*EVIDENCE FOR SEMICONSERVATIVE REPLICATION OF CIRCULAR POLYOMA DNA**

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DNA extracted from purified polyoma (Py) virus contains three components, I, II, and III, with sedimentation coefficients of 20*S*, 16*S*, and 14*S*.[1] Polyoma DNA I (Py DNA I), which in most preparations accounts for 80 per cent or more of the total DNA, is a circular helix with two separately continuous strands; it is infective[2] and shows spontaneous, monomolecular renaturation.[3] The introduction of a discontinuity into one of the strands converts Py DNA I into a circular helix with a sedimentation coefficient of 16*S*.[4] This conversion product, which in this article is referred to as Py DNA II′, exhibits a denaturation behavior similar to that of linear, double-stranded DNA. Its physicochemical properties are comparable to those of naturally occurring Py DNA II. Both I and II have molecular weights of about 3×10^6 and display in CsCl solutions a buoyant density of 1.709 gm cm^{-3}.[1]

The purpose of this work was to determine the mode of replication of circular Py DNA. The experimental results obtained are compatible with semiconservative replication.

Materials and Methods.—Mouse kidney (MK) cell cultures were prepared from CR 1 mice (Wander AG, Bern) and infected 1–3 days after confluence.[6]

Purification of Py virus: Crude lysate (infected cells together with culture medium) was frozen and thawed 3 times, incubated with pancreatic deoxyribonuclease in order to hydrolyze cellular DNA,[5] and then purified according to Winocour.[6] Py DNA showed essentially the same properties whether it had been extracted with phenol[7] or with sodium dodecyl sulfate (SDS).[8, 9]

Pulse-labeling with deoxythymidine-H³ (TdR-H³): Synthesis of viral and cellular DNA in infected MK cells was studied by following the incorporation of TdR-H³: at a given time the culture medium (modified Eagle's medium with 10% horse serum) was replaced by fresh medium containing TdR-H³ as specified in the text. At the end of the pulse, the monolayers were washed twice with culture medium, and at various times thereafter (chase period) aliquots were extracted with SDS. Pulse-labeling and chase were performed in a hood at 37°C in a water-saturated atmosphere containing about 5% CO_2.

Deoxythymidine-methyl-H³ (spec. act. 10–15 c/m*M*) and *5-bromodeoxyuridine-2-C¹⁴* (BUdR-C¹⁴; spec. act. 19 mc/m*M*) were obtained from New England Nuclear Corp. Radioactive samples were dried on filter paper, put in toluene scintillation fluid, and counted 2–4 times in a low-background beta-spectrometer (Nuclear-Chicago model 725).

5-fluorodeoxyuridine (FUdR), a generous gift from Hoffmann-La Roche, Basel, was used in all experiments at 6×10^{-5} *M* (15 μg/ml), a concentration found to inhibit thymidylate synthesis in more than 99% of Py-infected MK cells.[10] The use of FUdR was found to be necessary because in the absence of the analogue the substitution of TdR by BUdR in cellular and viral DNA was relatively poor. BUdR (Calbiochem) and BUdR-C¹⁴ were used in all experiments at a concentration of 1.3×10^{-5} *M* (4 μg/ml).

SDS extractions of viral and cellular DNA were performed according to Weil *et al.*[9] In some experiments the method was slightly modified in order to remove selectively most of the cellular DNA ("selective SDS extraction").[11]

Sedimentation velocity analyses by band centrifugation were performed in a preparative ultracentrifuge as described previously.[9, 12] Following centrifugation under standard conditions (Fig. 1), the position of Py DNA varied in different experiments by no more than ±5% (±1 fraction).

CsCl density gradient equilibrium centrifugation was performed according to Vinograd and Hearst.[13] For determinations of the density of CsCl solutions following ultracentrifugation, 2–4 fractions of 4 drops each were collected into tubes containing 0.5 ml paraffin oil. The density was obtained from the refractive index measured at 25°C in a Zeiss-Abbé refractometer and corrected by taking the buoyant density of *E. coli* DNA as 1.710 gm cm^{-3}.[14] If present, light Py DNA (LL) was used as a density marker (1.709 gm cm^{-3}).[1]

