Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (eds.) (1959). *Data for Biochemical Research*, p. 286. Oxford: Clarendon Press.
Dustin, J. P., Czajkowska, C., Moore, S. & Bigwood, E. J. (1953). *Analyt. chim. Acta*, **9**, 256.
Elson, L. A. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
Freudenberg, K., Walch, H. & Molter, H. (1940). *Naturwissenschaften*, **30**, 87.
Frisell, W. R., Meech, L. A. & Mackenzie, C. G. (1954). *J. biol. Chem.* **207**, 709.
Gibbons, R. A. & Morgan, W. T. J. (1954). *Biochem. J.* **57**, 283.
Gibbons, R. A., Morgan, W. T. J. & Gibbons, M. N. (1955). *Biochem. J.* **60**, 428.
Holborow, E. J. & Loewi, G. (1962). *Immunology*, **5**, 278.
Jankovic, B. D. & Waksman, B. H. (1962). *J. Immunol.* **89**, 606.
Jepson, J. B. & Smith, I. (1953). *Nature, Lond.*, **172**, 1100.
Kekwick, R. A. (1950). *Biochem. J.* **46**, 438.
Landsteiner, K. & Harte, R. A. (1940). *J. exp. Med.* **71**, 551.
Landsteiner, K. & Harte, R. A. (1941). *J. biol. Chem.* **140**, 673.
Ma, T. S. & Zuazaga, G. (1942). *Industr. Engng. Chem. (Anal.)*, **14**, 280.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Martin, C. J. & Axelrod, A. E. (1953). *Proc. Soc. exp. Biol. N.Y.*, **83**, 461.
Moore, S., Spackman, D. H. & Stein, W. H. (1958). *Analyt. Chem.* **30**, 1185.
Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 893.
Morgan, W. T. J. (1947). *Experientia*, **3**, 257.
Morgan, W. T. J. (1954–1955). *Scientific Basis of Medicine*, vol. 4, p. 92. University of London.
Morgan, W. T. J. (1959). *Int. Symp. on Biologically Active Mucoids*, p. 6. Warsaw: Polska Akademia Nauk.
Morgan, W. T. J. (1963). *Ann. N.Y. Acad. Sci.* **106** (2), 177.
Morgan, W. T. J. & King, H. K. (1943). *Biochem. J.* **37**, 640.
Morgan, W. T. J. & Pusztai, A. (1961). *Biochem. J.* **81**, 648.
Neuman, R. E. & Logan, M. A. (1950). *J. biol. Chem.* **184**, 299.
Pusztai, A. & Morgan, W. T. J. (1961). *Biochem. J.* **80**, 107.
Rees, M. W. (1946). *Biochem. J.* **40**, 632.
Rondle, C. J. M. & Morgan, W. T. J. (1955). *Biochem. J.* **61**, 586.
Ryle, A. P., Sanger, F., Smith, L. F. & Kitai, R. (1955). *Biochem. J.* **60**, 541.
Warren, L. (1959). *J. biol. Chem.* **234**, 1971.
Watkins, W. M. (1958). *Proc. 7th Congr. int. Soc. Blood Transfusion, Rome*, p. 206.
Watkins, W. M. (1959). *Ciba Found. Symp.: Biochemistry of Human Genetics*, p. 217. London: J. and A. Churchill Ltd.
Watkins, W. M. (1962). *Immunology*, **5**, 245.
Watkins, W. M. & Morgan, W. T. J. (1959). *Vox sang., Basel*, **4**, 97.

Biochem. J. (1963) **88**, 555

The Accumulation of Ribonucleic Acid by a Mutant of *Escherichia coli*

BY S. DAGLEY, G. TURNOCK AND D. G. WILD
Department of Biochemistry, The University, Leeds 2

(*Received* 21 *March* 1963)

In bacteria, protein synthesis is normally closely linked to the synthesis of RNA, so that, if the synthesis of one is diminished or stopped, that of the other is similarly affected. Under certain conditions, however, protein synthesis can be sharply retarded while synthesis of RNA is less affected; in these circumstances the bacteria therefore accumulate RNA. These abnormal conditions can be produced by treating *Escherichia coli* with chloramphenicol or puromycin; the RNA synthesized during incubation with either of these antibiotics has been extensively studied, e.g. by Hahn, Schaechter, Cezlowski, Hopps & Ciak (1957), Neidhardt & Gros (1957), Yarmolinski & de la Haba (1959), Takeda, Hayashi, Nakagawa & Suzuki (1960) and Aronson & Spiegelman (1961*a*, *b*).

Another instance in which cells can accumulate RNA has also been described. In general, bacterial mutants requiring an amino acid for growth no longer synthesize protein and RNA when the essential amino acid is withdrawn (Pardee & Prestridge, 1956). However, Borek, Ryan & Rockenbach (1955) discovered that, if the methionine-requiring mutant 58–161 of *E. coli* K12 is starved of methionine, protein synthesis stops but RNA continues to be made; Fleissner & Borek (1962) have investigated in some detail the 'transfer' RNA that is synthesized during methionine-starvation. Stent & Brenner (1961) have shown that the ability of this mutant to synthesize RNA in the absence of protein synthesis is genetically determined. By suitable conjugation experiments, mutants requiring amino acids other than methionine can be obtained which synthesize RNA when their 'new' amino acid requirement is withdrawn. Apparently there exists a locus on the bacterial chromosome which in this strain produces a less stringent control of RNA synthesis. The site of this locus in relation to known genetic markers has been established (Alfoldi, Stent & Clowes, 1962).

The present work was carried out with the same methionine-requiring mutant of *E. coli*. Although 'transfer' RNA is synthesized during methionine starvation, a large part of the RNA made under these conditions appeared in crude cell-free extracts as material detectable in the analytical ultracentrifuge and sedimenting at 14–16s. The isolation, composition and properties of this material are described and its relation to the RNA synthesized during treatment with chloramphenicol or puromycin, and to that normally present in cells, is discussed. A preliminary account of some of this work has been given (Dagley, Turnock & Wild, 1962*a*).

EXPERIMENTAL

Organisms. Two methionine-requiring mutants of *Escherichia coli* K12 were obtained from Dr W. Hayes, Microbial Genetics Research Unit, Hammersmith Hospital, London, W. 12. They were maintained by monthly subculture on nutrient–agar slopes. One mutant, 58–161, was that used by Borek *et al.* (1955), Stent & Brenner (1961) and Alfoldi *et al.* (1962); the other, H239, was used for purposes of comparison.

Cultural conditions. Batches (10 l.) of cells were grown at 30° with forced aeration in a medium adjusted to pH 7·0 with NaOH and containing, per litre: KH_2PO_4, 9 g.; $(NH_4)_2SO_4$, 1 g.; $MgSO_4,7H_2O$, 1·6 g.; DL-methionine, 0·05 g.; glucose, 0·4 g. The inoculum was 25 ml. of cells grown in a similar medium containing 2·5 g. of glucose/l. In the medium containing 0·4 g. of glucose/l., growth was limited by exhaustion of glucose from the medium: after overnight growth to a limiting population, 2·3 g. of glucose/l. was added and the cells were harvested 1 hr. later in a Laval continuous-flow centrifuge while in the exponential phase.

When it was required to incubate these cells in the absence of methionine, they were washed by centrifuging with 800 ml. of methionine-free medium containing 2·5 g. of glucose/l., resuspended in 3·8 l. of the same medium and vigorously aerated at 30°. In experiments involving the use of [^{35}S]sulphate, a 'low-sulphate' medium of pH 7·0 was used, which contained, per litre: KH_2PO_4, 9 g.; NH_4Cl, 2 g.; $MgCl_2,6H_2O$, 1·3 g.; $Na_2SO_4,10H_2O$, 0·13 g.; DL-methionine, 0·05 g.

