The Action of Streptomycin in a Mutant of *Escherichia coli* with Increased Sensitivity to the Antibiotic

By G. TURNOCK

Department of Biochemistry, University of Leicester, Leicester LE1 7RH, U.K.

(Received 20 March 1970)

A mutant of *Escherichia coli* with increased sensitivity to streptomycin has been studied. This strain differed from a normal *str^s* strain in that streptomycin produced inhibition of protein synthesis and loss of viability with almost no lag period. Chloramphenicol protected a normal *str^s* strain but not the mutant against the bactericidal action of streptomycin. The results obtained support the idea that access of streptomycin to its site of action in a normal cell is restricted, and that this restriction, which is much less effective in the mutant, probably involves a permeability barrier. Comparison of the inhibition of protein synthesis by streptomycin with concomitant changes in the distribution of polyribosomes in both strains suggested that the antibiotic can directly inhibit the translation of mRNA.

The antibiotic streptomycin completely inhibits protein synthesis in the bacterium *Escherichia coli*. This is believed to reflect the primary event that leads to death of the cell because mutation to resistance involves a change in a protein of the 30S ribosomal subunit (Cox, White & Flaks, 1964; Traub & Nomura, 1968). The bactericidal effect of streptomycin is due to its essentially irreversible binding to the ribosome. However, after addition of the drug to a culture of a sensitive strain there is a lag of several minutes before any cell death or inhibition of protein synthesis can be detected (Dubin, Hancock & Davis, 1963). Two explanations for the existence of the lag period have been proposed: the first states that the rate of entry of streptomycin into the cell is limited and, further, that it is dependent on active growth (Dubin *et al.* 1963), whereas the second suggests that streptomycin can only bind to the ribosome at one particular point in the 'ribosome cycle' (Luzzatto, Apirion & Schlessinger, 1968, 1969). Significant inhibition of protein synthesis in this case requires active metabolism for several minutes to expose a majority of the ribosome population to the antibiotic.

A mutation that modifies the degree of resistance to streptomycin of *str^r* strains of *E. coli* has been described (Turnock, 1969). Until more is known about its function the gene concerned has been designated as *b*, and the wild-type and mutant alleles are given as *b⁺* and *b⁻* respectively. The effect of the *b⁻* allele in an *str^s* strain has now been examined, and it was found that sensitivity to streptomycin is considerably increased. The results obtained sup-

port the hypothesis that the lag period before streptomycin has any effect on a normal strain is due to the slow entry of the antibiotic into the cell. As a corollary of this the experiments also show that streptomycin can directly inhibit the translation of mRNA, in agreement with the work of Modolell & Davis (1968, 1969), who studied the effect of streptomycin on protein synthesis *in vitro*.

MATERIALS AND METHODS

Strains of *E. coli*. The two strains that are designated L and M respectively in this paper were obtained by mating strain Hfr 15-5 with strain 15-28H (Turnock, 1969). Their full description is as follows: strain L, *b⁺str^{thy}-his⁻try⁻*; strain M, *b⁻str^{thy}-his⁻try⁻*. The important characteristics to note are that both strains carry the same *str^s* allele, derived from strain Hfr 15-5, and that the mutant allele of gene *b* in *str^r* strains is associated with an altered degree of resistance to streptomycin (Turnock, 1969). The abbreviations L and M refer to colony size (Large or Medium) under standard conditions. The difference between them is due to the *b⁻* allele, which in some way also diminishes the efficiency of growth of M strains.

Strains L1 (*b⁺str^r*) and M2 (*b⁻str^r*) were used in one experiment. These strains were also obtained in the course of the genetic analysis of the ribosome mutant 15-28H (Turnock, 1969).

Media and growth conditions. Liquid cultures were grown with aeration by shaking at 37°C. Extinctions of cultures were measured at 450nm with a Gilford 300 micro-sample spectrophotometer (light-path 1 cm). The minimal medium used contained (per l): Na₂HPO₄, 6g; KH₂PO₄, 3g; NaCl, 0.5g; NH₄Cl, 1g; CaCl₂, 11mg; MgSO₄, 246mg; glucose, 2g; thymine, 10mg; L-histidine,

50 mg; L-tryptophan, 50 mg. Cultures were grown overnight in minimal medium with a limiting concentration of glucose (0.6 g/l) to give E_{450} values of about 1.2 for strain L and 0.7 for strain M. Growth was restarted by diluting 100-fold into fresh medium with excess of glucose (2 g/l). Unless otherwise stated all experiments were carried out when the extinction had reached 0.2–0.25, and aeration was continued after addition of streptomycin. The mean generation times of strain L and strain M were 45 min and 55 min respectively.

