Ribonucleic Acid Synthesis by *Escherichia coli* C 3000/L after Infection by the Ribonucleic Acid Coliphage ZIK/1, and Properties of the Coliphage-Induced Double-Stranded Ribonucleic Acid

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1. The efficiency of extracting nucleic acids from *Escherichia coli* after five methods of obtaining cell lysis was determined. 2. The recovery of various nucleic acid species isolated after chromatography on methylated albumin-coated kieselguhr was also examined. 3. Double-stranded coliphage-induced RNA was isolated from infected bacteria and its resistance to ribonuclease digestion under various conditions determined. 4. The involvement of double-stranded RNA during the infection process was demonstrated. 5. The time-course of the syntheses in infected cells of double-stranded RNA, DNA, single-stranded coliphage and 16s ribosomal RNA, transfer RNA and ribosomal 23s RNA was examined. 6. It was demonstrated that the syntheses of DNA, transfer RNA and ribosomal RNA decreased 10–15min. after infection. 7. Synthesis of coliphage RNA commenced 10–15min. after infection and double-stranded RNA was also synthesized from about 10min. after coliphage adsorption.

It has recently been demonstrated that infection of male strains of *Escherichia coli* by single-stranded RNA bacteriophages (RNA coliphages) results in the formation of double-stranded RNA that is resistant to ribonuclease and, in the period immediately after adsorption, is composed of the parental phage RNA and a newly synthesized complementary strand (Weissmann & Borst, 1963; Weissmann, Borst, Burdon, Billeter & Ochoa, 1964*a*; Kelly & Sinsheimer, 1964; Erikson, Fenwick & Franklin, 1964; Kaerner & Hoffmann-Berling, 1964; Fenwick, Erikson & Franklin, 1964; Nonoyama & Ikeda, 1964; Kelly, Gould & Sinsheimer, 1965). Replication of the phage RNA involves an asymmetric semi-conservative mechanism whereby the parental RNA strand is replaced by an identical newly synthesized RNA (Weissmann, Borst, Burdon, Billeter & Ochoa, 1964*b*). Repetition of these processes results in an overall increase in not only parental phage RNA but also the double-stranded RNA (Weissmann *et al.* 1964*a*).

The recently isolated RNA coliphage ZIK/1 has been shown to differ from other RNA coliphages both in the characteristics of its RNA (Bishop & Bradley, 1965; Bishop, 1965) and in the composition of its coat protein (D. H. L. Bishop & M. Birnstiel, unpublished work). It has been found that cell division after adsorption of the RNA coliphage ZIK/1 continued for 40min. and cell lysis, which commenced 20min. later, was half complete 80min. after adsorption and 90–95% complete 40min. later (Bishop, 1965). Also, it was demonstrated that ribosome synthesis decreased 10–15min. after coliphage adsorption, bacteriophage RNA was synthesized 10–15min. after adsorption and intracellular bacteriophages were present 25min. later. Owing to the method of investigation it was not possible to demonstrate the presence of double-stranded RNA in infected cells.

The present paper demonstrates the involvement of double-stranded RNA during the infection of bacteria by coliphage ZIK/1 and also examines the time-course, during the infection process, of the syntheses of double-stranded RNA and bacterial DNA, as well as of single-stranded phage RNA, bacterial 23s ribosomal RNA and s-RNA.* MAK was used to separate the RNA and DNA species (Mandell & Hershey, 1960; Nonoyama & Ikeda, 1964). Some of the properties of the double-stranded RNA were investigated.

METHODS AND MATERIALS

Organisms. Small- and large-scale culture procedures for *E. coli* C3000/L and the RNA coliphage ZIK/1 have

* Abbreviations: s-RNA, 'soluble' or transfer RNA; MAK, methylated-albumin-coated kieselguhr.

been described previously (Bishop & Bradley, 1965; Bishop, 1965). Bacterial counts were determined from E_{600} of the suspension (as described by Bishop, 1965) in conjunction with an empirically derived graph of E_{600} plotted against the viable bacteria count.

Incorporation of [2-^{14}C]*uracil or* [2-^{14}C]*thymine into bacteria and coliphage* ZIK/1. Bacteria were inoculated into 500ml. of 2·4% (w/v) nutrient broth (Oxo Ltd., London, S.E. 1) and cultured at 37° with shaking until a concentration of 3×10^8 cells/ml. was reached. Then 5ml. of sterile 0·1M-$CaCl_2$ solution and 10ml. of a broth suspension of coliphages (15×10^{11} plaque-forming units, equivalent to ten times the concentration of bacteria; i.e. multiplicity of infection 10) were added and the culture was reincubated for 15min., at which time 0·1mc of [2-^{14}C]-uracil (specific radioactivity 40·6mc/m-mole; The Radiochemical Centre, Amersham, Bucks.) was added. The culture was either harvested after a further 35min. incubation (at the onset of lysis) or after a further 110min. (when lysis was complete). In the former case the bacteria were harvested by centrifugation at 15000*g* for 15min. (at 4°) in the Servall model SS–4 centrifuge, and washed by recentrifugation after resuspension in 50ml. of 2·4% (w/v) nutrient broth containing unlabelled uracil (0·1%). The bacteria were finally suspended at a concentration of $2·5 \times 10^8$ cells/ml. in TM1 buffer [0·01M-tris–HCl buffer (pH7·2)–1·0mM-$MgCl_2$] containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%), and the nucleic acids were extracted.

The ^{14}C-labelled coliphages were prepared from the bacterial lysate in a manner similar to that described by Bishop (1965). *E. coli* C3000/L was inoculated into 500ml. of nutrient broth and grown at 37° for 3hr. (3×10^8 cells/ml.), when 5ml. of sterile 0·1M-$CaCl_2$ solution and 15×10^{11} RNA coliphages were added. Then 15min. later 0·1mc of [2-^{14}C]uracil (40·6mc/m-mole) was added and incubation was continued until lysis was complete (about 120min. after phage addition). The ^{14}C-labelled coliphages were prepared from the lysate as described by Bishop (1965).

The incorporation of [2-^{14}C]thymine into bacteria was studied as follows. Bacteria in the exponential phase (30ml.; 1×10^8 cells/ml.) were inoculated with 5μc of [2-^{14}C]thymine (specific radioactivity 1·28mc/m-mole; The Radiochemical Centre) and incubated with shaking until a density of 5×10^8 cells/ml. was reached. The bacteria were then harvested by centrifugation, resuspended in 2·4% (w/v) nutrient broth containing unlabelled thymine (0·1%) and recentrifuged. They were finally suspended in 60ml. of TM1 buffer containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%), and the nucleic acids were extracted.

Incorporation of [^{32}P]*orthophosphate into bacteria infected by coliphage ZIK*/1. The incorporation of [^{32}P]orthophosphate into bacteria was followed by a method similar to that described by Bishop & Bradley (1965). *E. coli* C3000/L were inoculated from a liquid culture into 680ml. of 2·4% (w/v) nutrient broth and incubated at 37° with shaking for 90min. until a concentration of 2×10^8 cells/ml. was reached. Then 5ml. of sterile 0·1M-$CaCl_2$ solution and $1·4 \times 10^{12}$ RNA coliphages in 20ml. of 2·4% (w/v) nutrient broth were added (multiplicity of infection 10) followed by 1·0mc of carrier-free [^{32}P]orthophosphate (The Radiochemical Centre). Incubation of the culture was continued and the incorporation of ^{32}P into acid-insoluble material determined on 1ml. samples. At intervals, 50ml. portions were removed, mixed with 0·5ml. of M-KCN and poured on ice to cool to 4°. The bacteria were harvested by centrifugation at 15000*g* for 15min. (4°) in the Servall centrifuge and washed by centrifugation after suspending in 20ml. of 0·1M-sodium phosphate buffer, pH7·2, containing KCN (0·01M). The bacteria were finally resuspended in 10ml. of TM1 buffer containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%).

