interactions, etc.). Sonneborn, T. M., in *Evolving Genes and Proteins*, ed. J. Bryson and H. Vogel (New York: Academic Press, 1964); Woese, C., these PROCEEDINGS, **54**, 1546 (1965).

[8] For a general review of amino acid chromatography see Lederer, E., and M. Lederer, *Chromatography* (Amsterdam: Elsevier, 1957).

[9] Zubay, G., and P. Doty, *Biochim. Biophys. Acta*, **29**, 47 (1958). A number of other attempts have remained unpublished because the results were negative.

[10] Isherwood, F. A., and M. H. Jermyn, *Biochem. J.*, **48**, 515 (1951).

[11] Woese, C., C. Saxinger, and S. Dugre, in preparation.

[12] We use the now-customary three-letter abbreviations for the amino acids: phe for phenylalanine, gln for glutamine, ilu for isoleucine, etc. For the bases we use U for uracil, A for adenine, etc.

[13] We shall use "codon" here to mean some oligonucleotide which is simply related to, but not necessarily the same as, the trinucleotide in mRNA usually referred to as a "codon."

[14] $\Delta R_M = 1/\Delta R_F - 1$, where ΔR_F is the difference between R_F in any given P-DMP-water mixture and the R_F in DMP-water alone (same mole fraction of water in both cases).

[15] Although base-base interactions within the codon could in certain cases distort the geometry considerably.

[16] Ser and thr have a more complex type of chromatographic behavior than do other amino acids, so we cannot be absolutely certain of their relative ranking.

[17] Eichhorn, G., P. Clark, and E. Becker, in press.

[18] Nakajima, T., and A. Pullman, *J. Chim. Phys.*, **55**, 793 (1958); Pullman, B., *J. Chem. Phys.*, **43**, 5233 (1965).

---

*HETEROGENEOUS RNA'S OCCURRING DURING THE REPLICATION OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS**

BY T. SREEVALSAN AND R. Z. LOCKART, JR.

DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF TEXAS, AUSTIN

Communicated by Wilson S. Stone, January 24, 1966

Brown and Cartwright[1] reported recently that three peaks of virus-specific RNA were found in hamster kidney cells after infection with foot and mouth disease (FMD) virus. When the isolated RNA was centrifuged on sucrose gradients, the bulk of the infectivity was associated with the fastest-sedimenting RNA. However, the two slower-sedimenting RNA's were also infectious, and some of the infectivity associated with these RNA's remained after incubation with RNase, unlike that of the fast-sedimenting peak. The question whether both of the two slower-sedimenting peaks consisted of molecules of double-stranded RNA has not yet been settled. Thus, in addition to the two sedimentation kinds of RNA (i.e., presumably the major strand and the replicative form) found with several other viruses,[2-11] FMD virus added a third. We wish to report here studies performed with Western equine encephalomyelitis (WEE) virus, an RNA virus. Our findings also show that there are three species of infectious RNA formed during the replication of WEE virus.

Materials and Methods.—Cells and virus; Primary cultures of chick embryo (CE) cells were prepared according to the method of Dulbecco and Vogt,[12] and grown in Eagle's medium with 3% calf serum. The source and preparation of WEE virus, its storage, and the assay of infective titers have been described.[13] The WEE virus used was purified by two successive single-plaque

selections. Stock suspensions were produced by growth in chick embryo cells. The strain of Mengo virus used was obtained through the courtesy of Dr. D. Baltimore, and stock virus preparations were produced in the L line of mouse cells.

Infection of cells and labeling: Infection of the cells was accomplished as follows. Monolayers of cells were washed with phosphate-buffered saline (PBS), followed by the addition of 0.5 ml virus which had been diluted in PBS to obtain a multiplicity of 10 plaque-forming units (PFU) per cell (12×10^6 cells per culture). Incubation at 37° for 1 hr was permitted for virus adsorption. The cultures were then washed three times with warm PBS, and 5 ml of prewarmed Eagle's medium without antibiotics was added. Further incubation was at 37°.