Determination of viable counts. After appropriate dilution in the minimal salts medium 0.1 ml samples were mixed with 2.5 ml portions of soft agar (0.65%) at 45°C and poured on agar plates, followed by second 2.5 ml portions of soft agar to form a protective layer. The plates were incubated at 37°C for 24–30 h for determination of the number of colonies. The agar used was the Oxoid no. 1 product and both the soft agar and the plates were prepared in the standard minimal medium. The initial dilution was always at least 10-fold for strain L and 100-fold for strain M and was sufficient to decrease the amount of streptomycin in the samples below an inhibitory concentration.

Measurement of protein synthesis by incorporation of L-[14 C]phenylalanine. The minimal medium was supplemented with L-phenylalanine (12.5 mg/l). At the start of the experiment 1 μ Ci of L-[U- 14 C]phenylalanine (15.3 mCi/mmol) was added to 20 ml of culture and 0.5 ml samples were taken into 0.5 ml portions of ice-cold 10% (w/v) trichloroacetic acid containing L-phenylalanine (1 mg/ml). After being heated at 92°C for 20 min, the samples were cooled to room temperature and the precipitates were collected on membrane filters. The filters were washed with three 2 ml portions of 5% (w/v) trichloroacetic acid, dried at 70°C for 2 h and assayed for radioactivity in a liquid-scintillation spectrometer. The scintillator solution contained: toluene, 1 litre; 2,5-diphenyloxazole, 5 g; 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 0.3 g. Samples were counted for a minimum of 2000 counts.

Analysis of polyribosomes. At an E_{450} of 0.9–1.0, when growth is still exponential, 3–4 ml of culture was pipetted into an equal volume of half-frozen TMK buffer (0.01 M-tris chloride, pH 7.2, 10 mM-magnesium acetate, 100 mM-KCl) in ice. The bacteria were immediately collected by centrifuging, resuspended at 0°C in 0.08 ml of TMK buffer containing lysozyme (200 μ g/ml) and frozen in liquid N₂. To obtain lysis the temperature of the samples was allowed to rise to 0°C and then they were thawed briefly at room temperature before being placed back in ice. Then 10 μ l of 10% (w/v) Triton X-100 in TMK buffer and 5 μ l of deoxyribonuclease (200 μ g/ml in TMK buffer) were added. This method of lysis is based on that originally described by Ron, Kohler & Davis (1966).

To determine the distribution of ribosomes the samples were immediately layered from a broad-tipped pipette on to linear 15–30% (w/w) sucrose density gradients in TMK buffer. The gradients (total volume 17 ml) were prepared in $\frac{5}{8}$ in $\times$ 4 in tubes for the Spinco SW27 rotor, and were centrifuged for 150 min at 78 740 g at 2°C. An ISCO model 180 gradient fractionator and model 220 UV analyser were used to analyse the gradients.

Protein synthesis in vitro. The bacteria were grown in the minimal medium described above, supplemented with 1% vitamin-free casamino acids (casein hydrolysate), to

an E_{450} of 1.0. After being harvested by centrifuging at 2°C they were washed with 250 ml of TAMK buffer (10 mM-tris acetate, pH 7.5, 40 mM-KCl, 40 mM-ammonium acetate, 10 mM-magnesium acetate, 0.5 mM-CaCl₂, 0.01 mM-zinc acetate, 6 mM-2-mercaptoethanol)/g wet wt. and resuspended in 1 ml of the same buffer. A cell-free extract was prepared by 90 s exposure in an MSE ultrasonic disintegrator at 0°C, a fine probe being used. Cell debris was removed by centrifuging at 30 000 g for 30 min after addition of deoxyribonuclease (10 μ g/ml), and the clear supernatant (protein content 15–20 mg/ml) was dialysed for 5 h at 0°C against 500 vol. of TAMK buffer. Preparations were stored until required in 0.4 ml portions in liquid N₂.