Infection of E. coli *C*3000/*L by* ^{14}C-*labelled coliphage.* Bacteria were inoculated from a liquid culture into 450ml. of 2·4% (w/v) nutrient broth and cultured at 37° until a concentration of 2×10^8 cells/ml. was reached, when 5ml. of M-KCN, 50ml. of [2-^{14}C]uracil-labelled coliphage ($4·5 \times 10^{10}$ coliphage plaque-forming units, 1130counts/min. total) in 2·4% (w/v) nutrient broth and 5ml. of sterile 0·1M-$CaCl_2$ solution were added (multiplicity of infection 0·5), and the mixture was reincubated at 37° for 8min. The bacteria were harvested by centrifugation, washed free of unadsorbed coliphages by suspension in 100ml. of 2·4% (w/v) nutrient broth containing KCN (0·01M) and recentrifuged. The bacteria were finally resuspended in 500ml. of 2·4% (w/v) nutrient broth and reincubated at 37° with shaking. After resuspension less than 1×10^3 free coliphages were present in the medium, whereas approx. $3·8 \times 10^{10}$ bacteria gave rise to phage, as determined by plating the bacteria with *E. coli* C3000/L. Therefore at least 80% of the bacteria that could be infected had been infected. At intervals 50ml. portions were removed, mixed with 0·5ml. of M-KCN, cooled to 4° by pouring on ice, centrifuged and washed with TM1 buffer containing KCN (0·01M) and finally suspended in TM1 buffer containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%), and the nucleic acids were extracted.

Extraction of nucleic acids. The extraction of coliphage-ZIK/1 RNA from purified phage has been described (Bishop, 1965). The extraction of total nucleic acids from bacteria was similar in its initial stages. To 10ml. of a bacterial suspension (2×10^8–5×10^8 cells/ml.) in TM1 buffer containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%) were added 0·5g. of bentonite, prepared according to the procedure of Fraenkel-Conrat, Singer & Tsugita (1961), and 1ml. of 10% (w/v) sodium lauryl sulphate. Then 1min. later 10ml. of freshly redistilled phenol containing 8-hydroxyquinoline (0·1%) was added (Kirby, 1962). The mixture was stirred for 15min. and the emulsion centrifuged at room temperature at 6000*g* for 10min. in an MSE Magnum refrigerated centrifuge operated at room temperature. The aqueous layer was removed as completely as possible and 8ml. of the phenol layer discarded. The remainder ('interface fraction', containing some of the aqueous and phenol layer together with precipitated bacteria, bentonite and bacterial debris) was resuspended with TM1 buffer [containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%)], saturated with phenol containing 8-hydroxyquinoline (0·1%) (10ml. final volume) and extracted with 5ml. of phenol as described above. The aqueous layer from the second extraction was combined with that from the first extraction and, to remove phenol and sodium lauryl sulphate, the mixture was extracted twice with an equal volume of AnalaR diethyl ether [saturated with TM1 buffer containing NaCl (0·1M) and naphthalene-1,5-disulphonate (0·2%)]. The ether was removed after centrifugation at 6000*g* for 5min. and final

traces of ether were removed at 4° by evaporation in a stream of O_2-free N_2. The nucleic acids were then dialysed against TM1 buffer containing NaCl (0·1 M) (or TM1 buffer alone) for 24 hr. at 4° with five changes of liquid. After the dialysis, which removed residual traces of phenol and sodium lauryl sulphate, the absorption spectrum of a 20-fold dilution of the sample was taken to verify the absence of phenol (Table 3).

The efficiency of extracting nucleic acids by this method was examined to determine whether there was a substantial or selective loss of nucleic acids during the procedure and also to investigate the effects of a variety of methods for obtaining cell lysis. Bacteria grown in the presence of [^{32}P]orthophosphate, [2-^{14}C]uracil or [2-^{14}C]thymine were harvested and washed free of extracellular radioactive material as described above. The total and acid-insoluble radioactive material present in the cells was determined on samples after they had been resuspended in TM1 buffer containing NaCl (0·1 M) and naphthalene-1,5-disulphonate (0·2%) at a concentration of 2×10^8–4×10^8 cells/ml. Five methods of cell lysis were used. (1) The cells were lysed with sodium lauryl sulphate and extracted for nucleic acids as described above. (2) The cells were disintegrated for 2 min. at 4° with a Dawe Soniprobe (type 1130 A), setting 4, operated at 2 A (1% remaining viable cells; Bishop, 1965), and lysed and extracted as described above. (3) Cells were treated with lysozyme and EDTA at pH 8·0 in 0·5 M-sucrose to form protoplasts (90% efficiency; Bishop 1965), then lysed and extracted as described above. (4) Protoplasts were similarly prepared and lysed with 1% sodium lauryl sulphate for 20 hr. at 40° and then extracted with phenol. (5) Penicillin-produced spheroplasts were prepared from another culture (0·01% remaining viable bacteria; Bishop, 1965) according to the procedure of Bishop, Roche & Nisman (1964) and lysed and extracted as described above.

The nucleic acids were extracted with phenol and sodium lauryl sulphate and the total radioactivity in the phenol and aqueous layers was determined after the volume of each layer had been measured. The amount of radioactivity present is expressed as a percentage of the initial bacterial total radioactivity (Table 1). The interface fraction was resuspended with TM1 buffer [containing NaCl (0·1 M) and naphthalene-1,5-disulphonate (0·2%)], saturated with phenol containing 8-hydroxyquinoline (0·1%) (10 ml. final volume) and, after homogenization, samples were removed for dialysis and to determine the total radioactivity. The rest was re-extracted with phenol as described above. The total radioactivity in the three fractions, aqueous, phenol and interface, was determined as described above.

To determine the acid-insoluble radioactivity in the various fractions it was necessary to remove the phenol, which would otherwise partially dissolve the membrane filter. Samples from each fraction were therefore dialysed in the presence of chloroform against TM1 buffer containing NaCl (0·1 M) for 48 hr. at 4° with five changes of buffer. The volume of the solution was determined before and after dialysis. Phenol layers were dissolved in 5 vol. of TM1 buffer containing NaCl (0·1 M) before dialysis. After dialysis the acid-insoluble radioactivity was determined (Table 1), and, for nucleic acids labelled with [2-^{14}C]-uracil or [2-^{14}C]thymine, the amount of radioactivity present in RNA and DNA was determined after treatment with ribonuclease or deoxyribonuclease. It was found that at least 96% of the acid-insoluble [2-^{14}C]uracil-labelled material was rendered acid-soluble after ribonuclease treatment for all the fractions and about 3% after deoxyribonuclease treatment. Similarly for the [2-^{14}C]thymine-labelled material about 98% of the acid-insoluble material was rendered acid-soluble after deoxyribonuclease treatment and less than 0·5% after ribonuclease treatment. It was concluded therefore that most of the [2-^{14}C]thymine label was incorporated into DNA, whereas most of the [2-^{14}C]uracil was incorporated into RNA.

It was also found, after treatment of bacteria with ultrasonic vibrations, that about 92% of the [2-^{14}C]-thymine-labelled material, 5% of the [2-^{14}C]uracil-labelled material and 7% of the ^{32}P-labelled material was acid-soluble (Bishop, 1965). Treatment with ultrasonic vibrations was therefore unsuitable for the extraction of DNA.

