within the year correlates with similar spacing of the wet and dry periods and especially with the two points each year when almost daily rains cease. No other environmental stimuli were found which correlate in a similar suggestive way.

The most likely postulated mechanism for control of the nesting peaks involves the stimulus of the dry-out and sun exposure of the dry season to the onset of molt. The molt in turn reduces nesting because of a partial antagonism of this process and the culminative phase of breeding. Resumption of nesting at high frequency follows innate recovery processes of the adenohypophysis-gonad mechanism and thus falls well within the rainy period subsequent to each dry period.

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*THE ISOLATION OF T4-SPECIFIC RNA ON A DNA-CELLULOSE COLUMN**,†

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It has been proposed[1] that genetic information is carried from DNA to ribosomes, the site of protein synthesis, by "messenger" molecules. The messenger is presumed to be a polyribonucleotide having a molecular weight of several hundred thousand, a base sequence complementary to that of the DNA segment from which it originated, and a high rate of metabolic turnover.

The RNA synthesized in phage T2-infected *E. coli* very closely resembles the hypothetical messenger-RNA in many of its properties. Volkin and Astrachan reported that this phage-induced RNA was similar in base composition to T2 DNA[2] and metabolically unstable.[3] Subsequently, T2-specific RNA was shown to be associated with ribosomes[4, 5] and to have an average molecular weight of approximately 1.5×10^5 (ref. 4). Finally, this RNA was found to form specific complexes with heat-denatured T2-DNA, indicating a complementary sequence relationship.[6]

All investigations of messenger-RNA previously reported have been carried out with specific radioactive labeling, to permit the detection and study of messenger-RNA in the presence of large amounts of metabolically less active RNA. Some enrichment of T2 specific RNA has been obtained by zone electrophoresis and centrifugation, but purification was incomplete.[4]

In this paper, we report a chromatographic method for the isolation of T4-specific RNA, based upon RNA-DNA hybrid formation. Further, the method per-

mitted us to select, from the mixture of T4-specific RNA molecules, a fraction which appears to correspond to the *r*II region of the phage genome.

Materials and Methods.—Preparation of the DNA-cellulose columns: Phosphocellulose (40 gm, Schleicher and Schuell, 0.9 meq P/gm) was acetylated with 120 ml acetic anhydride in 200 ml of glacial acetic acid and 0.2 ml of concentrated sulfuric acid as catalyst. The resulting gel was precipitated by pouring into an excess of water, filtered, washed, and dried at room temperature *in vacuo.*

Phage DNA was obtained by growing 15 liters of *E. coli* BB in glucose-salts medium[7] for three generations to 5×10^8 cells/ml. Th bacteria were infected with T4-B-wild-type phages or the mutant *r*1272 at a multiplicity of 0.1, followed by vigorous aeration at 37° for 12–24 hr. When lysis was complete, a few ml of chloroform was added. The phage yield usually was between 5×10^{11} and 10^{12} particles per ml.

The phages were purified by differential centrifugation, and the DNA was isolated by the phenol method according to the procedure of Grossman *et al.*[8] Since it was not possible to dissolve DNA directly in pyridine (the solvent chosen for the condensation reaction), the DNA was dissolved in distilled water at a concentration of 2 mg/ml and dialyzed against increasing concentrations of methanol.[9] Finally, the solution of DNA in methanol was dialyzed against pyridine; the remaining methanol and water were distilled off at 40°C *in vacuo.*

A reaction procedure similar to that used in the preparation of synthetic polynucleotides[10] has been used to link the phage DNA to the acetylated phosphocellulose. To 0.1 gm of DNA in pyridine 1.5 to 2 gm acetylated phosphocellulose were added, and the mixture was heated to 110°C to keep water out of the reaction mixture. Subsequently, 1 gm of DCC was added and the mixture was refluxed for 1 hr at 115°C. After cooling, the product was added to water and the resulting slurry was filtered and freeze-dried. The material was ground in a mortar, water was added, and the slurry was ground in a tissue homogenizer. Repeated washing with distilled water was used to take out most of the condensing agent and the unreacted DNA. The percentage of phage DNA bound to cellulose was measured using P^{32}-labeled DNA. The reaction product was washed with distilled water until no P^{32}-labeled material could be detected in the wash water. The amount of P^{32} remaining in the phosphocellulose was found to be between 60 and 70% of the input in two different preparations.