Extinctions of cultures were determined at 540 mμ with a Unicam SP. 1400 spectrophotometer with cuvettes of 2 mm. light-path.

Preparation of cell-free extracts. Harvested cells were allowed to drain free of medium and were then disintegrated without abrasive in a Hughes (1951) bacterial press cooled to about −15°. The frozen disintegrated cells were taken up in buffer composed of 15 mM-KH_2PO_4 containing magnesium acetate (0·1 mM) and brought to pH 7·0 with 5N-NaOH, normally on the basis of 1 g. of crushed cells to 3 ml. of buffer. This buffer has been used extensively and is referred to below simply as 'phosphate–magnesium acetate solution'. Insoluble debris was then removed by centrifuging at 15 000 *g* for 60 min. at 5°.

Ultrasonic treatment of extracts. Cell-free extracts were exposed to the output (60 w) of a MSE–Mullard ultrasonic disintegrator at an average frequency of 20 kcyc./sec. The extract (1 ml.), contained in a thick-walled glass tube, was cooled in a beaker of ice during the exposure, and the stainless-steel probe (length 133 mm.; end diameters 19 mm.) was cooled in a deep-freeze before use. Under these conditions there was no appreciable rise in temperature during treatment.

Analytical methods. For determinations of RNA, DNA and protein on suspensions of cells, 5·0 ml. portions of the suspension were pipetted into centrifuge tubes containing 0·5 ml. of ice-cold 50% (w/v) trichloroacetic acid. The precipitate was collected by centrifuging in the cold in a bench centrifuge and was washed once with 5 ml. of ice-cold 5% (w/v) trichloroacetic acid. It was then extracted twice with 4 ml. of 5% trichloroacetic acid for 30 min. at 100°. The two supernatants were pooled and made up to 10·0 ml. with 5% trichloroacetic acid. This solution was used for the determination of RNA by the orcinol method (Schneider, 1957) and of DNA by the modification of the diphenylamine reaction described by Burton (1956). The precipitate remaining after the two extractions was assayed for protein by a modification of the biuret method (Stickland, 1951).

RNA was determined in crude extracts and fractions derived from them by the orcinol method; protein was determined with the combined biuret reagent of Sols (1947) or by the method of Lowry, Rosebrough, Farr & Randall (1951).

Crystallized bovine serum albumin was used as a standard for the protein determinations, and D-deoxyribose and D-ribose for DNA and RNA respectively. Values for ribose were expressed, where necessary, as RNA by multiplying by 4·42. This procedure takes into account the fact that only purine nucleotides enter into the orcinol reaction (Ashwell, 1957), and that the ratio of purines to pyrimidines in *E. coli* RNA is 1·3:1·0 (Spahr & Tissières, 1959).

Ultracentrifuge studies. Protein concentrations of crude cell-free extracts were adjusted to 1% before analytical ultracentrifuging, which was carried out in a Spinco model E ultracentrifuge equipped with schlieren optics and fitted with a rotor temperature and indicator control unit. Analytical runs were carried out at 20·0°. The same machine was used for preparative ultracentrifuging.

Isolation of 50s ribosomes and accumulated material. The procedures given below were used to isolate from cell-free extracts (*a*) 50s ribosomes and (*b*) the components sedimenting at 14–16s that appear when cells are incubated in the absence of methionine ('accumulated material'). The initial cell-free extracts were prepared by taking up 1 g. of crushed cells to 4 ml. of phosphate–magnesium acetate solution and all operations were carried out at 3–5°.

(*a*) Isolation of 50s ribosomes. A portion (50 ml.) of cell-free extract was centrifuged for 45 min. at 102 000 *g* in the preparative 'A' rotor. The pellets were taken up in a total of 10 ml. of phosphate–magnesium acetate solution and centrifuged for 30 min. at 124 000 *g* in the SW39 (swinging-bucket) rotor. The pellets were then taken up in 3·5 ml. of phosphate–magnesium acetate solution. When this preparation was examined in the analytical cell, it was seen to contain largely 50s ribosomes contaminated by coloured material that at 187 000 *g* sedimented rapidly to the bottom of the cell. Tissières, Watson, Schlessinger & Hollingworth (1959) report the association of bacterial cytochromes with impure ribosome preparations. The 50s preparations were therefore freed from this coloured material by centrifuging successive portions at 187 000 *g* in an analytical cell of long

(30 mm.) light-path until the coloured debris had sedimented to the bottom of the cell. The supernatants were then pooled and retained as the final preparation of 50s ribosomes; their homogeneity was confirmed by a further analytical ultracentrifuging.

(*b*) Isolation of accumulated material. It was essential to remove components that sedimented slowly completely from cell-free extracts before attempting to separate accumulated material from the 30s and 50s ribosomes. A portion (65 ml.) of cell-free extract was therefore centrifuged for 4·5 hr. at 102 000 *g* in the preparative 'A' rotor: the supernatant was used as a source of non-ribosomal protein; the pellets were taken up in an equal volume of phosphate–magnesium acetate solution and centrifuged twice for 4·5 and 4 hr. respectively. The pellets were then dissolved in 15 ml. of phosphate–magnesium acetate solution. At this stage the preparation consisted of 30s and 50s ribosomes plus accumulated material. A portion of the ribosomes was then removed by centrifuging at 124 000 *g* for 30 min. in the SW39 rotor. The yellow–brown pellets were discarded and the supernatants were centrifuged in successive portions at 187 000 *g* in each of two analytical cells of long (30 mm.) light-path until the 30s-ribosome boundary had almost reached the bottom of the cells. At this point centrifuging was stopped, and the top 0·3 ml. of the supernatant in a cell was carefully removed and rejected; most of the rest of the supernatant (about 1 ml.) was then retained for the final preparation. The combined supernatants from three such centrifugings (two cells being used each time) were pooled and the purity of this final preparation was checked by analytical ultracentrifuging.

Isolation of ribonucleic acid. The method used to isolate RNA from crude extracts or from purified preparations was essentially that of Kurland (1960). The solution, 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 solution. 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 NaCl (0·1 M). Traces of phenol were removed by carrying out two more ethanol precipitations. The acetate buffer was used in all sedimentation studies of RNA preparations.

Sucrose-density-gradient analysis of cell-free extracts. The technique was essentially that of McCarthy, Britten & Roberts (1962). Extracts for density-gradient analysis were prepared with 1 g. of crushed cells to 2·5 ml. of phosphate–magnesium acetate solution containing 5 μg. of deoxyribonuclease/ml. A drop (0·07 ml.) of extract was placed on a clean microscope slide near to another drop (0·07 ml.) of 4% (w/v) sucrose. A pipette (0·2 ml.) with the tip drawn out and slightly bent was used to suck up first the extract and then the sucrose solution; the sharp boundary between the solutions was abolished by slight tipping of the pipette. The contents of the pipette were then allowed to run slowly on to the top of 5·0 ml. of sucrose in phosphate–magnesium acetate solution contained in a plastic centrifuge tube. The sucrose was present in a linear gradient from 5% (w/v) at the top of the tube to 20% (w/v) at the bottom. This gradient was produced by using the mixer described by Britten & Roberts (1960). Layered tubes thus prepared were centrifuged in the SW39 rotor of the ultracentrifuge. They were then clamped and pierced by a hypodermic needle carefully pushed through the bottom of the tube. Leakage around the needle was prevented by means of a small piece of Parafilm (A. Gallenkamp and Co. Ltd.) coated with silicone grease. When the guard wire of the needle was removed, the contents of the tube could be collected in about seventy 5-drop fractions that were received alternately into (*a*) 2·0 ml. of phosphate–magnesium acetate solution for measurement of the extinction at 260 mμ: this served to locate the nucleic acids; and (*b*) 3·0 ml. of 5% (w/v) trichloroacetic acid: radioactivity measurements were then made on the precipitate.