Of the other methods of cell lysis investigated, the most efficient for DNA extraction was that involving protoplasts, sodium lauryl sulphate and phenol. A 1 min. treatment with sodium lauryl sulphate was as efficient as a 20 hr. treatment: the protoplast suspension became immediately water-clear and viscous after the addition of sodium lauryl sulphate and remained so during the 20 hr. incubation. The extraction of spheroplasts (penicillin-produced) was less efficient and although the suspension became viscous it was not water-clear. The difference in behaviour between lysozyme-produced protoplasts and penicillin-produced spheroplasts was presumably a result of the fact that, after treatment with lysozyme, the bacterial cell wall is solubilized, whereas during spheroplast induction by penicillin in the presence of bivalent ions the cell walls of bacteria are broken and shed but are not solubilized. The extraction of DNA from whole bacteria by sodium lauryl sulphate was the least efficient method examined, although about 72% of the total radioactivity was extracted into the aqueous phases. It was observed that the interface material from the first phenol extraction was more viscous than the aqueous layer, and in view of the observation that all the RNA could be extracted by sodium lauryl sulphate and phenol it was apparent that DNA, unlike RNA, was associated with the precipitated material of the interface layer.

The extraction of RNA was almost equally efficient whichever method of cell lysis was used.

Preparation of MAK columns. The procedure for preparing methylated bovine serum albumin was identical with that described by Mandell & Hershey (1960). The preparation of MAK columns (kieselguhr coated with methylated bovine serum albumin) was as follows. An 18 g. portion of acid-washed kieselguhr was added to 100 ml. of 0·4 M-NaCl–0·025 M-potassium phosphate buffer, pH 6·9, and stirred on a magnetic stirrer. Then 5 ml. of an aqueous 1% (w/v) solution of methylated bovine serum albumin was added and 5 min. later the mixture was transferred to a column (2 cm. diam.) and sedimented at 0·5 kg./cm.^{2} pressure. The column was washed under pressure with an additional 100 ml. of 0·4 M-NaCl—0·025 M-potassium phosphate buffer, pH 6·9. The void volume of the column was about 35 ml. and the height about 20 cm.

Fractionation of nucleic acids on MAK columns. A 1 ml. sample of nucleic acids (about 0·2 mg.) was applied to a MAK column and allowed to soak in. Then 5 ml. of 0·4 M-NaCl–0·025 M-potassium phosphate buffer, pH 6·9, was added and the nucleic acids were eluted at a rate of 15 ml./

Table 1. *Extraction of nucleic acids by sodium lauryl sulphate and phenol*

Bacteria were grown in the presence of [^{32}P]orthophosphate, [2-^{14}C]uracil or [2-^{14}C]thymine as described in the Methods and Materials section. Five methods of cell lysis were investigated. (1) Bacteria were lysed with sodium lauryl sulphate for 1 min., then extracted with phenol. (2) Cells were disintegrated by ultrasonic treatment, then extracted with sodium lauryl sulphate and phenol. (3) Protoplasts were derived from bacteria by lysozyme–EDTA treatment and lysed for 1 min. with sodium lauryl sulphate before phenol extraction. (4) Protoplasts were derived from bacteria by lysozyme–EDTA treatment and lysed for 20 hr. at 40° with sodium lauryl sulphate before phenol extraction. (5) Penicillin-produced spheroplasts were lysed with sodium lauryl sulphate and extracted with phenol as described in the Methods and Materials section. The total and acid-insoluble radioactivity, expressed as a percentage of the initial bacterial total radioactivity, for the aqueous, phenol and interface fractions of the first and second phenol extractions is given. The percentage of total counts (corrected) was calculated by taking into consideration the volume of the aqueous or phenol layers present in the interface fraction.

Nucleic acid precursor	Method of cell breakage	% acid-insoluble		First phenol extraction								
				Aqueous fraction			Interface fraction			Phenol fraction		
		Before lysis	After lysis	% of total counts	% of total counts (corrected)	% acid-insoluble	% of total counts	% of total counts (corrected)	% acid-insoluble	% of total counts	% of total counts (corrected)	% acid-insoluble
[^{32}P]Ortho-phosphate	(1)	98·8	—	76·4	84·0	73	22·0	13·3	18	1·6	2·7	1
	(2)	98·8	93·1	83·4	90·0	71	8·7	1·5	7	7·9	8·5	6
	(3)	98·8	—	78·7	88·3	74	15·1	4·8	14	6·2	6·9	6
	(4)	98·8	—	79·0	87·8	74	13·2	4·1	12	7·8	8·1	7
	(5)	88·7	—	81·6	89·9	71	13·9	4·5	10	4·5	5·6	3
[2-^{14}C]Uracil	(1)	96·3	—	78·5	87·0	75	19·0	8·0	18	2·5	5·0	2
	(2)	96·3	94·8	83·1	91·7	81	11·8	2·0	10	5·1	6·3	3
	(3)	96·3	—	80·0	90·0	78	17	4·5	16	3·0	5·5	3
	(4)	96·3	—	78·8	90·7	75	18·7	5·9	19	2·5	3·4	3
	(5)	89·8	—	80·1	94·1	71	16·8	2·3	16	3·1	3·6	3
[2-^{14}C]Thymine	(1)	97·1	—	53·8	59·2	50	42·6	36·1	40	3·6	4·2	3
	(2)	97·1	8·3	—	—	—	—	—	—	—	—	—
	(3)	97·1	—	77·3	83·4	76	20·6	12·8	19	2·1	3·8	2
	(4)	97·1	—	78·0	82·5	76	19·6	13·2	19	2·4	4·3	3
	(5)	91·9	—	66·8	83·9	62	31·0	12·8	28	2·2	3·3	2
				Second phenol extraction								
				Aqueous fraction			Interface fraction			Phenol fraction		
				% of total counts	% of total counts (corrected)	% acid-insoluble	% of total counts	% of total counts (corrected)	% acid-insoluble	% of total counts	% of total counts (corrected)	% acid-insoluble
[^{32}P]Ortho-phosphate	(1)	98·8	—	10·7	13·1	10	9·1	5·8	9	2·2	3·1	1
	(2)	98·8	93·1	6·2	6·7	6	1·3	0·6	1	1·2	1·4	1
	(3)	98·8	—	9·0	10·6	7	3·0	0·9	2	3·1	3·6	2
	(4)	98·8	—	9·1	10·1	5	1·9	0·6	2	2·2	2·5	2
	(5)	88·7	—	10·1	10·7	6	2·5	0·9	3	1·3	2·3	1
[2-^{14}C]Uracil	(1)	96·3	—	15·6	17·8	15	2·9	0·4	3	0·5	0·8	—
	(2)	96·3	94·8	10·0	10·8	10	1·1	0·1	1	0·7	0·9	1
	(3)	96·3	—	14·6	16·4	15	2·1	0·1	2	0·3	0·5	—
	(4)	96·3	—	15·9	18·0	14	2·3	0·1	2	0·5	0·6	—
	(5)	89·8	—	15·7	18·6	15	0·7	0·1	1	0·4	0·6	—
[2-^{14}C]Thymine	(1)	97·1	—	16·2	18·6	15	23·6	20·6	23	2·8	3·4	3
	(2)	97·1	8·3	—	—	—	—	—	—	—	—	—
	(3)	97·1	—	10·8	14·6	10	6·8	2·6	6	3·0	3·4	2
	(4)	97·1	—	11·1	13·8	10	5·7	2·6	6	2·8	3·2	2
	(5)	91·9	—	19·8	21·2	18	10·1	7·9	10	1·1	1·9	1

hr. with a linear gradient of NaCl (0·5–1·2 M) in 200 ml. of 0·025 M-potassium phosphate buffer, pH 6·9, saturated with chloroform. Fractions (about 3 ml.) were collected automatically, and 0·5 mg. of bovine serum albumin and an equal volume of 10% (w/v) trichloroacetic acid were added to each. Alternative gradients were used as described in the text.

