[24] Brimacombe, R., J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, and T. Jaouni, these PROCEEDINGS, **54**, 954 (1965).
[25] Söll, D., E. Ohtsuka, D. S. Jones, R. Lohrmann, H. Hayatsu, S. Nishimura, and H. G. Khorana, these PROCEEDINGS, **54**, 1378 (1965).
[26] Brenner, S., A. O. W. Stretton, and S. Kaplan, *Nature*, **206**, 994 (1965).
[27] Weigert, M. G., and A. Garen, *Nature*, **206**, 992 (1965).
[28] Ambler, R. P., *Biochem. J.*, **89**, 349 (1963).
[29] Munkres, K. D., N. H. Giles, and M. E. Case, *Arch. Biochem. Biophys.*, **109**, 397 (1965).
[30] Munkres, K. D., and F. M. Richards, *Arch. Biochem. Biophys.*, **109**, 466 (1965).
[31] Woodward, D. O., and K. D. Munkres, these PROCEEDINGS, **55**, 872 (1966).

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*PURIFICATION AND PROPERTIES OF THE REPLICATIVE INTERMEDIATE OF THE RNA BACTERIOPHAGE R17**

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Communicated by Theodore T. Puck, April 12, 1966

Replicative intermediate (RI) is a unique RNA structure found in cells infected with RNA viruses.[1] RI sediments as a broad band, is only partially digested by ribonuclease, and sediments as a sharp band at a lower *S* value (13*S*) after ribonuclease treatment.[1–3] It is termed replicative intermediate, since it is labeled with an RNA precursor, such as H^3-uridine, before any other viral-specific RNA,[1] since label from replicative intermediate can be chased into single-stranded viral RNA,[1] and since parental viral RNA is incorporated intact into RI.[2, 4] RI may be a double-stranded RNA template with nascent, partially completed, single-stranded viral RNA chains.[3] The double-stranded RNA with no nascent chains (13*S* component) is termed replicative form.[1, 3]

Since it has not been possible to purify this material until now, the properties of RI have been inferred from various indirect experiments. In general, RI has usually fractionated with ribosomal RNA. Thus, in a sucrose gradient, RI sediments as a broad band between approximately 23 and 16*S*, i.e., in the same region as the two ribosomal RNA components. Also, RI is precipitable with 1 *M* NaCl (along with ribosomal RNA), due to the single-stranded component.[5] It also fractionates with ribosomal RNA on a column of calcium phosphate according to the procedure of Bernardi and Timasheff,[6] and in a two-phase polymer system consisting of dextran sulfate and polyethylene glycol.[7] Although it can be partially separated from ribosomal RNA by sedimentaton in sucrose gradients of low ionic strength, the method has not proved practical for analysis or for bulk purification.[7] It was, therefore, particularly gratifying to effect a remarkable separation of RI by stepwise elution from a cellulose column. Exploiting the change in chemical activity of nucleic acids in buffers containing variable amounts of alcohol, Barber separated soluble RNA from ribosomal RNA on a cellulose column.[8] By further fractionation it was possible to separate RI from ribosomal RNA, as will be shown in this paper.

Materials and Methods.—Bacteria and virus: *E. coli* strain 3000, a nonlysogenic Hfr strain, was kindly provided by Professor R. L. Sinsheimer. R17 bacteriophage, free of λ, was used in all experiments.[9]

Media and solutions: Cells were grown in a modification of TPG medium[10] in which 0.2% glycerol was substituted for glucose, and casamino acids (Difco) were used at a final concentration of 0.15%. This medium was designated TCGl and was the basic medium for spheroplasts as well as cells.

STE buffer for chromatography had the following composition: 0.1 *M* NaCl, 0.001 *M* EDTA, and 0.05 *M* Tris, pH 6.98 or 6.85 at 25°C (reagent grade Trizma, Sigma Chemical Co.).

