C^{14}-malonyl ACP was also synthesized by the interaction of C^{14}-malonyl thiophenol with the polypeptide at pH 8.0 in a manner similar to the malonyl transfer from thiophenol to coenzyme A. For each mole of polypeptide at pH 8.0 was added a slight excess of the C^{14}-malonyl thiophenol, and the reaction mixture was placed in ice bath and mixed vigorously by bubbling N_2 through. The pH was maintained at 8.0 at all times. At the end of 3 hr, the pH was carefully lowered to 6.0 with HCl, and the mixture was extracted four times with diethyl ether and then lyophilized. The residue was dissolved in water and characterized as malonyl ACP by its hydrolysis to malonic acid. Interaction with hydroxylamine yielded the hydroamine acid derivative.

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*THE REPLICATIVE FORM OF MS2 RNA: AN X-RAY DIFFRACTION STUDY**

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During the multiplication of single-stranded RNA[1] viruses a specific replicative form of RNA, with the characteristics of a double helix, is synthesized.[2–10] Such a replicative form, first identified by Montagnier and Sanders[2] in animal cells infected with encephalomyocarditis virus, has since been reported for poliovirus[4] and RNA-containing bacteriophages such as MS2,[3, 5–8] R17,[9] and fr.[10] The replicative form consists of complementary RNA strands, one of which is of the parental type.[3, 5] It appears to be an obligatory intermediate in the reproduction of RNA viruses[3, 5] and accumulates in large quantities in the later stages of infection. In the case of phage MS2, it has been shown[3, 6] by *in vitro* experiments that the replicative form serves as template for the synthesis of MS2 RNA by the phage-induced RNA synthetase.[11]

In this paper we report on an X-ray diffraction study of the purified replicative form of MS2 RNA. The detailed patterns obtained, which are identical to those reported for the double-stranded RNA of reovirus,[12] conclusively establish the double-helical structure of the MS2 replicative form.

Materials and Methods.—Preparation of replicative form of MS2 RNA: A typical preparation was carried out as follows. *E. coli* Hfr 3000 infected with MS2 virus was homogenized with glass beads. The homogenate was treated with DNAase and fractionated with $MgCl_2$ exactly as described for the purification of RNA synthetase.[6] After addition of sodium dodecyl sulfate to a final concentration of 0.5%, this preparation, rich both in enzyme and replicative form,[6] was extracted three times with phenol, washed with ether, and the RNA was collected after precipitation with ethanol. The precipitate was dissolved in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate to give an RNA concentration not exceeding 1 mg/ml and incubated with 50 μg/ml of pancreatic ribonuclease A for 30 min at 25°. After addition of sodium dodecyl sulfate, the digest was concentrated by precipitation with ethanol. It was dissolved in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate, extracted four times with phenol, and the RNA then precipitated with

ethanol. Chromatography on Sephadex G-200 resolved this mixture into two components; the high molecular weight RNA contained in the first half of the small, front-running peak (which comprises about 4% of the UV-absorbing material applied to the column) was then banded in Cs_2SO_4 (mean density 1.62 gm/ml). The replicative form was recovered from the middle of the tube; a thick layer of material near the meniscus (containing polysaccharides) and a heavier layer containing some UV-absorbing, precipitated material were discarded. The fractions containing the replicative form were dialyzed against 0.15 *M* sodium chloride, 0.015 *M* sodium citrate, ethanol was layered on the aqueous phase, and the resulting fibrous precipitate wound up on a glass rod. The second half of the front-running peak resulting from the Sephadex chromatography was processed in an identical fashion, yielding material with somewhat lower sedimentation coefficient. Three hundred gm of cells yielded a total of 24 mg of pure replicative form. The overall yield of replicative form is about 10–15%. Details of the procedure will be published elsewhere.