Chemicals and isotopes: Actinomycin D was obtained through the courtesy of Merck, Sharp and Dohme Co., West Point, Pa. It was used at a concentration of 5 μg/ml throughout these studies. Three ml of Eagle's medium containing the drug was added to each culture. The cultures were incubated for 2 hr at 37°. C^{14} uridine was purchased from New England Nuclear Corp. H^3 uridine was obtained from Nuclear-Chicago Corp. An Ansitron liquid scintillation counter was used to determine radioactivity. One-half ml of the aqueous sample was mixed with 15 ml of cold Bray's[14] solution and the radioactivity determined.

Isolation of nucleic acids: Nucleic acid was isolated from cells or whole virus by extraction with phenol and sodium dodecyl sulfate (SDS).[15]

Assay of infectious viral RNA (iRNA): The assay of iRNA was performed by the plaque technique in CE cells essentially as described earlier.[16] Solutions of NaCl used for washing the cultures were made in 0.02 *M* phosphate buffer (pH 7.2) containing 0.04% EDTA.

Density gradient analysis: Two ml of a solution of the nucleic acid in 0.01 *M* acetate buffer (pH 5.0) was layered on a 28-ml density gradient of 20–5% (w/v) sucrose dissolved in 0.005 *M* Tris buffer (pH 5.0) with 0.04% EDTA. The whole was centrifuged at 24,000 rpm for 14 hr in a Spinco model L centrifuge with the rotor SW 25 at 4°. Unlabeled chick embryo cell RNA was added to each gradient to serve as a sedimentation standard. Equal fractions of about 0.75 ml were collected by puncturing the bottom of the tubes with a needle. One-half ml of each fraction was used for counting radioactivity and the remainder was used to estimate the optical density and infectivity.

Chromatography on methylated albumin kieselguhr (MAK): This was performed according to the method described by Sueoka and Cheng.[17] One mg of nucleic acid was used per 10 ml of the column. Stepwise elution was performed using NaCl of varying concentrations in 0.05 *M* phosphate buffer pH 6.7. Five-ml fractions were collected. The radioactivity, optical density, and infectivity of each fraction were estimated.

Results.—Actinomycin D inhibits DNA-dependent RNA synthesis in mammalian cells;[18, 19] however, it does not inhibit the synthesis of many RNA viruses, including WEE virus.[20, 21] The drug almost completely inhibits the synthesis of cellular RNA. Therefore, RNA synthesized in CE cells treated with actinomycin and infected with WEE virus is almost exclusively viral RNA.

Synthesis of WEE viral RNA in actinomycin-treated CE cells: Figure 1*a* shows the incorporation of H^3 uridine into the 29*S*, 18*S*, and 4*S*-RNA's of normal CE cells. However, when CE cells were incubated with actinomycin D for 2 hr, no radioactivity was found in 29*S* and 18*S* RNA's, while a considerable amount was found in the soluble RNA (Fig. 1*b*). These results agree with those obtained by other workers using different cells. Figure 1*c* shows the pattern of labeling in CE cells incubated with actinomycin and infected with WEE virus. There are three peaks of radioactivity which were absent in the material extracted from uninfected cells handled under similar conditions. We will refer to these as peaks 1, 2, and 3 from the bottom of the tube toward the top. Peak 2 had the highest amount of radioactivity, followed by peak 3 and then by peak 1. The majority of the total infectivity was in the fractions from the region of peak 1, coinciding with the distribution of the radioactivity. RNA from peaks 2 and 3 both contained several