In vivo incorporation: Uninfected cells, grown to a titer of 2×10^8/ml, were labeled with H^3-uridine, usually for 15 sec. The cells were then poured over frozen TCGl (30 ml ice per 100 ml cells) containing KCN (final concentration 0.01 *M*) and unlabeled uridine (final concentration 100 μg/ml). For infection, cells were also grown to a titer of 2×10^8/ml and harvested by centrifugation. Spheroplasts were produced from these cells using the procedures of Spiegelman *et al.*[11] The stock lysozyme solution was at 5 mg/ml, however, and the cells were only incubated 2 min at room temperature in order to form spheroplasts. The spheroplasts from 100 ml of cells were poured into 23 ml of TCGl medium containing 10% sucrose, a supplement of 0.1% $MgSO_4$, and 1% bovine serum albumin, fraction V (spheroplast medium). To this was added 0.6 ml of 0.1% protamine and enough bacteriophage to infect at an input multiplicity of 25–40. After allowing bacteriophage adsorption to take place for 5 min at room temperature, the cells were diluted into TCGl (the final volume was $1/2$ the original volume in which the cells had grown) supplemented as just described. Actinomycin D (kindly supplied by Merck, Sharpe and Dohme) was added to a final concentration of 0.25–1.0 μg/ml in order to suppress normal RNA synthesis partially or completely. The infected cells were labeled with H^3-uridine, usually for 15 sec at 30 min postinfection, in order to label the replicative intermediate.[1] The labeling period was terminated as just described for the uninfected cells. It should be noted that a normal growth cycle takes place in spheroplasts infected with RNA bacteriophage.

Nucleic acid: The chilled cells were centrifuged, resuspended in STE, and the nucleic acids were extracted with phenol in the presence of 1% sodium dodecyl sulfate, as previously described.[4] Three or four phenol extractions (room temperature) were followed by extraction with ether and removal of the ether with bubbling nitrogen. The nucleic acids were then adjusted to 0.2 *M* potassium acetate and precipitated once with 2 vol of alcohol, taken up in a small volume of STE, and then the replicative intermediate and ribosomal RNA were precipitated in 1 *M* NaCl.[5] This precipitate was again dissolved in STE and used in the work described here.

Isotope techniques: All samples were counted in a Tri-Carb liquid scintillation counter using the Millipore filter technique as previously described.[4] The amount of labeled RNA resistant to ribonuclease (ribonuclease A, Worthington Biochemical Corp.) was measured as described previously.[4] H^3-uridine of very high specific activity (20–30 c/mM) was obtained from the Radiochemical Centre, Amersham, England.

Chromatography: All chromatography was carried out at room temperature (25°C). Whatman cellulose CF11 was suspended in TSE, and fine particles were removed by repeated decantation. The cellulose was then poured into a column and washed with TSE containing 1% 2-mercaptoethanol and 0.01 *M* EDTA. The column was then washed with TSE: ethanol, 65:35 (v/v) until the effluent OD_{260} was the same as that of the buffer. The RNA sample was dissolved in TSE, and ethanol was added to a final concentration of 35%. For analytical work, columns 15–20 cm × 1.0 cm diameter were used. Up to 10 OD_{260} units of nucleic acids could be fractionated on such a column. The sample was usually applied to the column in a volume of 0.4–0.6 ml and stepwise elution proceeded with decreasing concentrations of ethanol. Fractions were collected by a fraction collector using a volumetric fractionating device. Drop counting was not feasible due to the change in drop volume with change in ethanol concentration.

Batch preparation of replicative intermediate: *E. coli* 3000 was grown in a 14-liter fermentor vat to a titer of 2–3 × 10^8/ml and then infected at a multiplicity of 20 PFU/cell. At 35 min p.i., the entire batch (usually 11 liters) was poured into a carboy containing 1 liter of frozen 0.1 *M* NaCl (frozen at −70°C), 120 ml of 1 *M* KCN, and 120 ml of 2-mercaptoethanol. The cells were collected using a continuous-flow centrifuge, homogenized in a small volume of cold TSE, and then extracted with phenol as described above. The nucleic acid was so concentrated that it was

possible to precipitate with 1 *M* NaCl immediately. The precipitate, containing ribosomal RNA and RI, was collected by centrifugation for 20 min at 10,000 rpm (Servall SS-34 rotor). The precipitate was taken up in TSE, adjusted to 35% ethanol, and chromatographed on cellulose. The complete elution of ribosomal RNA in 15% ethanol was monitored with a LKB Uvicord, then the fraction eluting in TSE was collected and concentrated by precipitation with 2 vol of ethanol or by lyophilization. This material was then chromatographed a second time on a smaller column of cellulose. The fraction eluting from the second column in TSE alone proved to be pure RI since a further cycle of chromatography did not alter its properties.