To destroy endogenous mRNA 0.3 ml of extract was mixed at 0°C with 0.9 ml of a stock solution of amino acids, nucleoside triphosphates and salts [prepared as described by Forchhammer & Kjeldgaard (1967) except that the concentration of L-phenylalanine was decreased to 10% of that of the other amino acids], 0.15 ml of tRNA from *E. coli* B (1.5 mg/ml in TAMK buffer) and 0.75 ml of TAMK buffer. After incubation at 35°C for 10 min the reaction mixture was chilled in ice and a further 0.15 ml of TAMK buffer and 0.03 ml of L-[U- 14 C]phenylalanine (1 mg/ml; 15.3 mCi/mmol) were added. A 75 μ l portion of the preincubated extract was used per reaction mixture. RNA from bacteriophage f2 (25 μ g in 15 μ l of TAMK buffer), kindly supplied by Dr S. Hardy, was added after the streptomycin sulphate (also dissolved in TAMK buffer) when this was present. All additions were made at 0°C and the volume in each case was made up to 100 μ l with TAMK buffer. The reaction mixtures were incubated at 35°C for 25 min, chilled in ice and 2.5 ml of 5% (w/w) trichloroacetic acid containing 1 mg of L-phenylalanine/ml was added. Processing of the samples from this point onwards was as described above under 'Measurement of protein synthesis by incorporation of L-[14 C]phenylalanine'.

Protein synthesis in the presence of bacteriophage f2 RNA was linear for at least 25 min. Samples incubated for this time without added mRNA had radioactivities of 150–160 c.p.m. With the addition of 25 μ g of bacteriophage f2 RNA the stimulation of incorporation above this background level was 1000–1500 c.p.m. for extracts from strains L and L1 and 500–800 c.p.m. for extracts from strains M and M2. The ribosome content of each extract was determined from measurement of the E_{260} by using a value for $E_{1\text{cm}}^{1\%}$ of 250 (Modolell & Davis, 1968). In all the experiments described in the present paper there was between 120 and 150 μ g of ribosomes per reaction (i.e. a concentration of 0.43–0.49 μ M assuming a molecular weight of 2.8×10^6). As the ribosome is known to be the site of action of streptomycin the amount of protein synthesized in the presence of the antibiotic has been expressed as a function of the streptomycin/ribosome ratio in each reaction.

RESULTS

Effect of streptomycin on viability. Fig. 1 compares the bactericidal effect of streptomycin (100 μ g/ml) on the normal strain, L, and the mutant strain, M, which carries the b^- allele (Turnock, 1969). With

Fig. 1. Effect of streptomycin (100 µg/ml), added at 0 min, on the viability of strain L (●) and strain M (○). The inset shows a separate experiment in which the loss of viability of strain M during the first 5 min after addition of streptomycin was investigated in more detail.

strain L there was no loss of viability for about 10 min whereas strain M started to die immediately after addition of streptomycin. This is shown in more detail in the inset to Fig. 1, which illustrates the kinetics of killing of strain M during the first 5 min after addition of the antibiotic.

The different sensitivities of the two strains to streptomycin are illustrated in another way in Fig. 2, which compares their survival at a series of concentrations of the antibiotic in a time approximately equal to one generation of normal growth. Strain M was killed by concentrations of streptomycin greater than 0.1 µg/ml whereas strain L was not significantly affected by 10 µg/ml. To test for the ability to grow in the presence of the antibiotic, cultures of the two strains were streaked on nutrient agar plates containing 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg of streptomycin/ml. Strain M grew only at the lowest concentration whereas strain L grew in the presence of up to 2.0 µg/ml.

Protection against the bactericidal action of streptomycin. There are several ways in which an *str^s* strain can be protected against the lethal action of streptomycin. The most important of these are (i) addition of chloramphenicol (Plotz & Davis,

Fig. 2. Effect of the concentration of streptomycin on the survival of strain L (●) and strain M (○). Cultures were subdivided into flasks containing a range of concentrations of the antibiotic and the viable counts were determined after 50 min. The initial viable counts/ml were 4.7×10^7 for strain L and 6.1×10^7 for strain M.

1962), (ii) starvation for glucose (Luzzatto *et al.* 1969) and (iii) anaerobiosis (Stern, Barner & Cohen, 1966). The effect of streptomycin on strains L and M under these various conditions was compared.

