The Synthesis of Ribonucleic Acid during Inhibition of *Escherichia coli* by Chlortetracycline

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1. During inhibition of *Escherichia coli* by chlortetracycline, protein synthesis was sharply reduced whereas synthesis of RNA was much less affected. 2. Most of the RNA made during inhibition was contained in particles that sedimented more slowly than ribosomes. 3. The particles were more sensitive than ribosomes to degradation by ultrasonic vibrations and ribonuclease and differed from ribosomes in their behaviour during chromatography on DEAE-cellulose. 4. The particles contained two species of RNA that differed slightly in their sedimentation properties from the two RNA components found in ribosomes. 5. The nature of the events taking place during inhibition by chlortetracycline is discussed with particular reference to the status of the particles that accumulate and to the mode of action of this and other antibiotics.

Although the antibiotic chlortetracycline has been shown to have a number of different effects on microbial metabolism (for review see Jackson, 1964), it has been suggested that the antibacterial action of this drug depends primarily on its inhibitory effect on protein synthesis (Hahn, 1958). In cell-free systems from *Escherichia coli*, chlortetracycline strongly inhibits the incorporation of amino acids from aminoacyl-transfer RNA complexes into protein bound to ribosomes (Franklin, 1963, 1964). However, Gale & Folkes (1953) reported that, although protein synthesis is strongly inhibited by chlortetracycline, the synthesis of nucleic acids is much less affected and that cells incubated with the antibiotic accumulate RNA.

This accumulation of RNA is unusual in that simultaneous synthesis of protein is normally a requisite for synthesis of RNA. Thus when an amino acid auxotroph is starved of its required amino acid, neither protein nor RNA is made (Pardee & Prestidge, 1956). Continued synthesis of RNA accompanied by a greatly reduced amount of protein synthesis has, however, been found to occur under special conditions. It is observed when *E. coli* is inhibited by other antibiotics, notably by chloramphenicol or puromycin (Gale & Folkes, 1953; Takeda, Hayashi, Nakagawa & Suzuki, 1960). The 'relaxed' methionine-requiring mutant 58-161 of *E. coli* (and related strains) is exceptional in its ability to synthesize RNA when starved of methionine (Borek, Ryan & Rockenbach, 1955).

The present investigation is concerned primarily with the nature of the RNA synthesized by *E. coli*

during inhibition by chlortetracycline. It is shown that a large part of this RNA is contained in particles that not only sediment more slowly than ribosomes but are distinct from them in several other properties. The nature of the events taking place during inhibition by chlortetracycline is discussed with particular reference to the mode of action of this and other antibiotics and to theories of the control of synthesis of RNA. A preliminary account of some of this work has been given (Holmes & Wild, 1965).

EXPERIMENTAL

Growth of bacteria. The organism used was *Escherichia coli* K12, maintained by serial monthly subculture on nutrient-agar slopes. Batches of cells were grown at 30° without aeration in flasks filled to the neck with medium adjusted to pH 7.0 with NaOH and containing (g./l.): KH₂PO₄, 18; (NH₄)₂SO₄, 1; MgSO₄·7H₂O, 0.4; glucose, 1.8; Difco Bactopeptone, 10. Under these conditions of culture, changes in pH are small and growth is limited by exhaustion of the glucose (Bowen, Dagley & Sykes, 1959). For experiments with chlortetracycline, cells were harvested and resuspended in an equal volume of similar medium containing 1.6 g. of MgSO₄·7H₂O/l. and 10 g. of glucose/l. Growth was allowed to take place for 30 min. at 30° and a freshly prepared solution (in water) of chlortetracycline hydrochloride was then added to give a final concentration 15 µg./ml. Cells were incubated with the drug for 2 hr.; if necessary, RNA synthesized in this period was labelled by the addition of 20 µC of [2-¹⁴C]uracil/l. together with 30 mg. of unlabelled uracil/l.

Extinctions of cultures were determined at 540 mµ with a Unicam SP.1400 spectrophotometer. Estimations of RNA, DNA and protein were carried out by using respectively

orcinol, diphenylamine and biuret methods, as described by Dagley, Turnock & Wild (1963).