Recovery of nucleic acids from MAK columns. The recovery of nucleic acids from MAK columns was investigated to determine whether there were losses of nucleic acids during the chromatography on MAK columns. Total nucleic acids were extracted from a culture of *E. coli* C3000/L infected with RNA coliphage and grown in the presence of [^{32}P]orthophosphate for 50 min. as described above. A 2 ml. sample of nucleic acids (E_{260} 4·66; acid-insoluble radioactivity 270220 counts/min./ml.) was chromatographed on MAK as described in the text (see Fig. 1). The total recovery of acid-insoluble radioactivity was 83·0%; 8·1% was present in the s-RNA peak, 6·9% in the DNA and double-stranded RNA peak, 45·2% in the ribosomal 16s RNA and single-stranded coliphage RNA peak (see below), and 19·7% in the 23s ribosomal RNA peak. Samples from each peak were put on fresh MAK columns and rechromatographed. The recoveries of s-RNA, DNA and double-stranded RNA, ribosomal 16s RNA and single-stranded coliphage RNA, and ribosomal 23s RNA were respectively 99·8, 74, 81 and 81%. Samples from each fraction were again rechromatographed and the respective recoveries were 99·5, 73, 82 and 81%, suggesting that the losses which occurred for each type of nucleic acid during the chromatography were a result of the chromatographic technique and not a selective loss of specific fractions of each nucleic acid species. Increasing the salt concentration to 3 M after the first or subsequent chromatographies eluted an additional 0·3% of the added acid-insoluble radioactivity. The recovery of [2-^{14}C]thymine-labelled DNA, prepared as described above, was similar: 73% was recovered on the first chromatography and 74% on the second and third chromatographies. Higher recoveries have been recorded for DNA by using stepwise salt elutions (Sueoka & Cheng, 1962). The recovery of double-stranded RNA, purified from DNA and other RNA as described below, was 71 and 73% on the first and second chromatographies.

Digestion of nucleic acids with ribonuclease and deoxyribonuclease. Unlike ribonuclease, deoxyribonuclease is substantially inhibited by 0·015 M-sodium citrate, trace amounts of phenol or sodium lauryl sulphate. Phenol and sodium lauryl sulphate were therefore removed from nucleic acid preparations before treatment with ribonuclease or deoxyribonuclease. To digest the DNA or RNA in a preparation, a 1 ml. sample of nucleic acid in TM1 buffer containing NaCl (0·1 M) was mixed with 20 μg. of deoxyribonuclease I (Sigma Chemical Co., St Louis, Mo., U.S.A.) or 5 μg. of ribonuclease A (Sigma Chemical Co.) in 0·1 ml. of TM1 buffer (or both) and incubated at 37° for 30 min. The remaining amount of acid-insoluble radioactivity was determined after the addition of 0·5 mg. of bovine serum albumin and 1 ml. of 10% (w/v) trichloroacetic acid.

Isolation of double-stranded RNA. The procedure of Ammann, Delius & Hofschneider (1964) was used to separate RNA from insufficiently digested DNA, and double-stranded RNA from other RNA without using ribonuclease see below). A 1 ml. sample of nucleic acids, extracted from bacteria 50 min. after bacteriophage addition, was digested first with 20 μg. of deoxyribonuclease in 0·1 ml. of TM1 buffer at 37° for 30 min. EDTA was then added (final concn. 2 mM) followed by 3 ml. of water and 4 ml. of ethanol; this mixture is similar to that described by Geiduschek (1962) for preventing the rapid intramolecular type-1 renaturation of heat-denatured DNA. (It is believed that this rapid renaturation is due to guanine-plus-uracil-rich nucleotide pair sequences, and is found in DNA from a variety of sources; Geiduschek, 1962.) The mixture was then heated at 78° for 10 min. and rapidly cooled in a stream of cold water. Then 2 vol. of ethanol was added, and the mixture was cooled at −10° for 30 min. and centrifuged at 20000*g* in the Servall centrifuge for 15 min. The precipitate was dissolved in 0·1 M-NaCl, and bacterial RNA and single-stranded coliphage RNA were removed by precipitation with NaCl (final concn. 1·7 M) and centrifugation at 20000*g* for 15 min. The supernatant, containing double-stranded RNA, was chromatographed on MAK. To determine the efficiency of this purification process, [2-^{14}C]uracil-labelled double-stranded RNA, prepared by this procedure, was mixed with unlabelled total nucleic acids from an uninfected culture and purified again as described above. The percentage of acid-insoluble radioactivity recovered was 84%. It was not possible to determine, however, if there was any selective loss of double-stranded RNA during the extraction.

Determination of the melting point of double-stranded RNA. A sample of ^{14}C-labelled double-stranded RNA was isolated from the total nucleic acids extracted from RNA-coliphage-infected bacteria grown in the presence of [2-^{14}C]-uracil. The double-stranded RNA was freed from DNA by deoxyribonuclease and separated from other RNA as described above before chromatography on MAK. The fractions containing double-stranded RNA (see below) were combined (35 ml.) and dialysed *in vacuo* against 0·15 M-NaCl–0·015 M-sodium citrate–0·01 M-$MgCl_2$ in 0·025 M-tris–HCl buffer, pH 7·2 (buffer–salts solution), until a 26 ml. volume was attained. Samples (1 ml.) of the RNA were sealed into small Pyrex glass tubes (0·5 cm. diam.) and heated at various temperatures in a bath of glycerol. Duplicate tubes, equilibrated for 20 min. at each temperature, were cooled by plunging into a stream of cold water and then into ice. The tubes were opened, 5 μg. of ribonuclease in 0·1 ml. of TM1 buffer was added and the mixture reincubated at 37° for 30 min. The residual acid-insoluble radioactivity was determined after the addition of 0·5 mg. of bovine serum albumin.

Measurement of radioactivity. The precipitation and preparation of acid-insoluble radioactive material and counting in a Packard Instrument Co. Tri-Carb liquid-scintillation spectrometer (model 3002) were as described previously (Bishop & Bradley, 1965). Determination of total radioactivity, the discrimination of ^{32}P from ^{14}C radiation and counting efficiency of the discriminated radioactivity have also been described previously (Bishop & Bradley, 1965; Bishop, 1965). The efficiency of counting ^{32}P alone was 95% and that of ^{14}C alone was 75%. All counts were corrected for background radiation and the natural loss of radioactivity during the time-course of an experiment.

Sedimentation of RNA in a sucrose gradient. The sedimentation of RNA in a sucrose gradient and subsequent fractionation were as described by Bishop *et al.* (1964).

RESULTS

Chromatography of nucleic acids on MAK. The elution sequence of nucleic acids from MAK columns has been shown to be dependent on molecular size, the extent of hydrogen-bonding and base composition (Mandell & Hershey, 1960; Sueoka & Cheng, 1962; Nonoyama & Ikeda, 1964). The sequence obtained for total nucleic acids [dialysed against TM1 buffer containing sodium chloride (0·1 M)] from RNA-coliphage-infected or uninfected *E. coli* C3000/L is shown in Fig. 1. The

