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*BIOPHYSICAL CHARACTERISTICS OF THE RNA-CONTAINING BACTERIAL VIRUS R17**

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The discovery of a bacteriophage containing RNA[1] has stimulated considerable interest in the isolation and characterization of other RNA-containing bacterial viruses. Among those which have subsequently been isolated[2] is R17.

The receipt of a sample of R17 and of its host, *E. coli* K-12 (Hfr, methionine$^-$), from Dr. Angus Graham of the Wistar Institute has enabled us to investigate physical and chemical properties of the virus and of its components. R17 appears to be eminently suitable for studies of virus structure and synthesis. It is stable, can be grown in considerable quantity, and is readily purified. It can be degraded to yield protein subunits and stable, high-molecular weight RNA.

This report concerns intact R17. Investigations of the viral protein and RNA will be reported elsewhere.

Methods.—Growth and purification: R17 was grown on *E. coli* K-12 (Hfr, methionine$^-$) in shake-flask cultures to virus concentrations of 10^{12} to 10^{13} plaque-forming units per ml. Cells and debris were removed by centrifugation. The pH of the supernatant liquid of the lysate was slowly lowered to 4 by the addition of 20% acetic acid, and the resulting precipitate was removed by centrifugation and discarded. Three hundred and fifty gm of ammonium sulfate were added for each liter of liquid. The material which precipitated was spun down, taken up in 0.05 *M* Na phosphate buffer, pH 7.0, and subjected to 3 cycles of centrifugation of 15 min at 15,000 rpm (discard sediment), and 150 min at 30,000 rpm (resuspend sediment) in the No. 30 rotor of a Spinco Model L preparative centrifuge. All procedures were performed at 4°–10°C. The final sediment, resuspended in buffer, gave a single symmetrical peak in the analytical centrifuge and contained 30–80% of the plaque-forming units present in the original lysate. Its homogeneity will be documented in the next section.

Sedimentation: Sedimentation velocity analyses were made in a Spinco Model E analytical centrifuge at 35,600 rpm. The temperature was regulated at 20°C throughout all runs. The observed sedimentation coefficients were corrected to standard conditions[3] and are designated $s_{20,w}$.

Diffusion: Diffusion was analyzed in a Spinco Model H electrophoresis-diffusion apparatus with Rayleigh optics. Creeth's[4] procedure for calculation was used. The diffusion coefficient was corrected to standard conditions and is designated $D_{20,w}$.

Ultraviolet absorption: Absorption spectra were determined with a Cary Model 15 spectrophotometer. Corrections for light scattering are small and were made by extrapolation from the neighboring nonabsorbing region of the spectrum. For most of the biophysical measurements reported here, concentration was determined by ultraviolet absorption at 260 mμ.

Partial specific volume: The partial specific volume, $\bar{v}$, of the virus was calculated assuming the validity of the additive procedure described by Cohn and Edsall.[5] Thus, $\bar{v} = \Sigma \bar{v}_i w_i + \bar{v}_{RNA} \cdot w_{RNA}$ where $\bar{v}_i$ is the partial specific volume of amino acid residue, i, $\bar{v}_{RNA}$ is the partial specific volume of RNA (taken as 0.53 ml/gm), w_i and w_{RNA} are the relative abundances in the virus of each of the amino acid residues and the RNA, as obtained from amino acid analyses and a determination of the RNA content of the virus.

Chemical analyses: For P determinations, virus samples, along with internal control samples containing known amounts of P, were oxidized with H_2SO_4 in the presence of H_2O_2, and then P was measured by Gomori's method.[6]

N was measured by the Nessler reaction procedure[7] after oxidation of the R17 samples to $(NH_4)_2SO_4$ with H_2SO_4 and H_2O_2.