Preparation of cell-free extracts. Harvested cells were disintegrated in a Hughes (1951) bacterial press cooled to about -15° . For analytical ultracentrifuging and sucrose-density-gradient analyses, the frozen disintegrated cells were taken up in 15 mM- KH_2PO_4 , pH 7.0, containing 0.1 mM-magnesium acetate (phosphate-magnesium acetate buffer), normally on the basis of 1 g. of crushed cells to 2.5 ml. of buffer. Insoluble debris was then removed by centrifuging at 15000g for 60 min. at 5° .

Ultrasonic treatment of extracts was carried out as described by Dagley *et al.* (1963).

Analytical ultracentrifuging. Protein concentrations of cell-free extracts were adjusted to 1% before analytical ultracentrifuging, which was carried out in a Spinco model E ultracentrifuge at 187000g and at laboratory temperature.

Isolation of RNA. RNA was isolated from crude extracts by the method of Kurland (1960). The extract, to which had been added 2 mg. of sodium dodecyl sulphate/ml., was shaken for 10 min. at room temperature with 1 vol. of phenol saturated with phosphate-magnesium acetate buffer. The mixture was centrifuged at low speed and the aqueous layer removed from the denser phenol layer with a pipette. The extraction was repeated twice more but with 0.5 vol. of phenol. The RNA was precipitated from the aqueous layer by the addition of 2 vol. of ethanol in the cold, collected on the centrifuge and dissolved in 10 mM-acetate buffer, pH 4.6, containing 0.1 M-NaCl. Traces of phenol were removed by two more ethanol precipitations. Some isolations were also carried out from cells which had been disrupted in the presence of bentonite (about 5 mg. of bentonite/g. of cells). In these cases, about 1 mg. of bentonite/ml. was also included in the phosphate-magnesium acetate buffer which was used to prepare the crude extracts and about 0.25 mg./ml. of aqueous layer was added after each phenol extraction. Bentonite for these purposes was prepared by suspending the commercial product (British Drug Houses Ltd., Poole, Dorset) in phosphate-magnesium acetate buffer. The suspension was centrifuged at 4300g for 3 min.; the fine particles remaining in the supernatant were collected by centrifuging at 6300g for 20 min., washed twice with phosphate-magnesium acetate buffer and finally dispersed in this buffer at a concentration of about 1 mg./ml. No differences were detected between preparations of RNA made in the presence and absence of bentonite.

Sucrose-density-gradient analysis. Cell-free extracts were prepared in phosphate-magnesium acetate buffer containing 5 μg . of deoxyribonuclease/ml. Clarified extract (0.07 ml.) was used for analysis in a sucrose gradient (5–20%, w/v, in 5 ml. of phosphate-magnesium acetate buffer), by the technique described by Dagley *et al.* (1963). After centrifuging at 124000g and 5° in the SW 39 rotor of the Spinco model L ultracentrifuge, 10-drop fractions were collected into 3.0 ml. of phosphate-magnesium acetate buffer for determination of the extinction at 260 μm , followed by precipitation with a final concentration of 5% (w/v) of trichloroacetic acid for the estimation of radioactivity.

Density-gradient analysis of preparations of RNA was carried out similarly, except that 10 mM-acetate buffer, pH 4.6, containing 0.1 M-NaCl was used in place of phosphate-magnesium acetate buffer both in the sucrose gradients and for reception of the 10-drop fractions.

DEAE-cellulose chromatography. Cell-free extracts were

prepared in 10 mM-tris chloride, pH 7.2, containing 20 mM- MgCl_2 (tris-magnesium chloride buffer) with the addition of 5 μg . of deoxyribonuclease/ml. They were clarified as before and then centrifuged for 4 hr. at 102000g and 5° in the 50 rotor of the model L ultracentrifuge to sediment ribosomes and accumulated material. The pellet was dissolved in a small volume of tris-magnesium chloride buffer and a portion, containing about 5 mg. of RNA, was applied to a column of DEAE-cellulose (1 cm. $\times$ 15 cm.) prepared as described by Turnock & Wild (1965). Elution was carried out with a linear gradient of NaCl (0–1.0 M) in 200 ml. of tris-magnesium chloride buffer. Fractions of volume of about 4 ml. were collected. After measurement of the extinction of a fraction at 260 μm , trichloroacetic acid was added to a final concentration of 5% (w/v) and the precipitate assayed for radioactivity.