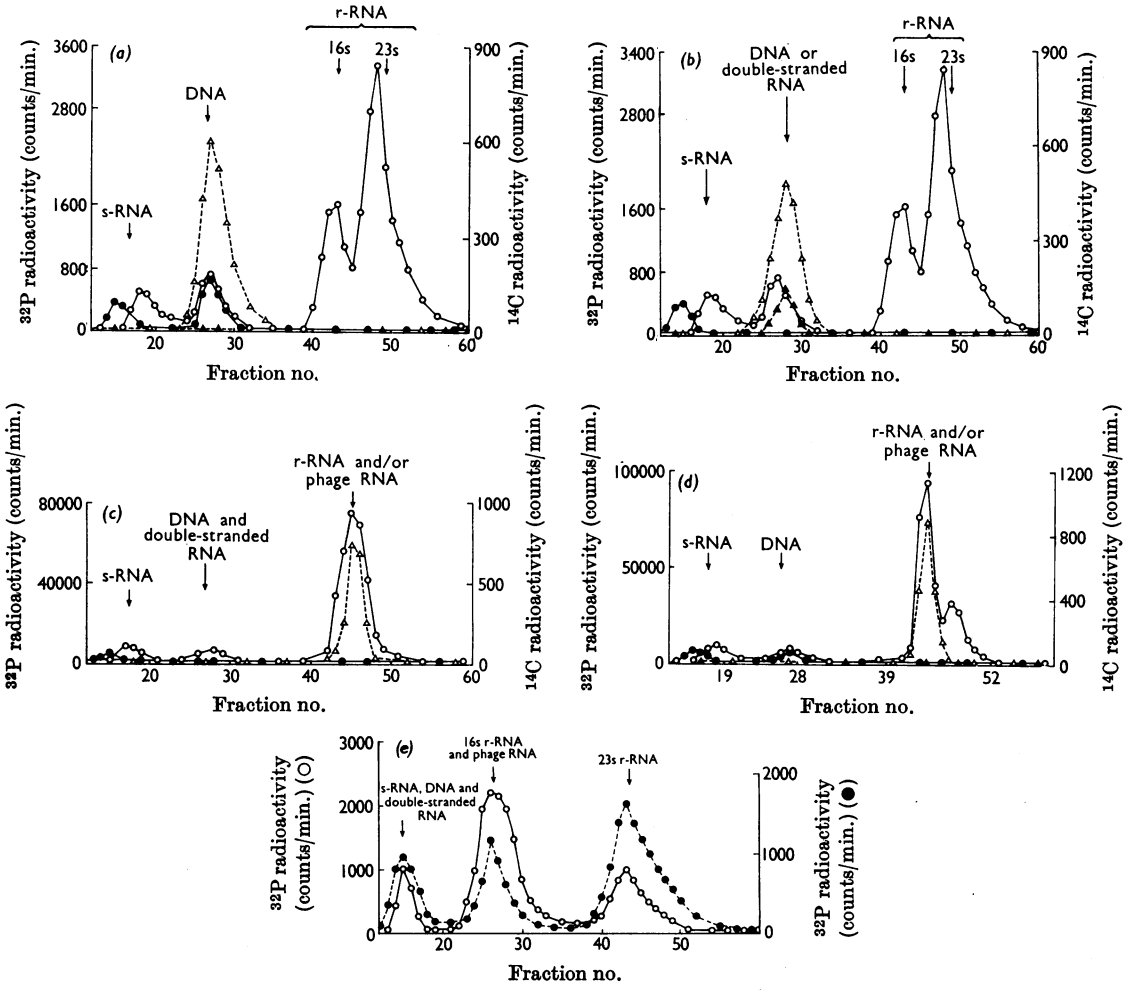

Fig. 1. Chromatography of nucleic acids on MAK columns. Experimental details are given in the Methods and Materials section. (*a*) ^{32}P-labelled total nucleic acids from uninfected bacteria (○), treated with ribonuclease before chromatography (●); [2-^{14}C]thymine-labelled total nucleic acids (△), treated with deoxyribonuclease before chromatography (▲). (*b*) ^{32}P-labelled total nucleic acids from uninfected cells (○), treated with deoxyribonuclease and ribonuclease before chromatography (●); [2-^{14}C]uracil-labelled purified double-stranded coliphage RNA (△), treated with ribonuclease before chromatography (▲). (*c*) ^{32}P-labelled total nucleic acids from infected bacteria before dialysis against TM1 buffer (○), treated with deoxyribonuclease and ribonuclease before chromatography (●); [2-^{14}C]uracil-labelled purified 28s coliphage RNA (△). (*d*) ^{32}P-labelled total nucleic acids from infected cells after dialysis against TM1 buffer (○), treated with ribonuclease before chromatography (●); [2-^{14}C]uracil-labelled purified coliphage RNA, heat-denatured as described in the text (△), treated with ribonuclease (▲). (*e*) ^{32}P-labelled total nucleic acids from infected cells dialysed against TM1 buffer, chromatographed on MAK with a 0·8–1·25 M-NaCl salt elution gradient (○); ^{32}P-labelled total nucleic acids from uninfected cells chromatographed similarly (●). r-RNA, ribosomal RNA.

identification of bacterial DNA and single-stranded coliphage RNA was obtained by co-chromatography of ^{32}P-labelled total nucleic acids from infected cells with bacterial nucleic acids from uninfected cells grown in the presence of [2-^{14}C]-thymine, or with RNA extracted from purified [2-^{14}C]uracil-labelled coliphage. The identification of double-stranded coliphage RNA was obtained by a similar co-chromatography with [2-^{14}C]uracil-labelled double-stranded RNA (see below). Ribosomal RNA and bacterial s-RNA were identified by comparison with the results of Nonoyama & Ikeda (1964).

The result of incubating purified single- or double-stranded [2-^{14}C]uracil-labelled coliphage RNA, [2-^{14}C]thymine-labelled bacterial DNA or ^{32}P-labelled total nucleic acids from infected or uninfected cells with ribonuclease or deoxyribonuclease before chromatography on MAK is illustrated in Fig. 1. Most of the nucleic acid species were digested almost completely by their respective nucleases, although there were some residual acid-insoluble oligonucleotides that were eluted directly from the column after digestion of bacterial RNA with ribonuclease. The recovery of double-stranded RNA, after ribonuclease treatment, was approx. 20% of the added material, presumably owing to slow ribonuclease digestion (see below).

The effect of heating coliphage RNA on its subsequent behaviour on chromatography on MAK is also shown in Fig. 1. A sample of purified [2-^{14}C]uracil-labelled 28s coliphage RNA, suspended in 0·1M tris–hydrochloric acid buffer, pH 7·2, was heated at 86° for 20min. and cooled by plunging in a stream of cold water. After chromatography on MAK with ^{32}P-labelled total nucleic acids from uninfected bacteria it was found that the coliphage RNA was eluted with the bacterial 16s ribosomal RNA. When the elution gradient was changed in an attempt to differentiate the coliphage RNA from the ribosomal RNA it was still found to be impossible to resolve these two RNA species (Fig. 1).

Similarly when the total nucleic acids were dialysed after extraction against TM1 buffer alone and chromatographed on MAK the single-stranded coliphage RNA was eluted with the bacterial 16s ribosomal RNA, and so allowed an estimate to be obtained of the 23s ribosomal RNA (see below).

Identification and properties of double-stranded RNA. Digestion of total nucleic acids with deoxyribonuclease and ribonuclease before chromatography on MAK results in a substantial loss of double-stranded RNA (see the preceding section). Another method was therefore used for the purification of double-stranded RNA that did not involve ribonuclease (see the Methods and Materials section). The properties of the resulting material with regard to its digestion by deoxyribonuclease and ribonuclease under various conditions are illustrated in Table 2. The material resisted

Table 2. *Properties of the* [2-^{14}C]*uracil-labelled single-stranded coliphage RNA or double-stranded RNA isolated from* E. coli *infected with the RNA coliphage ZIK/1*

Double-stranded RNA was isolated from *E. coli* C3000/L infected with coliphage ZIK/1 in the presence of [2-^{14}C]uracil as described in the Methods and Materials section. Single-stranded coliphage RNA was isolated from purified [2-^{14}C]uracil-labelled coliphage. Samples of the nucleic acid (900 counts/2min.) were equilibrated with various buffer and salt solutions for 10min. at 37° before addition of the enzyme, namely 5μg. of ribonuclease/ml. (R) or 20μg. of deoxyribonuclease/ml. (D). Incubation, precipitation and counting of the residual acid-insoluble radioactivity were carried out as described in the Methods and Materials section.