Fibers: Before the fibers were prepared, the RNA preparation was twice precipitated with ethanol in the cold, winding the precipitate around a glass rod of 2.5 mm diameter, resuspending between precipitations in 0.15 *M* sodium chloride, 0.015 *M* sodium citrate, dialyzing against two changes of the same buffer, each for 3 hr, and centrifuging for 15 min in an International clinical centrifuge before precipitation. The alcohol precipitate was washed in 80% and 100% ethanol, and air-dried. It was then pried loose from the glass rod. Two glass rods of about 1 mm diameter were mounted in holders which could be moved apart slowly and steadily by means of a fine lead screw. The tips of the glass rods were aligned approximately 1 mm apart, and a drop of glass-distilled water was placed between them. A small piece of the RNA was then placed in the drop. The water was rapidly taken up by the RNA, and a sticky gel formed between the tips of the glass rods. The glass rods were then slowly moved apart with continual microscopic observation. The gel, as it slowly dried, usually reached a stage at which slow stretching was possible without breakage under conditions of about 40% relative humidity and a temperature of 21°C. Fibers were made in this way of a diameter of approximately 0.05–0.10 mm. The fibers were cut free of the glass rods and observed in a Leitz polarizing microscope using a Leitz Berek compensator. The best fibers had a negative birefringence of 0.08, which compares well with that given by the best DNA fibers.

For the X-ray diffraction examination, pieces of the fiber about 1 mm long and containing the highly birefringent regions were selected. Using red nail varnish as adhesive, these were cemented to brass stretchers of a type similar to those previously described.[13] (Colored varnish was used to help distinguish the adhesive from the fiber.) Improved orientation could then be obtained by breathing gently on the fibers and allowing them to buckle, then taking up the slack by screwing the attachment points of the fiber slowly apart.

X-ray diffraction apparatus: X-rays were generated by Jarrell-Ash line focus units with a spot size of 1.4 mm by 0.1 mm and viewed at 5° take-off angle. Copper targets and 0.016-mm Ni filters yielded predominantly Cu Kα radiation of 1.54 Å wavelength. The cameras used collimators of lead glass 1 cm in length and 0.06 mm internal diameter. Sheet lead 0.5 mm thick with a hole 0.5 mm in diameter was placed at each end of the collimator, and as a further guard a platinum electron microscope aperture, 0.1 mm hole diameter, was placed at the exit. Suitable regions of the fiber specimen were aligned with the collimator using a microscope illuminator. The distance from specimen to film was 27 mm. The humidity within the cameras was controlled by bubbling helium through appropriate saturated salt solutions. Most experiments were done using saturated sodium chlorate, giving a relative humidity of 75%, and saturated sodium tartrate, giving 92% humidity. Ilford Industrial G X-ray film was used with a development time of 7 min at 20°C.

Results and Discussion.—Purified replicative form: The properties of this material, to be discussed in detail elsewhere, can be summarized as follows. As determined by the annealing assay,[5] typical preparations are at least 95 per cent pure in regard to nucleotide material. The replicative form gives a positive orcinol reaction[14] (corresponding to an equivalent amount of yeast RNA) and a negative cysteine test,[15] indicating the absence of deoxyribose (less than an amount corresponding to 5% contamination by DNA). Degradation with KOH gives rise to 2′ −(3′)-ribonucleotides. The ratio of purines to pyrimidines

is one. The replicative form is resistant to RNAase and DNAase under conditions where single-stranded viral RNA and calf thymus DNA, respectively, are rapidly and completely depolymerized. After heat denaturation it becomes as sensitive to RNAase as single-stranded viral RNA. If the hyperchromicity is examined as a function of temperature,[16] a sharp transition is found, the T_m depending on the salt concentration. In 0.015 M sodium chloride, 0.0015 M sodium citrate, the T_m is 87°, no increase of absorbancy being observed between 20° and 80°. In ten times higher salt concentration the T_m is above 100°. The buoyant density in Cs_2SO_4 is 1.609 gm/ml as compared with the value of 1.626 gm/ml for single-stranded MS2 RNA.[5] In the ultracentrifuge the UV-absorbing material sediments with a sharp boundary and a sedimentation coefficient ($s_{20,w}$) of 9.5 S; this is lower than the 12–15 S expected for a double helix with twice the molecular weight (2×10^6) of MS2 RNA.[17] This may be due to limited damage resulting from the intensive ribonuclease treatment used in the isolation procedure.[18] The purified replicative form is not infectious in an *E. coli* protoplast system sensitive to MS2 RNA; moreover, no evidence could be obtained for infectivity of the replicative form present in carefully prepared extracts of infected *E. coli*. This agrees with the findings of Kaerner and Hoffmann-Berling[10] with fr phage and contrasts with the results obtained for the replicative form of encephalomyocarditis virus[2] and poliovirus[19] that were reported to be infectious.