Results and Discussion.—(1) *Chromatography of labeled RNA from uninfected and infected cells:* Barber demonstrated the separation of soluble RNA and ribosomal RNA on a cellulose column.[8] The soluble RNA was eluted with the 35 per cent ethanol–65 per cent buffer front, and ribosomal RNA was then eluted in distilled water. In the STE buffer used here, ribosomal RNA can be eluted in 15 per cent ethanol–85 per cent STE (Fig. 1*a*). A small amount of OD_{260} elutes with the 35 per cent ethanol front. This probably represents soluble RNA and some native DNA (see below), whereas the remainder of the OD_{260} elutes in 15 per cent ethanol. Practically all of the 15-sec pulse label of uninfected cells is in ribosomal and messenger RNA.[12] A very small amount of label eluted in STE alone. Of the total label eluted, 2.8 per cent eluted with the 35 per cent ethanol front, 92.7 per cent in 15 per cent ethanol, and 4.5 per cent in STE alone. All of these fractions were rendered acid-soluble by ribonuclease.

The mixture of ribosomal RNA and RI (1 *M* NaCl precipitate) from infected cells presented a very different pattern of elution (Fig. 1*b*). Since soluble RNA and DNA were not present, all of the detectable OD_{260} eluted in 15 per cent ethanol. But now only 26.7 per cent of the radioactivity eluted in 15 per cent ethanol, and 73.3 per cent eluted in STE alone. When tested for resistance to ribonuclease A (0.1 μg/ml, 10 min, 37° C), the label eluted in 15 per cent ethanol was 6 per cent resistant and that eluted in STE was 65 per cent resistant.

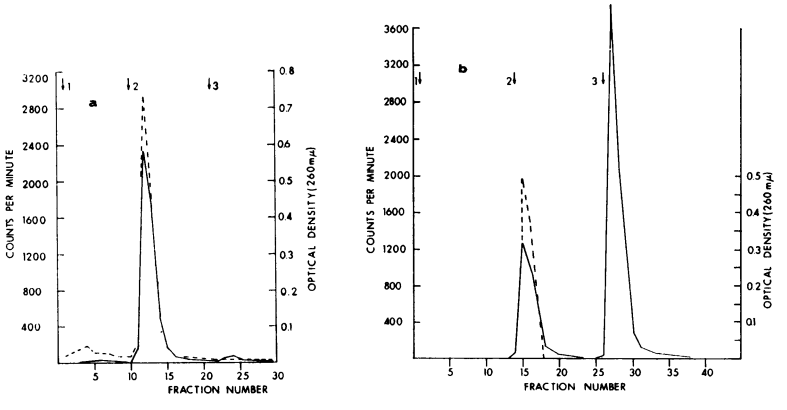

FIG. 1.–(*a*) Chromatography on cellulose of the total RNA from *E. coli* 3000, grown to a titer of 2.3×10^8/ml in TCGl and labeled with H^3-uridine at 5 μc/ml (specific activity, 24.4 c/mM) for 15 sec. The sample was in STE adjusted to 35% alcohol and applied to the column at *1*. Elution with 15% alcohol was started at *2*, and with buffer alone at *3*. Fraction volume was approximately 3.5 ml. *Solid line*, acid-precipitable cpm; *dotted line*, optical density at 260 mμ. (*b*) Chromatography on cellulose of the RNA in the 1 *M* NaCl precipitate from *E. coli* spheroplasts infected for 30 min with R17 bacteriophage in the presence of 0.25 μg/ml of actinomycin D and then labeled for 15 sec with H^3-uridine at 15 μc/ml (specific activity, 24.4 c/mM). At the time of harvest the cells were at a titer of 1.8×10^8/ml. Chromatography data as in (*a*).

The sucrose gradient sedimentation patterns of the eluates in 15 per cent ethanol and STE alone (from Fig. 1*b*) are shown in Figures 2*a* and *b*. The pattern in Figure 2*a* is similar to that of uninfected cells and represents the residual normal synthesis in the presence of the comparatively low concentration of actinomycin used here (0.25 μg/ml). Both ribosomal RNA components were labeled, as well as the region in the gradient where messenger RNA is usually found. The typical optical density pattern of the ribosomal RNA demonstrated that RNA was not degraded after adsorption onto and elution from the cellulose column.