Experiments and Results.—Incorporation of deoxythymidine-H^3 (TdR-H^3) into viral and cellular DNA of Py-infected mouse kidney (MK) cells: Replication of Py virus in confluent MK cells proceeds in two phases. The first one corresponds to the "eclipse"; during phase 2 the actual replication of progeny virus takes place, accompanied by virus-induced cellular DNA synthesis.[9, 15, 16] Pulse labeling (see *Materials and Methods*) for 2.5 or 5 min was performed during the period of high rate of Py DNA synthesis, i.e., between 20 and 40 hr post infectionem (p.i.). Viral and cellular DNA were extracted with SDS at various lengths of time after the pulse and sedimented by band centrifugation under conditions leading to separation of the slower-moving Py DNA I (20*S*) and II (16*S*) from the faster-sedimenting cellular DNA[9] (Fig. 1*C*).

Figure 1*A* shows the distribution of TdR-H^3 in an SDS extract derived from infected MK cells which had been pulse-labeled for 2.5 min and where the reaction was stopped on ice (no chase): essentially no TdR-H^3 is found to be associated with 20*S* or 16*S* Py DNA. A relatively small quantity of TdR-H^3 is present in polydis-

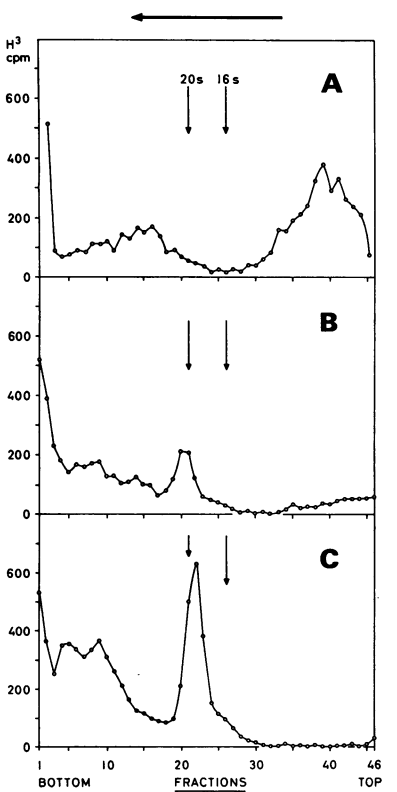

FIG. 1.—Sedimentation velocity analysis by band centrifugation of pulse-labeled (TdR-H^3) DNA extracted with SDS from Py-infected MK cells. TdR-H^3 pulse at 30 hr p.i. (25 μc/ml). (*A*) Pulse 2.5 min, reaction stopped on ice, no chase. (*B*) Pulse 5 min, 10-min chase. (*C*) Pulse 5 min, 60-min chase. Lamella: 0.2 ml SDS extract. Bulk solution: 3 ml CsCl, ρ = 1.505 gm cm^{-3}, pH 7.5. Centrifuged in Spinco model L 50, rotor SW 39, at 35,000 rpm for 3.5 hr at 24°C. Following centrifugation, fractions of two drops were collected. Arrows indicate expected positions of Py DNA I (20*S*) and II (16*S*; see Table 1). Radioactive material in upper third of tube consists of free thymidine phosphates and TdR-H^3. On filter paper, counting efficiency for H^3 associated with mononucleotides is about half that of H^3 incorporated into DNA. Data in Fig. 1 are not corrected for counting efficiency.

perse DNA with sedimentation coefficients > 20S, part of which might be viral DNA *in statu nascendi.*

Radioactive Py DNA I and II become first detectable about 10 min after the start of the TdR-H^3 pulse. Thereafter, the radioactivity associated with Py DNA increases and reaches a maximum after about 30 min (Figs. 1*B* and *C*). Sedimentation velocity analyses of SDS extracts in sucrose gradients (10–40%) gave comparable results. In the experiments reported in this paper, no radioactive Py DNA III (14S) could be observed.

Incorporation of 5-bromodeoxyuridine (BUdR) into intracellular Py DNA: Meselson and Stahl[17] incorporated the heavy isotope N^{15} into newly synthesized DNA of *E. coli* and separated the labeled heavy from the unlabeled light DNA by density gradient equilibrium centrifugation in CsCl solutions. In this way they obtained evidence for semiconservative replication of *E. coli* DNA, a process leading to daughter molecules which contain an intact parental and a newly synthesized complementary strand.