Chromatography on phage DNA-cellulose: The columns were made 15 to 25 cm long and 1.3 to 1.5 cm in diameter, and contained between 100 and 200 mg DNA. The decreased flow rates which resulted from a high DNA content could be improved by adding varying amounts of coarse grade cellulose. Each column was surrounded with a jacket heated by a circulating water bath.

The RNA samples were applied to the column in about 1 ml of 2 × SSC and incubated for 1 hr in the top part of the column; then 1.5 ml 2 × SSC was added to the column, allowing the RNA to move down into a zone of new DNA-cellulose; the mixture was incubated again, and so forth until the RNA sample was incubated with every part of the column. Optimal hybrid formation was achieved by stepwise incubation at 55°C for a total of 12 to 15 hr. After removal of unadsorbed RNA from the column with 2 × SSC, the eluent was changed to 0.01 × SSC, and the temperature was raised to 65°C to dissociate the DNA-RNA complexes. The radioactivity in the RNA of the effluent fractions was determined as described below.

Incorporation of P^{32} and H^3 into RNA: P^{32} orthophosphate was used to label all RNA preparations uniformly before and after infection. RNA synthesized after phage infection was labeled specifically with H^3-uracil (New England Nuclear Corporation, 1 C/mM). For the labeling experiments, the bacteria were grown at 37°C in medium C of Roberts *et al.*,[11] modified by including 5 gm NaCl and 0.37 gm KCl per liter and lowering the phosphate concentration to 10^{-3} *M*; 0.1 *M* tris (pH 7.3) was used as buffer. To 2×10^7 bacteria per ml, P^{32} orthophosphate was added at a concentration of 2.5 μC/ml in Experiment 1 and 100 μC/ml in Experiment 2. When the bacteria had grown to 5×10^8 cells/ml (generation time: 60 min), they were infected with 5×10^9 phage particles/ml in the presence of 10 μg/ml of L-tryptophan, required for adsorption of the phage. The efficiency of infection was checked by assaying the number of surviving bacteria after 3 min. The survival was 0.02% in Experiment 1 and 0.1% in Experiment 2.

H^3-uracil was added to 100 ml of the infected culture at a concentration of 0.5 μg/ml. In Experiment 1, the H^3-uracil was incorporated from 12 to 15 min after infection and in Experiment 2 from 3 to 10 min and from 3 to 15 min. At the end of the specified period, the culture was

chilled by pouring it onto 40 ml of crushed frozen buffer (10^{-2} M tris, pH 7.3, containing 10^{-2} M $MgCl_2$). All subsequent operations were performed at 0–2°C. The bacteria were sedimented by centrifugation for 5 min at 10,000 × g, and the pellet was resuspended in 2 ml of 10^{-2} M tris buffer (pH 7.3) containing 5×10^{-3} M $MgCl_2$ and 10^{-2} M Na azide. This suspension was frozen in dry ice-methanol and stored at −10°C.

Extraction and purification of RNA: After the frozen cells were thawed in a 20°C water bath, lysozyme was added at a concentration of 600 μg/cc. After 2 min, 10 μg/cc of DNAase was added and incubation was continued for 2 min at 20°C. The pH was then adjusted to 5 by the addition of 0.02 M acetic acid (0.2 ml). Sodium dodecyl sulfate was added to a concentration of 5 mg/ml and the tube was incubated at 37°C for 3 min. The solution was shaken for 5 min at room temperature with an equal volume of distilled phenol saturated with 0.05 M acetate buffer (pH 5.2) containing 10^{-2} M $MgCl_2$. The aqueous and phenol phases were separated by centrifugation for 10 min at 10,000 × g.

The phenol-extracted RNA was further purified by passage over an IR-120 Sephadex column. A 15 cm column of Sephadex G-25 (Pharmacia, Uppsala, Sweden), coarse grade, was packed into a glass tube 1 cm in diameter, and on top of this was placed a 20 cm column of Amberlite IR-120, in the K^+ form. The column was washed before use with an excess of 0.01 M potassium acetate-acetic acid buffer at pH 5.2.