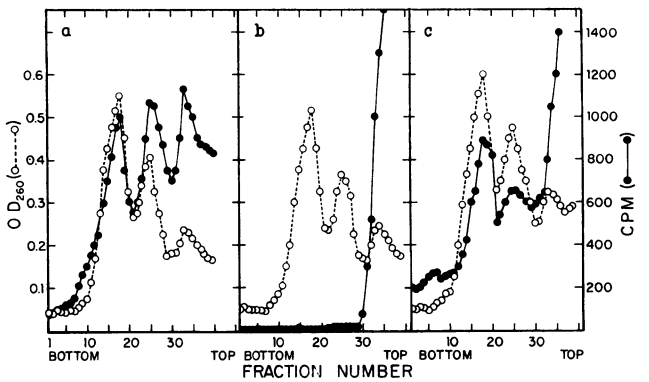

FIG. 1.—Sucrose gradient analysis of RNA from uninfected CE cells and CE cells infected with WEE virus. H^3 uridine (2 μc/ml) was present as indicated below. The total RNA was extracted and analyzed by sucrose gradients as described in *Materials and Methods*. (*a*) Uninfected cultures labeled for 2 hr. (*b*) Uninfected cultures incubated for 2 hr with 5 μg/ml of actinomycin D and then in the presence of H^3 uridine for an additional 11 hr. (*c*) Cultures incubated for 2 hr with actinomycin D (5 μg/ml) and then infected with WEE virus. H^3 uridine was added at 1 hr after the addition of virus and allowed to remain an additional 11 hr.

hundred PFU of infectious RNA, however. In relation to the marker CE cell RNA, RNA in peak 1 was heavier than the 29*S* ribosomal RNA. Peak 2 sedimented between the 29*S* and 18*S* RNA peaks of cellular origin. Peak 3 sedimented between the 18*S* and 4*S* peaks of cellular RNA. Peak 3 was not clearly distinguished in several experiments but appeared as a shoulder on the soluble material. The fractions collected from the gradient (Fig. 1*c*) were incubated with 0.01 μg/ml of RNase for 10 min at 25° and titrated for infectious RNA. None of the fractions retained their infectivity under these conditions. In another experiment the RNA from infected cells was similarly incubated with RNase prior to density gradient analysis under similar conditions. Fractions containing material with optical density and radioactivity were found only at the top of the tube. Thus, we found no RNase-resistant material in any one of the kinds of virus-specific RNA.

Analysis of the three kinds of virus-specific RNA's: Attempts were made to determine whether the infectivity associated with each of the three peaks of radio-

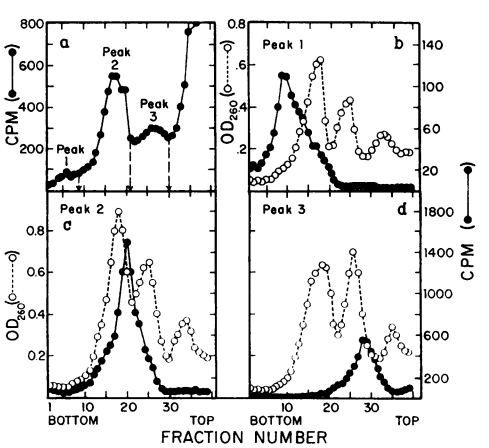

FIG. 2.—Sucrose gradient analysis of RNA obtained from cells 12 hr after infection with WEE virus. RNA was isolated from cultures incubated with actinomycin D and infected with WEE virus. H^3-labeled uridine (2 μc/ml) was added to cultures 1 hr after virus addition. The RNA was layered on a sucrose gradient as described in *Materials and Methods*. (*a*) Distribution of radioactivity in the gradient. (*b*) Fractions 1–9 as shown in (*a*) were pooled, dialyzed against 100 vol of 0.005 *M* Tris pH 5.0 with 0.03% EDTA, then reprecipitated, along with RNA from uninfected cells. The reprecipitated RNA was dissolved in buffer and centrifuged on a sucrose density gradient as before. (*c*) Fractions 10–22 as shown in (*a*) were pooled and processed as above. (*d*) Fractions 23–31 as shown in (*a*) were pooled and processed as above.

TABLE 1

DISTRIBUTION OF INFECTIVITY AND RADIOACTIVITY OF THE THREE TYPES OF WEE VIRAL RNA

		Infectivity, PFU/fraction	Cpm/fraction
Pool before recentrifugation		5.6×10^4	7×10^3
Pool after recentrifugation			
Peak	Fraction		
1	5	4.0×10^2	4×10^1
	9	2.9×10^3	1.1×10^2
	20	1.3×10^2	2.2×10^1
	35	Nil	5
Pool before recentrifugation		5.4×10^3	4×10^4
Pool after recentrifugation			
Peak	Fraction		
2	9	5.0×10^1	1.1×10^2
	20	3.0×10^2	1.5×10^3
	25	4.0	3.8×10^2
Pool before recentrifugation		1.3×10^3	1.6×10^4
Pool after recentrifugation			
Peak	Fraction		
3	9	Nil	2.5×10^1
	20	Nil	1.0×10^2
	26	4.0×10^1	5.8×10^2
	35	Nil	8×10^1