The pattern in Figure 2*b* is very similar to that already described for replicative intermediate.[1] Since cellular RNA is now absent from this gradient, the distribution pattern and the distribution of the ribonuclease-resistant component (the double-stranded component)[1, 3] can be interpreted in terms of a molecular structure. Components of higher *S* value have been interpreted as double-stranded templates with very long nascent single strands, whereas those of lower *S* value are believed to have shorter nascent single strands.[1, 3] This interpretation is supported by the skewness toward lower *S* values of the ribonuclease-resistant component. Presumably the entire RI structure is not labeled in a short pulse such as is used in this experiment and, therefore, a quantitative analysis of the distribution is reserved for studies on uniformly labeled RI, to be reported in a later publication.

Most RI can be eluted in 10 per cent ethanol, but elution is not complete. The per cent RNase resistance and the sedimentation patterns of RI eluted in 10, 5, and 0 per cent ethanol are all the same, i.e., there is no subfractionation of RI in the 10–0 per cent ethanol eluates.

One aliquot of RNA in the 1 *M* NaCl precipitate from infected cells was dissolved in 0.1 *M* NaCl, 0.05 *M* Tris, pH 6.98, 0.001 *M* magnesium acetate, and treated with

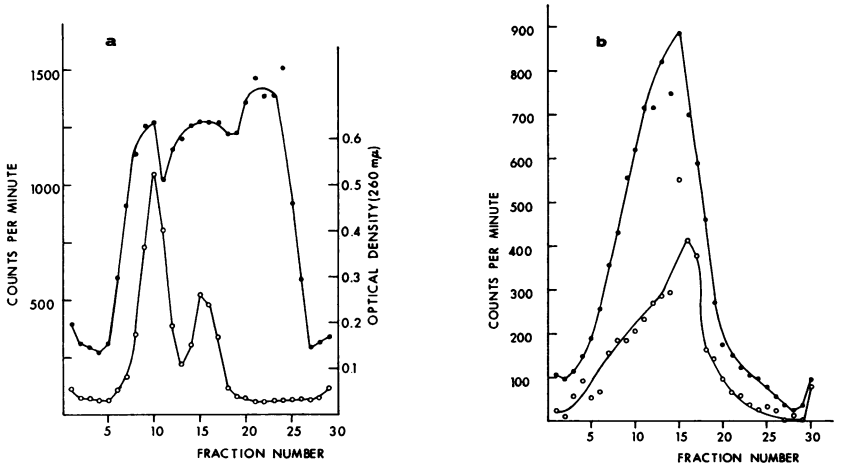

FIG. 2.—(*a*) Sucrose gradient sedimentation pattern of the RNA shown in Fig. 1*b* (infected cells) to be eluted from cellulose in 15% ethanol. The gradients were 20–5% sucrose in STE. The SW39 rotor was run in the Spinco model L-2 centrifuge at 36,000 rpm for 5 hr at 5°C. *Open circles*, optical density at 260 mμ; *closed circles*, acid-precipitable radioactivity (H^3) in cpm. (*b*) Sucrose gradient sedimentation pattern of the RNA shown in Fig. 1*b* to be eluted from cellulose in STE alone. Centrifugation as in Fig. 2*a*, but at 39,000 rpm for 4.5 hr at 5°C. *Closed circles*, acid-precipitable H^3; *open circles* acid-precipitable H^3 after treatment with ribonuclease A (0.1 μg/ml, 10 min, 37°C).