(i) Chloramphenicol. Fig. 3 shows that addition of chloramphenicol (100 µg/ml) immediately before streptomycin completely protected strain L, whereas strain M still exhibited considerable loss of viability, albeit at a lower rate than the control culture without chloramphenicol. This is an important difference between the two strains. As a control the effectiveness of chloramphenicol as an inhibitor of protein synthesis was determined by measuring its effect on the incorporation of L-[14 C]phenylalanine into total cell protein. In both strain L and strain M protein synthesis was completely inhibited by chloramphenicol (100 µg/ml) with no detectable lag. Chloramphenicol by itself did not kill either strain.

(ii) Glucose starvation. Both strains were completely unaffected by streptomycin (100 µg/ml) added after starvation for glucose for the equivalent of one generation time, although there was loss of viability in the normal way when glucose was restored at the time of the addition of streptomycin.

(iii) Anaerobiosis. Addition of streptomycin to cultures growing anaerobically in glucose-minimal medium provided an interesting contrast between strains L and M, in that under these conditions the

Fig. 3. Effect of chloramphenicol on the action of streptomycin. At 0 min cultures were divided between two flasks, one of which contained chloramphenicol to give a concentration of $100\mu\text{g/ml}$. At 5 min streptomycin ($100\mu\text{g/ml}$) was added to both flasks. Strain L plus streptomycin: ▲, with chloramphenicol; ●, without chloramphenicol; strain M plus streptomycin: △, with chloramphenicol; ○, without chloramphenicol.

mutant was less sensitive than the normal strain (Fig. 4). The protection afforded to strain L was not very marked.

Effect of streptomycin on protein synthesis. The kinetics of inhibition of protein synthesis by streptomycin ($100\mu\text{g/ml}$) in strains L and M are illustrated in Fig. 5. The results mirror the effect of the antibiotic on the viability of the two strains in that strain L was unaffected for 8–10 min whereas protein synthesis in strain M was inhibited within 2 min.

Luzzatto *et al.* (1969) have studied the metabolism of polyribosomes in *E. coli* after addition of streptomycin and have found that there is a gradual decline in the polyribosome content of the cells with a concomitant increase in the number of 70S monoribosomes. The kinetics of these changes led them to postulate that streptomycin can only inhibit

Fig. 4. Action of streptomycin under anaerobic conditions. Cultures were bubbled slowly with N_2 in 50 ml conical flasks filled to the neck with minimal medium, with the addition of L-glutamic acid (350mg/l) to compensate for loss of CO_2 in the stream of N_2 . The mean generation times were: strain L, 58 min; strain M, 72 min. Streptomycin ($100\mu\text{g/ml}$) was added at 0 min, and samples were taken for determination of the viable count. ●, Strain L; ○, strain M.

protein synthesis by interfering with initiation, and that the lag before there is any effect on the rate of synthesis is a measure of the time required for a significant number of ribosomes to be made available by the normal process of chain termination. The rapidity with which protein synthesis is inhibited by streptomycin in strain M therefore made it important to analyse polyribosome metabolism in the presence of the antibiotic.

Sedimentation analyses of extracts of strains L and M, prepared by gentle lysis in order to preserve polyribosomes, are shown in Fig. 6. The positions of the 70S monoribosomes, diribosomes and tri-ribosomes and the larger polyribosomes are indicated. Peaks due to the 30S and 50S ribosomal subunits can be seen between the 70S monoribosomes and the soluble proteins and tRNA at the top of the gradient. Extracts of both strains were prepared at different times after addition of streptomycin and analysed by sedimentation in sucrose density gradients for the distribution of ribosomes between 70S monoribosomes, diribosomes + tri-ribosomes and the large polyribosomes. Peak areas were measured with a planimeter. Accurate values could not be obtained for the ribosomal subunits,

although their contribution to the total ribosomal population in control cultures is clearly quite small (Fig. 6), and there was no significant change in the

Fig. 5. Effect of streptomycin on the synthesis of protein, measured by the incorporation of L-[^{14}C]phenylalanine (see the Materials and Methods section), in strain L (Fig. 5a) and strain M (Fig. 5b). ●, Control culture; ○, streptomycin (100 µg/ml) added at 0 min.

presence of streptomycin. Fig. 7 shows the changes in the distribution of ribosomes in extracts of the two strains prepared after addition of streptomycin (100 µg/ml) to cultures in exponential growth. In both strains there was progressive loss of polyribosomes and accumulation of 70S monoribosomes and, to a smaller extent, diribosomes and triribosomes, although the changes began immediately with strain M but only after a lag with strain L.