RNA	Nuclease	Concn. of tris–HCl buffer, pH 7·2 (M)	Concn. of NaCl (M)	Concn. of $MgCl_2$ (M)	% of radioactivity acid-insoluble after digestion
Double-stranded	None	0·025	0·15	—	100
Double-stranded	R	0·025	0·15	0·01	87
Double-stranded	R	0·002	0·15	—	86
Double-stranded	R	0·002	0·10	—	36
Double-stranded	R	0·002	0·05	—	4
Double-stranded	R	0·002	0·01	—	2
Double-stranded	R	0·002	0·01	0·01	86
Double-stranded	R	0·002	—	0·01	83
Double-stranded	R	0·01	—	0·001	7
Double-stranded	D	0·035	0·15	0·01	100
Double-stranded	D	0·002	0·01	0·01	101
Double-stranded	D	0·002	0·01	0·001	101
Single-stranded	R	0·025	0·15	0·01	1
Single-stranded	D	0·025	0·15	0·01	101

digestion by deoxyribonuclease but was almost completely (93%) digested by ribonuclease in TM2 buffer [0·01 M-tris–hydrochloric acid buffer (pH 7·2)–0·1 mM-magnesium chloride]. In buffer–salts solution only 13% was digested after 30 min. at 37°. The time-course of digestion in TM2 buffer or buffer–salts solution is shown in Fig. 2. Almost identical properties have been demonstrated for double-stranded RNA from other coliphage-infected bacteria (Nonoyama & Ikeda, 1964; Ammann *et al.* 1964).

The effect of heat and rapid cooling on its sensitivity to ribonuclease digestion was also examined (Fig. 3). Almost all (98%) of the nucleic acid was digested by ribonuclease in buffer–salts solution after heating at 110° and rapid cooling. The midpoint of melting, T_m, was 99° and the melting range 94–104°. None of the nucleic acid was digested by deoxyribonuclease under identical conditions. When the material in buffer–salts solution was heated at 102° for 10 min. and slowly cooled in an oven, only 28% was subsequently digested by ribonuclease, indicating that substantial renaturation of the double-stranded molecule had taken place.

It was apparent from these results that the nucleic acid was not double-stranded DNA nor, on account of the sharpness of the melting transition, just single-stranded coliphage RNA, which has been shown to have T_m 60° in 0·14 M-sodium chloride and a melting range 40–80° (Bishop, 1965), and is sensitive to ribonuclease digestion in buffer–salts solution (Table 2). The possibility that the nucleic acid was an RNA–DNA hybrid was also excluded in view of the observations that DNA–RNA hybrids are digested completely by deoxyribonuclease and partially by ribonuclease, depending on the concentration of salt (Nygaard & Hall, 1964). Contamination with a small amount of single-stranded coliphage RNA was also ruled out because the method of preparation involved chromatography on MAK, which has been shown to separate single-stranded RNA from double-stranded RNA (Fig. 1). The possibility that the material represents an association of single-stranded RNA is unlikely in view of the sharp melting transition (Ammann *et al.* 1964). It was assumed therefore that the nucleic acid was double-stranded RNA.

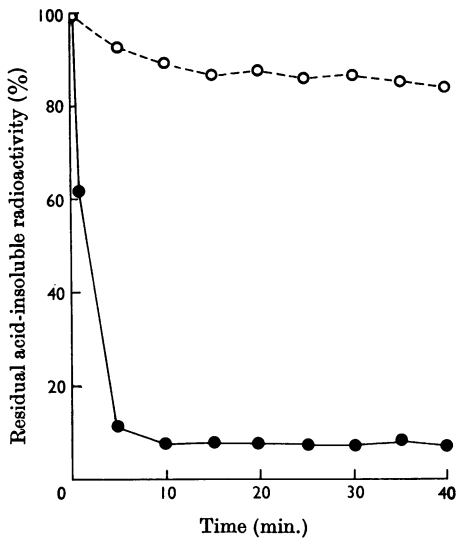

Fig. 2. Ribonuclease digestion of double-stranded coliphage RNA. Double-stranded [2-^{14}C]uracil-labelled coliphage RNA was isolated from DNA and single-stranded RNA by the procedure of Ammann *et al.* (1964) and suspended in buffer–salts solution (0·15 M-NaCl–0·015 M-sodium citrate–0·01 M-$MgCl_2$ in 0·025 M-tris–HCl buffer, pH 7·2) (○) or TM2 buffer (●) and incubated with 5 μg. of ribonuclease/ml. at 37° for various times. The residual acid-insoluble radioactive material was determined as described in the Methods and Materials section and is expressed as a percentage of the original added material.

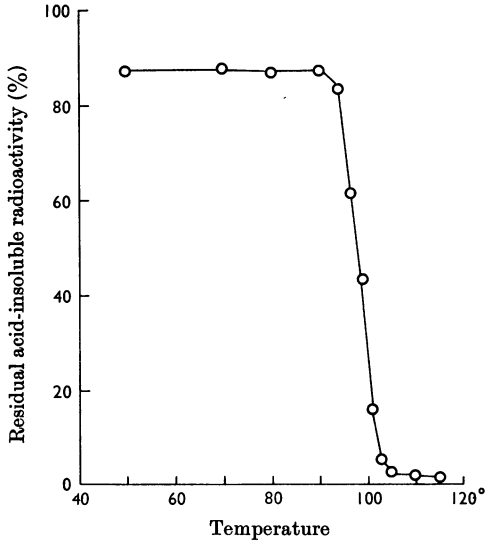

Fig. 3. Effect of heating on the sensitivity of double-stranded RNA to ribonuclease digestion. Double-stranded [2-^{14}C]uracil-labelled coliphage RNA was isolated and suspended in buffer–salts solution as described in Fig. 2. Samples were sealed in Pyrex tubes and equilibrated at various temperatures for 20 min. in a bath of glycerol. They were then cooled by plunging in a stream of cold water and digested with 5 μg. of ribonuclease/ml. at 37° for 30 min. The percentage of residual acid-insoluble radioactivity was determined as described in the Methods and Materials section and is expressed as a percentage of the original added material.

Fate of the infecting coliphage RNA. To demonstrate that the RNA from the infecting coliphage becomes incorporated into the ribonuclease-resistant double-stranded RNA, purified [2-^{14}C]uracil-labelled coliphages ZIK/1 were allowed to infect bacteria in the presence of 0·01 M-potassium cyanide, then unadsorbed coliphage and potassium cyanide were removed by centrifugation and the bacteria reincubated at 37°. Under these conditions during the period of adsorption bacterial metabolism is inhibited and the coliphage development consequently retarded. On reincubation the population of cells behave for a short period as if they had been infected synchronously. [Similar apparent synchronous infection can be obtained by the use of chloramphenicol (Kelly *et al.* 1965).] At intervals bacteria were removed, the acid-insoluble radioactivity was determined on a sample and the total nucleic acids were extracted. The percentage of ribonuclease-resistant ^{14}C-labelled material recovered during the time-course of the experiment is shown in Fig. 4. After sedimentation (in a