Results.—Sedimentation: Invariably, preparations of purified R17 exhibited a single, symmetric schlieren peak in analytical centrifugation indicative of an homogeneous macromolecular material (Fig. 1). A set of runs was made on a

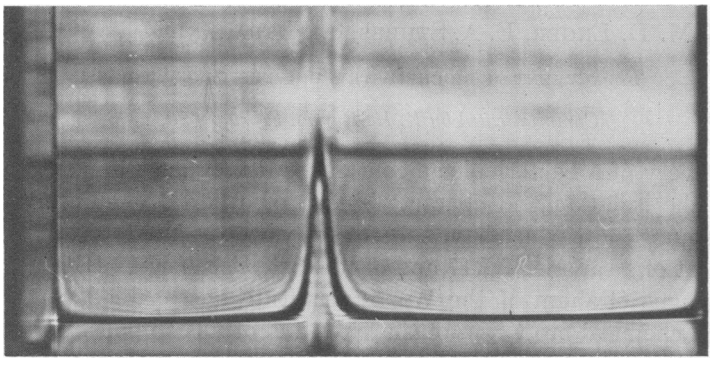

FIG. 1.—Sedimentation pattern of purified R17 showing a single symmetrical peak with no evidence of material at the meniscus.

sample in 0.05 M Na phosphate buffer, pH 7.0, at concentrations of 0.408, 0.306, 0.204, and 0.102%. The corrected sedimentation rate as a function of concentration, c (in %), was found to be

$$s^c_{20,w} = 78.9 - 7.35\,c.$$

Diffusion: The sample above, at concentration 0.408% in 0.05 M Na phosphate buffer, was used for a diffusion experiment. Pictures of Rayleigh fringes were taken every 6 hr over a period of 11 days. The temperature was maintained at 2°C throughout the run. Assays made at the beginning and the end of the experiment showed that no loss of infectivity had occurred. The observed apparent diffusion coefficients, calculated from representative fringe pictures, showed no time dependence. Furthermore, apparent diffusion coefficients calculated from various fringe pairs showed only a small (and linear) dependence upon Creeth's parameter $(Z_j^*)^2$ indicating that the macromolecular material in the diffusion sample was essentially homogeneous.

The observed diffusion coefficient was 0.728×10^{-7} cm²/sec. Correction to standard conditions gave at the midpoint of the diffusing boundary

$$D^{0.204\%}_{20,w} = 1.328 \times 10^{-7}\ \text{cm}^2/\text{sec}.$$

Chemical analyses: The ratio of N to P was 5.39. It may be calculated from its composition that R17 RNA contains 9.63% P and 16.02% N. It may be

calculated from its composition that R17 protein contains 16.5% N (assuming that aspartic acid and asparagine content are equal, and that glutamic acid and glutamine content are equal). Thus, from the N/P ratio R17 virus contains 31.7% RNA.

Refractive index increment: The refractive index increment was found to be 1.77×10^{-4} ml/mg at wavelength 546 mμ. For this determination R17 concentration was measured by N analysis.

Ultraviolet absorption: Figure 2 shows the ultraviolet absorption spectrum of R17. The absorbancy of a 1 mg/cc virus solution (1 cm path length) is 8.20 at 260 mμ. For this determination R17 concentration was measured by counting Rayleigh fringes in the diffusion apparatus.

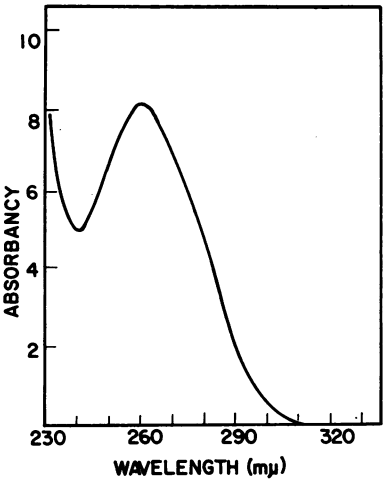

FIG. 2.—Absorbancy plotted against wavelength for purified R17 at a concentration of 1 mg/ml in 0.05 M sodium phosphate buffer, pH 7.