activity which appeared in sucrose gradient analysis of labeled RNA from infected cells was actually due to a distinct RNA species and was not a result of RNA trailing from peak 1. Labeled RNA's from peaks 1, 2, and 3 (Fig. 2*a*) were isolated and recentrifuged on separate sucrose gradients. Figures 2*b*, *c*, and *d* show the results. The radioactivity from pooled fractions from each different peak (Fig. 1*a*) was reisolated and centrifuged separately. Radioactivity was found at the same positions as was found after the first centrifugation. Furthermore, each peak retained its infectivity. Table 1 shows the radioactivity and infectivity of some selected fractions obtained from the density gradient analysis of each of the peaks shown in Figures 2*b*, *c*, and *d*. Peaks of infectious material were found to coincide with the peaks of radioactivity. These results show (1) that peak 1 contained most of the infectious RNA, and (2) that peaks 2 and 3 both contained infectious RNA, though much less. These results are in complete accord with those obtained after the initial centrifugation on a sucrose gradient. The infectivity and radioactivity of all three kinds of RNA obtained from the gradients were sensitive to treatment with 0.01 μg/ml of RNase for 10 min at room temperature.

Fractionation of nucleic acid from WEE virus-infected CE cells by chromatography on MAK column: Chromatography of nucleic acid on MAK columns has been found to give efficient separation of viral RNA from host cell RNA. This method was therefore employed to separate labeled viral RNA from cellular RNA. Nucleic acid isolated from CE cells previously incubated with actinomycin D and labeled with H^3 uridine separated into four components as judged by optical density (Fig. 3*a*). The peaks were found to elute at 0.4, 0.75, 0.8, and 0.85 *M* concentrations of sodium chloride. These components have been identified by others as sRNA, DNA, 18*S*, and 29*S* ribosomal RNA, respectively.[17] Only the peak eluting at 0.4 *M* concentration of sodium chloride possessed radioactivity. However, when nucleic acid from infected cells under similar conditions was chromatographed, peaks of radioactivity appeared at 0.8, 0.85, 0.9, and 0.95 *M* concentrations of sodium chloride, in addition to that found at 0.4 *M* concentration. Analysis of

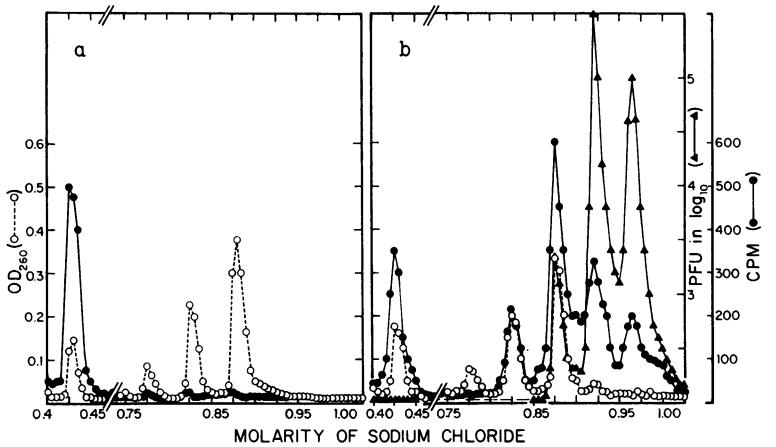

FIG. 3.—Chromatographic analysis of RNA by use of a methylated albumin-kieselguhr (MAK) column. The RNA was obtained as described in *Materials and Methods*. To a glass tube (1.5 cm diameter × 2.5 cm) sufficient MAK to make a 20-ml volume column was added. Twenty-four OD units (at a concentration of 1.5 OD units per ml) were dissolved in 0.1 *M* sodium chloride buffered with 0.05 *M* phosphate to pH 6.7, and added to the colunm. Elution was achieved by the stepwise addition of increasing concentrations of sodium chloride buffered with 0.05 *M* phosphate at pH 6.7. (*a*) Nucleic acid obtained from uninfected cultures which had been incubated for 2 hr with actinomycin D (5 μg/ml), then, after its removal, an additional 12 hr in the presence of H^3 uridine. (*b*) Same as in (*a*), except that the cells were infected with a large multiplicity of WEE virus at the time the actinomycin was removed.

