Despite its key role in the transfer of genetic information, the physical structure of the enzyme RNA polymerase remains relatively unexplored. The only published data comes from the work of Zillig and his collaborators. They first reported that in deoxyribonuclease-treated extracts of *E. coli*, the enzyme sediments on a sucrose gradient at 20–25S. More recently they have examined some of the properties of highly purified preparations of the enzyme. A sedimentation coefficient of 24S was measured in the analytical ultracentrifuge while electron microscopy revealed particles apparently made of subunits and having an aggregate diameter of 120 Å. Thus, they estimated its molecular weight as between 6 × 10^6 and 9 × 10^6. Since the purified enzyme had the same sedimentation coefficient as in the crude extracts, they believed that they had isolated RNA polymerase in its native form. They suggested, however, that it could dissociate into active subunits, and that other workers finding lower sedimentation values may have isolated such subunits.

The experiments reported below show that highly purified RNA polymerase dissociates into subunits in buffers with high ionic strength and that the subunits can be reassociated by dialysis into buffers with low ionic strength. This is shown by both sedimentation velocity and sedimentation equilibrium measurements.

**Materials and Methods.—Biochemicals:** Unlabeled nucleoside triphosphates were purchased from Pabst Laboratories (P-L Biochemicals, Inc.). ATP-S-C^14 was purchased from Schwarz BioResearch, Inc. Parantitrophophenyl phosphate was purchased from K and K Laboratories, Inc. Calf thymus DNA was isolated by the procedure of Kay, Simmonns, and Dounce. T7 DNA was prepared as described in another paper. Catalase (beef liver) and alkaline phosphatase (*E. coli*), purchased from Worthington Biochemical Corp., were dialyzed overnight against a solution containing 0.2 M KCl and 0.01 M potassium phosphate buffer, pH 7.0, before use.

**Solutions:** 0.1 M KCl buffer: 0.1 M KCl, 0.01 M potassium phosphate buffer, pH 7.0, 1 mM 2-mercaptoethanol and 0.5 mM EDTA; 0.5 M KCl buffer: 0.5 M KCl, 0.01 M potassium phosphate buffer, pH 7.0, 1 mM 2-mercaptoethanol, and 0.5 mM EDTA.

**Miscellaneous:** Hydroxyapatite prepared by the method of Tiselius, Hjertén, and Levin is a generous gift from Dr. Bruce Alberts. Dialysis tubing was boiled twice for 15 min in 5% Na2CO3, rinsed thoroughly with water, then boiled for 15 min in 0.05 M EDTA, pH 7. Boiled dialysis tubing was stored at 2°C in 0.05 M EDTA, pH 7.

**Calf thymus DNA assay:** The calf thymus DNA assay reaction mixture, in 0.25 ml, contained 0.04 M tris-HCl buffer, pH 7.9, 0.05 KCl, 5 mM MgCl2, 2 mM MnCl2, 0.16 mM each of UTP, CTP, and GTP, 0.12 mM ATP-8-C^14, 120 μg/ml calf thymus DNA (350 μmolecules/ml in DNA phosphorus), 4 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.5 mg/ml bovine serum albumin (BSA), and enzyme. After incubation for 10 or 20 min at 37°C, the reaction was stopped by adding 3 ml of cold 5% trichloroacetic acid. After 10 min at 0°C, the precipitate was collected and washed two times with 3-ml portions of cold 2% trichloroacetic acid on a Millipore HA filter. The filter was affixed to an aluminum planchet with a drop of 1% BSA, dried and counted in an end-window, low background, gas-flow counter.

**T7 DNA assay:** The T7 DNA assay was the same as the calf thymus DNA assay except that 60 μg/ml of T7 DNA was used in place of calf thymus DNA, and MnCl2 was omitted. With purified enzyme, the T7 DNA assay gave from two- to threefold higher rates of incorporation than the calf thymus DNA assay. A unit of enzyme activity is defined as the incorporation of 1 μmole of total nucleotide into an acid-insoluble product in 1 min at 37°C.