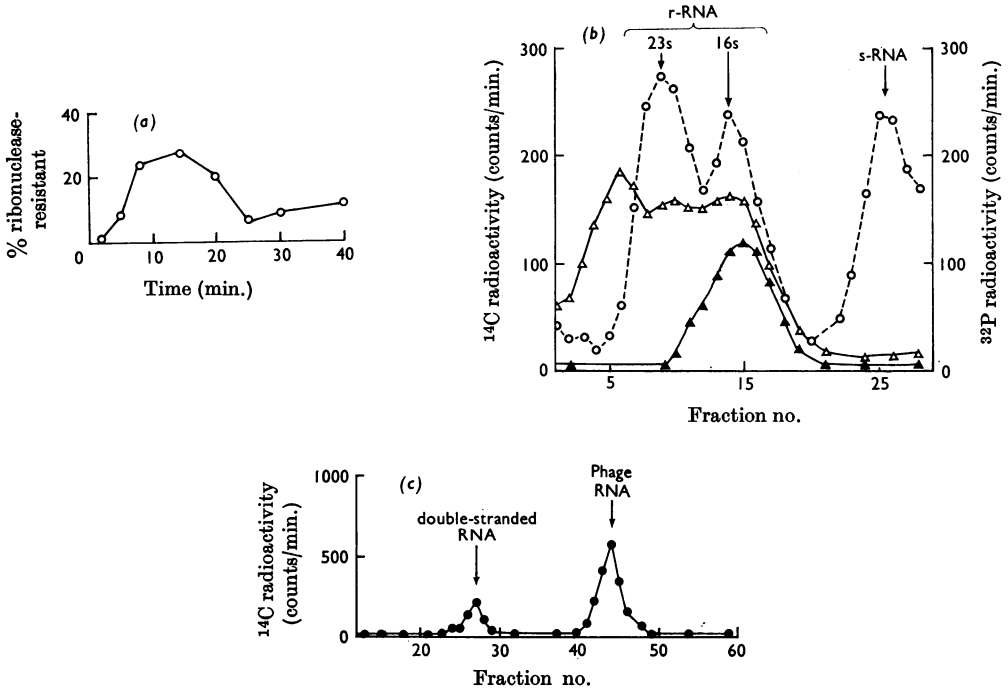

Fig. 4. Incorporation of infecting RNA into double-stranded RNA. [2-^{14}C]Uracil-labelled purified coliphages were allowed to infect bacteria in the presence of 0·01 M-KCN. Unadsorbed bacteriophages were removed by centrifugation and the infected bacteria cultured at 37°. At intervals samples were removed, the total nucleic acids extracted and the acid-insoluble ^{14}C radioactivity was determined to calculate the efficiency of extraction. Ribonuclease digestion of the total nucleic acids in buffer–salts solution (Fig. 3) and chromatography on MAK were carried out as described in the Methods and Materials section. The nucleic acids sample extracted 14 min. after reinoculation was dialysed against TM1 buffer containing NaCl (0·1 M) and sedimented in a gradient of sucrose (20–5%, w/v) in 0·1 M-tris–HCl buffer at 13° for 4 hr. at 37 500 rev./min. in the Spinco model L centrifuge (SW 39 rotor). The gradient was fractionated and the acid-insoluble radioactive material determined as described by Bishop *et al.* (1964). Half of each fraction was mixed with NaCl (final concn. 0·15 M) and digested with ribonuclease (5 μg./ml.) for 30 min. at 37°. The residual acid-insoluble radioactivity was determined as described in the Methods and Materials section. ^{32}P-labelled total nucleic acids from uninfected bacteria were extracted as described in the Methods and Materials section. Gradient profiles obtained for parallel centrifugations are superimposed. (*a*) Percentage of ribonuclease-resistant ^{14}C-labelled acid-insoluble material in the total nucleic acids extracted from infected bacteria at various times after reinoculation and corrected for the efficiency of extraction. (*b*) Sedimentation of the ^{14}C-labelled total nucleic acids extracted 14 min. after reinoculation (△), treated with ribonuclease after fractionation (▲); ^{32}P-labelled total nucleic acids from uninfected bacteria sedimented similarly (○). (*c*) Chromatography on MAK of the ^{14}C-labelled total nucleic acids extracted 14 min. after reinoculation. r-RNA, ribosomal RNA.

gradient of sucrose in 0·1 M-tris–hydrochloric acid buffer, pH 7·2) of a sample from a similar experiment extracted 14 min. after reincubation, it was found that (by comparison with the 23s, 16s and 4s RNA extracted from uninfected ^{32}P-labelled bacteria), of the three main RNA peaks, the one with a sedimentation coefficient of about 15s was ribonuclease-resistant (Fig. 4). Chromatography of the 14 min. sample on MAK also demonstrated that the parental infecting coliphage RNA had become incorporated into a double-stranded ribonuclease-resistant form (Fig. 4).

Analysis of RNA synthesized after bacteriophage infection. To examine the nucleic acid synthesis after infection of *E. coli* C3000/L with the RNA coliphage ZIK/1, bacteria were inoculated with coliphage and [^{32}P]orthophosphate and incubated with shaking for 120 min. At intervals, bacteria and bacterial debris were removed by centrifugation and the incorporation of ^{32}P into acid-insoluble material was determined (Table 3). The bacteria were extracted for total nucleic acids, which were then dialysed against TM2 buffer to remove phenol and sodium lauryl sulphate. The absorption spectrum of the nucleic acids and acid-insoluble radioactivity were determined (Table 3). A portion from each sample was chromatographed on MAK and the recovery of the acid-insoluble radioactive material calculated (Table 3). Also, the amount of acid-insoluble radioactivity present in the s-RNA, DNA and double-stranded RNA, 16s ribosomal RNA and coliphage single-stranded RNA, and the 23s ribosomal RNA were estimated. After correction for the respective losses on MAK chromatography (see the Methods and Materials section), the natural loss of radioactivity during the time-course of the experiment and the losses incurred during the extraction process (see the Methods and Materials section), the computed amounts of the various nucleic acid species were plotted (Fig. 5).

The synthesis of s-RNA and ribosomal 23s RNA was exponential for the first 10–15 min. after coliphage addition, when the rates of synthesis for both RNA species were similarly decreased. In contrast, the synthesis of single-stranded coliphage RNA together with ribosomal 16s RNA was exponential from the time of coliphage addition until 30–40 min. later. Though it was not possible to differentiate the synthesis of coliphage single-stranded RNA from that of the ribosomal 16s RNA under the conditions employed, if it is assumed that the amount of 16s ribosomal RNA was never more than 48% of the amount of the 23s ribosomal RNA at any time, then it is possible to obtain an estimate of the synthesis of single-stranded coliphage RNA alone (Fig. 5). The ratio 48/100 for the amount of 16s to 23s ribosomal RNA was obtained after chromatography on MAK

Table 3. *Extraction of* ^{32}P*-labelled nucleic acids after coliphage ZIK/1 infection*

E. coli C3000/L in the exponential phase were inoculated with $CaCl_2$, RNA coliphage (multiplicity of infection 10) and [^{32}P]orthophosphate and incubated with shaking. At intervals samples were removed and the acid-insoluble radioactivity was determined (column 2). The total nucleic acids were extracted, dialysed free from phenol and sodium lauryl sulphate (volume about 18 ml.) and the acid-insoluble radioactivity was determined (column 3) to calculate the efficiency of the extraction process (column 4). Also, E_{230}, E_{260} and E_{280} (columns 5–7) and the specific activity of each sample (column 8) were determined. The specific activity is expressed in terms of the ^{32}P counts/min./ml./E_{260} unit.

Time after [^{32}P]phosphate addition (min.)	$10^{-5}\times$ Bacterial acid-insoluble radioactivity (counts/min./50 ml.)	$10^{-4}\times$ Total nucleic acids acid-insoluble radioactivity (counts/min./ml.)	% of ^{32}P extracted	E_{230}	E_{260}	E_{280}	$10^{-3}\times$ Specific activity
10	4·73	2·13	83·1	2·20	4·70	2·26	4·56
15	8·92	4·02	82·0	2·78	5·78	2·83	6·96
20	15·00	6·88	82·1	2·76	5·96	2·90	11·54
25	23·10	10·32	80·9	3·26	6·75	3·40	15·30
30	34·72	15·48	81·6	3·34	6·59	3·07	23·49
40	62·32	27·97	80·8	3·72	7·77	3·72	36·00
50	95·93	42·65	82·7	3·58	7·36	3·54	57·99
60	126·11	56·06	80·0	3·79	7·59	3·62	73·86
70	141·90	65·24	82·3	4·20	9·23	4·39	70·69
80	143·10	64·90	83·0	4·30	8·51	4·37	76·24
90	136·31	61·61	79·1	4·10	8·31	4·15	74·17
110	79·53	—	—	—	—	—	—
130	63·76	—	—	—	—	—	—