Experiments involving radioisotopes. (i) [^{35}S]Sulphate. Carrier-free [^{35}S]sulphate (10 μc/l.) was included in the 'low-sulphate' medium. (ii) DL-[^{35}S]Methionine (10 μc/l.) was added to the normal medium in which the concentration of methionine had been decreased to 30 mg./l. (iii) [2-^{14}C]-Uracil. This compound was used to label RNA before analysis of cell-free extracts by density-gradient centrifuging. The scale of the initial stages of the experiment was decreased so that 1·5 l. of cells were grown overnight. To label the RNA made before washing and starvation, 10 mg. of uracil/l. and 13 μc of [2-^{14}C]uracil/l. were included in the medium. Unlabelled uracil at the same concentration was included in the media used for washing and resuspension. In the converse type of experiment, RNA synthesized during methionine starvation was labelled by including 10 mg. of uracil/l. and 40 μc of [2-^{14}C]uracil/l. in the starvation medium.

For the determination of radioactivity, material precipitated by 5% (w/v) trichloroacetic acid at 0° was collected on Oxoid (Oxo Ltd.) membrane filters by using a stainless-steel filter tower (Tracerlab Inc.). The filters were gummed to aluminium planchets, dried over phosphorus pentoxide in a desiccator and counted for a minimum of 1000 counts with a thin-window gas-flow counter (Nuclear–Chicago Corp.). Samples prepared as described were 'infinitely thin'.

Chemicals. Radiochemicals were obtained from The Radiochemical Centre, Amersham, Bucks. Crystalline ribonuclease and deoxyribonuclease were from L. Light and Co. Ltd., and from the Nutritional Biochemicals Corp. respectively; crystalline bovine serum albumin was from the Armour Laboratories, Eastbourne.

RESULTS

Most of the results described below were obtained with the methionine-requiring mutant, 58–161, of *E. coli*. Since this strain was used so extensively it is referred to simply as 'the mutant' unless comparisons are being made with the second methionine-requiring mutant, H 239.

Accumulation of ribonucleic acid by starved cells. Fig. 1 shows changes in extinction and in the RNA, DNA and protein contents of cell suspensions of both of the methionine-requiring mutants, 58–161 and H 239, that had been grown in the presence of methionine, washed once and then resuspended in aerated methionine-free medium. *E. coli* H 239 synthesized no protein and no RNA during the incubation but there was an increase of about 30% in

DNA in 3 hr. *E. coli* 58–161 also synthesized no protein but the RNA content of the culture increased considerably and had doubled after 3 hr. DNA was also made during starvation and increased by about 25% in 3 hr.

Since about 50–85% of the RNA in bacterial cells is contained in their ribosomes (McQuillen, 1961), cell-free extracts of both these organisms were examined in the ultracentrifuge to see if any changes had taken place during the starvation.

Changes in schlieren diagrams. Figs. 2 (*a*) and 2 (*b*) compare schlieren diagrams of extracts prepared from starved cells of *E. coli* 58–161 at zero time and after starvation for 3 hr. The 50s and 30s ribosomes appear to be unaffected by starvation but two major changes have taken place in the diagrams. First, a very sharp boundary sedimenting at about 6s has appeared; this was completely removed by treating the extract with 5 μg. of deoxyribonuclease/ml. at room temperature and is therefore presumably caused by the DNA synthesized during starvation. A slight and rather variable shoulder (sedimentation coefficient about 6s) to the large slow-moving boundary was apparent in extracts that were prepared at zero time from the mutant 58–161. This shoulder was removed by similar treatment with deoxyribonuclease. DNA sedimenting in bacterial extracts as a very sharp boundary (the 'DNA-spike') at about 8s has been reported by other workers (Schachman, Pardee & Stanier, 1952; Siegel, Singer & Wildman, 1952). Treatment with deoxyribonuclease had no other effects on the schlieren diagrams. Secondly, additional material has appeared, with boundaries between the DNA-spike and the 30s ribosomes. At early times of centrifuging (Fig. 2*b*) this was visible as a single boundary, but later (Fig. 2*c*) consisted of two components having sedimentation coefficients, in these crude extracts, of about 14s and 16s.

Examination in the ultracentrifuge of extracts from cells incubated for periods up to 3 hr. showed that during this time the 14–16s material gradually accumulated. After incubation for 3 hr. little further RNA synthesis took place and after 4·5 hr. schlieren diagrams were similar to those after 3 hr. After starvation for 6 hr. some slight breakdown of

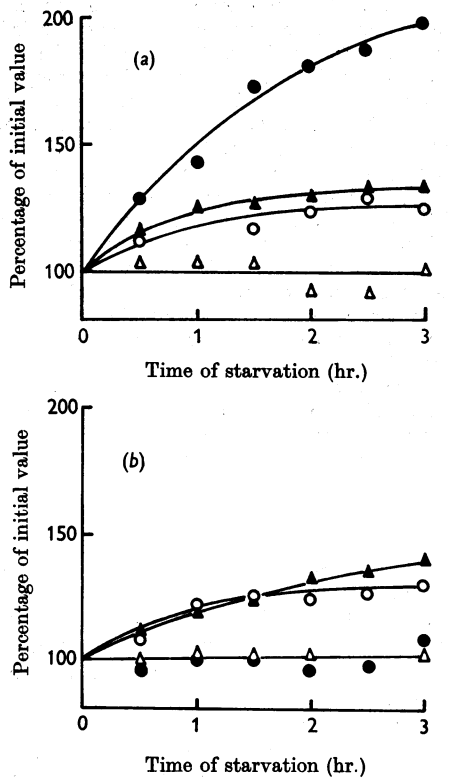

Fig. 1. Variation in protein (△), RNA (●) and DNA (○) contents, and the extinction at 540 mμ (▲) of cultures of two methionine-requiring *E. coli* mutants aerated in methionine-free medium at 30°. Experimental details are given in the text. Results are expressed as percentages of the values at zero time. (*a*) Mutant 58–161; (*b*) mutant H239.

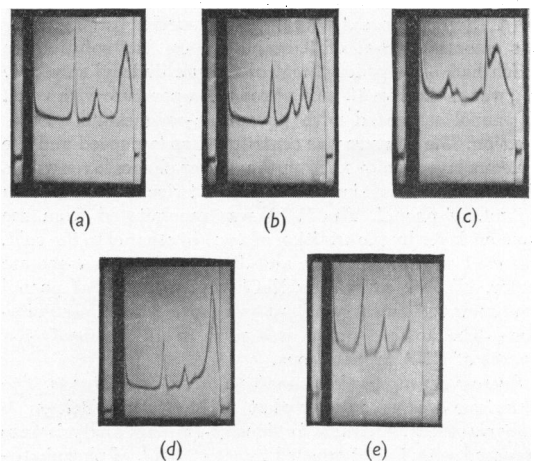

Fig. 2. Schlieren diagrams of cell-free extracts prepared from mutant 58–161 of *E. coli*. Sedimentation is to the left and was at 187 000 *g*. All photographs except (*c*) were taken after 16 min. Bar angles were: (*a*) and (*b*), 45°; (*c*), 35°; (*d*), 60°; (*e*), 40°. (*a*) Control: cells harvested before starvation; the leading boundaries are caused by 30s and 50s ribosomes. (*b*) Cells harvested after starvation for 3 hr. (*c*) As for (*b*) but after centrifuging for 48 min.; the ribosome boundaries have reached the bottom of the cell. (*d*) An extract giving a diagram similar to (*b*) but after treatment with ultrasonic vibrations for 4 min. (*e*) An extract giving a diagram similar to (*b*) but after incubation with ribonuclease (1 μg./ml.) at room temperature.

accumulated material apparently took place, although the 50s and 30s ribosomes were not appreciably different in concentration. Accordingly, a standard incubation time of 3 hr. was used to study accumulating material sedimenting at 14–16s.