BUdR, an analogue of TdR, has been used successfully as a density label for viral,[18] bacterial,[19] and mammalian[20] DNA. Substitution of TdR by BUdR in both strands leads to heavy DNA (HH) which exhibits in CsCl density gradients a markedly increased buoyant density. Substitution of TdR in only one of the strands yields hybrid DNA (HL) with a buoyant density exactly between those of light (LL) and heavy (HH) DNA.

The incorporation of BUdR into newly synthesized, intracellular Py DNA was studied as follows: At 24 or 36 hr p.i. the monolayers were pulse-labeled for 20 min

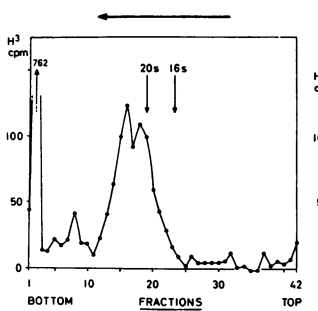

FIG. 2

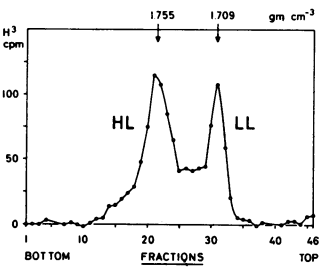

FIG. 3

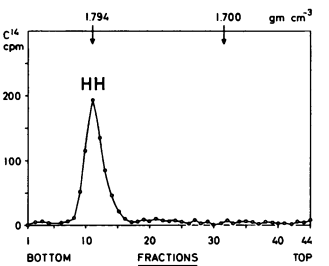

FIG. 4

FIG. 2.—Sedimentation velocity analysis by band centrifugation of radioactive (TdR-H^3) hybrid and light Py DNA extracted with SDS from infected MK cells. At 24 hr p.i. TdR-H^3 pulse (20 μc/ml) for 20 min. Thereafter, monolayers incubated for 2 hr in the presence of BUdR + FUdR. Py DNA extracted by "selective SDS method." Centrifuged as described in Fig. 1. It should be noted that Py DNA containing BUdR has increased sedimentation coefficients. Analysis by CsCl density gradient (see Fig. 3).

FIG. 3.—CsCl density gradient equilibrium centrifugation of radioactive hybrid (HL) and light (LL) Py DNA extracted with SDS from infected MK cells. Fractions 11–23 of Fig. 2 pooled and diluted in 0.001 *M* Na phosphate, pH 7.9. Solid CsCl (Merck) added to adjust the density to 1.75 gm cm^{-3} and the volume to 3 ml. Centrifuged in Spinco model L 50, rotor SW 39, at 35,000 rpm for 40 hr at 24°C. Following centrifugation, fractions of two drops were collected. In one strand of the hybrid DNA (HL) TdR is substituted by BUdR.

FIG. 4.—CsCl density gradient equilibrium centrifugation of heavy Py DNA (HH; BUdR-C^{14}) extracted from purified virions. At 12 hr p.i. BUdR + FUdR added. At 24 hr p.i. medium replaced by fresh medium containing the same concentration of FUdR. The unlabeled BUdR was substituted by BUdR-C^{14} (0.1 μc/ml) at the same molar concentration. The virus was harvested 86 hr p.i. and then purified. The phenol-extracted DNA was centrifuged as described in Fig. 3.

with TdR-H^3. Then they were washed twice, covered with culture medium containing BUdR + FUdR (1.3×10^{-5} *M* BUdR; 6×10^{-5} *M* FUdR to inhibit thymidylate synthesis), and incubated at 37°C for 2 hr. Py DNA was extracted by the "selective SDS method" and sedimented by band centrifugation (Fig. 2).

Analysis by CsCl density gradient equilibrium centrifugation showed that the radioactive DNA consists mainly of light (LL), i.e. "normal," and hybrid (HL) Py DNA with buoyant densities of 1.709 and 1.755 gm cm^{-3} (Fig. 3). About two thirds of the TdR-H^3 is found in DNA which shows an increased density because of incorporated BUdR. This indicates that the synthesis of Py DNA continued in most cells after the addition of BUdR + FUdR.

The material at intermediate densities (Fig. 3) probably represents Py DNA which replicated shortly after the addition of BUdR + FUdR. If prior to the addition of BUdR the thymidylate pool was exhausted by incubation with FUdR, only light and hybrid Py DNA were found.