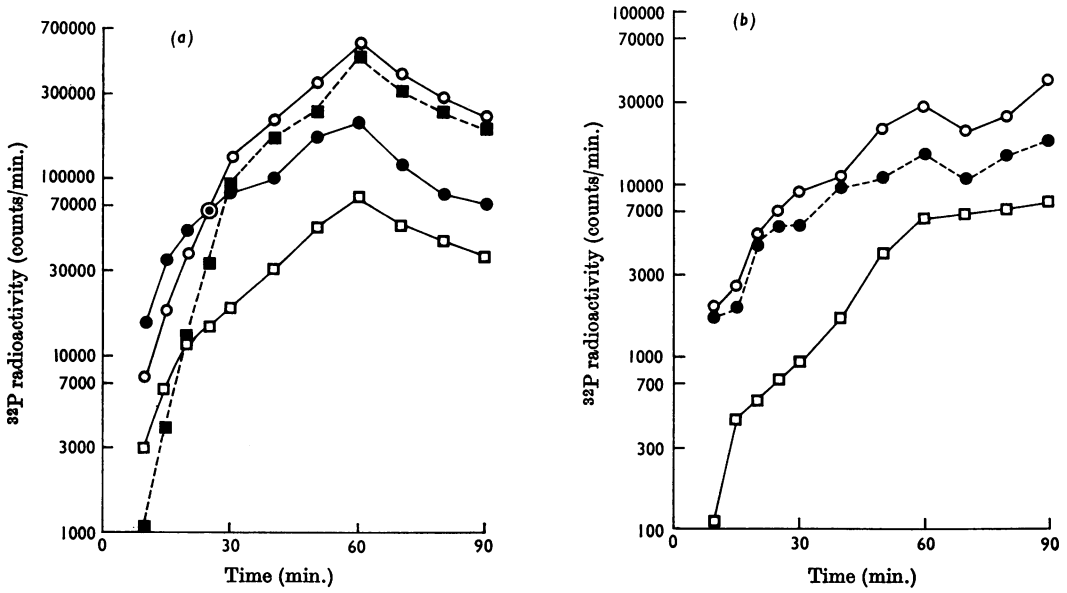

Fig. 5. Analysis of the nucleic acid synthesis in infected cells. Total nucleic acids were extracted at intervals from cells infected by bacteriophage and grown in the presence of [^{32}P]orthophosphate as described in the Methods and Materials section. The efficiency of extraction was determined for each sample (Table 3), and the nucleic acids, after dialysis against TM1 buffer, were chromatographed on MAK (Fig. 1). The amounts of acid-insoluble radioactive material in the s-RNA, DNA and double-stranded RNA, 16s ribosomal RNA and coliphage single-stranded RNA, and 23s ribosomal RNA peaks were determined and corrected for the efficiency of extraction, the loss due to chromatography and loss of activity during the time-course of the experiment (Methods and Materials section). Deoxyribonuclease digestion before chromatography was also carried out as described in the Methods and Materials section. Double-stranded RNA was extracted from the total nucleic acids as described by Ammann *et al.* (1964), and the amounts at each time were determined after correcting for the efficiency of extraction and loss of activity during the experiment. (*a*) 23s Ribosomal RNA (●), coliphage single-stranded RNA and 16s ribosomal RNA (○), s-RNA (□) and coliphage single-stranded RNA calculated as described in the text (■). (*b*) DNA and double-stranded RNA (○), DNA calculated after deoxyribonuclease digestion of the total nucleic acids and chromatography on MAK (●) and double-stranded RNA extracted as described by Ammann *et al.* (1964) (□).

of the total nucleic acids extracted from uninfected bacteria grown in the presence of [^{32}P]orthophosphate. On this basis it was found that the coliphage single-stranded RNA was not synthesized until 10–15min. after bacteriophage addition.

It was impossible, on the basis of chromatography on MAK, to differentiate DNA synthesis from that of double-stranded RNA.

Another method was therefore employed to estimate the synthesis of both species of nucleic acid. The total nucleic acid was treated with deoxyribonuclease and rechromatographed on MAK. The difference between the amount of acid-insoluble radioactivity in the DNA and double-stranded RNA region of the chromatogram before and after treatment was taken as an estimate of the amount of DNA (Fig. 5). It was apparent that this estimate was not very reliable in view of the scatter of the results, and that the residual radioactivity after deoxyribonuclease treatment was even more unreliable as an estimate of double-stranded RNA (Fig. 5). Another method, which removed DNA by treatment with deoxyribonuclease and single-stranded RNA without treatment with ribonuclease, was used to obtain an estimate of the amount of double-stranded RNA (Ammann *et al.* 1964). The result, corrected for the loss of material during the extraction process (see the Methods and Materials section), is shown in Fig. 5. It was observed that double-stranded RNA synthesis commenced about 10min. after addition of the bacteriophage and continued to be synthesized exponentially until about 50min. after bacteriophage addition, when the rate of synthesis decreased.

It was also found that, whereas the recovery of

single-stranded RNA decreased from about 60min. after bacteriophage addition, the amounts of DNA and double-stranded RNA increased slightly (Fig. 5). This could have been due to the fact that bacteria and bacterial debris were extracted for nucleic acids and no account was taken of the material liberated from lysed cells; it has already been suggested that DNA (and presumably double-stranded RNA), in contrast with single-stranded RNA, tends to adhere to bacterial debris (see the Methods and Materials section). Another possible explanation of the observation is that cells for which lysis is delayed synthesize more double-stranded RNA and DNA than those which lyse earlier.

DISCUSSION

Properties of the coliphage double- and single-stranded RNA. The properties of the induced double-stranded coliphage RNA are very similar to those of other RNA coliphages (Weissmann *et al.* 1964*a*; Kelly & Sinsheimer, 1964; Nonoyama & Ikeda, 1964; Erikson *et al.* 1964; Kaerner & Hoffmann-Berling, 1964; Kelly *et al.* 1965).

Process of infection by an RNA coliphage. The initial sequence of events of the infection process has become much clearer in the last two years. It is apparent that RNA coliphages, like the filamentous bacteriophages, are specific to male strains (Hfr or F^+) of *E. coli* (Loeb & Zinder, 1961; Bradley, 1964). The RNA coliphage attaches to the male-specific fimbriae and injects its RNA into the bacterium (Brinton, Gemski & Carnaham, 1964). The RNA ('plus' strand) becomes associated periodically during the first few minutes of infection with ribosomes from which it can be extracted as 20s RNA (Erikson *et al.* 1964; Kelly *et al.* 1965). The enzyme RNA-primed RNA-polymerase (RNA replicase) is synthesized during this period, and by about 10min. after coliphage adsorption replication of the infecting RNA 'plus' strand takes place to give, according to the theory proposed by Weissman *et al.* (1964*b*), a double-stranded RNA composed of the 'plus' and a complementary 'minus' strand. This polymerase is specific to the RNA that induced it (Haruna & Spiegelman, 1965). Replication of the double-stranded molecule is asymmetric, resulting in 'plus' strands alone (Weissmann *et al.* 1964*b*). It is apparent that these processes are repeated so that the amounts of both double-stranded and single-stranded coliphage RNA are increased inside the infected cell. The synthesis of the RNA replicase has been shown to be continuous throughout the infection process (Weissmann, Simon, Borst & Ochoa, 1963), and, though the time-course of the synthesis of coat protein has not yet been defined, however, it has been demonstrated for the RNA coliphage ZIK/1 that complete progeny coliphage are present by 40min. after coliphage adsorption, some 20min. before cell lysis commences (Bishop, 1965). In conjunction with these observations it has been found that syntheses of bacterial DNA, ribosomes, ribosomal RNA and s-RNA are decreased 10–15min. after coliphage adsorption (Bishop, 1965).

Though the experiment designed to examine the fate of parental RNA in the infected bacterium demonstrated that it became incorporated into double-stranded RNA, it does not in itself preclude the possibility of a breakdown of the infecting RNA and the synthesis of a non-specific type of double-stranded RNA from the breakdown products. However, double-stranded RNA isolated from infected bacteria, although non-infectious itself, contains infectious RNA (D. H. L. Bishop, unpublished work). It would seem unlikely therefore that the parental RNA is broken down before becoming incorporated into double-stranded material. Whether, however, the double-stranded RNA is an intermediate of the replication process or a side product is not proven in these experiments.

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