It was possible to obtain results with strain L that were similar to those obtained with the mutant strain by increasing the concentration of streptomycin from 100 to 500 µg/ml. Two experiments in which the effect of 500 µg of streptomycin/ml on strain L was investigated are illustrated in Fig. 8. The first (Fig. 8a) shows that cell death and inhibition of protein synthesis commence much earlier with the higher concentration of the antibiotic (cf. Figs. 1 and 5), whereas the second (Fig. 8b) indicates that this is also true for the changes that occur in the distribution of ribosomes (cf. Fig. 7).

Effect of streptomycin on protein synthesis in vitro. The results presented so far clearly show that strain M is much more sensitive to streptomycin than strain L, although both carry the same *str^r* allele. As the site of action of streptomycin is known to lie within the cell and to be specifically associated with the ribosome, it is important to know if the difference between the two strains is also apparent in their response to streptomycin *in vitro*.

Fig. 6. Sedimentation analysis in sucrose density gradients of extracts, prepared by gentle lysis, of strain L (a) and strain M (b). See the Materials and Methods section for details. The direction of sedimentation is from right to left and the major ribosome peaks are identified.

Fig. 7. Changes in the distribution of ribosomes in the presence of streptomycin. Extracts were prepared after addition of streptomycin (100 μ g/ml) at 0 min and analysed by sedimentation in sucrose density gradients (as Fig. 6). Peak areas were measured with a planimeter after tracing in the baseline from a blank gradient. Results for the 70S monoribosomes, diribosomes+triribosomes and the larger polyribosomes are expressed as a percentage of the total ribosome population. ●, Strain L; ○, strain M.

Fig. 9 compares the inhibition of protein synthesis by streptomycin in cell-free extracts of strains L and M supplied with RNA (as mRNA) from bacteriophage f2. The results are expressed in terms of the streptomycin/ribosome ratio in the reaction mixtures and show that extracts of the two strains did not differ significantly in their sensitivity to streptomycin, in contrast with the behaviour of the intact bacteria.

Measurements were made on unfractionated cell-free extracts so that the response to streptomycin of the complete system for protein synthesis in each strain could be compared. However, when ribosomes from extracts of strains L and M were purified by differential centrifugation, and their sensitivity to streptomycin was determined with bacteriophage f2 RNA and a supernatant enzyme system from strain 15TP (Turnock, 1969), very similar results were again obtained. Protein synthesis was inhibited by 50% at a concentration of streptomycin of 0.4 μ M and a streptomycin/ribosome ratio 0.5–0.6 in each case.

Fig. 8. (a) Effect of streptomycin (500 μ g/ml added at 0 min) on viability and protein synthesis in strain L. □, Viable cells/ml; incorporation of L-[14 C]phenylalanine was measured in the absence (●) and presence (○) of the antibiotic. (b) Changes in the distribution of ribosomes in strain L in the presence of 500 μ g of streptomycin/ml added at 0 min. Measurements were made as described in the Materials and Methods section and in the legend to Fig. 7. ▲, 70S monoribosomes; ○, diribosomes+triribosomes; ●, large polyribosomes.

The binding of streptomycin by ribosomes from a normal *str^s* strain is very efficient and it is conceivable that a mutation that conferred increased affinity for the antibiotic might not make any observable difference in an experiment *in vitro* of the type described in Fig. 9. However, ribosomes from *str^r* strains have a decreased affinity for streptomycin (Petitpas-Dewandre, Barbason & Verly, 1969) and so cell-free extracts of strains M2 (*b⁻ str^r*) and L1 (*b⁺ str^r*) were used as another assay for the possible effect of the *b⁻* allele *in vitro*. The growth rate of strain M2 is lowered in the presence of streptomycin (100 μ g/ml) whereas strain L1 is indifferent to the antibiotic (Turnock, 1969). The

Fig. 9. Protein synthesis in cell-free extracts of strain L (●) and strain M (○) in the presence of streptomycin (for details see the Materials and Methods section). As the concentration of ribosomes was closely similar in the two experiments (0.43 and $0.49 \mu\text{M}$ for strain M and strain L respectively) the amount of protein synthesized is expressed as a function of the streptomycin/ribosome ratio in each reaction.