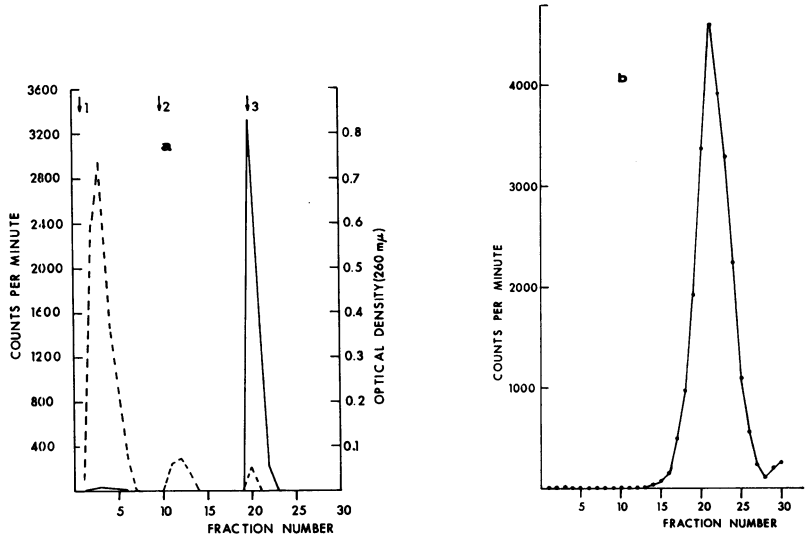

FIG. 3.—(*a*) Chromatography on cellulose of the RNA from *E. coli* 3000 infected and labeled with H^3-uridine as described in Fig. 1*b*, but treated with ribonuclease A (0.1 μg/ml, 10 min, 37°C) prior to chromatography. After ribonuclease treatment, the RNA was re-extracted once with phenol and precipitated in 2 vol of ethanol in the presence of 0.2 *M* potassium acetate. Chromatography data as in Figs. 1*a* and *b*. (*b*) Sucrose gradient sedimentation pattern of the H^3-labeled RNA shown in Fig. 3*a* to be eluted from cellulose in STE alone. Centrifugation as described in Fig. 2*a*.

5 μg/ml of DNase (electrophoretically purified, free of RNase, Worthington Biochemical Corp.) for 15 min at 37°C. After phenol extraction and precipitation with alcohol, the RNA was resuspended in STE and chromatographed. The pattern was the same as that shown in Figure 1*b*, and the sucrose gradient sedimentation pattern of the STE eluate was the same as that shown in Figure 2*b*. Therefore, the properties of replicative intermediate are not due to any complex of RNA with DNA.

When replicative intermediate from the 23–16*S* region of a sucrose gradient is treated with ribonuclease, the RNase-resistant core is found to sediment at 13*S*, and its structure is compatible with that of the so-called replicative form, i.e., double-stranded RNA of 2×10^6 mol wt.[2] The 15-sec pulse-labeled RNA described in Figures 1*b* and 2*a*, *b* was treated with ribonuclease and then chromatographed on cellulose. The chromatographic pattern (Fig. 3*a*) was much different from that of Figure 1*b*. Most of the optical density eluted with the 35 per cent ethanol front. This was presumably degraded ribosomal RNA. Since only acid-precipitable radioactivity was measured, little radioactivity accompanied this optical density peak. Only a small amount of OD_{260} and no radioactivity was found in the 15 per cent ethanol eluate. Finally, almost all of the radioactivity eluted in the STE fraction. This was collected, concentrated by precipitation with 2 vol of alcohol, taken up again in a small volume of STE, and run on a sucrose gradient. A sharp peak sedimenting at about 13*S* indicated that replicative form made up all of this fraction (Fig. 3*b*).

Single-stranded R17 viral RNA was also chromatographed. When 2.0 OD_{260} units of this RNA (*S* value = 27*S*) were applied to the cellulose column, all eluted

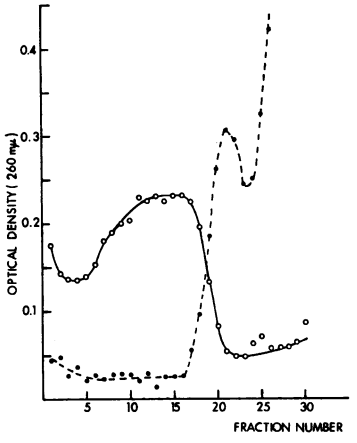

FIG. 4.—Sucrose gradient sedimentation of optical density amounts of replicative intermediate (nonlabeled) isolated from the STE eluate of the cellulose column. Centrifugation as described in Fig. 2a. *Open circles*, untreated replicative intermediate; *closed circles*, treated with 0.1 μg/ml of ribonuclease A (10 min, 37°C) and then re-extracted with phenol before centrifugation.