In experiments where following the TdR-H^3 pulse the cells were incubated with BUdR-C^{14} instead of unlabeled BUdR, a third radioactive band containing heavy Py DNA (HH) was found at a density of 1.794 gm cm^{-3} (see below).

Results of control experiments suggest that the SDS method extracts from MK cells with about the same efficiency free viral DNA and Py DNA contained in virions. The yield remains unaffected when TdR is substituted by BUdR.

In all experiments a fraction of the TdR-H^3 pulse-labeled Py DNA retained its original density (LL). This could be explained by the finding that a fraction of newly synthesized viral DNA is encased into viral capsids and is thus removed from the pool of replicating viral DNA.[21]

Evidence for the enclosure of BUdR-labeled Py DNA into viral capsids: The following experiments demonstrate that intracellular light, hybrid, and heavy Py DNA are encased in viral capsids, and that DNA extracted from purified virus has essentially the same properties as viral DNA extracted directly from MK cells.

Properties of heavy Py DNA (HH): In experiments where BUdR + FUdR or BUdR-C^{14} + FUdR had been added to the cultures prior to the onset of the synthesis of viral DNA (<13 hr p.i.),[11] the infective titer of crude lysates harvested 3–4 days p.i. amounted only to about 1 per cent and the hemagglutinating titer to about 10 per cent of controls grown in the absence of the analogues. Py virions of such lysates exhibit in CsCl a slightly increased buoyant density.[11] DNA extracted from these virions forms in CsCl gradients a uniform radioactive band at a density of 1.794 gm cm^{-3} (Fig. 4 and Table 1). It contains heavy Py DNA (HH) where, judging from the buoyant density, about 85 per cent of TdR has been substituted by BUdR-C^{14}. This estimate is based on the difference in buoyant density (0.2 gm cm^{-3}) of poly AdR-TdR and poly AdR-BUdR.[22] If hybrid Py DNA (HL) was the immediate precursor of heavy Py DNA, at any given time a fraction of the newly synthesized hybrid DNA would be enclosed into viral capsids and would thus be preserved. The absence of detectable amounts of radioactive hybrid DNA therefore implies—if semiconservative replication is assumed—that heavy Py DNA is able to replicate.

The pattern of sedimentation of heavy Py DNA in CsCl solutions (Fig. 5) is similar to that of light Py DNA (LL): about 80 per cent of the molecules sediment quickly (28*S*) and the remainder slowly (23*S*). The 28*S* and 23*S* heavy Py DNA

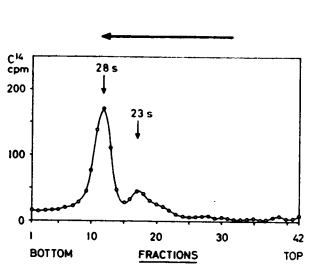

FIG. 5

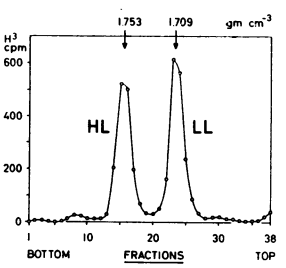

FIG. 6

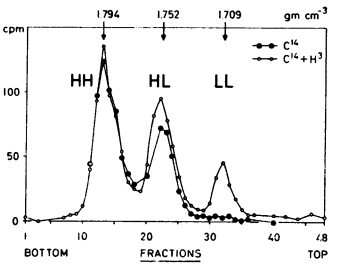

FIG. 7

FIG. 5.—Sedimentation velocity analysis by band centrifugation of radioactive heavy Py DNA (HH) extracted from purified virions. Heavy Py DNA (I_{HH} and II_{HH}; BUdR-C^{14}), pooled fractions 12–15 of the CsCl density gradient shown in Fig. 7, sedimented through a CsCl solution (ρ = 1.505 gm cm^{-3}) as described in Fig. 1. The indicated sedimentation coefficients are relative to 20S Py DNA I_{LL} (see Table 1).

FIG. 6.—CsCl density gradient equilibrium centrifugation of radioactive hybrid (HL) and light (LL) Py DNA extracted from purified virions. At 22 hr p.i. TdR-H^3 pulse (5 μc/ml) for 30 min. Monolayers washed and fresh medium added containing FUdR. Seventy min later BUdR added to the medium. The virus was harvested 91 hr p.i. and purified. The phenol-extracted DNA was centrifuged as described in Fig. 3.