The aqueous phase from the phenol extraction was applied to this column at 25°C, and the RNA was washed through with the 0.01 M acetate buffer under sufficient pressure to give a flow rate of 1 ml/min. RNA passes through both portions of the column with the solvent front and may be located by its ultraviolet absorption with a maximum at 260 mμ. Across the RNA peak, perfect coincidence was found between absorbance at 260 mμ and acid-precipitable H^3 and P^{32} activity. Several fractions later, another ultraviolet-absorbing peak, containing phenol, was eluted from the column. This second peak shows an absorption maximum at 270 mμ.

Counting of H^3- and P^{32}-RNA: Preparation of the RNA samples for liquid scintillation counting was described previously.[6] P^{32} and H^3 activity were determined by simultaneous counting in a Packard Model 314 EX liquid scintillation spectrometer. Suitable adjustment of the photomultiplier voltage and the gain of each of the two amplifiers made it possible to count each isotope at a balance point, with no more than 1% cross-contamination in the results for either P^{32} or H^3.

Base analysis: The base composition of uniformly P^{32}-labeled RNA was determined by a procedure based upon isotope dilution. The P^{23}-labeled RNA ($2–8 \times 10^5$ cpm, s.a., 1×10^5 cpm/μg) was mixed with 1.5 mg of *E. coli* ribosomal RNA. The mixture was precipitated with 10% TCA at 0°C and washed once with cold ethanol-ether (1:3). The RNA precipitate was dissolved in 0.4 ml of 0.3 N KOH and hydrolyzed for 16 hr at 30°C.

The resulting solution of 2′,3′-ribonucleotides was then applied to a column of Dowex-1-formate, X-8 (diameter 1 cm, height 0.8 cm). After loading, the column was washed with 30 ml H_2O, followed by 30 ml of 0.005 N formic acid. Approximately 50 ml of each of the following eluents were passed through the column: (1) 0.025 N formic acid for CMP, (2) 0.2 N formic acid for AMP, (3) 0.01 N formic acid + 0.05 N ammonium formate for UMP, and (4) 0.1 N formic acid + 0.2 N ammonium formate for GMP.

The effluent was collected in 3.5 ml fractions and the ultraviolet absorbance was measured at 260 and at 280 mμ. For assay of radioactivity 0.5 ml of each fraction was mixed with 6 ml ethanol and 8 ml toluene containing 0.8 mg POPOP and 32 mg PPO.

In calculating the base composition, the specific activity of each nucleotide (the weighted mean value for the five peak fractions of each) was multiplied by the mole fraction of that nucleotide in the carrier RNA. The quantities resulting (cpm /nucleotide) were normalized to give the nucleotide composition of the P^{32} RNA. The base composition of the carrier, determined by anion exchange separation and spectrophotometric analysis of the nucleotides, was: AMP: 24.6 ± 0.7%; UMP: 20.5 ± 0.8%; CMP: 22.5 ± 0.4%; and GMP: 32.4 ± 1.0%. The values are in good agreement with the base composition of *E. coli* ribosomal RNA reported by Spahr and Tissières.[12]

Results.—Purification of T4-specific RNA: The first experiments were undertaken to determine whether the DNA-cellulose columns would separate RNA made after phage infection (T4-specific RNA) from *E. coli* RNA. For this purpose, doubly

labeled RNA (Preparation I) was used. The P^{32} label was distributed throughout all RNA molecules and the H^3 label represents RNA made after infection.

One mg RNA was applied to a column containing 50 mg T4-wild type DNA linked to cellulose. The column was developed stepwise, washed, and eluted at low ionic strength and high temperature to release quantitatively the adsorbed RNA (see *Materials and Methods*). From each of the 2 ml fractions of column effluent, 0.1 ml was used for analysis of TCA-precipitable H^3 and P^{32} activity.

The elution diagram (Fig. 1) shows a clear separation of the H^3-labeled molecules