Radioactivity measurements. Material precipitated by 5% (w/v) trichloroacetic acid at 0° was collected on Oxoid (Oxo Ltd., London, E.C. 4) membrane filters by using a stainless-steel filter tower. The filters were gummed to planchets, dried in air and counted for a minimum of 1000 counts with a thin-window gas-flow counter (Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.). Samples were always 'infinitely thin'.

Chemicals. [$2\text{-}^{14}\text{C}$]Uracil was from The Radiochemical Centre, Amersham, Bucks. Crystalline ribonuclease and deoxyribonuclease were from the Nutritional Biochemicals Corp. Chlortetracycline hydrochloride was supplied by Lederle Laboratories, London, W.C.2.

RESULTS

Accumulation of RNA during inhibition. Cells were grown overnight in the peptone medium, harvested and resuspended in an equal volume of fresh medium. Growth was allowed to take place for 30 min. and then chlortetracycline (15 μg ./ml.) was added to a portion of the culture. At this concentration the antibiotic caused considerable inhibition of growth (Fig. 1*a*); changes in the protein, RNA and DNA contents of the inhibited culture are shown in Fig. 1*b*). During incubation with the antibiotic, DNA was made and increased by about 60% in 2 hr. In this same time the RNA content of the culture doubled, although protein increased by only about 15%. In the experiments described below, cells were always inhibited for 2 hr. with 15 μg . of chlortetracycline/ml. Under these conditions, preferential synthesis of RNA was consistently observed, increases of RNA from 60 to 120% being accompanied by small increases in protein content of 4–15%.

Since most of the RNA in a bacterial cell is in its ribosomes, cell-free extracts were examined in the analytical ultracentrifuge to see if changes in the ribosome fraction could be detected consequent upon inhibition.

Changes in schlieren diagrams. Figs. 2(*a*) and 2(*b*) show schlieren diagrams obtained during the ultracentrifuging of clarified extracts prepared from cells before and after incubation with chlortetracycline.

Fig. 1. Effects of adding (at zero time) 15 μ g. of chlortetracycline/ml. to *E. coli* growing at 30° in peptone medium. (a) Extinction at 540 $m\mu$ of control (○) and inhibited (●) cultures. (b) Variation in protein (■), RNA (▲) and DNA (□) contents of the inhibited culture in (a). Results in (b) are expressed as percentages of the contents at zero time (protein, 175 μ g./ml.; RNA, 72 μ g./ml.; DNA 7.4 μ g./ml.).

Major differences between the diagrams are apparent.

For these experiments, extracts were made in phosphate-magnesium acetate buffer. Under these conditions, the diagram for the control extract (Fig. 2a) shows boundaries caused by the 50s and 30s ribosomes, together with a large slow-moving boundary produced by the 'soluble' cell contents. In the extract from the inhibited cells (Fig. 2b) an additional sharp boundary sedimenting at about 6s has become visible. This was removed by incubation of the extract at room temperature with 1 μ g. of deoxyribonuclease/ml. (Fig. 2c), as was also the small 'spike' (sedimentation coefficient about 6s) to the large slow-moving boundary of the control extract. The sharp boundary in the diagrams from inhibited cells is thus caused by the

Fig. 2. Schlieren diagrams, after centrifuging at 187000g, of cell-free extracts prepared from *E. coli*. Sedimentation is to the left. The bar angle was 40°. All photographs except (a) were taken after 20 min. (a) Control: cells harvested before addition of chlortetracycline. Photograph taken after 16 min. (b) Cells harvested after inhibition for 2 hr. by 15 mg. of chlortetracycline/l. (c) The extract giving (b) but after incubation with 1 μ g. of deoxyribonuclease/ml. (d) The extract giving (b) but after incubation with 1 μ g. of ribonuclease/ml. (e) The extract giving (b) but after treatment for 2 min. with ultrasonic vibrations.

DNA synthesized during inhibition. A similar boundary is also visible in extracts from cells of the 'relaxed' methionine-requiring mutant 58-161 of *E. coli* that have synthesized DNA (and RNA) in the absence of protein synthesis (Dagley *et al.* 1963). DNA sedimenting in bacterial extracts as a 'spike' at about 8s has been observed by other workers (Siegel, Singer & Wildman, 1952).