**Sedimentation velocity measurements of RNA polymerase activity on sucrose gradients:** Sedimen-
tation coefficients were determined by zone centrifugation as described by Martin and Ames. RNA polymerase activity in the sucrose gradient fractions was determined by the calf thymus DNA assay. Alkaline phosphatase activity was measured by the increase of optical density at 410 μm after incubating 75 μl of each fraction with 3 ml of a solution containing 0.2 mg/ml paranitrophenyl phosphate and 0.1 M tris buffer, pH 7.9, for 5 min at 23°C. Catalase activity was measured by the decrease in optical density at 240 μm after incubating 50 μl of each fraction with 3 ml of 0.02 M H₂O₂ in 0.01 M potassium phosphate buffer, pH 7.0, for 1 min at 23°C.

Analytical ultracentrifugation: A Spinco model E analytical ultracentrifuge was used for sedimentation velocity and sedimentation equilibrium measurements. Photographs were taken on Eastman Kodak metallocraphic plates and measured with a Gaertner microcomparator. Sedimentation velocity measurements were made using cells with 12-mm Kel-F centerpieces.

Molecular weights from sedimentation equilibrium data: Molecular weights of RNA polymerase were determined by centrifugation of the enzyme to sedimentation equilibrium using the short column methods described by Van Holde and Baldwin. These methods give accurate values with small amounts of material and with short times for attaining equilibrium.

With one of these methods, the equilibrium conditions are chosen such that the concentration of the protein at the midpoint of the column is within 1% of the initial concentration. With this approximation a weight average molecular weight \( M_w = \sum_n \frac{c_i M_i}{\sum_i c_i} \), where \( c_i \) and \( M_i \) are the concentration and molecular weight, respectively, of the \( i \)th species is determined with:

\[
M_w = \frac{y'}{A_0 T r^2} \frac{RT}{\omega^2 (1 - \bar{\rho})},
\]

(1)

where \( y' \) is the displacement of the schlieren pattern of the enzyme from the baseline at the midpoint of the column as measured on the microcomparator (in arbitrary units), \( r^2 \) is the radial distance at the midpoint of the column (in cm), \( R \) is the gas constant, \( T \) is the temperature in degrees Kelvin, \( \rho \) is the density of the solution, \( \bar{\rho} \) is the partial specific volume of the protein, \( \omega \) is the angular velocity of the rotor, and \( A_0 \) is the initial concentration of the enzyme solution determined by numerical integration of the photograph of the schlieren pattern of the concentration gradient formed between the enzyme solution and the buffer in a double-sector, capillary-type synthetic boundary cell. The units of \( A \) are arbitrary (cm).

By measuring \( y \) at several different points in the column, the molecular weight can be determined by two other methods. The weight average is also determined with the following formula:

\[
M_w = \frac{4.60 \; RT}{\omega^2 (1 - \bar{\rho})} \frac{d(\log_{10} A_r)}{d(r^2)},
\]

(2)

where \( A_r = A_0 + \int_r^\infty y \; dr \). The value of \( d(\log_{10} A_r)/d(r^2) \) is determined from the slope of \( \log_{10} A_r \) vs. \( r^2 \). This method is also dependent upon the measurement of \( A_0 \) and the same approximation used for equation (1); consequently, it is probably no more accurate than the single-point method.

However, it uses measurements from several points in the cell and gives some idea of the distribution of protein at equilibrium.

The z-average molecular weight \( M_z = \sum_n \frac{c_i M_i^2}{\sum_i c_i M_i} \) is determined by method II of Van Holde and Baldwin with the following formula:

\[
M_z = \frac{RT}{\omega^2 (1 - \bar{\rho})} \frac{d(y/r)}{d(A_r)}.
\]

(3)

The value of \( d(y/r)/d(A_r) \) is determined from the slope of \( y/r \) vs. \( A_r - A_m \), where \( A_m \) is the value of \( A \) at the meniscus. This method has the advantage of being independent of the approximations used in the other two methods and is also a more sensitive indicator of heterogeneity.