FIG. 7.—CsCl density gradient equilibrium centrifugation of radioactive heavy (HH), hybrid (HL), and light (LL) Py DNA extracted from purified virions. At 23 hr p.i. TdR-H^3 pulse (5 μc/ml) for 30 min. Monolayers washed and fresh medium added containing FUdR. Forty-five min later BUdR-C^{14} (0.25 μc/ml) added to the medium. The virus was harvested 77 hr p.i. and purified. The phenol-extracted DNA was centrifuged as described in Fig. 3. Heavy (HH) and light (LL) Py DNA contain only C^{14} and H^3, respectively, while hybrid DNA (HL) contains both, C^{14} + H^3.

are structurally analogous to light Py DNA I_{LL} (20S) and II_{LL}(16S); they are therefore designated as Py DNA I_{HH} and II_{HH}. Their increased sedimentation velocity in CsCl solutions can be explained by their higher density.

Properties of hybrid Py DNA (HL): In the presence of BUdR + FUdR, hybrid Py DNA is synthesized which can be extracted from MK cells with SDS (Fig. 3). A fraction of it is encased in viral capsids and can be extracted from purified Py virions. In the experiment shown in Figure 6 the intracellular pool of thymidylate had been exhausted (FUdR) prior to the addition of BUdR. The absence of detectable amounts of radioactive DNA at intermediate densities shows that recombination either occurs at too low a frequency or involves regions of Py DNA too small to be detected by this method (see also Fig. 10).

If after a TdR-H^3 pulse the monolayers are incubated with BUdR-C^{14} instead of

TABLE 1

BUOYANT DENSITY AND SEDIMENTATION COEFFICIENTS OF NORMAL AND OF BUdR-SUBSTITUTED POLYOMA DNA

			$\rho_{25°}$† (gm cm^{-3})	Sedimentation Coefficient$_{CsCl}$‡ Polyoma DNA I	II
Light	LL	○*	1.709	20	16
Hybrid	HL	○	1.753	24	20
Heavy	HH	○	1.795	28	23

* The drawings symbolize the light and the heavy strands of Py DNA. Neither a possible "linker" nor the quaternary structure[4] is considered.

† $\rho_{25°}$: buoyant density in CsCl. The values represent the mean of five independent preparations analyzed in the preparative ultracentrifuge, using light Py DNA as a density marker.

‡ Sedimentation coefficients determined in the preparative ultracentrifuge in CsCl ρ- = 1.505 gm cm^{-3} under the conditions specified in Fig. 1. Py DNA I_{LL} used as a velocity marker. The values given in this table are relative to the $S^{\circ}_{20,w}$ of Py DNA I_{LL}[1] taken as 20.

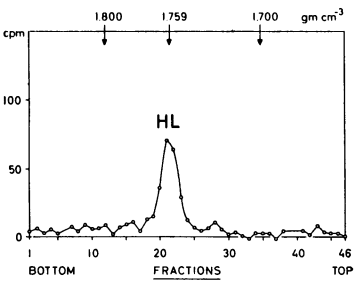

FIG. 8.—CsCl density gradient equilibrium centrifugation of thermally denatured and spontaneously renatured radioactive hybrid Py DNA I_{HL}. Hybrid Py DNA (HL) containing C^{14} + H^3 (pooled fractions 21–23 shown in Fig. 7) was dialyzed for 3 days against 0.001 *M* Na phosphate, pH 8.0. The hybrid, which consisted mainly of I_{HL}, was heated for 3.5 min at 89°C and cooled rapidly in ice-water. The DNA was centrifuged as described in Fig.3. The buoyant density of the heated corresponds to that of the native hybrid DNA. The same result was obtained when the hybrid DNA had been boiled (97°C) for 3 min. The conditions of heating and cooling used are sufficient to denature BUdR-substituted, linear DNA of comparable base composition and size.

BUdR, radioactive heavy (HH), hybrid (HL) and light (LL) intracellular Py DNA are formed and a fraction of the three species gets enclosed into viral capsids (Fig. 7).

Sedimentation velocity analysis of hybrid Py DNA (HL) by band centrifugation in CsCl reveals a major and a minor component with sedimentation coefficients of 24*S* and 20*S* which are designated as Py DNA I_{HL} and II_{HL} (Table 1).