the distribution of radioactivity showed that the peak eluted by 0.85 *M* concentration of sodium chloride had the highest amount, followed by that eluted by 0.9 and 0.95 *M* concentrations of sodium chloride, respectively. Infectivity determinations showed that the material eluted by 0.9 and 0.95 *M* concentrations of sodium chloride had the majority of the infectivity of the viral RNA (Fig. 3*b*). There was infectivity eluted by 0.85 *M* concentration of sodium chloride. These results again show the heterogeneity of the infectious viral RNA and confirm the results obtained using sucrose gradients.

To rule out the possibility that the heterogeneity of the viral RNA resulted from the isolation procedure, the following experiment was performed: Radioactive RNA corresponding to peak 1 was obtained by centrifugation on a sucrose gradient as before. Part of the labeled RNA was added to CE cultures treated with actinomycin D and infected with WEE virus 12 hr previously. This mixture was subjected to phenol extraction as described in *Materials and Methods*. The rest of the labeled RNA from peak 1 was also extracted a second time with phenol as a control. Both samples of re-extracted RNA were then individually recentrifuged on density gradients as before. Figure 4*a* shows the distribution of radioactivity after the second centrifugation. The peak of radioactivity was in the same fractions as before, and no new peaks were found. This demonstrates that neither the extraction procedure nor enzymes from infected cells caused any breakdown of WEE virus RNA. These results therefore rule out the possibility that breakdown of high-molecular-weight viral RNA was responsible for the heterogeneity of the viral RNA.

Comparison of RNA's induced by WEE virus and Mengo virus in L cells: Mengo virus RNA has been reported to sediment faster than the 29*S* ribosomal RNA on a

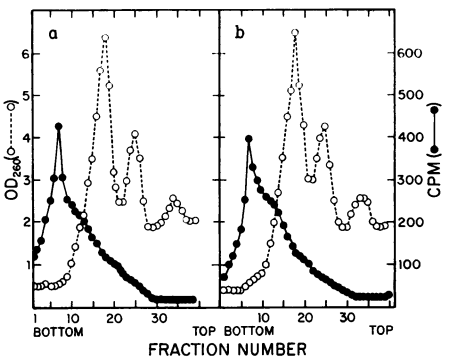

FIG. 4.—Sucrose gradient analysis of RNA associated with peak 1. H^3-labeled RNA associated with peak 1 was obtained by pooling and reprecipitating radioactive fractions 1–12. (*a*) Part of this material was re-extracted and recentrifuged. (*b*) The remainder of the original pooled RNA was mixed with cultures of CE cells infected 12 hr previously, then re-extracted and recentrifuged.

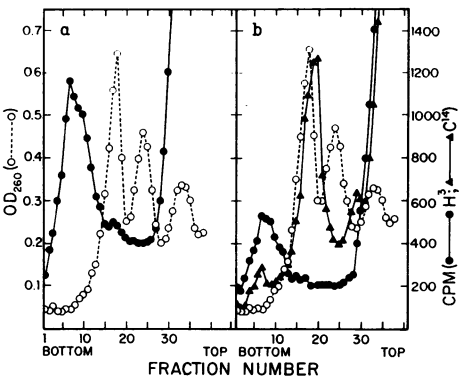

FIG. 5.—Comparison of WEE viral RNA and Mengo viral RNA after centrifugation on sucrose gradients. L cells were incubated with 5 μg/ml of actinomycin D and then infected with Mengo or WEE virus. The label (C^{14} or H^3 uridine) was added an hour after virus addition. The cultures were harvested at 12 hr after the addition of virus, and RNA was isolated. (*a*) H^3-labeled RNA from Mengo virus-infected cells. (*b*) A mixture of H^3-labeled RNA from Mengo virus-infected cells with C^{14}-labeled RNA from WEE virus-infected cells.

sucrose gradient.[22] Only one peak of viral RNA sedimenting below the 29*S* RNA was found on sucrose gradients when labeled nucleic acid from Mengo virus-infected L cells was fractionated.