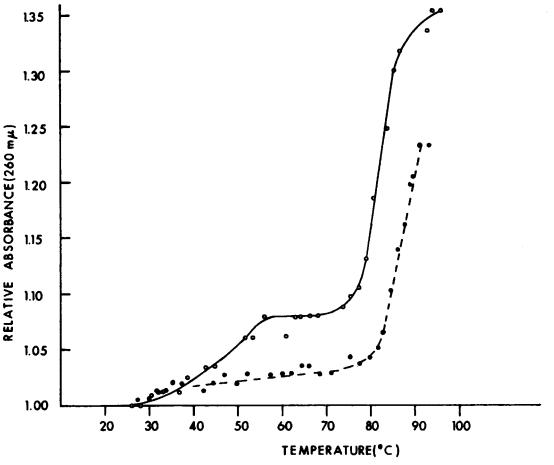

FIG. 5.—Thermal denaturation of replicative intermediate. *Open circles*, replicative intermediate in 0.001 *M* EDTA, 0.05 *M* Tris buffer, pH 6.98 (25°C); *closed circles*, replicative intermediate treated with ribonuclease A (0.1 μg/ml, 10 min, 37°C), re-extracted with phenol, and dialyzed against a 1000-fold excess of 0.001 *M* EDTA, 0.05 *M* Tris buffer, pH 6.98 (25°C) prior to measuring thermal denaturation. Correction was made for the thermal expansion of water.

in 15 per cent ethanol. H^3-labeled double-stranded RNA isolated from reovirus (kindly provided by Dr. W. Iglewski) using a Cs_2SO_4 equilibrium gradient[13] eluted completely in STE alone. The R17 viral-specific RNA in a 1 *M* NaCl supernate (replicative form)[14] also eluted in STE alone. Thus, the following generalizations can be made: soluble RNA and small fragments of RNA elute from cellulose with the 35 per cent ethanol front; single-stranded RNA's (ribosomal, messenger, viral) elute in 15 per cent alcohol; double-stranded RNA's (replicative intermediate, replicate form, reovirus RNA) elute in buffer alone. DNA did not elute as well as RNA, but native DNA (highly polymerized calf thymus DNA, Worthington Biochemical Corp.) eluted predominantly in the 35 per cent ethanol front and denatured DNA (heated 1.5 min at 95°C in STE, then immediately frozen in dry ice: alcohol; relative increase in absorbance at 260 mμ = 1.16) eluted predominantly in 15 per cent ethanol.

(2) *Some further properties of replicative intermediate:* Milligram amounts of RI can be isolated from 10- to 12-liter batches of infected cells according to the procedures described under *Materials and Methods*. The sucrose gradient sedimentation pattern of unlabeled RI is shownin Figure 4. The extremely broad peak is to be expected from the heterogeneous nature of RI. As mentioned above, the material of higher *S* values is believed to have longer single-stranded chains and, therefore, chains closer to completion. The sharp cutoff in the distribution at about 16*S* is noteworthy. Perhaps double-stranded templates with very short single-stranded components may not precipitate in 1 *M* NaCl; that is to say, there may be some critical length of single strand necessary to precipitate RI in 1 *M* NaCl. The com-

ponent sedimenting to the bottom of the tube may be aggregated RNA. These points will be treated further in a subsequent paper.

As has been shown for labeled RI, treatment of optical density amounts of this material with RNase prior to centrifugation resulted in conversion to a band which sediments at 13S (Fig. 4). This is believed to be the double-stranded template from which the ribonuclease-sensitive single strands have been removed, i.e., replicative form.

Thermal denaturation of RI clearly demonstrated the single-stranded and double-stranded components of the population (Fig. 5). The single-stranded component is denatured over a broad temperature band, as is usual for single-stranded RNA.[15, 16] This is followed by a very sharp increase in OD_{260} at a much higher temperature, corresponding to the thermal denaturation curve for replicative form as described by Ammann *et al.*[14] By comparing the OD_{260} after denaturation of the single-stranded component with that after denaturation of the double-stranded component, the amount of single-stranded component was estimated to be 22.2 and 22.5 per cent on two separate batches of RI. Similar data has been obtained on R17 replicative intermediate isolated by another chromatographic procedure, molecular sieving on agarose.[17] The fractionation on agarose appears to be based on molecular weight differences, whereas that on cellulose appears due to differences in molecular structure. Thus, the isolation of RI by two very different procedures emphasizes the stability and uniqueness of this population of RNA molecules.

The thermal denaturation curve of RI, which had been treated with RNase and then dialyzed before heating, exhibited only the component attributable to the double-stranded structure, as might be expected (Fig. 5).

A further estimate of the amount of single-stranded component was made by measuring the RNase-resistant fraction of RI which had been uniformly labeled with H^3-uridine. This RI was purified by two successive adsorption-elution cycles from cellulose. In this case 28 per cent was sensitive to 0.1 μg RNase/ml and 31.5 per cent to 1.0 μg RNase/ml (both incubated 10 min at 37°C). Thus, there appears to be between 22 and 28 per cent single-stranded component in the population of RI.

