubated for 3 hours at 4°C with yeast RNA before electrophoresis. As with *E. coli*, the presence of the added RNA caused little, perhaps 5%, of the radioactive bacterial RNA to move into the forward peak. Again, the free RNA showed a lower mobility in the presence of extract than when run alone, but also a small amount was found in the area of the electrophoresis diagram occupied by the major bacterial component.

These experiments clearly demonstrate that the bacterial RNA in the major electrophoretic peak is not readily displaced by the added yeast RNA. Unfortunately no conclusions can be drawn from these experiments regarding the firmness or specificity of binding of RNA of the minor component to protein.

Metabolic relationship of the two RNA bands

Since the nucleic acids in the two peaks seem to be of a different nature, chemically and physically, the possibility should be considered that one serves as a precursor of the other. To test this hypothesis growing *E. coli* were exposed to radioactive orthophosphate for 10 minutes, and an extract was prepared by sonic treatment. After starch electrophoresis, determinations of both total nucleic acids and radioactive nucleic acids were made. No significant difference in specific activity was found between any parts of the two bands (Table II). It can be calculated that if one band were to act as

TABLE II

SPECIFIC ACTIVITIES OF PHOSPHORUS INTRODUCED IN 10 MINUTES INTO RNA

Fraction, cm	*O.D.*$_{260}$	*Sp. Act.**	*Fraction, cm*	*O.D.*$_{260}$	*Sp. Act.**
11	0.14	108	18	0.10	81
12	0.27	80	19	0.21	100
13	0.54	65	19.5	0.24	82
14	0.79	70	20	0.20	81
15	0.39	76	21	0.12	82
17	0.06	86	22	0.08	80

* Ratio of counts per minute to optical density at 260 mμ (O.D.$_{260}$) in arbitrary units.

References p. 172/173.

a precursor of the other, and both increase in proportion to cell mass, at least a twofold difference between the specific activities of the two should exist for at least 1 hour. Therefore, a direct precursor-product relation seems very unlikely.

Dependence of electrophoresis pattern of age of cells

For comparison with bacteria grown to the late logarithmic phase ($6 \cdot 10^8$/ml), extracts of older and younger cells were prepared. An extract of resting bacteria ($3 \cdot 10^9$/ml) prepared by 10 minutes treatment with the Mickle disintegrator showed an electrophoresis pattern with a single broad nucleic acid zone over the entire region in which nucleic acids were found from the control culture. Younger bacteria ($2 \cdot 10^8$/ml) gave a pattern similar to the control, although the two bands were somewhat better separated.

EXPERIMENTS ON CHLOROMYCETIN-TREATED CELLS

Results of electrophoresis

Extracts of cells exposed to chloromycetin, which inhibits the synthesis of protein but not of nucleic acids, contain a new electrophoretic component. Fig. 4a shows the results obtained with a culture of *E. coli* which had been exposed to 10 μg/ml of chloromycetin for 2 hours. In the presence of chloromycetin the amount of bacterial protein remained unchanged and the nucleic acid content increased 68%. To distinguish the newly formed nucleic acids, radioactive orthophosphate was introduced 2 minutes subsequent to the chloromycetin.

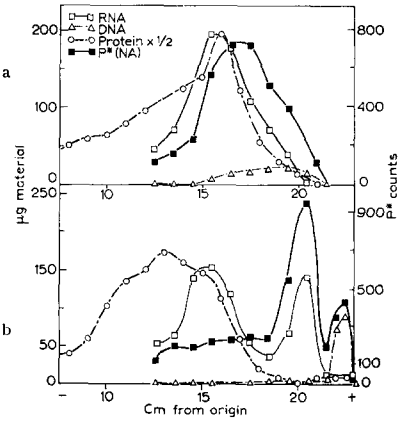

Fig. 4a. Starch electrophoresis pattern of an extract prepared by alumina grinding of *E. coli* B exposed to chloromycetin.
Fig. 4b. Starch electrophoresis patterns after sonic treatment of the extract used to obtain Fig. 4a.

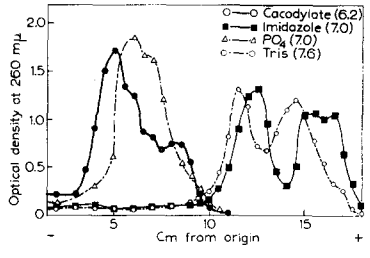

Fig. 5. Starch electrophoresis patterns of an extract prepared by alumina grinding of *E. coli* B exposed to chloromycetin. Run in various buffers.