Similar experiments were also carried out with the methionine-requiring mutant H 239. When extracts prepared from cells that had been incubated for 3 hr. in methionine-free medium were examined in the ultracentrifuge, a sharp boundary appeared, removed by treatment of the extracts with deoxyribonuclease; otherwise the schlieren diagrams were similar to those for extracts from unstarved cells. It therefore appeared likely that the material sedimenting at 14–16s that accumulated in the mutant 58–161 was associated with the RNA synthesized in the absence of protein synthesis. The rest of the work was therefore concerned with this mutant (*E. coli* 58–161) and with the material that accumulated during methionine-starvation.

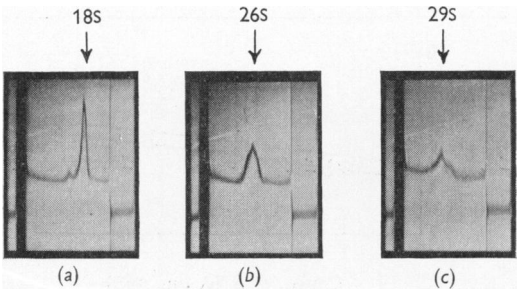

Fig. 3. Schlieren diagrams, after centrifuging for 16 min. at 187 000 *g*, for purified preparations of accumulated ribonucleoprotein in phosphate buffer with: (*a*) no magnesium acetate; (*b*) 5 mM-magnesium acetate; (*c*) 10 mM-magnesium acetate. Sedimentation is to the left. The bar angle was 45°. Numbers above the diagrams show sedimentation coefficients of boundaries at 20·0°.

Effect of ultrasonic vibrations and ribonuclease. When ultrasonic waves were passed through an extract containing accumulated material, there was a considerable decrease in the amount of accumulated material after 1 min.; after 4 min. the 14–16s particles had almost completely broken down, even though the 50s and 30s ribosomes were largely unaffected (Fig. 2*d*). The DNA-spike no longer appeared in sedimentation patterns of extracts so treated.

Accumulated material thus differs from the ribosomes in its stability to ultrasonic vibrations and further experiments showed that it also appeared to be more rapidly degraded by ribonuclease. This is shown in Fig. 2 (*e*): when an extract was incubated at room temperature with 1 μg. of ribonuclease/ml., there was no apparent change in the concentrations of the ribosomes but almost complete breakdown of accumulated material. The latter must therefore contain RNA but in a form more accessible to the enzyme than the RNA of ribosomal ribonucleoprotein. Free RNA is very sensitive to ribonuclease, but so are certain ribonucleoprotein particles that accumulate in cells treated with chloramphenicol or puromycin (Nomura & Watson, 1959; Turnock, White & Wild, 1962). Accordingly, the particles that accumulate during methionine-starvation were isolated and their composition was studied.

Isolation and composition of accumulated material. The method of differential centrifuging used to isolate accumulated material has been described above. A typical preparation is shown in Fig. 3 (*a*): resolution into two components was evident later in the centrifuge run and it was shown that isolated purified material was still sensitive to degradation by ribonuclease (1 μg./ml.).

Purified accumulated material was analysed for protein and RNA, and the results, together with similar analyses of 50s ribosomes, are given in Table 1. They show that accumulated material

Table 1. *Analyses of* 50s *ribosomes and accumulated material of* Escherichia coli *mutant* 58–161

The analyses were of preparations isolated and purified in separate experiments as described in the text. In Expts. 1 and 2, 50s ribosomes were obtained from cell-free extracts containing accumulated material.

Expt. no.	Preparation	RNA (mg./ml.)	Protein (mg./ml.)	Percentage composition	
				RNA	Protein
1	50s Ribosomes	1·12	0·86	57	43
2	50s Ribosomes	2·38	1·50	61	39
3	50s Ribosomes	1·42	0·98	58	42
4	50s Ribosomes	1·89	1·59	54	46
			Average	58	42
5	Accumulated material	3·36	2·50	57	43
6	Accumulated material	7·35	5·90	55	45
7	Accumulated material	4·28	3·00	59	41
			Average	57	43

contains both RNA and protein; further, the ratio of these two components is about the same as in 50s ribosomes. Only traces of DNA were found in accumulated material, which must therefore be ribonucleoprotein containing about 57 % of RNA.

Values of S^0_{20} for the two components of accumulated material were determined by measuring the sedimentation coefficients of purified material at different dilutions (Fig. 4). Values of 20s and 23s were obtained.

Further examination of the ribonucleic acid and protein of accumulated ribonucleoprotein. The material that accumulated during methionine-starvation was therefore present as ribonucleoprotein particles of about the same composition as 50s ribosomes, but differing from them in sensitivity to ribonuclease and ultrasonic vibrations as well as in sedimentation coefficients. The problem now arose as to whether the RNA and protein of the accumulated ribonucleoprotein had any resemblance to the RNA and protein of the ribosomes.

(*a*) Ribonucleic acid. RNA isolated from 30s ribosomes has a sedimentation coefficient, S^0_{20}, of 16s; RNA from 50s ribosomes appears to sediment as two species, having sedimentation coefficients of 16s and 23s (Kurland, 1960). Thus, when RNA was isolated from a cell-free extract of the mutant grown in the presence of methionine, three species of RNA were seen in schlieren diagrams. The two faster-moving components corresponded to the ribosomal RNA, the slow-moving component to the 'transfer' RNA, of the cells. When RNA was isolated from starved cells that contained accumulated ribonucleoprotein, no additional boundaries were seen. This suggested that the RNA contained in accumulated material is 'ribosomal' as far as its sedimentation properties are concerned. This was confirmed in experiments in which RNA was isolated from purified preparations of accumulated ribonucleoprotein. Two components were seen in schlieren diagrams; their sedimentation coefficients, S^0_{20}, are 16s and 23s (Fig. 5). These values compare well with the values obtained by Kurland (1960) for ribosomal RNA and with values of 17s and 24s that were obtained in the present work when RNA was isolated from a purified 50s-ribosome preparation.

(*b*) Protein. The cyst(e)ine and methionine content of protein from accumulated material was determined. These two amino acids were chosen for two reasons. First, comparisons of the cysteine and methionine contents of accumulated material, 50s ribosomes and non-ribosomal protein could readily be made by experiments with ^{35}S-labelled materials; secondly, ribosomes have been reported to have a low content of cysteine (Roberts, Britten & Bolton, 1958; Spahr, 1962); a low methionine content has also been reported by Roberts *et al.* (1958) although this has not been confirmed. Since this particular mutant requires methionine for growth, it was of additional interest to see whether the protein associated with the accumulated material is deficient in this amino acid.

The cysteine contents of ribosomal protein, accumulated ribonucleoprotein and 'soluble' (i.e. non-ribosomal) protein in cell-free extracts were separately determined by growing, washing and starving the cells in the 'low-sulphate' medium containing [^{35}S]sulphate. Under these conditions only the cysteine of protein becomes radioactive. The different fractions were isolated in the same experiment and their specific radioactivities compared. Table 2 shows that ribosomal protein contains about 52 % of the cysteine in 'soluble' protein and that accumulated ribonucleoprotein has a similar (57 %) low cysteine content. Table 2 also gives the radioactivity in the different fractions when the cells were grown, before washing and starvation, in a medium containing [^{35}S]methionine: under these conditions, little radioactive cysteine should be formed (Roberts, Abelson, Cowie, Bolton & Britten, 1955). The results show that the frac-

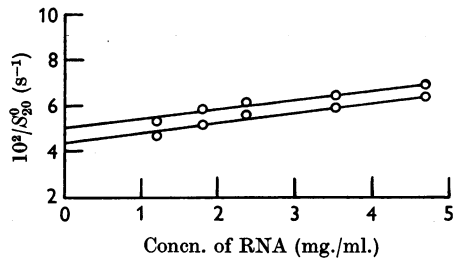

Fig. 4. Determination of values of S^0_{20} for the two components of accumulated ribonucleoprotein. A purified preparation of accumulated material was made as described in the text and different dilutions were centrifuged at 187 000 *g* and 20·0°. Sedimentation coefficients were calculated at each dilution. Values of 20s and 23s are given by extrapolation to zero concentration.