An additional difference between the ultracentrifuge patterns is in the appearance of new boundaries in the schlieren diagram for the inhibited culture (Fig. 2b): these are caused by components that sediment more slowly than the 30s ribosomes and, in these crude extracts, at about 15s. The convection which tended to occur during ultracentrifuging of extracts from inhibited cultures was reduced after treatment of the extracts with 1 μ g. of deoxyribonuclease/ml. In diagrams for such extracts (Fig. 2c), the material accumulated during inhibition can be more clearly seen to sediment as two components which partially resolve in the course of the centrifugation.

The particles accumulated during inhibition contain RNA but are more sensitive than ribosomes

to treatment with either ribonuclease or ultrasonic vibrations. Fig. 2(d) shows that accumulated material was degraded when an extract was incubated with $1\mu\text{g.}$ of ribonuclease/ml. at room temperature, conditions which left the ribosome boundaries unaffected. A similar instability was observed when an extract containing these particles was subjected to ultrasonic vibrations for 2 min. (Fig. 2e).

Sucrose-density-gradient analysis of extracts containing accumulated material. Analysis of extracts from inhibited cells on sucrose-density gradients showed that the particles accumulated during inhibition contained most of the RNA synthesized in this time. For these experiments, RNA made during incubation with chlortetracycline was labelled by the addition of $[2\text{-}^{14}\text{C}]\text{uracil}$ to the culture together with the antibiotic. Fig. 3 shows the results of a typical analysis: the RNA made during inhibition gives rise to two peaks of radioactivity in the sucrose gradient. About 25% of the RNA had sedimented very little during the centrifuging and thus had sedimentation properties similar to those of transfer RNA; the remainder of the radioactive RNA occupied a position in the sedimentation diagram corresponding to that expected for the particles visible in the schlieren diagrams. The 50s ribosomes were scarcely radioactive and although the 30s ribosomes were not well resolved from accumulated material by this technique, it is likely that these too were not labelled

appreciably. Thus few new ribosomes were formed during inhibition and most of the RNA that was made was in the particles that accumulate in this period.

Chromatography on DEAE-cellulose. Chromatography on DEAE-cellulose is a method which has been much used for the separation of ribosomes from components concerned in their synthesis (Britten, 1963). For the experiment shown in Fig. 4, the RNA made by inhibited cells was labelled by the addition of $[2\text{-}^{14}\text{C}]\text{uracil}$ to the medium together with the chlortetracycline. A cell-free extract was then made with tris-magnesium chloride buffer and ribosomes and accumulated particles were collected by centrifuging the extract. The pellet from the centrifuging was dissolved in tris-magnesium chloride buffer, a portion containing about 5 mg. of RNA was applied to a column of DEAE-cellulose and elution carried out with a concentration gradient of sodium chloride. Fig. 4 shows measurements of the extinction and radioactivity of fractions of the eluate. Ribosomes were eluted from the column when the sodium chloride concentration was about 0.40 M and, in confirmation of the results of the analyses on sucrose-density gradients, were scarcely

Fig. 3. Sucrose-density-gradient analysis of an extract prepared from cells incubated for 2 hr. in a peptone medium containing $[2\text{-}^{14}\text{C}]\text{uracil}$ and 15 mg. of chlortetracycline/l. The general procedure is described in the text. Sedimentation in the gradient was for 3 hr. at 124000g. Measurements were of extinction at $260\text{ m}\mu$ (○) and 5% trichloroacetic acid-insoluble radioactive material (●).

Fig. 4. Chromatography on DEAE-cellulose of a fraction containing ribosomes and accumulated material prepared from cells incubated for 2 hr. in a peptone medium containing $[2\text{-}^{14}\text{C}]\text{uracil}$ and 15 mg. of chlortetracycline/l. Preparation of the sample and general experimental details are described in the text. A portion of the preparation was loaded on the column and eluted with a concentration gradient of NaCl (0–1.0 M) in 200 ml. of tris-magnesium chloride buffer. Measurements of extinction at $260\text{ m}\mu$ (○) and 5% trichloroacetic acid-insoluble radioactive material (●) were made on fractions of volume about 4 ml.

radioactive; almost all the radioactivity was eluted at a higher salt concentration (about 0.55M) and although the radioactive material contributed to the extinction profile in this region, it was well resolved from fractions containing ribosomes. The particles accumulated during inhibition thus differed appreciably from ribosomes in their behaviour on columns of DEAE-cellulose. In studies of the precursors of ribosomes which have been detected in exponentially growing cells, the order of elution of the different components from DEAE-cellulose seems to depend primarily on the ratio of RNA to protein in the material, components with a lower percentage of protein than ribosomes being eluted at higher salt concentrations (Britten, 1963). This suggests that the particles formed during the considerable inhibition of protein synthesis by chlortetracycline might contain less protein than ribosomes.