Sedimentation equilibrium measurements on several samples were made simultaneously using a multichannel cell described by Yphantis. The sample channels of the cell contained 10 μl of the enzyme solutions and 7 μl of fluorocarbon FC-43 (Minnesota Mining and Manufacturing Co., Minneapolis, Minn.). The reference channels contained 20 μl of the appropriate buffer. The
height of the sample columns was 0.6 mm. Using a rotor speed of 5,227 rpm, all samples were within 5% of sedimentation equilibrium after 5 hr as judged by the fact that identical values of y' were measured on photographs taken after 4 and 5 hr.

**Purification of RNA polymerase:** RNA polymerase was purified from *E. coli* B grown as described by Richardson, Schildkraut, Apostian, and Kornberg and purchased from the Grain Processing Corp., Muscatine, Iowa. The purification procedure was based on the one described by Chamberlin and Berg, with the following modifications: fraction 1 was centrifuged for 2.5 hr at 30,000 rpm instead of 4 hr, and the amount of protamine sulfate necessary to precipitate 90% of the activity was determined by titration of an aliquot of the streptomycin supernatant fraction.

After the DEAE fractionation, the enzyme was purified further by chromatography on hydroxylapatite. The following operations were all at 0–4°C. Approximately 30 mg of the DEAE fraction in 15 ml of 0.01 M tris buffer pH 7.9 containing 1 mM 2-mercaptoethanol was first dialyzed for 4 hr against 1 liter of 0.04 M potassium phosphate buffer, pH 7.0, containing 1 mM 2-mercaptoethanol, then applied to a column of hydroxylapatite (1.8 cm × 13 cm) previously washed further with 70 ml of the same buffer. The protein was eluted with a linear gradient with 0.04 M and 0.4 M potassium phosphate buffer, pH 7.0, as limiting concentrations. The total volume of the gradient was 600 ml, and 1 mM 2-mercaptoethanol was present throughout. The flow rate was 30 ml/hr and 7-ml fractions were collected. As a precaution, 0.1 mM EDTA was added to each fraction. Enzyme activity appears at about 0.18 M potassium phosphate. Fractions with specific activities within 95% of the specific activity of the fraction with the highest activity were pooled and the protein was precipitated by adding solid ammonium sulfate to 60% saturation. For best yield this is done as soon as possible after elution of the enzyme from the column. After 60 min, the precipitates were collected by centrifugation for 30 min at 15,000 × g and the pellets dissolved in enough of a 10% solution of glycerol containing 0.2 M KO, 0.01 M tris buffer, pH 7.9, 5 mM glutathione, and 0.1 mM EDTA to give a final protein concentration of 4–6 mg/ml. Enzyme solutions were frozen in a dry ice acetone bath and stored at −20° or −70°C in 0.5-ml lots. From 10 to 20% of the activity was lost upon freezing, but once frozen, the enzyme was stable for at least 6 months. After being thawed, the enzyme could be kept for nearly a month at 2°C before losing a significant amount of activity.

In the best preparations, the specific activity of the hydroxylapatite fractions was 0.5 μmole total nucleotide incorporated per minute per mg of protein in the T7 DNA assay, and 6 mg of protein with such activity has been isolated from 100 gm of cells. The yield from the hydroxylapatite step was about 80% with a 20% increase in specific activity over the DEAE fraction.

**Properties of purified polymerase:** The enzyme is stable to dialysis at 0–4°C for at least 12 hr and can be incubated at 57°C for 3 hr with less than 10% loss of activity (with total protein concentration at least 0.1 mg/ml). At 55°C, however, the half life of the enzyme is about 1 min.