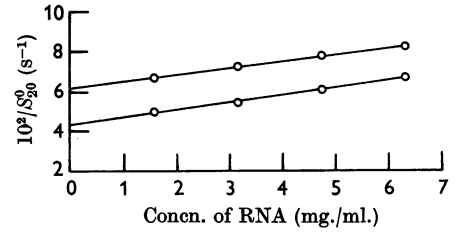

Fig. 5. Determination of values of S^0_{20} for the two RNA components obtained from accumulated ribonucleoprotein. RNA was isolated from a purified preparation of accumulated ribonucleoprotein and sedimentation coefficients determined at a series of dilutions by centrifuging at 187 000 *g* and 20·0°. Values of 16s and 23s are given by extrapolation to zero concentration.

Table 2. *Specific radioactivity of protein in fractions from cell-free extracts of* Escherichia coli *mutant* 58–161

Cells were grown in the presence of either [^{35}S]sulphate or [^{35}S]methionine (10 μc/l.), and cell-free extracts were made after washing and starvation in the absence of methionine. The extracts were centrifuged, as described in the text, to give preparations of 50s ribosomes, accumulated ribonucleoprotein and a 'soluble' fraction, containing non-ribosomal protein. Radioactivity measurements were on 5% (w/v) trichloroacetic acid-insoluble precipitates. Growth in a medium containing [^{35}S]sulphate will label the cyst(e)ine in protein.

Fraction	Specific radioactivity (counts/min./mg. of protein)	
	Growth on [^{35}S]-sulphate	Growth on [^{35}S]-methionine
50s Ribosomes	590	8200
Accumulated ribonucleoprotein	650	8700
'Soluble' fraction	1140	9100

tions do not differ significantly in their methionine content and, in particular, that the protein that accumulates in ribonucleoprotein particles when the cells are starved of methionine contains a normal methionine complement.

Experiments with labelled ribonucleic acid. Although the material that accumulates during starvation is ribonucleoprotein, it remained to be decided whether it contained most of the RNA synthesized during starvation and also whether there was any appreciable synthesis of 30s and 50s ribosomes during this period. Accordingly, extracts were prepared from cells that had been exposed to [2-^{14}C]uracil during starvation, thus labelling the RNA made during this time, and the cell-free extracts were centrifuged in a sucrose-density-gradient. Fig. 6 (*a*) shows the result of an experiment in which sedimentation in the gradient was for 165 min. Little radioactivity was associated with the 50s ribosomes and, although the 30s ribosomes were not resolved from accumulated material, the radioactivity in this region was associated with material sedimenting slowly. The radioactivity associated with material that has sedimented very little shows that during starvation a considerable amount of 'transfer' RNA is also synthesized. Attempts were made to resolve the 30s ribosomes from accumulated material by allowing a longer time (265 min.) for sedimentation. These were only partially successful. Fig. 6 (*c*) does, however, again show the association of radioactivity in this region with material that sediments relatively slowly and suggests that little synthesis of 30s ribosomes took place during starvation.

This was confirmed by treating a portion of the extract used in the experiment of Fig. 6 (*a*) with ribonuclease (1 μg./ml.) and then carrying out a further density-gradient centrifuging: examination of this extract in an analytical cell showed that ribonuclease treatment had almost completely removed the accumulated material without any appreciable effect on the ribosomes. The result (Fig. 6*b*) of the analysis of the fractions from the density gradient shows that there is little radioactivity in the ribosomes and therefore that no appreciable ribosome synthesis took place during starvation.

From these experiments it appears that almost all the RNA synthesized during starvation (except that associated with 'transfer' RNA) appears in accumulated material and that there is very little synthesis of the ribosomes. There still remained the possibility, however, that during starvation some breakdown of the ribosomes might occur to give material sedimenting with accumulated ribonucleoprotein. The result of the experiment shown in Fig. 7 suggests that this breakdown does not take place to any considerable extent. Cells were labelled with [^{14}C]uracil before they were starved in non-radioactive medium. The ribosomes were therefore radioactive and, if they were degraded, radioactivity should have appeared in accumulated material. There was little radioactivity in these fractions and it is apparent that no appreciable breakdown of the ribosomes took place.

Effect of magnesium ions. If the Mg^{2+} ion concentration in a bacterial extract is increased by the addition of magnesium salts, then aggregation of 50s and 30s ribosomes occurs, and ribosomes sedimenting at 70s are formed. At very high Mg^{2+} ion concentrations, ribosomes sedimenting at 100s may be formed by the aggregation of two 70s particles (Tissières *et al.* 1959).

Fig. 8 (*a*) shows the schlieren diagram of a cell-free extract that contained accumulated material and was prepared in phosphate buffer with no added Mg^{2+} ions. When the Mg^{2+} ion concentration of this extract was raised to 5 mM by the addition of magnesium acetate some few 70s ribosomes were formed, but in addition the accumulated ribonucleoprotein now sedimented much closer to and was less well resolved from the 30s ribosomes (Fig. 8*b*). This effect was enhanced with 10 mM-magnesium acetate (Fig. 8*c*). Similar effects were observed when, instead of adding magnesium salts to cell-free extracts, the extracts were initially prepared with buffer containing high Mg^{2+} ion concentrations.

The behaviour of purified accumulated ribonucleoprotein at different Mg^{2+} ion concentrations was also investigated. The appearance in schlieren diagrams of the material in phosphate buffer with no magnesium acetate present is shown in Fig. 3 (*a*): at

a concentration of 5 mM (Fig. 3*b*) the boundary associated with these components was broader and, as the concentration was raised, the accumulated ribonucleoprotein became more and more polydisperse, until at a concentration of about 10 mM (Fig. 3*c*) precipitation from solution began to take place.

Stability of accumulated ribonucleic acid. If *E. coli* is treated with chloramphenicol, protein synthesis is greatly diminished or stopped, RNA synthesis continues and the cells accumulate RNA, some of which appears in the cells as ribonucleoprotein particles sedimenting in crude extracts at about 16–18s (Nomura & Watson, 1959; Dagley & Sykes, 1959). The RNA accumulated by the cells under these conditions is unstable in that it is rapidly degraded when they are resuspended in buffer solutions (Neidhardt & Gros, 1957); during this period the 16–18s particles are broken down (Turnock *et al.* 1962).