We also showed that a small plaque mutant of WEE virus capable of multiplying in L cells induced all the three kinds of virus-specific RNA's. The three kinds of WEE viral RNA's were therefore not limited to one kind of cell or to one strain of WEE virus. We compared the sedimentation rates of Mengo viral RNA and WEE viral RNA prepared and centrifuged under similar conditions. Mengo viral RNA labeled with H^3 uridine was prepared from actinomycin D-treated L cells infected with Mengo virus. WEE viral RNA with C^{14} uridine was obtained from actinomycin-treated L cells infected with WEE virus. The nucleic acids obtained were centrifuged on density gradients of sucrose as follows: (1) H^3-labeled Mengo viral RNA alone and (2) a mixture of H^3-labeled Mengo viral RNA and C^{14}-labeled WEE viral RNA. The results are in agreement with published reports.[22] The majority of the radioactive RNA from Mengo virus-infected cultures sedimented faster than the 29*S* ribosomal RNA (Fig. 5*a*). However, Figure 5*b* shows the distinct radioactive peaks due to C^{14} uridine which represents the WEE virus-specific RNA, and the single peak due to H^3 uridine which represents the Mengo viral RNA when the two types of viral RNA were centrifuged in the same gradient. These experiments ruled out the possibility that the techniques used during the extraction and centrifugation of WEE viral RNA was responsible for the presence of the three types found. Moreover, the heterogeneity observed for WEE viral RNA was virus-specific as Mengo viral RNA made in the same kind of cells was quite different.

Discussion.—The preceding studies indicate that three kinds of newly synthesized and infectious virus-specific RNA's are found in CE and L cells infected with WEE

virus. The RNA associated with peak 1 accounted for 99 per cent of the infectivity found in unresolved RNA. The infectivity of the RNA's associated with peaks 2 and 3 was not due to contamination of RNA from peak 1 shown by two methods. The first involved the isolation and recentrifugation of each RNA species on sucrose gradients. The peak fractions contained the highest amounts of infectious material. The second kind of evidence that infectious viral RNA exists heterogeneously came from the experiments in which MAK columns were used to fractionate the RNA's. Again, three peaks of infectious RNA were found. Contamination of the later-sedimenting RNA by the larger-molecular-weight material that might be encountered in separations on sucrose gradients would not be expected in this case as lower-molecular-weight materials are eluted first from the MAK columns.[17]

Repeated attempts to find ribonuclease-resistant RNA from all three kinds of RNA were unsuccessful. Infectivity was lost, and all radioactivity incorporated in macromolecules was found in soluble RNA when RNase treatment was done. Such treatment included the incubation of the three kinds of RNA with various concentrations of RNase and the incubation of RNA with the enzyme in citrate buffer containing 0.15 *M* sodium chloride. The ribonuclease sensitivity test was carried out with the three kinds of RNA obtained after centrifugation of RNA isolated from infected cells in the presence of 0.15 *M* NaCl. Sodium chloride was included in the buffer in some of the experiments because of the reported requirement of a certain ionic strength in the buffer for RNA to maintain resistance to RNase.[23] The possibility that any of the peaks are double-stranded cannot be entertained in view of the results reported here.

In the work of Brown and Cartwright[1] with the RNA of FMD virus from infected hamster cells, their peaks A, B, and C correspond to peaks 1, 2, and 3 as described here. However, WEE virus RNA's are different from those from FMD virus in two respects. First, none of the three peaks from WEE virus-infected cells was resistant to RNase, while peaks B and C from FMD virus were partially resistant. Second, the amount of radioactivity in peak 1 from WEE virus-infected cells was always less than that found in peak 2 or 3 at any time after infection. Peak A from FMD virus-infected cells had the highest amount of radioactivity, even though the authors reported that this was variable from experiment to experiment.

In all the experiments reported, the WEE virus used originated from a single plaque isolation. Also, virus which originated from the infectious RNA of each of the three kinds of RNA again yielded all three kinds of RNA in a second cycle of growth. The possibility that the three kinds of RNA originated from a heterogeneous population of the virus can thus be rejected.