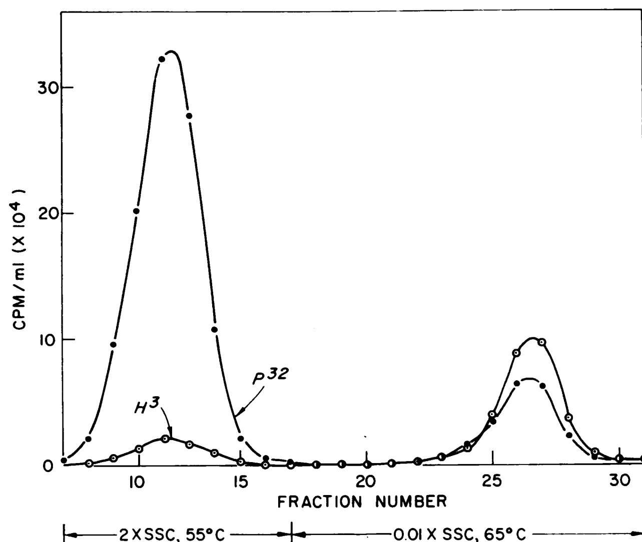

FIG. 1.—Chromatography of RNA on DNA-cellulose. One mg RNA with a specific activity of 3.5×10^3 cpm/μg of P^{32} (labeled before and after infection) and 700 cpm/μg of H^3 (labeled from 12–15 min after infection) was incubated on DNA-cellulose with stepwise development at 55°C for a total of 15 hr. The first peak was eluted with $2 \times$ SSC at 55°C. At fraction 18, the eluent was changed to $0.01 \times$ SSC, and the temperature was raised to 65°C. From each fraction, 0.1 ml was taken for analysis of radioactivity. Fraction size = 2 ml.

from the bulk of the P^{32}-RNA. Whereas the first peak is high in P^{32} and low in Hs activity, the second has a H^3/P^{32} ratio eight times that of the starting material and contains 82 per cent of the RNA made subsequent to T4 infection (H^3-RNA).

Base composition of adsorbed RNA: Base composition analysis was used to characterize the RNA fraction adsorbed by DNA-cellulose.

RNA from Experiment 2, having a specific activity of 1×10^5 cpm P^{32}/μg RNA, was used for these studies. The length of time (four generations) for which the P^{32} was present in the bacterial culture before infection should have insured a uniform distribution of radioactive phosphorus atoms within all RNA and RNA precursor molecules. Therefore, the base composition can be directly determined from the percentage of the P^{32} in the molecule associated with the nucleotides in an alkaline hydrolysate.

The base analysis given in Table 1 shows that the DNA-cellulose column has

TABLE 1

BASE COMPOSITION OF P^{32}-LABELLED RNA ADSORBED BY T4 DNA-CELLULOSE

	Preparation II-10			Preparation II-15	
	A	B	C	A	C
CMP	22.6	17.5	15.4	23.2	16.4
AMP	23.6	30.9	30.6	24.4	29.4
UMP	20.0	29.6	32.9	21.0	32.3
GMP	33.8	21.9	21.1	31.4	21.9

The values are given as moles per 100 moles of the four nucleotides. A = Total RNA before chromatography. B = RNA fraction adsorbed by DNA-cellulose-column. C = RNA adsorbed upon rechromatography of fraction B. Preparation II-10 = RNA isolated 10 min after infection. Preparation II-15 = RNA isolated 15 min after infection.

specifically adsorbed from the original mixture of RNA molecules those having an average composition of 16% CMP, 30% AMP, 33% UMP, and 21% GMP. These figures agree closely with those obtained by others using specific P^{32} labeling after T2 infection. Thus it appears that here very effective separation between T4-specific RNA and *E. coli* RNA has been achieved. Moreover, the separation is almost complete after one passage through the DNA-cellulose column, since the base ratio does not change markedly upon rechromatography.

The effect of adsorption and elution upon the physical state of RNA: When examined by CsCl gradient centrifugation, RNA eluted from the DNA-cellulose column was found to have an anomalously low density (Fig. 2*A*). In previous studies, T2-specific RNA was observed always to have its maximum concentration within the first 0.5 ml emerging from the bottom of the centrifuge tube.[6] After dialysis against 0.01 × SSC, heating for 5 min at 100°C and rapid cooling in ice, the density of this RNA became normal (Fig. 2*B*).

The RNA preparation originally applied to the column and the material emerging in the solvent front were also examined by density gradient centrifugation. In each case, the RNA exhibited a normal density; the concentration distribution was indistinguishable from that shown in Figure 2*B*.

These experiments demonstrate that the adsorption and/or desorption process causes a reversible decrease in the density of RNA. Whether the diminished density results from a conformational change in RNA, from attachment to a small amount of DNA, or from some other factor, is unknown.