Fig. 10. Protein synthesis in cell-free extracts of strain L1 (●) and strain M2 (○) in the presence of streptomycin (for details see the Materials and Methods section). The ribosome concentration was $0.45 \mu\text{M}$ in both cases and the amount of protein synthesized is expressed as a function of the streptomycin/ribosome ratio in each reaction.

effect of streptomycin on protein synthesis in cell-free extracts of the two strains is shown in Fig. 10, and it is clear that both preparations responded in the same way. The concentration of streptomycin required to give 50% inhibition of protein synthesis was 1000-fold greater than that required with the extracts prepared from the *str^s* strains (cf. Fig. 9).

DISCUSSION

Strain M (*b⁻ str^s*) is much more sensitive to streptomycin (Figs. 1 and 2) than strain L (*b⁺ str^s*), although both carry the same *str^s* allele. A mutation

in another ribosomal protein could modify the *Str* phenotype (Turnock, 1969), but investigation of the effect of streptomycin on protein synthesis *in vitro* (Figs. 9 and 10) gave no indication of any intrinsic difference between preparations from *b⁺* and *b⁻* strains. This being so the results are most easily explained on the basis of a greater ease of penetration of streptomycin into *b⁻* cells. The implication of this hypothesis is that the mutation directly affects a membrane component.

The uptake of streptomycin by *E. coli* has been studied by Davis and his co-workers (Anand, Davis & Armitage, 1960; Plotz, Dubin & Davis, 1961; Dubin *et al.* 1963). There is an essentially instantaneous primary uptake with the characteristics of an electrostatic interaction at the cell surface. Thus the electrophoretic mobility of bacteria is immediately affected by streptomycin (McQuillen, 1951) and the binding may be reversed by the addition of salts (Anand *et al.* 1960; Plotz *et al.* 1961). After the onset of cell death and inhibition of protein synthesis there is further uptake of streptomycin that may be an indirect effect of membrane damage (Dubin *et al.* 1963).

The rapid binding of streptomycin to the cell surface makes it difficult to assay the entry of the antibiotic into the cytoplasm. However, the delay before there is any inhibition of protein synthesis in a normal strain (Fig. 5a) suggests that penetration of the membrane by streptomycin may be quite slow. The protection afforded by chloramphenicol and glucose starvation implies that streptomycin may only be able to enter the cell at the point where active synthesis of membrane is taking place. In the case of treatment with chloramphenicol it has been shown that the uptake of streptomycin is in fact greatly diminished (Anand *et al.* 1960; Hurwitz & Rosano, 1962). For the *b⁻* strain, which responds to streptomycin almost instantaneously, the presence of chloramphenicol no longer has a protective effect. This is compatible with the idea that streptomycin can penetrate a *b⁻* cell much more easily.

Both *b⁺* and *b⁻* strains were protected against the bactericidal action of streptomycin by starvation for glucose. The similarity in behaviour, in contrast with the differential effect of chloramphenicol, suggests that entry of streptomycin may normally be dependent not only on active membrane synthesis but also on energy metabolism. In a *b⁻* cell the first, but not the second, requirement is abolished. This is further emphasized by the effect of streptomycin on cultures growing anaerobically (Fig. 4), a situation in which strain M was actually less sensitive than strain L.

An entirely different explanation for the relatively slow response of protein synthesis to the presence of streptomycin has been proposed by

Luzzatto *et al.* (1968, 1969). This requires interference with protein synthesis only at the point of initiation, so that continued protein synthesis, after addition of streptomycin, is required for a majority of the ribosome population to be exposed to the antibiotic. The results of Modolell & Davis (1968, 1969), however, have demonstrated that streptomycin *in vitro* immediately inhibits the translation of mRNA.

The loss of polyribosomes in the presence of streptomycin (Luzzatto *et al.* 1969), which has been confirmed in the present work (Figs. 7 and 8), is more reasonably ascribed to slow breakdown after binding of streptomycin rather than to interference with initiation. For both strain L and strain M there was still a significant population of large polyribosomes present at a time when the rate of protein synthesis had decreased to a very low value (cf. Figs. 5 and 7). When strain L was treated with 500 μ g of streptomycin/ml (Fig. 8), the inhibition of protein synthesis, loss of viability and changes in the distribution of ribosomes all began more quickly than at the lower (100 μ g/ml) concentration used in other experiments. Luzzatto *et al.* (1969) also observed that these effects are dependent on the concentration of streptomycin in the medium, a result entirely compatible with the idea that entry of streptomycin into a normal cell is restricted. If, on the other hand, the lag period depended primarily on the time required for the completion of translation of mRNA in progress at the time of addition of streptomycin, the concentration of the antibiotic in the medium would not be expected to have any significant effect. That it does have an effect, at concentrations several orders of magnitude greater than that required to give significant inhibition of protein synthesis *in vitro*, is further evidence that the breakdown of polyribosomes is a secondary consequence rather than a precursor to the inhibition of protein synthesis. This conclusion contradicts the hypothesis of Luzzatto *et al.* (1969), whose data were, however, obtained with a fragile mutant of *E. coli* that has to be grown under abnormal conditions. Nevertheless Fig. 9 of their paper does suggest that in their minimal medium the rate of protein synthesis in the presence of streptomycin declined more rapidly than would be expected from the kinetics of appearance of 70S monoribosomes, if this species represents that fraction of the ribosome population that has been effectively exposed to the antibiotic. Another criticism of their hypothesis is that chloramphenicol completely protects a normal *str^s* strain against the bactericidal effect of streptomycin (Plotz & Davis, 1962), although mRNA continues to enter polyribosomes via, it is presumed, the normal route in the presence of chloramphenicol (Gurgo, Apirion & Schlessinger, 1969). Thus the

lethal event, the formation of the aberrant initiation complexes (Luzzatto *et al.* 1969), would be expected to occur in the presence of chloramphenicol, and this clearly does not happen. It has already been argued that the protection afforded by chloramphenicol reflects the need for growth of the cell to permit uptake of streptomycin, a requirement that is abolished in the *b⁻* strain.

The results obtained with both strain L and strain M therefore support the view that there is a permeability barrier to streptomycin (Anand & Davis, 1960; Dubin *et al.* 1963) and that streptomycin can directly inhibit the translation of mRNA (Modolell & Davis, 1968, 1969). In a *b⁻ str^r* strain it is probable that the ready access of streptomycin is sufficient to give an internal concentration high enough to partially lower the rate of protein synthesis (Turnock, 1969).

Part of this work was carried out in the Department of Genetics, University of Leicester, and was supported by a grant to Professor R. H. Pritchard from the Science Research Council and by the Jane Coffin Childs Memorial Fund for Medical Research (Fund Project no. 229). The author is grateful to Dr S. Hardy for much invaluable discussion and to Mrs B. Birch for technical assistance.

REFERENCES

- Anand, N. & Davis, B. D. (1960). *Nature, Lond.*, **185**, 22.
- Anand, N., Davis, B. D. & Armitage, A. K. (1960). *Nature, Lond.*, **185**, 23.
- Cox, E. C., White, J. R. & Flaks, J. G. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 703.
- Dubin, D. T., Hancock, R. & Davis, B. D. (1963). *Biochim. biophys. Acta*, **74**, 476.
- Forchhammer, J. & Kjeldgaard, N. O. (1967). *J. molec. Biol.* **24**, 459.
- Gurgo, G., Apirion, D. & Schlessinger, D. (1969). *J. molec. Biol.* **45**, 205.
- Hurwitz, C. & Rosano, C. L. (1962). *J. Bact.* **83**, 1193.
- Luzzatto, L., Apirion, D. & Schlessinger, D. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 873.
- Luzzatto, L., Apirion, D. & Schlessinger, D. (1969). *J. molec. Biol.* **42**, 315.
- McQuillen, K. (1951). *Biochim. biophys. Acta*, **7**, 54.
- Modolell, J. & Davis, B. D. (1968). *Proc. natn. Acad. Sci. U.S.A.* **61**, 1279.
- Modolell, J. & Davis, B. D. (1969). *Nature, Lond.*, **224**, 345.
- Petitpas-Dewandre, A., Barbason, H. & Verly, W. G. (1969). *Eur. J. Biochem.* **7**, 307.
- Plotz, P. H. & Davis, B. D. (1962). *J. Bact.* **83**, 802.
- Plotz, P. H., Dubin, D. T. & Davis, B. D. (1961). *Nature, Lond.*, **191**, 1324.
- Ron, E. Z., Kohler, R. E. & Davis, B. D. (1966). *Science, N.Y.*, **153**, 1119.
- Stern, J. L., Barner, H. D. & Cohen, S. S. (1966). *J. molec. Biol.* **17**, 188.
- Traub, P. & Nomura, M. (1968). *Science, N.Y.*, **160**, 198.
- Turnock, G. (1969). *Molec. gen. Genet.* **104**, 295.