Following thermal denaturation under conditions sufficient to disrupt all hydrogen bonds, hybrid Py DNA I_{HL} renatures spontaneously (Fig. 8) as does normal Py DNA I_{LL}.[3] Py DNA I_{LL} (20*S*) can be converted into 16*S* Py DNA II'_{LL} by the introduction of a discontinuity into one of the strands.[4] Similarly, hybrid Py DNA I_{HL} can be transformed into II'_{HL}. Thermal denaturation of II'_{HL} leads to the physical separation of the complementary strands which band in CsCl gradients at the densities expected for single-stranded light (L; TdR-H^3) and single-stranded heavy (H; BUdR-C^{14}) Py DNA (Fig. 9).

Absence of experimental evidence supporting a conservative mode of replication: The experimental results reported above are consistent with semiconservative replication. They do not, however, exclude a conservative model of replication according to which the infecting parental DNA, its double-stranded structure being conserved, acts as a template for the production of single-stranded Py DNA molecules, each of which primes the synthesis of one complementary strand. The newly formed helices might—as did the infecting parental Py DNA—direct the synthesis of single strands, and so forth.

If duplex formation took place shortly after the single strands had been synthesized at a given time *A*, then several hours later, i.e., at time *B*, no Py DNA

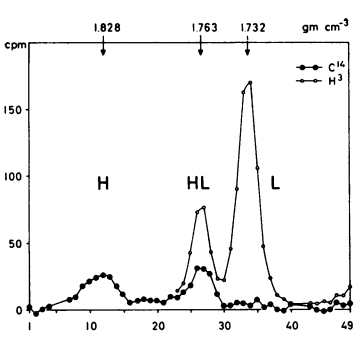

FIG. 9.—CsCl density gradient equilibrium centrifugation of thermally denatured hybrid Py DNA II'_{HL}. Separation of the heavy (H; BUdR-C^{14}) from the light (L; Td-RH^3) strand. Hybrid Py DNA (HL) was treated with hydroquinone[4] so as to convert about 60% of I_{HL} to II'_{HL}. The preparation was dialyzed, heated, cooled, and centrifuged as described in Fig. 8. The remaining Py DNA I_{HL} renatured spontaneously and contains, as expected, C^{14} + H^3, while the single-stranded heavy (H) and light (L) DNA contain only C^{14} or H^3, respectively. The density differences between the radioactive peaks correspond to the predicted values, while the measured densities are slightly higher. They are, however, within the limits of experimental error.

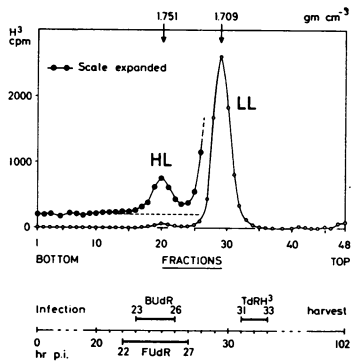

FIG. 10.—Evidence that a strand of Py DNA which was synthesized at time *A* (23–26 hr p.i.) is able to form a double-stranded helix at a later time *B* (31–33 hr p.i.). CsCl density gradient equilibrium centrifugation of radioactive Py DNA extracted from purified virions. FUdR, BUdR, and Td-RH³ (5 μc/ml) pulses indicated according to the timetable. The phenol-extracted DNA was centrifuged as described in Fig. 3. At a density of 1.751 a small band containing radioactive hybrid DNA (HL) can be seen. The heavy strand (BUdR) was synthesized at time *A* and the light, complementary strand (TdR-H³) at time *B*. For a better resolution of the hybrid region the scale of the radioactivity was expanded by a factor of 10 and the base line changed.

should remain single-stranded and able to direct the formation of duplexes. Figure 10, however, shows that heavy Py DNA strands (H; BUdR) which had been synthesized at time *A* (23–26 hr p.i.) primed at time *B* (31–33 hr p.i.) the synthesis of complementary, light strands (L; TdR-H³) leading to radioactive, hybrid Py DNA (HL). Due to the removal of most of the Py DNA formed at time *A* from the pool of replicating DNA by its being encased in viral capsids, only a small fraction of Py DNA formed at time *A* replicated at time *B*. If the strands of heavy Py DNA (H) which directed the synthesis of hybrid duplexes at time *B* had remained single-stranded up to this time, then at least 10 per cent of the pool of replicating Py DNA molecules should be single-stranded. So far, however, no single-stranded Py DNA could be found in infected MK cells. The experimental evidence thus fails to support the outlined model of conservative replication; it is in agreement, however, with semiconservative replication.