Sucrose-density-gradient analyses of RNA from inhibited cells. In bacteria, ribosomal RNA is of two sizes with sedimentation coefficients of 16s and 23s (Kurland, 1960). Transfer RNA sediments more slowly: a sedimentation coefficient of 4s has been reported (Tissières, 1959).

RNA was isolated from cells that had been labelled with [2-¹⁴C]uracil during incubation with chlortetracycline, to see whether species corresponding to these three components were made during inhibition. Fig. 5(a) shows the results of an analysis of this RNA on a sucrose-density gradient. The extinction profile locates the total RNA from the cells; the three peaks of radioactivity show the position of the newly synthesized RNA. The close correspondence between the two profiles suggests that the components made during inhibition are transfer RNA and the two species of ribosomal RNA.

However, during incubation with chlortetracycline the RNA content of a culture approximately doubles; the RNA made during inhibition therefore contributes appreciably to the extinction profile of the analyses and could mask slight differences in sedimentation properties between the RNA present before and that synthesized during the inhibition. An additional experiment was therefore carried out in which cells that had been incubated with chlortetracycline and [2-¹⁴C]uracil were diluted with about 20 times their number of non-radioactive cells that had not been in contact with the antibiotic. RNA was isolated from the mixed culture and analysed on a sucrose gradient. The results are shown in Fig. 5(b). Under these conditions, the radioactive RNA contributes little to the extinction profile and slight differences are apparent between the RNA synthesized during inhibition and ribosomal RNA from the non-radioactive cells. Fig. 5(b) shows that one of the radioactive species sediments a little further in the gradient than 16s RNA and the

Fig. 5. Sucrose-density-gradient analyses of RNA prepared from cells after incubation in a peptone medium containing [2-¹⁴C]uracil and 15 mg. of chlortetracycline/l. Preparation of RNA and the general experimental procedure are described in the text. Centrifugation in the gradient was for 7 hr. at 124000g. Measurements were of extinction at 260 mμ (○) and 5% trichloroacetic acid-insoluble radioactive material (●). In (a) RNA was isolated from cells after incubation for 2 hr. with chlortetracycline, 20 μC of [2-¹⁴C]uracil/l. and 30 mg. of unlabelled uracil/l. For (b), 20 ml. of cell suspension was incubated for 2 hr. with chlortetracycline, 1 μC of [2-¹⁴C]uracil/ml. and 100 μg. of unlabelled uracil/ml. After incubation, these cells were diluted into 400 ml. of the same cell suspension (containing 100 μg. of unlabelled uracil/ml.), which had not been in contact with the antibiotic and had been kept at 0° from zero time. RNA was then isolated from the mixed culture.

other slightly less than 23s RNA. These small differences were consistently observed in a number of different experiments. Their possible significance is discussed below.

DISCUSSION

Our results confirm the observation of Gale & Folkes (1953) that, during the strong inhibition of growth and protein synthesis produced by chlor-

tetracycline, considerable synthesis of RNA occurs. Under the conditions we have used, about 20% of this RNA is likely to be transfer RNA; the rest is contained not in ribosomes but in particles sedimenting more slowly than 30s ribosomes and which additionally differ from ribosomes in their sensitivity to ribonuclease and ultrasonic vibrations and in their behaviour during chromatography on DEAE-cellulose.

Apparently similar particles have been observed to accumulate in *E. coli*, when, under other circumstances, RNA continues to be made in the absence of much, or any, synthesis of protein. The relaxed methionine-requiring mutant 58-161 of *E. coli* continues to synthesize RNA when starved of methionine; this strain (and other related ones) has a genetic defect in the mechanism by which RNA synthesis is controlled by amino acids (Stent & Brenner, 1961). Most of the RNA synthesized by mutant 58-161 during starvation appears in particles whose sedimentation coefficients in crude extracts are about 14-16s and which are sensitive to ribonuclease and ultrasonic vibrations (Dagley *et al.* 1963). The particles are eluted from columns of DEAE-cellulose at higher concentrations of sodium chloride than ribosomes (Turnock & Wild, 1965).