No difference in the ability to be adsorbed by DNA-cellulose could be detected between light and heavy T4-specific RNA. Rechromatography of an adsorbed peak gave rise to the same percentage of complex formation, whether it was loaded directly onto the next column or heated and fast-cooled prior to reapplication.

rII-RNA: The isolation of a single molecular species of messenger-RNA would appear to require the most selective fractionation procedures. Chromatography on DNA-cellulose, because it is based upon the interaction of complementary base sequences, offers the hope of such selectivity. Using DNA from the T4 mutant *r*1272 in the DNA-cellulose columns, we have attempted to isolate RNA complementary to the *r*II region of the T4 genome. Since *r*1272 appears to lack the entire *r*II region, an *r*1272 DNA-cellulose column should not adsorb *r*II RNA, while still adsorbing all other types of T4-specific RNA. Since the *r*II region amounts to 1 per cent of less of the phage genome,[13, 14] and since the DNA-cellulose column adsorbs RNA with an efficiency of 80–90 per cent, isolation of *r*II-RNA by one passage through the column was not anticipated. However, one might expect to en-

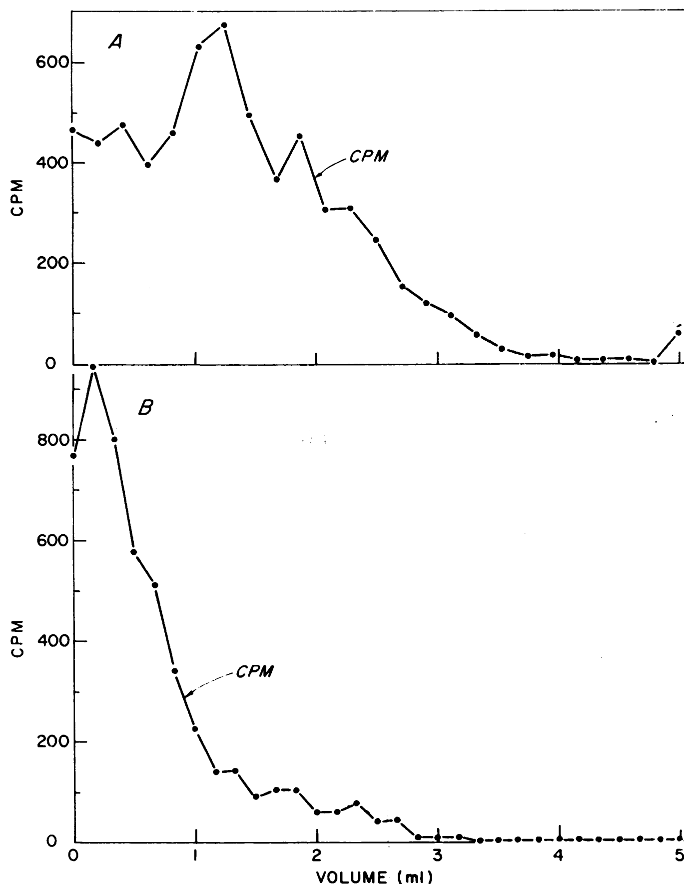

Fig. 2.—Centrifugation of RNA in a CsCl density gradient. (A) H^3-RNA eluted from DNA-cellulose. (B) H^3-RNA eluted from DNA-cellulose and subsequently heated for 5 min at 100°C with fast cooling. In each case, the sample (in 0.01 × SSC) was saturated with CsCl at 25°C and then layered under 4 ml of a CsCl solution of density = 1.73. Centrifugation was for 60 hr at 33,000 rpm in a Spinco Model L ultracentrifuge. Sampling and analysis were as previously described.[6] The left side of each diagram represents the bottom of the centrifuge tube.

rich for *r*II-RNA by repeatedly applying to the column those molecules which are not adsorbed.