A comparison of Fig. 4a with Fig. 1a, which was obtained with an equivalent extract of cells unexposed to chloromycetin, shows that the newly formed nucleic acids appeared just ahead of the original major band. In some electrophoresis runs the two bands were clearly differentiated, *e.g.*, Fig. 5. Protein moved forward with the new nucleic acid, from an original position around 7 cm.

Sonic treatment of the alumina-ground extract used to obtain Fig. 4a and subsequent starch electrophoresis gave the results shown in Fig. 4b. The results of a similar treatment of the control extract are shown in Fig. 1b. The major RNA band, which

References p. 172/173.

persists after exposure to chloromycetin, was unaffected by sonic treatment. In contrast, the newly synthesized RNA was released from its linkage to protein and exhibited an increase in mobility, in a manner analogous to that of the minor component of normal cells. With both the normal and chloromycetin-exposed cells the protein released from RNA and DNA by sonic treatment appeared in a region of lower mobility (between 10 and 14 cm).

The results of these experiments indicate that even under conditions where the cells show no net synthesis of protein the RNA produced by *E. coli* does not exist as free nucleic acid but is associated with protein. It appears that pre-existing protein is utilized for this purpose. This new material, however, differs from the major RNA-protein of normal cells in that it has a somewhat higher mobility and is labile to sonic treatment. In these respects it is similar to the minor RNA-protein of normal cells.

Unfortunately, since the mobilities of the new RNA component and that of free RNA were very similar, it was not possible to perform successfully an experiment, analogous to that done with normal extracts, to determine the firmness of the nucleic acid to protein linkage in the new component.

Duration of exposure

The results described above proved to be independent of the duration of exposure to chloromycetin. An experiment was performed in which rapidly growing *E. coli* (at $7 \cdot 10^8$/ml) were exposed to 10 μg/ml chloromycetin for 1, 2, or 3 hours. Protein synthesis was almost completely blocked during exposure to chloromycetin, while nucleic acid synthesis continued for about 1 to 2 hours and then became very slow. Extracts of these bacteria made by sonic oscillation were subjected to starch electrophoresis, and the patterns of nucleic acid distribution obtained from chloromycetin-grown cells were similar to that shown in Fig. 4b for all times of exposure to the antibiotic.

Results of centrifugation

Bacterial extracts show a typical pattern in the ultracentrifuge characterized by peaks with sedimentation coefficients of 40 *S*, 29 *S* and 5 *S*[2]. A comparison was therefore made of ultracentrifugal patterns of extracts of normal *E. coli* as compared with *E. coli* exposed to 10 μg/ml chloromycetin for 2 hours. Extracts were made by either grinding with alumina or sonic treatment. The alumina and sonic extracts gave identical patterns except for the disappearance of the DNA peak following sonic treatment. The latter effect has been noted previously[2]. The principal effect of exposure to chloromycetin was the complete disappearance of the 29 *S* peak (Fig. 6).

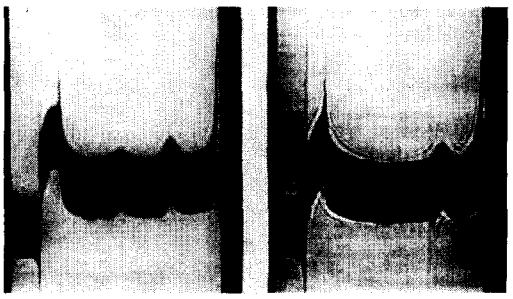

Fig. 6. Ultracentrifuge patterns of extracts prepared by alumina grinding of *E. coli* B. (a) control bacteria, (b) bacteria exposed to chloromycetin.

Since no new peak was detected, the newly formed nucleic acid must be either large (40 *S*) or of small size (5 *S* or less). To determine its location, an extract of *E. coli* exposed to chloromycetin and radiophosphate, and prepared by grinding with alumina, was centrifuged to sediment the 40 *S* particles (an average field of 100,000 *g* for

References p. 172/173.

160 minutes at 0°C). Analyses made on the original extract, supernatant, and resuspended precipitate are shown in Table III. It is seen that while most of the RNA was sedimented, most of the radioactive nucleic acids remained in the supernatant. Since in this experiment more than 80% of the NA formed was actually RNA, it appears that the newly formed RNA was either of small size, or was easily disrupted upon extraction. Further, since the radioactive RNA was precipitated by various concentrations of ethanol or $(NH_4)_2SO_4$ to approximately the same extent as the original RNA, it cannot be simply a collection of very small fragments.