Discussion and Summary.—The results reported are compatible with the assumption that Py DNA replicates semiconservatively as do other double-stranded DNA's.[17, 18, 20, 23, 24] No experimental evidence could be obtained in support of a conservative model of replication. Hybrid Py DNA where TdR is substituted by BUdR in one strand and also heavy Py DNA where TdR is replaced in both strands are able to replicate. In contrast, cellular DNA of infected[9] and of dividing, uninfected[11] MK cells, though able to form a hybrid, is unable to undergo a second cycle of replication leading to heavy DNA.

Sedimentation velocity analyses and melting behavior suggest that the structures of hybrid and heavy Py DNA are analogous to that of normal, light Py DNA.

Py DNA I which accounts for most of the viral DNA is a circular helix with separately continuous strands. Semiconservative replication therefore requires the introduction of at least one discontinuity into one of the strands. Following duplication, the continuity of the strands of the daughter molecules is restored. The process engaged in strand opening and closing remains unknown.

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Abbreviations: AdR, deoxyadenosine; BUdR, 5-bromodeoxyuridine; BUdR + FUdR, 1.3×10^{-5} *M* BUdR and 6×10^{-5} *M* FUdR; c, curie; cpm, counts per minute; DNA, deoxyribonucleic acid; FUdR, 5-fluorodeoxyuridine; *M*, mole or molar; MK, mouse kidney; p.i., post infectionem;

Py, polyoma; rpm, revolutions per minute; *S*, Svedberg; SDS, sodium dodecyl sulfate; TdR, deoxythymidine.

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[1] Weil, R., and J. Vinograd, these PROCEEDINGS, **50**, 730 (1963).
[2] Dulbecco, R., and M. Vogt, these PROCEEDINGS, **50**, 236 (1963).
[3] Weil, R., these PROCEEDINGS, **49**, 480 (1963).
[4] Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis, these PROCEEDINGS, **53**, 1104 (1965).
[5] Ruschmann, G., and T. Brunner, in preparation.
[6] Winocour, E., *Virology*, **19**, 158 (1963).
[7] Weil, R., *Virology*, **14**, 46 (1961).
[8] Watson, J. D., and J. W. Littlefield, *J. Mol. Biol.*, **2**, 161 (1960).
[9] Weil, R., M. R. Michel, and G. K. Ruschmann, these PROCEEDINGS, **53**, 1468 (1965).
[10] Pétursson, G., and R. Weil, in preparation.
[11] Hirt, B., in preparation.
[12] Vinograd, J., R. Bruner, R. Kent, and J. Weigle, these PROCEEDINGS, **49**, 902 (1963).
[13] Vinograd, J., and J. Hearst, *Progr. Chem. Org. Nat. Prod.*, **20**, 372 (1962).
[14] Schildkraut, C. L., J. Marmur, and P. Doty, *J. Mol. Biol.*, **4**, 430 (1962).
[15] Dulbecco, R., L. H. Hartwell, and M. Vogt, these PROCEEDINGS, **53**, 403 (1695).
[16] Winocour, E., A. M. Kaye, and V. Stollar, *Virology*, **27**, 156 (1965).
[17] Meselson, M., and F. W. Stahl, these PROCEEDINGS, **44**, 671 (1958).
[18] Kaplan, A. S., and T. Ben-Porat, *Virology*, **23**, 90 (1964).
[19] Baldwin, R. L., and E. M. Shooter, *J. Mol. Biol.*, **7**, 511 (1963).
[20] Chun, E. H. L., and J. W. Littlefield, *J. Mol. Biol.*, **3**, 668 (1961).
[21] Michel, M., in preparation.
[22] Wake, R. G., and R. L. Baldwin, *J. Mol. Biol.*, **5**, 201 (1962).
[23] Meselson, M., and J. J. Weigle, these PROCEEDINGS, **47**, 857 (1961).
[24] Kozinski, A. W., and P. B. Kozinski, *Virology*, **20**, 213 (1963).