The procedure used for purification of *r*II-RNA is outlined in Table 2. Chromatography on r^+ DNA-cellulose was done twice (W-1 and W-2) to insure complete removal of *E. coli* RNA. Repeated chromatography on *r*1272 DNA-cellulose produced an apparent enrichment of RNA unable to bind to the *r*1272 column. This is shown by the progressive increase in the percentage of RNA unadsorbed in successive runs (Table 2, bold-face numbers). Figure 3*A* shows the last run (R-3) in which a large portion did not adsorb to the *r*1272 column. One explanation for this might be a decreased ability of the *r*1272 column to bind RNA. This was tested by applying the RNA fraction adsorbed in run R-3 again to the same DNA-cellulose column. The result (Fig. 3*B*) shows that the column had retained its

TABLE 2

PURIFICATION OF *r*II-RNA

No. of run	Type of DNA linked to column	RNA applied to column	H^3-RNA applied to column (cpm)	Total recovery (%)	Fraction of H^3-RNA unadsorbed (%)	Fraction of H^3-RNA adsorbed (%)
W-1	r^+	Total from r^+-infected cells	700,000	88	18	82
R-1	*r*1272	Adsorbed RNA from run W-1	390,000	95	15	85
W-2	r^+	Unadsorbed RNA from run R-1	42,500	76	20	80
R-2	*r*1272	Adsorbed RNA from run W-2	21,000	93	34	66
R-3	*r*1272	Unadsorbed RNA from run R-2	6,800	95	55	45

Initially, 1 mg of Preparation I (700 cpm $H^3/\mu g$ RNA) was loaded onto the r^+ column (W-1). For the succeeding columns, prior to rechromatography, the four peak fractions of the adsorbed or unadsorbed RNA peak were concentrated to a volume of 1 ml by dialysis against a concentrated solution of polyethylene glycol (Carbowax 6,000). Following dialysis against 2 × SSC, the material was loaded onto the column indicated. Incubation and elution of the column were done as described in Fig. 1.

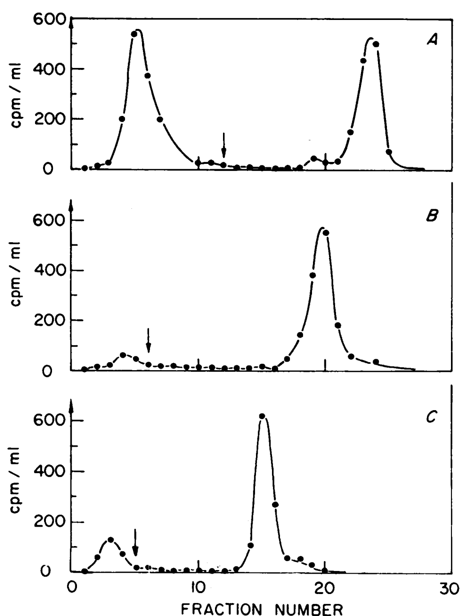

FIG. 3.—Chromatographic analysis of purified RNA. (A) Run R-3 (see Table 2). Column material *r*1272. (B) Rechromatography of the adsorbed RNA peak from A on the same *r*1172 column. (C) Chromatography of the unadsorbed RNA peak from A on an r^+ column. In each diagram, the RNA peak on the left emerged with the solvent front and the peak on the right is adsorbed RNA. The arrow indicates the point at which the eluent was changed from 2 × SSC to 0.01 × SSC and the temperature from 55° to 65°C. The fractions were concentrated prior to application to the column as described in Table 2.

ability to bind non-*r*II-RNA. Furthermore, the unadsorbed RNA from column R-3 fully retained its ability to be adsorbed by r^+ DNA (Fig. 3*C*).

Clearly, the fractionation procedure has brought about a considerable enrichment (perhaps 50-fold) of RNA capable of being adsorbed by r^+ DNA, but not by *r*1272 DNA. If the binding affinities of these polynucleotides are a true reflection of homologies between genetic regions and their respective messengers, this amounts to the purification of the messenger for a restricted genetic segment—the two cistrons of the *r*II region.

Discussion.—Nature of the interaction between RNA and DNA-cellulose: DNA linked to cellulose was chosen as a chromatographic material with the expectation that it might effect a fractionation of RNA molecules according to base sequence

as distinguished from simple bulk properties such as molecular weight or base composition. Those RNA molecules complementary in base sequence to the DNA of the column should be selectively adsorbed as a result of extensive RNA-DNA base-pair hydrogen bonding. Under the conditions employed to couple DNA to cellulose, the DNA is denatured[9] and should be capable[6] of forming specific complexes with RNA. To discuss the extent to which these expectations have been fulfilled, the following observations are relevant:

1. Complex formation required a prolonged incubation at elevated temperature (50–60°C). Very little adsorption of RNA was achieved at lower temperature (40°C) or with continuous passage of T4-specific RNA through the column, even at low flow rates.