TABLE III

FRACTIONATION BY CENTRIFUGATION OF AN EXTRACT OF CHLOROMYCETIN-TREATED *E. coli*

Fraction	*Protein*	*% of material**		
		DNA	*RNA*	*NA***
Extract	100	100	100	100
Supernatant	56	73***	38	72
Precipitate	57	12	74	34

* Supernatant plus precipitate total more than 100% owing to contamination of the latter by the former.

** Radioactive NA made in the presence of chloromycetin.

*** Some loss of material from these samples.

GENERAL OBSERVATIONS

Patterns obtained with various buffers or at different pH

The electrophoretic pattern obtained depended considerably on the buffer employed. In Fig. 5 are shown patterns obtained in different buffers, all from a single extract made by alumina-grinding *E. coli* exposed to chloromycetin. The negatively charged buffers cacodylate or phosphate (and also succinate, not shown) gave almost a single band in contrast to the cationic buffers imidazole or tris which caused two bands to appear. These bands all contained protein. The separation of the two bands was variable from one extract to another (compare extreme cases in Fig. 4a and 5).

Variations of pH showed less marked effects than did variations in the type of buffer employed. Similar results were obtained at pH 7 or 8, but at pH 6 two peaks, incompletely separated, appeared in phosphate buffer.

Location of specific proteins

The broad zone of protein observed in these starch electrophoresis patterns could result from a diversity of proteins with different mobilities, or could indicate that the method does not give good resolution with proteins. The latter possibility would seem unlikely since starch electrophoresis has been used very effectively in the purification of β-galactosidase[28] and to distinguish strains of tobacco mosaic virus[12]. However, to test sharpness of resolution under the present conditions, ability of the method to separate distinct enzymes was tested. An extract of *E. coli*, grown to contain D-serine deaminase and β-galactosidase, was prepared with the Mickle disintegrator and run in tris buffer, pH 7.0. The protein extended over at least a 20 cm range, in which β-galactosidase was almost all located at 8.0 to 8.5 cm, and D-serine deaminase was at 6 cm (with poor recovery of activity) while the main NA band was at about 12 cm. Single proteins thus appear in distinct locations.

Recoveries of materials

Grinding with 2.5 or 3.0 weights of alumina per packed wet weight of *E. coli* brought approximately 75% of the protein, the 260 mμ light-absorbing material, or ^{32}P radioactivity into the extract. Alumina did not adsorb appreciable amounts of nucleic acids or protein under the conditions used for grinding, as indicated by the failure of alumina to adsorb material from sonic extracts. Sonic treatment released over 90% of the cell contents into the extract.

Total recoveries from starch electrophoresis experiments were approximately 90% of the nucleic acids and 70% of the protein (about 2 and 12 mg put into the starch respectively). No

References p. 172/173.

attempt was made to recover material which moved more than 3 cm toward the cathode, so that an indeterminate fraction of the protein was lost because it had too low a mobility to overcome electroosmotic flow. Starch mixed with an extract showed no tendency to absorb protein or nucleic acids.

DISCUSSION

The present results using starch electrophoresis confirm and extend earlier experiments using the ultracentrifuge which demonstrated that bacterial RNA exists in at least two readily distinguishable forms. Most of the RNA is present as large ribonucleoprotein particles, but some is present in a relatively low molecular weight form.

The large nucleoprotein particles of *E. coli* possess a fairly uniform electrophoretic mobility. The protein and RNA are firmly held together, since they were stable to sonic treatment or repeated electrophoresis and the nucleic acid was not displaced by added yeast RNA. The size of these particles and their composition sets an upper limit of approximately $4 \cdot 10^5$ to the molecular weight of *E. coli* RNA.

The relatively low molecular weight RNA comprised 10 to 20% of the total and differed from the large nucleoprotein particles in a number of respects. It had a higher electrophoretic mobility and some of the protein associated with this material was bound firmly enough to move with the RNA on repeated electrophoresis, but did not remain attached after sonic treatment. Its base composition differed from that of the nucleoprotein particles, and depended on the portion of the band sampled, indicating that it is a heterogeneous mixture. The results obtained with cells of *B. megatherium* make it seem unlikely that the low molecular weight component is an artifact of preparation of extracts. Although more of this type of material is found in resting bacteria than in rapidly growing cells, ^{32}P-labeling experiments failed to reveal any metabolic relationship between it and the large nucleoprotein particles. At the present no evidence for its metabolic function (assuming it to have a function) is available.