In the experiment whose results are shown in Fig. 9, starved cells of the mutant containing accumulated ribonucleoprotein were resuspended in buffer (73 mM-potassium dihydrogen phosphate brought to pH 7·0 with 5N-sodium hydroxide); there was only a slight decrease in the RNA content of the cells in the first 0·5 hr.; the RNA concentration then remained constant, and comparison in the ultracentrifuge of extracts prepared from cells at zero time and after incubation for 2 hr. showed no change in the content of accumulated material or 50s ribosomes; some degradation of the 30s ribo-

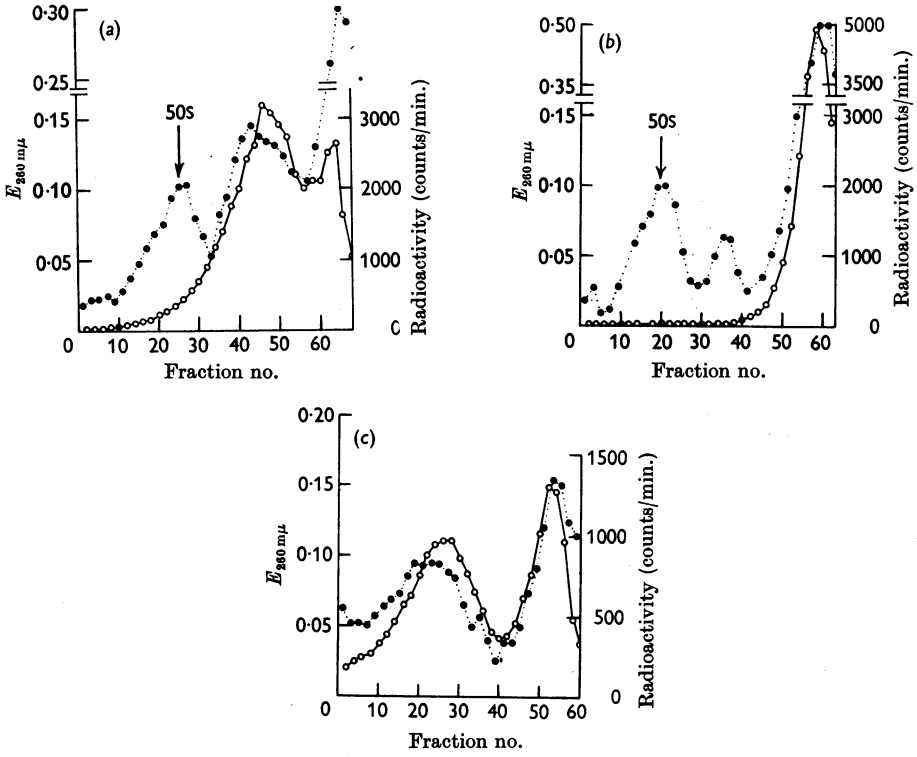

Fig. 6. Density-gradient analysis of extracts containing accumulated ribonucleoprotein prepared from mutant cells incubated with [2-^{14}C]uracil during starvation for 3 hr. in a methionine-free medium. The general procedure is described in the text. 4% (w/v) Sucrose solution (0·07 ml.) and extract (0·07 ml.) were layered on top of 5·0 ml. of sucrose in phosphate–magnesium acetate solution in a plastic centrifuge tube. The sucrose was in a linear gradient from 5% (w/v) at the top of the tube to 20% (w/v) at the bottom. After ultracentrifuging in the SW39 rotor at 124 000 *g* and 4°, the bottom of the tube was pierced and alternate 5-drop fractions were collected into phosphate–magnesium acetate solution (2·0 ml.) for measurement of extinction at 260 mμ (●) and into 3·0 ml. of 5% (w/v) trichloroacetic acid for measurement of the radioactivity of the precipitate (○). (*a*) Extract centrifuged for 165 min. (*b*) A portion of the extract that gave (*a*) was incubated with ribonuclease (1 μg./ml.) at room temperature; accumulated ribonucleoprotein was removed by this treatment and a sample (0·07 ml.) was then centrifuged for 165 min. (*c*) An extract centrifuged for 265 min.; the 50s ribosomes sedimented to the bottom of the centrifuge tube and were not collected.

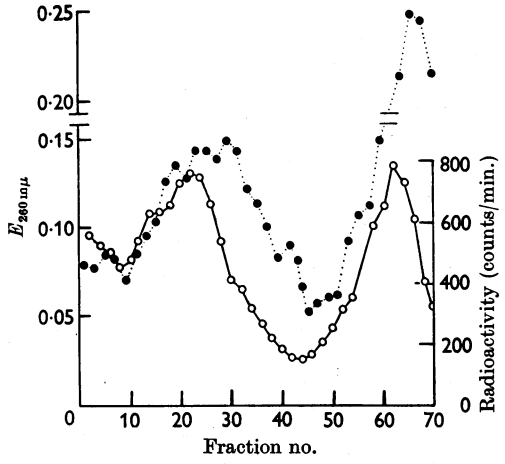

Fig. 7. Density-gradient analysis of an extract containing accumulated ribonucleoprotein prepared from mutant cells grown in a medium containing [2-^{14}C]uracil and then starved for 3 hr. in a methionine-free medium with no added radioactivity. The general procedure is described in the text. 4% (w/v) Sucrose solution (0·07 ml.) and extract (0·07 ml.) were layered on top of 5·0 ml. of sucrose in phosphate–magnesium acetate solution in a plastic centrifuge tube. The sucrose was in a linear gradient from 5% (w/v) at the top of the tube to 20% (w/v) at the bottom. Ultracentrifuging in the SW39 rotor was for 265 min. at 124000g and 4°. After centrifuging, the bottom of the tube was pierced and alternate 5-drop fractions were collected into phosphate–magnesium acetate solution (2·0 ml.) for measurement of extinction at 260 mμ (●) and into 3·0 ml. of 5% (w/v) trichloroacetic acid for measurement of the radioactivity of the precipitate (○). The 50s ribosomes sedimented to the bottom of the centrifuge tube and were not collected.

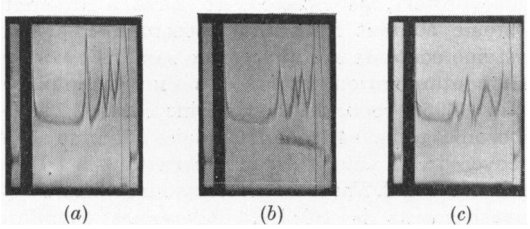

Fig. 8. Schlieren diagrams, after centrifuging for 12 min. at 187 000g, for cell-free extracts containing accumulated ribonucleoprotein in phosphate buffer with: (*a*) no magnesium acetate; (*b*) 5 mM-magnesium acetate; (*c*) 10 mM-magnesium acetate. Sedimentation is to the left. The bar angle was 45°.

somes had taken place. Thus accumulated ribonucleoprotein particles are stable under conditions in which rather similar particles produced by chloramphenicol action break down.

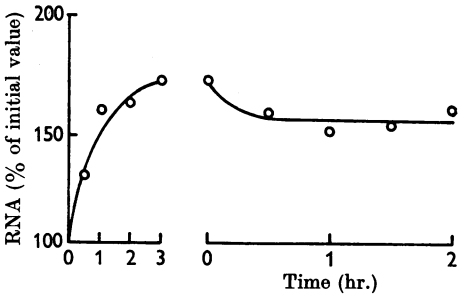

Fig. 9. Stability of the RNA accumulated by mutant cells. Cells were allowed to accumulate RNA by starvation for 3 hr. in methionine-free medium; they were then harvested and resuspended in the same volume of 73 mM-KH_2PO_4 brought to pH 7·0 with 5N-NaOH. RNA and protein determinations were made on 5·0 ml. samples as described in the text. There was no significant change in the protein content of the culture throughout the experiment. Changes in RNA are expressed as percentages of the value before starvation.

DISCUSSION

The normal behaviour of a bacterial mutant that requires an amino acid for growth and is starved of this amino acid is shown by the methionine auxotroph *E. coli* H239: no protein and no RNA are synthesized and the DNA content of the culture rises by about 30%. A similar increase in DNA has been observed, for example, during the starvation of a leucine auxotroph of *E. coli* K12 (Goldstein, Goldstein, Brown & Chou, 1959) and also when other strains of *E. coli* are placed in nitrogen-free minimal medium. This is about the increase that would be expected merely from the completion of already initiated cycles (Schaechter, 1961). The increase in DNA of about 25% found in the methionine auxotroph *E. coli* 58–161 is therefore not abnormal. What is abnormal, however, is the ability of mutant 58–161 to synthesize RNA in the absence of protein synthesis. This behaviour has been known for some time, and Borek and coworkers (Borek *et al.* 1955; Borek, Rockenbach & Ryan, 1956; Borek & Ryan, 1958; Mandel & Borek, 1961*a*, *b*; Fleissner & Borek, 1962) have examined some of the properties of this strain and of the RNA that is made in the absence of protein synthesis. They found that the 'transfer' RNA, which accounts for a considerable part of the RNA synthesized during methionine-starvation, lacks the methylated purines and pyrimidines that are normally present as minor components. The fact that this 'transfer' RNA may be partially nonfunctional may account for the slow rate at which protein synthesis resumes when methionine is added back to starved cultures.