The purification of replicative intermediate, as described here, depends on the single-stranded component in the first step (precipitation in media of high ionic strength), and on the double-stranded component in the second step (elution from cellulose). This is a clear demonstration of the association of single-stranded RNA (presumably the nascent viral RNA) with double-stranded RNA (presumably the template) to form the population of molecules termed replicative intermediate. The fractionation procedure described here should find wide application in studies on the mechanisms of RNA virus synthesis. It is inexpensive, very simple, and can be used at the tracer level or for isolation of large amounts of RI. The method appears so reliable and clear-cut that it might even be used in a search for unknown RNA viruses, such as human tumor viruses.

Our present extensive studies on the physical, chemical, and biological properties of replicative intermediate should soon lead to a more precise formulation of the mechanism of viral RNA replication.

The author gratefully acknowledges the superb technical assistance of Miss Marianne Salditt and Miss Elizabeth Hinckley.

* Supported by grant no. AI-05320 VR from the U.S. Public Health Service and by grant no. B-14646 from the National Science Foundation.

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[1] Fenwick, M. L., R. L. Erikson, and R. M. Franklin, *Science*, **146**, 527 (1964).

[2] Erikson, R. L., M. L. Fenwick, and R. M. Franklin, *J. Mol. Biol.*, **13**, 399 (1965).

[3] Erikson, R. L., and R. M. Franklin, *Bacteriol. Rev.*, in press.

[4] Erikson, R. L., M. L. Fenwick, and R. M. Franklin, *J. Mol. Biol.*, **10**, 519 (1964).

[5] Franklin, R. M., and M. L. Fenwick, unpublished observations.

[6] Bernardi, G., and S. N. Timasheff, *Biochem. Biophys. Res. Commun.*, **6**, 58 (1961).

[7] Franklin, R. M., unpublished observations.

[8] Barber, R., *Biochim. Biophys. Acta*, **114**, 422 (1966).

[9] Franklin, R. M., and N. Granboulan, *J. Bacteriol.*, **91**, 834 (1966).

[10] Sinsheimer, R. L., B. Starman, C. Nagler, and S. Guthrie, *J. Mol. Biol.*, **4**, 142 (1962).

[11] Spiegelman, S., I. Haruna, I. B. Holland, G. Beaudreau, and D. Mills, these PROCEEDINGS, **54**, 919 (1965).

[12] Gros, F., W. Gilbert, H. H. Hiatt, G. Attardi, P. F. Spahr, and J. D. Watson, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 26 (1961), p. 111.

[13] Shatkin, A. J., these PROCEEDINGS, **54**, 1721 (1965).

[14] Ammann, J., H. Delius, and P. H. Hofschneider, *J. Mol. Biol.*, **10**, 557 (1964).

[15] Gesteland, R. F., and H. Boedtker, *J. Mol. Biol.*, **8**, 496 (1964).

[16] Strauss, J. H., Jr., and R. L. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).

[17] Gordon, J., and R. L. Erikson, personal communication; Erikson, R. L., and J. Gordon, *Biochem. Biophys. Res. Commun.*, in press.

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*REACTIONS OF QUININE, CHLOROQUINE, AND QUINACRINE WITH DNA AND THEIR EFFECTS ON THE DNA AND RNA POLYMERASE REACTIONS**

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Communicated by Colin M. MacLeod, April 14, 1966

The synthetic antimalarial compounds, chloroquine (Resochin[1]) and quinacrine (Atebrin[2]), as well as the tetra-amine, spermine, which is structurally related to the aliphatic side chain of the two drugs,[3] form complexes with DNA[3–5] and interact with the double helix prominently by ionic attraction. We are reporting that quinine also interacts with DNA but binds to the polymer apparently through the formation of urea-sensitive hydrogen bonds.

A variety of effects can be attributed to the formation of complexes of DNA with these substances, for example: stabilization of the double helix by spermine,[5] chloroquine,[3] and quinacrine;[6] inhibition of bacterial type transformation by chloroquine;[7] antimutagenic effects of spermine[8] and quinacrine;[9] inhibition of nucleic acid biosynthesis in two plasmodia by quinine, chloroquine, and quinacrine[10] as well as in bacteria by chloroquine[11] and quinacrine;[12] and finally, inhibition by chloroquine of DNA-primed polymerase reactions *in vitro*.[13]

We distinguish two types of interactions between DNA and these complexing agents. One involves the chromophoric quinoline or acridine moieties of these