Of some interest is the RNA-containing material which accumulates in chloromycetin-inhibited cells. It is, in several respects, similar to the minor fast moving component of normal cells. They have similar mobilities and susceptibilities to disruption upon exposure to sonic oscillation. They do however appear to differ in size. The normal component is relatively small, its nucleic acid portion has an average molecular weight below 5,000 and possibly consists of a mixture of oligonucleotides. The material accumulating in cells exposed to chloromycetin is apparently larger than this as judged by its precipitability at various concentrations of ethanol and ammonium sulfate. Also, the base composition of RNA made in the presence of chloromycetin is similar to that of the major component[29].

Although the RNA synthesized in the presence of chloromycetin is bound to protein there is no net protein synthesis under these conditions. It is possible, although there is no direct evidence to support the hypothesis, that the RNA is coupled to the protein of the 29 *S* component, since this component disappears following exposure of the cells to chloromycetin. It is not known to what extent this protein-nucleic acid linkage is specific, but the linkage is easily disrupted by sonic treatment, in contrast to normal RNA nucleoprotein linkages. However, this attraction is firmer than that of bacterial protein for added high molecular weight RNA, since added RNA shows no tendency to bind bacterial protein under the conditions of electrophoresis.

The key question concerning the RNA produced by inhibited cells is whether it is

References p. 172/173.

a normal intermediate in RNA biosynthesis whose further metabolism has been blocked by chloromycetin, directly or indirectly. Alternatively it might be a by-product, a form in which the cell deposits its incompletely synthesized RNA under these conditions of totally blocked protein synthesis. That it is probably a metabolic by-product is suggested by the finding that in cells released from inhibition by chloromycetin this RNA does not act at once as a precursor to the normal nucleoproteins[30].

These observations are consistent with the conclusion reached elsewhere that most of the bacterial RNA is formed as a nucleoprotein[29].

It is not possible to decide from the present results whether bacterial DNA exists free or is present in the form of nucleoprotein. In starch electrophoresis both high molecular weight free DNA and DNA-nucleoprotein could appear in an area containing protein, owing to the relatively low mobility of undegraded DNA on starch. Appearance of a free, high mobility bacterial DNA band, following sonic treatment, can equally well be attributed to the breakage of bonds in free DNA or to disruption of a nucleoprotein. Previous workers have presented evidence that bacteria can synthesize both free and bound[5] DNA.

ACKNOWLEDGEMENTS

We wish to thank Professor W. M. STANLEY for making the facilities of the Virus Laboratory available to one of us (K.P.) during the tenure of a fellowship, Dr. H. K. SCHACHMAN for assistance in performing several ultracentrifuge runs, Dr. G. S. STENT for gifts of radioactive phosphate, and Drs. F. W. ALLEN, A. CRESTFIELD, H. FRAENKEL-CONRAT and Mr. D. E. HOARD for gifts of nucleic acids.

SUMMARY

1. Bacterial RNA migrates in only two bands on starch electrophoresis. The major band consists of the two kinds of nucleoprotein particles previously found by ultracentrifugation. RNA in the two bands is bound with different affinities to protein; these two forms of RNA have different base ratios and molecular sizes, and do not appear to be metabolically related.

2. Most of the RNA of rapidly growing *E. coli* or *B. megatherium* is in the form of nucleoproteins. Less than 15%, if any, is present as protein nucleates.

3. RNA made in the presence of chloromycetin is bound to protein but has a different mobility than does most of the RNA of normal *E. coli*. In contrast to normal RNA it is readily freed from protein by sonic treatment. During exposure to chloromycetin one of the two kinds of normal nucleoprotein particles disappears.

4. The mobility of DNA in the starch electrophoresis apparatus depends on molecular size. Conclusions regarding the attachment of DNA to proteins could not be made in these experiments.

5. The effects of variables such as methods of preparation of extracts, pH, buffers, and age of cells upon the starch electrophoresis patterns of bacterial extracts have been investigated.