The present results show that ribonucleoprotein particles, distinct from the 30s and 50s ribosomes and from 'transfer' RNA, are formed in the cells during starvation. Two new components appear in schlieren diagrams of cell-free extracts: their sedimentation coefficients, S^0_{20}, are 20s and 23s. It seems clear that most of the RNA contained in this 20–23s material is that synthesized during starvation; accordingly, under our experimental conditions, the supposition of Fleissner & Borek (1962) that most of the newly made RNA sediments with 'transfer' RNA is not correct. In the experiment in Fig. 6 (*a*), for example, about 70 % of the RNA synthesized during starvation for 3 hr. appears in components other than 'transfer' RNA.

It is difficult to exclude the possibility that a small proportion of the ribonucleoprotein that appears in ultracentrifuge diagrams during starvation is derived from pre-existing ribosomes, although there are several indications that this conversion does not take place to any considerable extent. When the RNA of the cells was labelled before they were starved in a medium free from radioactivity, little radioactivity entered that region of the gradient occupied by accumulated material (Fig. 7). Further, during starvation few ribosomes are synthesized and their concentrations as revealed by the peaks in the schlieren diagrams do not decline significantly. Nakada & Smith (1962) showed that, when the methionine-requiring mutant of *E. coli* K 12–4000 is starved of methionine, neither synthesis of new ribosomes nor turnover of ribosomal RNA and protein occurs. Similar conditions may exist in the mutant we have studied.

The changes in schlieren diagrams observed with the mutant are very similar to those found when *E. coli* is incubated with chloramphenicol or puromycin. Both these antibiotics greatly decrease protein synthesis, while permitting RNA synthesis to continue at a more nearly normal rate: schlieren diagrams show that with puromycin two components appear which sediment in crude extracts at about 14–18s (Dagley, White, Wild & Sykes, 1962*b*). With chloramphenicol, new ribonucleoprotein, sensitive both to ribonuclease and to ultrasonic vibrations, appears, which has been variously described as sedimenting in crude extracts as a number of components at about 14–18s (Dagley & Sykes, 1959) or as one component resolving into two when the concentration of the material is greatly decreased. These two components have sedimentation coefficients, S^0_{20}, of 18s and 25s. The RNA isolated from them sediments at 16s and 23s (Kurland, Nomura & Watson, 1962). Our results show that the shape of the boundaries and the sedimentation coefficients of the ribonucleoprotein accumulated by the mutant are sensitive to alterations in the concentration of Mg^{2+} ions; similar effects have been observed with the ribonucleoprotein accumulated by chloramphenicol-treated cells (Nomura & Watson, 1959).

In spite of the general similarity between the particles produced during the action of chloramphenicol and methionine-starvation, there are significant differences. The RNA accumulated by chloramphenicol-treated cells is rapidly degraded when they are incubated in buffer: the 'chloramphenicol particles' also disappear. The ribonucleoprotein accumulated by the mutant is stable under these same conditions, as are the particles formed in puromycin treatment (Turnock *et al.* 1962). The reasons for this difference are not clear.

The ribonucleoprotein accumulating in the mutant also appears to differ in composition from that formed in chloramphenicol-treated cells. Nomura & Watson (1959) reported that chloramphenicol particles contain 75 % of RNA and 25 % of protein: Dagley & Sykes (1960) similarly found that particles accumulated under chloramphenicol action contained more RNA and less protein than ordinary ribosomes. The composition of the material studied in the present paper, on the other hand, is apparently nearly the same as that of the 50s ribosomes, which contain 58 % of RNA and 42 % of protein. Ribonucleoprotein particles can readily absorb additional protein (Petermann & Pavlovec, 1961) and it would therefore be possible for protein to become bound to the particles from the mutant during the procedures for its isolation, so giving it an apparently 'ribosomal' composition. Were this to have happened, unless this protein was derived from ribosomes degraded during the isolation procedures, it would be expected to have the cysteine content of non-ribosomal protein, and the ribonucleoprotein, as isolated, to have a different cysteine content from the ribosomes. That the cysteine contents are almost the same is evidence that contamination of this sort has not taken place. Spahr (1962) reported the cysteine content of 50s ribosomes to be 0·49 mole/100 moles of amino acids recovered: his value for cell supernatant is 1·10, a ratio of 1·0:2·2. The ratio given by our radioactivity measurements is 1·0:1·9. Ribosomes therefore appear to be relatively deficient in cysteine, although not as deficient as originally reported (Roberts *et al.* 1958).

The origin of the protein of ribonucleoprotein particles accumulating during methionine-starvation presents unsolved problems, since it appears under conditions in which no net synthesis of protein is taking place. The same situation arises when cells are incubated with chloramphenicol; the accumulation of ribonucleoprotein is here accompanied by a considerable breakdown of the 30s ribosomes, but it has been calculated that this

breakdown will release only about half the protein that is associated with accumulated material (Kurland & Maaløe, 1962). In the mutant there appears to be no appreciable breakdown of the ribosomes during starvation, and the particles that accumulate appear to contain more protein than those produced by chloramphenicol action. This protein might be provided by the 'turn-over' taking place in starved cells (Mandelstam, 1960), although Kurland & Maaløe (1962) suggested that cells may contain a pool of ribosomal protein that could be used for this purpose.

There is as yet little evidence whether the material that accumulates in methionine-starvation, or under the action of chloramphenicol or puromycin, is also present in the cells at a much lower concentration under normal conditions; however, it has been suggested, both by Kurland *et al.* (1962) and by Dagley *et al.* (1962*b*), that this ribonucleoprotein may be closely related to precursors of the ribosomes. The particles produced by chloramphenicol action and by the mutant contain RNA having the same sedimentation coefficients as ribosomal RNA, and the protein of the particles in the mutant has a 'ribosomal' cysteine content. When cells are washed free from chloramphenicol they recover, and in sedimentation patterns the accumulated material is replaced by a large single boundary due to 30s ribosomes. A similar situation seems to be found when methionine is added back to starved cultures of the mutant: as protein synthesis resumes, the boundaries due to accumulated material diminish and there is an increase in area of the peak due to the 50s ribosomes (Dagley *et al.* 1962*b*). These experiments suggest, but do not prove, a precursor–product relationship between accumulated material and the ribosomes.

If growing bacteria are exposed to [^{14}C]uracil or [^{32}P]phosphate for a short time, the bulk of the radioactivity very rapidly enters RNA-containing material which is rather heterogeneous but which sediments at about 14s. At longer times, radioactivity appears in the ribosomes (McCarthy *et al.* 1962). Midgley & McCarthy (1962) showed that this 'pulse-labelled' RNA appears to contain components of two types: one, in which the base composition of the RNA approximates to that of the DNA of the cell (with thymine replaced by uracil) can possibly be equated with 'messenger' RNA (Jacob & Monod, 1961); the other, in which the base composition is that of the ribosomes, appears to contain ribosome precursors, which have been given the name 'eosomes' (McCarthy *et al.* 1962). The material accumulating in the mutant is similar to pulse-labelled RNA-containing material in its sensitivity to ribonuclease and its behaviour when magnesium salts are added to cell-free extracts; however, the sedimentation coefficients of accumulated ribonucleoprotein are rather larger than those of pulse-labelled material, and the RNA isolated from the latter has a sedimentation coefficient of 8s (Gros *et al.* 1961; McCarthy *et al.* 1962) in contrast with the 16s and 23s components that we have found in the accumulated material. If this ribonucleoprotein represents a stage in the biosynthesis of the ribosomes, it may represent a later and more ill-defined stage than the production of eosomal material.