Differential synthesis of RNA also occurs in cells of *E. coli* inhibited by chloramphenicol or puromycin. During incubation with either of these antibiotics, cells accumulate particles that sediment more slowly than ribosomes, that are sensitive to ribonuclease and that contain the bulk of the RNA synthesized during inhibition (Nomura & Watson, 1959; Dagley, White, Wild & Sykes, 1962). These particles are thus similar to those produced by chlortetracycline action.

It has been suggested that the particles that accumulate during starvation of the 'relaxed' mutant 58-161 or during inhibition by chloramphenicol or puromycin are related to the precursors of the ribosomes detected in exponentially growing cells (Kurland, Nomura & Watson, 1962; Dagley *et al.* 1962). These particles have been reported to contain RNA with the same sedimentation properties as ribosomal RNA (Kurland *et al.* 1962; Sells, 1964; Mandel & Borek, 1963). When methionine is added to starved cultures of the mutant 58-161, preferential synthesis of ribosomal protein takes place and ribosomes are formed from the RNA contained in the particles without apparent degradation and by a process akin to that described for the synthesis of ribosomes by exponentially growing cells (Turnock & Wild, 1965). Similar events occur in cells recovering from inhibition by puromycin (Sells, 1964). The particles accumulated during inhibition by chlortetracycline may be in this same category, although their fate when cells

recover from the action of the antibiotic remains to be investigated.

Differences in sedimentation properties similar to those between ribosomal RNA and two of the species of RNA made during inhibition by chlortetracycline have been found in other circumstances. Kono, Otaka & Osawa (1964) examined the RNA synthesized by *E. coli* that had been grown in a minimal medium, starved of glucose and then transferred to a broth containing peptone. Cells in the early stages of growth in the broth were briefly exposed to [^3H]-adenosine. The radioactivity was found to be incorporated into two species of RNA which corresponded to ribosomal RNA except that the slower moving component sedimented rather more quickly than 16s RNA derived from pre-existing ribosomes. Particles accumulating during inhibition by chloramphenicol or 5-fluorouracil were similarly shown to contain two species of RNA, one indistinguishable in its sedimentation properties from 23s ribosomal RNA, the other sedimenting slightly more quickly than the 16s component. Suzuki & Hayashi (1964) observed a small difference between ribosomal RNA and two species of RNA made during the recovery of *E. coli* from the effects of starvation of Mg^{2+} ions. The newly synthesized species had sedimentation coefficients of about 17s and 22.5s and also differed from ribosomal RNA in their hyperchromicity. Both groups of workers suggest that the differences in properties that they describe are a consequence of differences in secondary structure between the RNA from fully formed ribosomes and that from incompleting precursors. A similar explanation may be valid for the RNA accumulated during inhibition by chlortetracycline.

The preferential synthesis of RNA which occurs during inhibition by chlortetracycline, puromycin or chloramphenicol is explicable in terms of the hypothesis advanced by Kurland & Maaløe (1962) to explain the control of RNA synthesis by amino acids. They postulate that this control is mediated by a set of repressors, each repressor being neutralized when in combination with an amino acid specific for it. When all the repressors are so linked, the repression is lifted and synthesis of RNA can occur. The most likely candidates for the repressor molecules are the different species of transfer RNA, each of which is capable of combination with a specific amino acid. During protein synthesis, transfer RNA is present largely in the form of aminoacyl-transfer RNA complexes; if, for example, an amino acid auxotroph is starved of its required amino acid, uncombined transfer RNA specific for that amino acid accumulates and the synthesis of RNA is halted.

The precise modes of action of chlortetracycline and chloramphenicol on protein synthesis are obscure, although chloramphenicol bound to ribo-

somes may interfere in the functioning of messenger RNA (Wolfe & Hahn, 1965). Yarmolinsky & de la Haba (1959) first pointed out the structural similarity between puromycin and aminoacyl-transfer RNA and suggested that this antibiotic may interfere with protein synthesis by displacing transfer RNA from growing polypeptide chains. It is, however, clear that chlortetracycline, puromycin and chloramphenicol act on protein synthesis at a stage subsequent to the formation of aminoacyl-transfer RNA complexes, since synthesis of the complexes is not inhibited by the presence of these antibiotics (Franklin, 1963; Lacks & Gros, 1959; Yarmolinsky & de la Haba, 1959). Thus formation of RNA might be expected to continue in cells in which protein synthesis has been inhibited by their action.

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