REFERENCES

1 B. MAGASANIK, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, 1955, p. 373.
2 H. K. SCHACHMAN, A. B. PARDEE AND R. Y. STANIER, *Arch. Biochem. Biophys.*, 38 (1952) 245.
3 D. SZAFARZ, *Biochim. Biophys. Acta*, 6 (1951) 562.

[4] E. L. HESS AND H. D. SLADE, *Biochim. Biophys. Acta*, 16 (1955) 346.
[5] E. CHARGAFF, in E. CHARGAFF AND J. N. DAVIDSON, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, 1955, p. 330.
[6] E. F. GALE AND J. P. FOLKES, *Biochem. J.*, 53 (1953) 493.
[7] C. L. WISSEMAN, JR., J. E. SMADEL, F. E. HAHN AND H. E. HOPPS, *J. Bacteriol.*, 67 (1954) 662.
[8] A. B. PARDEE, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 263.
[9] A. D. HERSHEY AND M. CHASE, *J. Gen. Physiol.*, 36 (1952) 39.
[10] H. MCILWAIN, *J. Gen. Microbiol.*, 2 (1948) 288.
[11] H. MICKLE, *J. Roy. Microscop. Soc.*, 68 (1948) 10.
[12] K. PAIGEN, *Anal. Chem.*, 28 (1956) 284.
[13] H. G. KUNKEL AND A. TISELIUS, *J. Gen. Physiol.*, 35 (1951) 89.
[14] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
[15] W. C. SCHNEIDER, *J. Biol. Chem.*, 161 (1945) 293.
[16] G. CERIOTTI, *J. Biol. Chem.*, 198 (1952) 297.
[17] D. E. METZLER AND E. E. SNELL, *J. Biol. Chem.*, 198 (1952) 363.
[18] A. D. HERSHEY, J. DIXON AND M. CHASE, *J. Gen. Physiol.*, 36 (1953) 777.
[19] A. M. CRESTFIELD, K. C. SMITH AND F. W. ALLEN, *J. Biol. Chem.*, 216 (1955) 185.
[20] R. SIGNER AND H. SCHWANDER, *Helv. Chim. Acta*, 32 (1949) 853.
[21] G. H. LATHE AND C. R. L. RUTHVEN, *Biochem. J.*, 60 (1955) xxxiv.
[22] A. R. PEACOCKE AND H. K. SCHACHMAN, *Biochim. Biophys. Acta.*, 15 (1954) 198.
[23] E. G. PICKELS, W. F. HARRINGTON AND H. K. SCHACHMAN, *Proc. Natl. Acad. Sci. U.S.*, 38 (1952) 943.
[24] R. JEENER, *Nature*, 163 (1949) 837.
[25] V. ALLFREY, M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 37 (1953) 157.
[26] M. FLEMING AND D. O. JORDAN, *Discussions Faraday Soc.*, 13 (1953) 217.
[27] A. KLECZKOWSKI, *Biochem. J.*, 40 (1946) 677.
[28] B. ROTMAN AND S. SPIEGELMAN, *J. Bacteriol.*, 68 (1954) 419.
[29] A. B. PARDEE AND L. S. PRESTIDGE, *J. Bacteriol.*, 71 (1956) 677.
[30] A. B. PARDEE, unpublished data.

Received May 9th, 1956

PURIFICATION DE L'OVOMUCOÏDE PAR ÉLECTROPHORÈSE DE ZONE

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Deux méthodes permettent actuellement la préparation de l'ovomucoïde biologiquement actif à l'état de pureté très avancée: l'une est due à LINEWEAVER ET MURRAY[1], l'autre à FREDERICQ ET DEUTSCH[2]. Ces méthodes consistent l'une et l'autre en l'élimination des protéines étrangères accompagnant l'ovomucoïde dans le blanc d'oeuf par précipitation à pH 3.5 en présence d'acide trichloracétique.

LINEWEAVER ET MURRAY[1], puis FREDERICQ ET DEUTSCH[2] avaient déjà remarqué l'hétérogénéité à l'électrophorèse de leurs préparations. BIER et coll.[3] ont repris plus tard l'étude électrophorétique détaillée de ces deux préparations d'ovomucoïde, et sont arrivés à la conclusion que ce que l'on considère en général comme l'ovomucoïde, serait en fait un complexe de protéines très semblables, et dont les différents constituants, tous doués d'activité antitrypsique, ne présenteraient entre eux qu'une faible différence, d'un groupe chargé en plus ou en moins par exemple.

Bibliographie p. 180.