From this absorbancy index, RNA content of the virus may be determined provided that absorbancy indices for R17 protein and R17 RNA are known. The former was calculated from the abundance of tryptophan, tyrosine, and phenylalanine in the protein (known from amino acid analyses). The RNA absorbancy index appropriate for calculation of RNA content is that of the RNA *as it exists in the virus.* This was determined by hyperchromicity. The absorbancy of a solution of R17 was measured at pH 7, 20°C in Na phosphate buffer. The absorbancy was measured for an identical amount of virus in which the RNA was converted to the free nucleotide form by heating for 24 hr at 37°C in 0.3 N NaOH. The ratio of these absorbancies (after correction for protein absorbancy) immediately yields the absorbancy index of the RNA, inasmuch as the absorbancy index of the residue portion of the free nucleotides in alkaline solution is known. The latter[8] is 31.5 cm^2/mg for the case of R17 RNA whose bases are present in the ratios[9] G:A:U:C = 26:23:26:25. Thus, the absorbancy index of the RNA as it exists in the intact virus is 22.8 cm^2/mg. From this the per cent RNA in the virus is 34.1. These calculations are shown in Table 1. Since the RNA content determination from absorbancy indices is more circuitous, we will accept the value from N/P ratio for the subsequent discussion.

Molecular weight of R17: Molecular weight is given by the Svedberg equation in terms of the diffusion coefficient, D, the sedimentation coefficient, s, the partial specific volume, $\bar{v}$, the absolute temperature, T, the gas constant, R, and the solvent density, ρ.

$$M = \frac{RTs}{D(1 - \bar{v}\rho)}.$$

The Svedberg equation holds strictly only at infinite dilution. We make the usual assumption that the sedimentation and diffusion coefficients have a similar concentration dependence and insert their values at 0.204%:

$D_{20,w}^{0.204\%} = 1.328 \times 10^{-7}$ cm²/sec, and $s_{20,w}^{0.204\%} = 77.4 \times 10^{-13}$ sec.

The partial specific volume is 0.67 cc/gm. R, T, and ρ are referred to water at 20°C. Thus, $M = 4.19 \times 10^6$.

Since the RNA content is 31.7%, the RNA weighs 1.3×10^6 atomic mass units.

TABLE 1

HYPERCHROMICITY MEASUREMENTS AND CALCULATIONS FOR R17 VIRUS

1.	Measured absorbancy of intact R17 virus at 260 mμ	1.000 arbitrary units
2.	Less protein absorbancy* 1.000 − 0.50	0.950
3.	Measured absorbancy of alkali degraded R17 virus	1.390
4.	Less protein absorbancy* 1.390 − 0.080	1.310
5.	Ratio 2/4	0.726
6.	Calculated absorbancy index of R17 nucleotide residues (at pH 12)	31.5 cm²/mg
7.	Calculated absorbancy index of R17 RNA as it exists in the intact virus 31.5 × 0.726	22.8 cm²/mg
8.	Measured absorbancy index of R17 virus	8.20 cm²/mg
9.	Less protein absorbancy* 8.20 × 0.950	7.79 cm²/mg
10.	Per cent RNA in the virus (7.79/22.8) × 100 =	34.1%

* The absorbancy index of R17 protein (calculated from the amino acid composition) is 0.612 cm²/mg at 260 mμ at neutral pH and is 1.6 times this value in alkali. Protein absorption is calculated from these values and from the per cent protein in the virus, which is initially assumed, and then replaced with the value from line 10. This procedure converges, inasmuch as the protein absorbancy is much smaller than the RNA absorbancy.

Summary.—The RNA-containing bacterial virus, R17, has been purified. Its molecular weight, based on sedimentation and diffusion, is 4.19×10^6. It contains 1.3×10^6 atomic mass units of RNA. Its absorbancy index is 8.20 cm²/mg.

We would like to thank Dr. Angus Graham who gave us R17 virus and its host.

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