The ratio of absorbance at 280 μm to that at 260 μm was 1.8. Using the data of Warburg and Christian, such a ratio indicates that the contamination with nucleic acid was less than 0.1%. The specific absorbance was measured by three methods, all dependent upon a comparison with bovine serum albumin, the concentration of which was determined from its absorption coefficient of 0.66/mg/ml at 278 μm. By the biuret and Lowry procedures, RNA polymerase had a specific absorbance of 0.59/mg/ml at 280 μm. Using the synthetic boundary cell in the Spinco model E ultracentrifuge and assuming that RNA polymerase and BSA have identical refraction increments (refractive increments of proteins in aqueous solvents generally differ by less than 2%), a value of 0.67/mg/ml was obtained for the specific absorbance of RNA polymerase at 280 μm. For the work in this paper a compromise of 0.65/mg/ml at 280 μm is used to determine enzyme concentrations. The uncertainty of this value was about 10%.

When subjected to starch gel electrophoresis for 16 hr at a field of 4 volts/cm in 0.012 M tris buffer, pH 7.9, the purified enzyme protein migrated as a single band 22 cm toward the anode.

**Results.—Sedimentation properties of the purified enzyme:** The sedimentation coefficient of RNA polymerase depends very strongly on the ionic strength of the buffers used for the measurements. Freshly prepared enzyme sediments at 24S on a sucrose gradient in a buffer containing 0.05 M KCl, 0.01 M tris, pH 7.9, and 5 mM MgCl2 as based on extrapolations to the markers catalase (11S) and alkaline phosphatase (7.8S) (Fig. 1). When measured in the model E analytical ultracentrifuge...
in the same buffer and in the 0.1 M KCl buffer, the sedimentation coefficients were 22S and 21S, respectively (Table 1). However, when centrifuged in buffers with ionic strengths of 0.5 or higher, the same preparation gave sedimentation coefficients that were 13 ± 1S. Furthermore, the enzyme activity sedimented at 12 ± 1S on a sucrose gradient in the 0.5 M KCl buffer (initial concentration was 1.6 mg/ml).

Figure 2 is the schlieren photograph of enzyme sedimenting in the 0.1 M KCl buffer (wedge cell, upper pattern) and in the 0.5 M KCl buffer (normal cell, lower pattern). The change from one form to the other is reversible; the 13S form can be converted to the 21S form by dialysis into a buffer with an ionic strength of 0.1, and the 21S form can be converted to the 13S form merely by raising the ionic strength of the buffer to 0.5. The presence of 5 mM Mg++ appeared to have no effect on the sedimentation properties of the enzyme.

The discrepancy between the 24S measured on the sucrose gradient and 21S measured in the same buffer in the analytical ultracentrifuge could be a result of concentration dependence of the sedimentation coefficient. Initially the concentration of enzyme was 0.45 mg/ml in the zone for the sucrose gradient and 4 mg/ml for the analytical ultracentrifugation. However, the sucrose gradient determination was based on extrapolation to markers that moved less than one half as fast and could be in error by as much as 20 per cent. The measurements made with initial

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Buffer Description</th>
<th>Concentration (mg/ml)</th>
<th>Temp. (°C)</th>
<th>S_{rn}, w (S)</th>
<th>Ionic strength (T/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ionic strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 M KCl, 5 mM MgCl₂, 0.01 M tris, pH 7.9</td>
<td>4.0</td>
<td>21.1</td>
<td>21.7 ± 1.1</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5 M KCl buffer</td>
<td>3.6</td>
<td>8.4</td>
<td>20.6 ± 0.6</td>
<td>0.12</td>
</tr>
<tr>
<td>High ionic strength</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 M KCl buffer</td>
<td>4.1</td>
<td>8.4</td>
<td>13.6 ± 0.3</td>
<td>0.52</td>
</tr>
<tr>
<td>0.2 M KCl, 0.2 M (NH₄)₂SO₄, 0.01 M tris, pH 7.9</td>
<td>1.0</td>
<td>20.0</td>
<td>13.4 ± 0.4</td>
<td>0.52</td>
</tr>
<tr>
<td>Same as above plus 5 mM MgCl₂</td>
<td>4.4</td>
<td>21</td>
<td>12.8 ± 0.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>4.3</td>
<td>19.6</td>
<td>13.4 ± 0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

All buffers contained 1 mM 2-mercaptoethanol and 0.5 mM EDTA.