2. The complexes form at high ionic strength and are dissociated at low ionic strength.

3. The base composition of the RNA adsorbed resembles that of the DNA in the column.

In the light of available information,[15, 16] these considerations strongly suggest that the adsorption of RNA to the column involves complementary base-pair hydrogen bonding.

If, as appears likely, the DNA-cellulose linkages are formed by condensation of the glucosylic hydroxyls of T4 DNA with the phosphates of the cellulose, then the procedure in its present form can only be used with the DNA of T-even phages. However, DNA might be immobilized equally well by the reaction of another functional group, common to all DNA. Chromatographic preparation of complementary RNA might then be feasible for a variety of organisms.

The base composition of T4-specific RNA: Previously reported[2–4] studies of P^{32} incorporation into phage-specific RNA have all involved "pulse" labeling—that is, exposure of the infected culture to P^{32} for a time which is of the order of one generation time or less. Inferences as to the base composition of phage-specific RNA based upon these studies are made uncertain because phosphorus atoms in the various RNA precursors (CTP, ATP, UTP, GTP, or related compounds) might not be equally labeled during a period of metabolic transition such as occurs after bacteriophage infection. The values reported here for the nucleotide composition of T4-specific RNA are not subject to this uncertainty because the P^{32} was uniformly incorporated into all RNA molecules and RNA precursors.

Nonetheless, the nucleotide composition obtained by our method for T4-specific RNA and the one obtained by Volkin and Astrachan[2, 3] for T2-specific RNA are in close agreement. Both show a general pattern of high values for AMP and UMP and low values for CMP and GMP. Both indicate a base composition for phage-specific RNA close to that of T2 or T4 DNA and far from that of *E. coli* RNA. At a more detailed level, the agreement is also striking—in both cases GMP is considerably higher than CMP, whereas AMP and UMP occur to a similar extent. In view of the agreement on this point, it is tempting to attribute real significance to the nonequivalence of CMP (16%) and GMP (21%) in T4-specific RNA and the near-equivalence of their mean (18.5%) to the percentage of each of the corresponding bases, guanine (18%) and 5-hydroxymethylcytosine (17%) in T4 DNA.[17] Such a relation is to be expected if one strand of T4 DNA contains more guanine (21%) than cytosine (16%) (the other strand has the complementary

composition) and T4-specific RNA is synthesized using only strands of the latter type as template.

r-II-RNA: The mutant *r*1272 used in our studies covers the entire *r*II region, extending over both cistrons A and B, and represents a deletion by genetic criteria.[13, 14] However, the available physical methods were insufficiently sensitive to determine whether or not a corresponding segment of DNA is actually deleted from the genome of *r*1272. If this mutant is not a deletion, its failure to yield recombinants with any point mutant of the *r*II region implies that the DNA of the entire region is nonhomologous in base sequence to the DNA of wild-type phages. In either case, the DNA of the mutant *r*1272 would be unable to bind *r*II-RNA synthesized by a wild-type phage, since the complementary base sequence is not present in the DNA. The isolation of a small fraction of the T4-specific RNA which binds to r^+ DNA, but not to *r*1272 DNA, is consistent with the view that *r*1272 is truly a deletion but does not necessarily prove it.

Summary.—T4-specific RNA has been quantitatively separated from *E. coli* RNA by chromatography on columns made of T4 DNA linked to cellulose. Apparently, the basis of the fractionation is specific hydrogen-bond formation between the DNA of the column and RNA molecules complementary in base sequence. A DNA-cellulose column containing DNA from the T4 deletion mutant *r*1272 has been used to purify a type of RNA which appears to be specific for the *r*II region of the phage genome.

We wish to thank Seymour Benzer for giving us a stock of *r*1272.

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† Abbreviations used in this paper include the following: DCC: dicyclohexylcarbodiimide (Eastman); tris: tris (hydroxymethyl)amino-methane; POPOP: 1,4-bis-2(5-phenyloxazolyl) benzene; PPO: 2,5-diphenyloxazole; AMP: 2′,3′-adenylic acid; UMP: 2′,3′-uridylic acid; CMP: 2′,3′-cytidylic acid; GMP: 2′,3′-guanylic acid; SSC: 0.015 *M* Na citrate, 0.15 *M* NaCl.

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