These findings bring into question the previously held view that only the RNA strands found in mature virus particles contain sufficient information for infectivity; however, until more is known concerning the chemical composition, configuration, and size of each of the three moieties of RNA we have described here, no definite conclusions can be drawn. Work on the further characterization of the three kinds of viral RNA from WEE virus-infected cells is under way.

* Supported by grants AI-03538 and 5-K3-AI-19, 385 issued by the National Institutes of Health, Department of Health, Education and Welfare.

[1] Brown, F., and B. Cartwright, *Nature*, **204**, 955 (1964).

[2] Fraenkel-Conrat, H., *Trans. Faraday Soc.*, **55**, 494 (1959).
[3] Hausen, P., and S. W. Schafer, *Z. Naturforsch.*, **17b**, 15 (1962).
[4] Schaffer, F. L., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 89.
[5] Fraenkel-Conrat, H., and B. Singer, in *Proc. Intern. Congr. Biochem., 5th, Moscow, 1961* (1962).
[6] Montagnier, L., and F. K. Sanders, *Nature*, **199**, 664 (1963).
[7] Baltimore, D., *Science*, **143**, 1034 (1964).
[8] Weissmann, C., P. Borst, R. H. Burdon, M. A. Billeter, and S. Ochoa, these PROCEEDINGS, **51**, 682 (1964).
[9] Ralph, R. K., R. E. F. Mathews, A. I. Matus, and H. G. Mandel, *J. Mol. Biol.*, **11**, 202 (1965).
[10] Kaerner, H. C., and H. Hoffman-Berling, *Nature*, **202**, 1012 (1964).
[11] Shipp, W., and R. Haselkorn, these PROCEEDINGS, **52**, 401 (1964).
[12] Dulbecco, R., and M. Vogt, *J. Exptl. Med.*, **99**, 193 (1964).
[13] Lockart, R. Z., Jr., *J. Bacteriol.*, **85**, 556 (1963).
[14] Bray, G. A., *Anal. Biochem.*, **1**, 279 (1960).
[15] Scherrer, K., and J. E. Darnell, *Biochem. Biophys. Res. Commun.*, **7**, 486 (1962).
[16] Lockart, R. Z., Jr., and T. Sreevalsan, in *Viruses, Nucleic Acids, and Cancer*, 17th Annual Symposium on Fundamental Cancer Research at the University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas (Baltimore; Williams and Wilkins, 1963), pp. 447–461.
[17] Sueoka, N., and T. Y. Cheng, *J. Mol. Biol.*, **4**, 161 (1962).
[18] Reich, E., and R. M. Franklin, these PROCEEDINGS, **47**, 1212 (1961).
[19] Franklin, R. M., and D. Baltimore, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 175.
[20] Shatkin, A. J., *Biochim. Biophys. Acta*, **61**, 310 (1962).
[21] Lockart, R. Z., *Biochem. Biophys. Res. Commun.*, **15**, 513 (1964).
[22] Tobey, R. A., *Virology*, **23**, 10 (1964).
[23] Ochoa, S., in *Proc. Robert A. Welch Found. Conf. Chem. Res., 8th* (1964), p. 9.

---

*THE OPTICAL ROTATORY PROPERTIES OF THE β-CONFIGURATION IN POLYPEPTIDES AND PROTEINS**

BY P. K. SARKAR† AND PAUL DOTY

DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY

Communicated February 28, 1966

While the rotatory properties of the α-helical and disordered (or randomly coiled) forms of the polypeptide chain are now rather well characterized and understood both in the ultraviolet and the visible spectral regions,[1–4] the next most frequently occurring form, the β-configuration, has been much more elusive. Moreover, the number of proteins whose optical behavior cannot be explained in terms of particular proportions of α-helical and disordered residues grows,[5, 6] and the possibility that these contain significant amounts of the β-configuration deserves careful evaluation. The recent X-ray structure determination of lysozyme[7] reveals the presence of all three configurations, and hence the need to deal with the rotatory characterization of the β-form is no longer academic.

Actually, attempts to define the optical properties of polypeptides in the β-form span a decade,[8–12] but they have been severely limited by being carried out only in organic solvents. This was necessitated by solubility considerations, but it re-