---

**Fig. 1**—Determination of the sedimentation coefficient of RNA polymerase by zone centrifugation on a sucrose gradient. A 0.1-ml sample containing 45 µg of hydroxylapatite fraction RNA polymerase was layered on a 4.5-ml linear gradient of 20-5% sucrose in a solution containing 0.05 M KCl, 0.01 tris buffer, pH 7.9, 5 mM 2-mercaptoethanol, and 0.5 mM EDTA. Another 0.1-ml sample containing 40 µg of catalase and 50 µg of alkaline phosphatase was layered on a sucrose gradient identical to the one used for RNA polymerase, except that the EDTA was omitted. After centrifugation for 10 hr at 25,000 rpm at 0-4°C, fractions were collected and analyzed as described in the Methods section. RNA polymerase activity: C¹⁴ cpm, O-O-O; catalase activity: OD₄₅₀, O-O-O; and alkaline phosphatase activity: OD₄₀₅ ×-×-×.
Fig. 2.—Sedimentation pattern of RNA polymerase. Schlieren photograph taken approximately 32 min after reaching a speed of 35,000 rpm. Enzyme concentrations were 3.6 mg/ml in the 0.1 M KCl buffer (wedge cell, upper pattern) and 4.1 mg/ml in the 0.5 M KCl buffer (normal cell, lower pattern). The temperature was 8.4°C and the phase plate angle was 70°.

concen-trations of 4 mg/ml and 1 mg/ml in the 0.5 M KCl buffer suggest that there is no marked concentration dependence in that buffer, but more extensive measurements are needed before a firm conclusion is made.

Molecular weights of the two forms: The large change in the sedimentation coefficient with the increase in the salt concentration suggests that the higher ionic strength causes the enzyme to dissociate into subunits. Since the two forms are relatively homogeneous with respect to centrifugation (Fig. 2), it is possible to measure the molecular weights of the enzyme in the two buffers and thus show that the change in form is due to a dissociation.

Aliquots and twofold dilutions of the samples shown in Figure 2 were centrifuged to sedimentation equilibrium in a Yphantis cell. The molecular weights determined by the three different methods described in the Methods sections are given in Table 2. The graphical representations of the data needed for equations (2) and (3) are in Figure 3 for the enzyme in the 0.1 M KCl buffer and in Figure 4 for the enzyme in the 0.5 M KCl buffer. The weight averages were not determined for the diluted samples because their concentrations were not known accurately enough. The sample with an initial concentration of 2 mg/ml in the 0.5 M KCl buffer had an abnormal distribution with y' equal to 0. This abnormality was observed in three runs using freshly diluted enzyme each time, and could reflect a further dissociation that is strongly concentration-dependent and occurs during centrifugation. However, sedimentation coefficients of the enzyme in the 0.5 M KCl buffer measured with initial concentrations of 1 mg/ml and 4 mg/ml were similar (Table 1), suggesting that at least under the conditions of the sedimentation velocity measurements the enzyme does not dissociate further in the range of concentrations that would have been expected if the sample with the initial concentration of 2 mg/ml had reached sedimentation equilibrium. This finding has made it difficult to explain the abnormality, but it is still possible that the enzyme could dissociate under the conditions of the equilibrium centrifugation.

Discussion.—In both buffers the weight averages determined using equations (1) and (2) agree very well, which is not unexpected because both methods use the same

### Table 2

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Initial concentration (mg/ml)</th>
<th>$M_w \times 10^4$</th>
<th>$M_x \times 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equation (1)</td>
<td>Equation (2)</td>
<td>Equation (3)</td>
</tr>
<tr>
<td>0.1 M KCl</td>
<td>3.6</td>
<td>9.0 ± 0.9</td>
<td>9.1 ± 0.9</td>
</tr>
<tr>
<td>0.1 M KCl</td>
<td>1.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.5 M KCl</td>
<td>4.1</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>0.5 M KCl</td>
<td>2.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* $\omega^2 = 2.99 \times 10^5$ min $^{-2}$, $\rho = 0.73$ cc/gm (assumed), $T = 284.5^\circ$K.

* No value was determined because of the abnormal distribution. See text.
approximation and the same determination of \( A_0 \). With the conditions used, the concentration at the midpoint of the columns should have been within 1 per cent of the initial concentration for the enzyme in the 0.5 M KCl buffer and within 4 per cent for the enzyme in the 0.1 M KCl buffer. The agreement with the \( z \)-average is more significant because it is not dependent upon the knowledge of the absolute value of the concentration at any point in the column. In the 0.1 M KCl buffer, values obtained for \( M_w \) are within 10 per cent of the value of the \( M_z \), and in the 0.5 M KCl buffer the agreement is even closer.

Usually the fact that the \( M_z \) is larger than the \( M_w \) is an indication of heterogeneity in a preparation because the \( z \)-average places greater emphasis on the higher molecular weight components than the weight average. Curvature in the plots used for the \( z \)-average determination is also an indication of heterogeneity. However, the method used is very sensitive to optical distortions that occur near the meniscus and the base of a column. Because the columns used for the determinations presented here were very short (0.6 mm), much of the curvature seen in Figures 3b and 4b could be a result of distortions rather than heterogeneity. Such distortions could make the error in the molecular weight determinations as high as 10 per cent, which is one of the major drawbacks of using the very short columns.

Moreover, it is evident from the sedimentation velocity patterns (Fig. 2) that the preparation was not completely homogeneous. In the 0.1 M KCl buffer (upper pattern), 10–15 per cent of the material sedimented faster and another 10–15 per cent sedimented slower than the major, 21S component. Some of the slow component in the 0.1 M KCl buffer may be the same as the material that is moving just ahead of

**Fig. 3.**—Sedimentation equilibrium of RNA polymerase in the 0.1 M KCl buffer. Hydroxylapatite fraction RNA polymerase at an initial concentration of 3.6 mg/ml \( (A_s = 0.454) \). (a) Plot of \( \log_{10} A_r \) vs. \( r^2 \) for determination of \( M_w \) by equation (2). (b) Plot of \( y/r \) vs. \( A_r - A_m \) for determination of \( M_z \) by equation (3).

**Fig. 4.**—Sedimentation equilibrium of RNA polymerase in the 0.5 M KCl buffer. Hydroxylapatite fraction RNA polymerase at an initial concentration of 4.1 mg/ml \( (A_s = 0.519) \). (a) Plot of \( \log_{10} A_r \) vs. \( r^2 \) for determination of \( M_w \) of equation (2). (b) Plot of \( y/r \) vs. \( A_r - A_m \) for determination of \( M_z \) by equation (3).
the main, 13S peak in the 0.5 $M$ KCl buffer (lower pattern). This 16–18S material which is common to both solutions is probably nonenzymatic protein. However, the minor component that is sedimenting faster than the main, 21S material in the 0.1 $M$ KCl buffer is possibly a larger aggregate of the enzyme because it is not present at all in the 0.5 $M$ KCl buffer. The distribution of sedimenting material in the 0.5 $M$ KCl buffer, which shows less than 5 per cent contamination with some faster-moving material is probably a better indication of the purity of the enzyme than the more heterogeneous distribution in the 0.1 $M$ KCl buffer.

The sedimentation coefficient of 21S in the low-salt buffers is in close agreement with the results of Fuchs et al.\textsuperscript{2} who measured a value of 24S in a solution containing 0.022 $M$ NH$_4$Cl, 0.01 $M$ magnesium acetate, 0.01 $M$ tris acetate buffer, pH 7.4, and 1 mM 2-mercaptoethanol, which is a low-ionic-strength buffer. However, other workers (J. Milligan and M. Chamberlin; J. Hurwitz, personal communications) have found that the sedimentation coefficient of highly purified RNA polymerase both in low- and high-salt buffers is 11S. This discrepancy could be the result of the presence of an extra factor in some preparations, such as a small amount of nucleic acid, which could be responsible for the association. Such an explanation is more likely for the preparation of Fuchs et al. because they reported that the S$_{20,w}$ to 260-m$\mu$ absorbance ratio for their enzyme is 1.1, which would indicate a significant contamination (1–2\%) with nucleic acid. However, the S$_{20,w}$ to 260-m$\mu$ absorbance ratio of the preparation used in this laboratory was 1.8, which would indicate that the nucleic acid contamination was less than 0.1 per cent, or less than enough for a trinucleotide for each 21S unit. This amount of contamination would make such an explanation less likely, but even a very small amount of nucleic acid could be enough to cause the aggregation. It is also possible that some nonenzymatic protein is responsible for the dimerization.

The difference between 24S measured by Fuchs et al.\textsuperscript{2} and 21S measured in the analytical ultracentrifuge in this laboratory could be significant. However, they stated that the concentration dependence of the sedimentation coefficient was negligible, implying that at 4 mg/ml their preparation should be more nearly 24S than 21S. One possible explanation for the difference is that interactions with the nucleic acids contaminating their preparation may cause the enzyme to sediment faster. Another possibility is that the sedimentation coefficient of the enzyme in the low-salt buffer could change with the age and activity of the preparation. This possibility was suggested by the following preliminary observation. When fresh, the enzyme sedimented as a sharp peak with a sedimentation coefficient of 24S on a sucrose gradient (Fig. 1). After losing 60 per cent of its activity, the same preparation sedimented as a broader peak with S$_{20,w}$ = 17S under the same conditions.

In order to estimate the actual molecular weights of the various forms of the enzyme, the uncertainty in the value used for the partial specific volume must be considered. The value used was assumed to be 0.73 cc/gm. which is a common value for proteins. However, proteins are known to have values that range from 0.69 to 0.75 cc/gm. Such an uncertainty would affect the molecular weights of RNA polymerase by as much as 10 per cent. The recent finding by Hurwitz (personal communication) that the amino acid composition of E. coli RNA polymerase is similar to the amino acid composition of the average $E. coli$ protein suggests that the value used may not be much in error.
Using the values for $M_r$ and $M_z$ of RNA polymerase in the 0.5 M KCl buffer and assuming that the contaminant in that buffer is less than 5 per cent, the estimate of the molecular weight of the 13S form is $4.4 \pm 0.8 \times 10^6$ daltons. Thus, the 21S form, which is the primary component present at low ionic strength, would have a molecular weight of $8.8 \pm 1.6 \times 10^6$ daltons.

The results presented here represent a start on a study of the physical properties of RNA polymerase which has been limited by difficulties in obtaining large quantities of very pure enzyme. Work now is proceeding on more complete measurements of the effect of ionic conditions on the sedimentation properties of the enzyme. The above experiments are presented now because they do show quite clearly that the ionic conditions strongly affect the form of the enzyme and raise the question of what form combines with the DNA. Other work to be presented shows that the high salt conditions which produce the 13S form completely inhibit RNA polymerase activity, whereas the low salt conditions favorable for observing the 21S form are the optimal conditions for enzymatic activity. This suggests that the 21S form is biologically active. However, the role of DNA in determining the structure of the enzyme during synthesis is necessarily neglected in studies with purified enzyme. Thus, further work will be necessary to clarify this point.

The author wishes to thank Prof. James D. Watson for helpful advice and discussion. He is indebted to Dr. John Armstrong for advice on the sedimentation equilibrium measurements. He also wishes to acknowledge the skillful technical assistance of Miss Lislott Voegelin.

* This work was supported in part by research grant GM 09541-03 of the National Institutes of Health.

† Predoctoral fellow of the National Institutes of Health. Present address: Institut de Biologie Physico-Chimique, 13 Rue Pierre Curie, Paris V, France.

12 Chamberlin, M., and P. Berg, these PROCEEDINGS, 48, 81 (1962).
13 Warburg, O., and W. Christian, Biochem. Z., 310, 384 (1941).