X-ray diffraction: The most detailed diffraction patterns so far obtained from

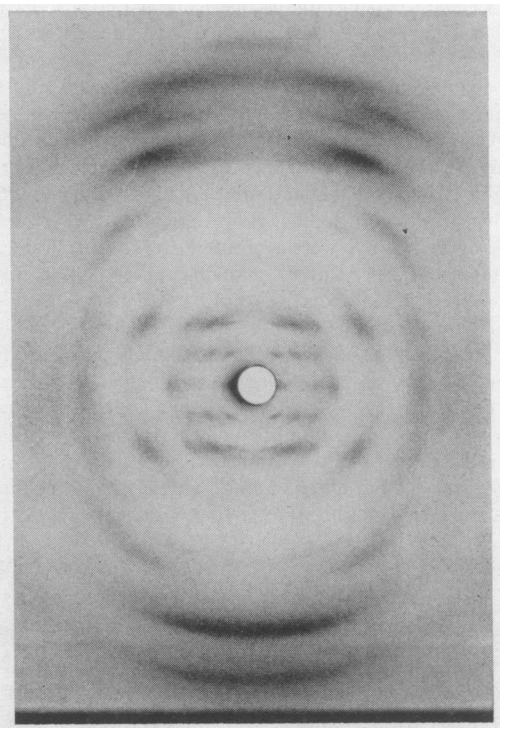

FIG. 1.—X-ray diffraction pattern from a fiber of MS2 RNA replicative form, 0.08 mm diameter, 92% relative humidity, exposure 17 hr. Fiber axis tilted approximately 15° from the perpendicular to the X-ray beam.

double-helical RNA are given by reovirus RNA.[12] The X-ray diffraction patterns from MS2 RNA replicative form and from reovirus RNA under similar conditions are illustrated in Figures 1–3. There are minor differences in crystallinity and orientation, but in all significant respects the patterns are identical.

This identity implies that over large regions (of the order of 100 helical repeats) the structures of reovirus RNA and the MS2 RNA replicative form are identical. The sugar phosphate chains must, therefore, have configurations which cannot differ by more than one or two tenths of an angstrom, since molecular model building and calculations show that the higher layer line intensity distributions are very sensitive to the precise position of the chains, and these intensities appear identical in the two diffraction patterns. It should be noted, however, that provided regular base pairing occurs and the glycosidic bonds are regularly placed (i.e., they have identical positions and orientation relative to the helix axis) the precise sequence of bases has no influence on the patterns obtained.

Such detailed comparisons cannot yet be made with the patterns given by preparations of fragments from transfer RNA[20] and from wound tumor virus RNA,[21] but within the limits of the comparison these also appear to be identical.

Furthermore, all preparations of RNA (viral, ribosomal, native transfer RNA) give diffuse patterns which imply that there are *regions* within them which have a similar configuration to reovirus RNA and to the MS2 RNA replicative form.[12, 20] The structure therefore appears to be general, and just as all double-helical DNA's

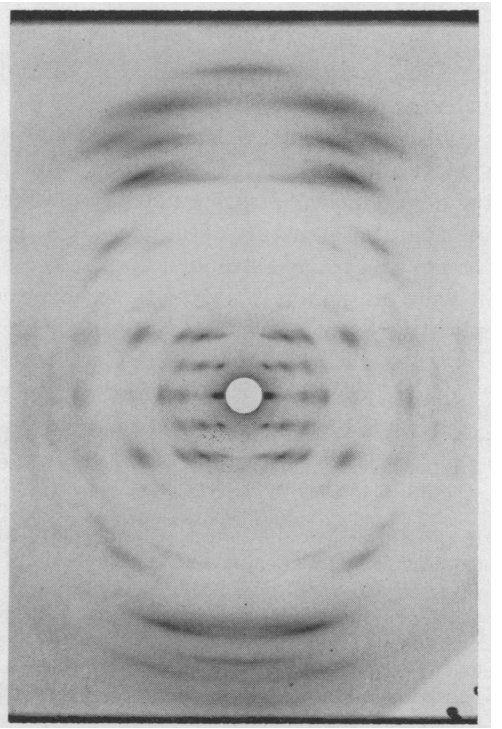

FIG. 2.—X-ray diffraction pattern from a fiber of reovirus RNA, 0.07 mm diameter, 92% relative humidity, exposure 21 hr. Fiber axis tilted approximately 15° from the perpendicular to the X-ray beam.