SUMMARY

1. Cells of the methionine-requiring mutant, 58–161, of *Escherichia coli* synthesized ribonucleic acid, but not protein, when incubated in methionine-free medium. During starvation material appeared in the cells which was detectable when crude cell-free extracts were examined in the analytical ultracentrifuge.

2. The material that accumulated was more sensitive to degradation by ultrasonic vibrations and ribonuclease than were the ribosomes. It was isolated and shown to be ribonucleoprotein, sedimenting as two components with S^0_{20} values of 20s and 23s.

3. Ribonucleic acid isolated from accumulated ribonucleoprotein had S^0_{20} values of 16s and 23s. The protein had the same low cysteine content as had 50s ribosomes.

4. Accumulated ribonucleoprotein particles contained ribonucleic acid synthesized during starvation. They did not contain appreciable amounts of ribonucleic acid derived from breakdown of the ribosomes. During starvation, little synthesis of the ribosomes took place.

5. The effects of the addition of magnesium salts to cell-free extracts containing accumulated material was investigated.

6. When starved cells were incubated in buffer, little ribonucleic acid was lost and the accumulated ribonucleoprotein particles were stable.

7. The relationship of the ribonucleoprotein that accumulates during methionine-starvation to the ribonucleic acid synthesized during treatment of *E. coli* with chloramphenicol or puromycin and to the ribonucleic acid normally present in the cells is discussed.

We thank the Department of Scientific and Industrial Research for the award of a Research Studentship (to G.T.).

REFERENCES

Alfoldi, L., Stent, G. S. & Clowes, R. C. (1962). *J. molec. Biol.* **5**, 348.

Aronson, A. I. & Spiegelman, S. (1961*a*). *Biochim. biophys. Acta*, **53**, 70.

Aronson, A. I. & Spiegelman, S. (1961*b*). *Biochim. biophys. Acta*, **53**, 84.

Ashwell, G. (1957). In *Methods in Enzymology*, vol. 3, p. 90. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Borek, E., Rockenbach, J. & Ryan, A. (1956). *J. Bact.* **71**, 318.
Borek, E. & Ryan, A. (1958). *J. Bact.* **75**, 72.
Borek, E., Ryan, A. & Rockenbach, J. (1955). *J. Bact.* **69**, 460.
Britten, R. J. & Roberts, R. B. (1960). *Science*, **131**, 32.
Burton, K. (1956). *Biochem. J.* **62**, 315.
Dagley, S. & Sykes, J. (1959). *Nature, Lond.*, **183**, 1608.
Dagley, S. & Sykes, J. (1960). *Biochem. J.* **74**, 11P.
Dagley, S., Turnock, G. & Wild, D. G. (1962*a*). *Biochem. J.* **83**, 3P.
Dagley, S., White, A. E., Wild, D. G. & Sykes, J. (1962*b*). *Nature, Lond.*, **194**, 4823.
Fleissner, E. & Borek, E. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1199.
Goldstein, A., Goldstein, D. B., Brown, B. J. & Chou, S. (1959). *Biochim. biophys. Acta*, **36**, 163.
Gros, F., Hiatt, H., Gilbert, W., Kurland, C. G., Risebrough, R. W. & Watson, J. D. (1961). *Nature, Lond.*, **190**, 581.
Hahn, F. E., Schaechter, M., Cezlowski, W. S., Hopps, H. E. & Ciak, S. (1957). *Biochim. biophys. Acta*, **26**, 469.
Hughes, D. E. (1951). *Brit. J. exp. Path.* **32**, 97.
Jacob, F. & Monod, J. (1961). *J. molec. Biol.* **3**, 318.
Kurland, C. G. (1960). *J. molec. Biol.* **2**, 83.
Kurland, C. G. & Maaløe, O. (1962). *J. molec. Biol.* **4**, 193.
Kurland, C. G., Nomura, M. & Watson, J. D. (1962). *J. molec. Biol.* **4**, 388.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall R. J. (1951). *J. biol. Chem.* **193**, 265.
McCarthy, B. J., Britten, R. J. & Roberts, R. B. (1962). *Biophys. J.* **2**, 57.
McQuillen, K. (1961). In *Protein Biosynthesis*, p. 263. Ed. by Harris, R. J. C. London: Academic Press (Inc.) Ltd.
Mandel, L. R. & Borek, E. (1961*a*). *Biochem. biophys. Res. Commun.* **4**, 14.
Mandel, L. R. & Borek, E. (1961*b*). *Biochem. biophys. Res. Commun.* **6**, 138.
Mandelstam, J. (1960). *Bact. Rev.* **24**, 289.
Midgley, J. E. M. & McCarthy, B. J. (1962). *Biochim. biophys. Acta*, **61**, 696.
Nakada, D. & Smith, I. (1962). *Biochim. biophys. Acta*, **61**, 414.
Neidhardt, F. C. & Gros, F. (1957). *Biochim. biophys. Acta*, **25**, 513.
Nomura, M. & Watson, J. D. (1959). *J. molec. Biol.* **1**, 204.
Pardee, A. B. & Prestridge, L. S. (1956). *J. Bact.* **71**, 677.
Petermann, M. L. & Pavlovec, A. (1961). *J. biol. Chem.* **236**, 3235.
Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T & Britten, R. J. (1955). *Publ. Carneg. Instn, no.* 607: *Studies in Biosynthesis in* Escherichia coli.
Roberts, R. B., Britten, R. J. & Bolton, E. T. (1958). *Microsomal Particles and Protein Synthesis*, p. 84. Ed. by Roberts, R. B. Washington: Washington Academy of Sciences.
Schachman, H. K., Pardee, A. B. & Stanier, R. Y. (1952). *Arch. Biochem. Biophys.* **38**, 245.
Schaechter, M. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **26**, 53.
Schneider, W. C. (1957). In *Methods in Enzymology*, vol. 3, p. 680. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Siegel, A., Singer, S. J. & Wildman, S. G. (1952). *Arch. Biochem. Biophys.* **41**, 278.
Sols, A. (1947). *Nature, Lond.*, **160**, 89.
Spahr, P. F. (1962). *J. molec. Biol.* **4**, 395.
Spahr, P. F. & Tissières, A. (1959). *J. molec. Biol.* **1**, 237.
Stent, G. S. & Brenner, S. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 2005.
Stickland, L. H. (1951). *J. gen. Microbiol.* **5**, 698.
Takeda, Y., Hayashi, S.-I., Nakagawa, H. & Suzuki, F. (1960). *J. Biochem., Tokyo*, **48**, 169.
Tissières, A., Watson, J. D., Schlessinger, D. & Hollingworth, B. R. (1959). *J. molec. Biol.* **1**, 221.
Turnock, G., White, A. E. & Wild, D. G. (1962). *Biochem. J.* **84**, 107P.
Yarmolinski, M. D. & de la Haba, G. L. (1959). *Proc. nat. Acad. Sci., Wash.*, **44**, 189.

Biochem. J. (1963) **88**, 566

The Metabolism of Glutamate in Homogenates and Slices of Brain Cortex

By R. J. HASLAM* and H. A. KREBS
Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford

(*Received* 18 *March* 1963)

Glutamate is a highly active metabolite in the cerebral cortex. It can be converted into α-oxoglutarate by transamination or dehydrogenation; it can be decarboxylated to γ-aminobutyrate, and its carbon skeleton can be synthesized from glucose and intermediates of glucose metabolism (see Waelsch & Lajtha, 1961; Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari, 1955; Kini & Quastel, 1959; Chain, Cohen & Pocchiari, 1962; Sellinger, Catazaro, Chain & Pocchiari, 1962; Krebs & Bellamy,

* Present address: Sir William Dunn School of Pathology, University of Oxford.