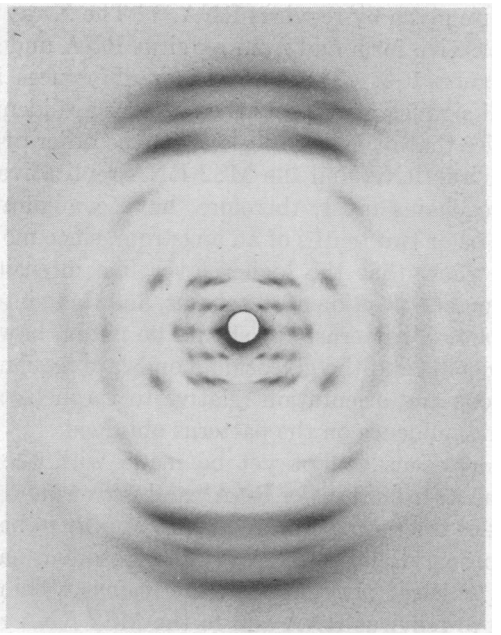

FIG. 3.—X-ray diffraction pattern from a fiber of MS2 RNA replicative form, 0.065 mm diameter, 92% relative humidity, exposure 22 hr, fiber axis tilted approximately 10° from the perpendicular to the X-ray beam.

adopt similar configurations, so do all double-helical RNA's, though the structures of the two types of nucleic acid are clearly different.

Summary.—X-ray diffraction patterns of the purified replicative form of bacteriophage MS2 RNA are identical to those given by the double- stranded reovirus RNA. The patterns prove the double-helical structure postulated for the replicative form and confirm the generality of the structure of double-helical RNA. It seems likely that the replicative form of other single-stranded viral RNA's described so far have the same structure in view of the similarity of their physicochemical properties with those of MS2 replicative form.

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‡ Fellow of the Rockefeller Foundation.

[1] Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; RNAase, pancreatic ribonuclease; DNAase, pancreatic deoxyribonuclease.

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[18] A replicative form isolated by Kaerner and Hoffmann-Berling (ref. 10) from *E. coli* infected with the RNA phage fr shows the same melting profile, T_m, RNAase resistance, and buoyant density in Cs_2SO_4 as the replicative form of MS2. Although the replicative form of phage fr, purified by the method A of these authors, also sediments with an s_{20} of about 8.5, their improved method B yielded a preparation containing a minor component with an s_{20} of 14.5 *S*. This is the value expected for a duplex containing twice as much RNA as the viral strand. A similar value, of 12–16 *S*, was obtained by Fenwick *et al.* (ref. 9) for material tentatively identified as the replicative form of the RNA phage R17 by sucrose gradient analysis of infected *E. coli* extracts.

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*HYDROSTATIC PRESSURE AND OSMOTIC POTENTIAL IN LEAVES OF MANGROVES AND SOME OTHER PLANTS**

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It has long been known that mangroves and other halophytes have a salt concentration in their cells which as a rule exceeds that of the sea water.[1–3] Only recently, however, has it been found that these plants run essentially fresh water in their xylem vessels.[4] They maintain, therefore, at all times a difference in osmotic potential between the sea water and xylem sap equivalent to 20–30 atm. It is generally held that the living protoplasm of plant cells is surrounded by a semipermeable membrane which separates it from the more or less rigid cellulose wall. The micellar structure of the wall is pervious to the solutes of the xylem sap, and forms a micropore "free space" extension of the vascular system.[5–9] These premises indicate equivalence between hydrostatic pressure and osmotic potential. The latter is always negative, and hence the relations can be expressed in the following simple equation: