*MOUSE CELLULAR DNA ENCLOSED IN POLYOMA VIRAL CAPSIDS (PSEUDOVIRIONS)**

By M. R. Michel, B. Hirt, and R. Weil

SWISS INSTITUTE FOR EXPERIMENTAL CANCER RESEARCH, LAUSANNE, SWITZERLAND

Communicated by V. Prelog, July 21, 1967

The DNA extracted from purified preparations of polyoma (Py) virus consists of three components, I, II, and III, with sedimentation coefficients of 20, 16, and ~14*S*. I and II are circular DNA molecules with a molecular weight of 3×10^6 and a buoyant density in CsCl of 1.709 gm cm^{-3}.[1, 2] Both I and II are infective.[3]

Earlier observations suggested that Py DNA III might be derived from mouse cellular DNA.[4, 5] In this paper we will show that most of the DNA molecules in III are linear fragments of mouse cellular DNA enclosed in Py viral capsids. Viral particles that contain mouse DNA instead of the viral genome are not infective and are referred to as *Py pseudovirions*.

Materials and Methods.—Wild-type virus was grown on confluent MK cell cultures[4, 6] and was harvested at various times after infection. The crude viral lysates were extensively treated with pancreatic DNase[18] and RNase[18] and then purified by differential and CsCl density gradient equilibrium centrifugation.[6] If viral preparations were subjected to a second cycle of purification, the relative amounts of Py DNA I, II, and III remained unchanged.[18]

Plaque-forming titer was generally determined on MK cultures[7] and in some comparative experiments on mouse embryo cultures.[8] *Hemagglutination titer* was determined according to Eddy *et al.*[9, 18] DNA was extracted from purified viral preparations with 0.6% SDS–0.01 *M* EDTA.[4]

For *radioactive labeling* of viral and cellular DNA deoxythymidine-methyl-H^3 (TdR-H^3, spec. act. 10–17 c/mM) and deoxythymidine-2-C^{14} (spec. act. 30 mc/mM) were used.[18] Radioactivity was measured on filter papers in a liquid-scintillation counter (Nuclear Chicago 6851). Unless otherwise indicated, the counting background was not subtracted.

Sedimentation velocity analysis by band centrifugation and CsCl density gradient equilibrium centrifugation were performed according to methods used in earlier work.[10, 11] The buoyant densities for the DNA's were determined by using *E. coli* DNA as a standard (ρ = 1.710 gm cm^{-3}).[12] The buoyant density of fractions (single drops) collected from CsCl density gradient of viral preparations was obtained from measurements of the refractive index at 25°C. The value of 1.330 gm cm^{-3} for Py virions (peak fraction) is the average from the measurement of 58 different viral preparations. DNA was examined by *electron microscopy* according to Kleinschmidt and Zahn.[13] Spherules of polystyrene latex (Dow Chemical) were added to the preparations and their shadow was used to calibrate the magnification.[14] The preparations were photographed at a magnification of 10^4.

"Natural" mouse RNA for hybridization experiments was extracted with hot phenol[15, 16] from uninfected MK cultures (2 days after plating) which had been labeled for 30 min with uridine-5-H^3 (5 μc/ml; spec. act. 4.4 c/mM) prior to the extraction.

"Synthetic" mouse RNA was prepared in a cell-free system with DNA-dependent *E. coli* RNA polymerase (sed. const. 13*S*; *E. coli* MRE 600; RNase I^-) according to Pettijohn and Kamiya[17] using highly purified DNA from MK cultures[18] as the primer. Under the conditions used, the attachment of the enzyme to the DNA was specific and enzymatic activity was optimal.[17]

DNA and radioactive mouse RNA were *hybridized* in solution. The amount of DNA-RNA hybrids resistant to RNase was determined on nitrocellulose filters (Schleicher and Schuell, Bac-T-Flex).[16, 19] Highly purified *E. coli* DNA was a gift of Dr. Bruce Alberts.

Results.—(1) *The behavior of Py virions and of pseudovirions in CsCl equilibrium density gradients:* In CsCl density gradients Py viral hemagglutinin is present in two main bands (Fig. 1): The dense band contains infective Py virions (*"virus band,"* ρ = 1.330 gm cm^{-3}) while the light one consists mainly of viral capsids devoid

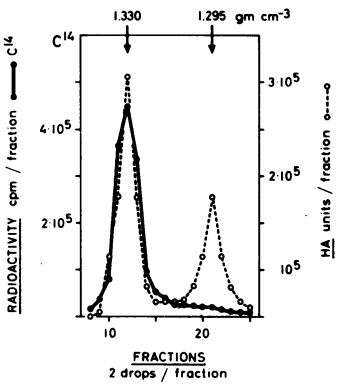

FIG. 1.—CsCl density gradient equilibrium centrifugation of a preparation of TdR-C^{14}-labeled Py virus. *"Virus band":* ρ = 1.330 gm cm^{-3}; *"capsid band":* ρ = 1.295 gm cm^{-3}. TdR-C^{14} (0.5 μc/ml) in culture medium 18–75 hr p.i. Virus (20 Petri dishes) harvested 75 hr p.i. and purified as described in *Materials and Methods*. CsCl density gradient (3 ml): Centrifuged in Spinco model L-50; rotor SW 39; 35,000 rpm; 20 hr; 25°C. Fractions collected from the bottom of the tube in 0.10 ml 0.001 *M* Na phosphate, pH 8.0.

of DNA (*"capsid band,"* ρ = 1.295 gm cm^{-3}).[6, 18] If DNA was labeled by growing the virus in the presence of radioactive TdR, then, as expected, radioactivity is mainly associated with the virus band. However, in 58 different viral preparations examined, the radioactive band was skewed (to a varying degree) toward the light side and extended into the capsid band. This skewness is mainly due to the pres-

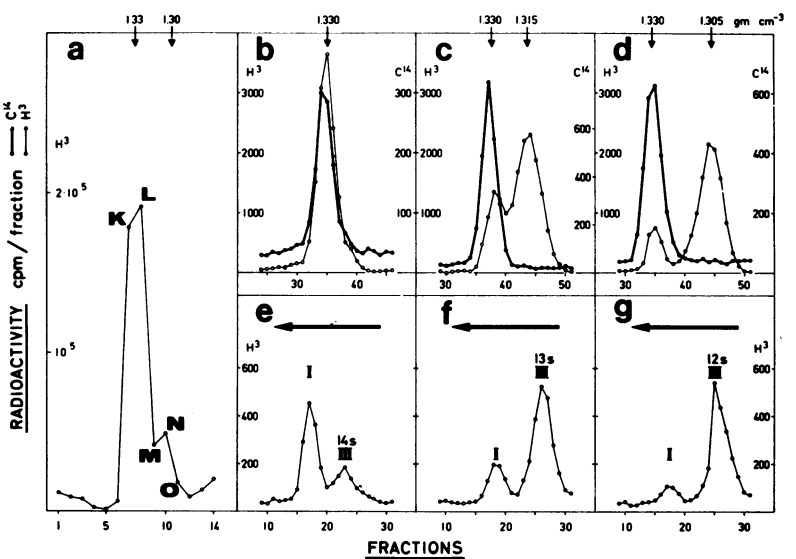

FIG. 2.—Buoyant density in CsCl of Py virions and of pseudovirions. Presence of Py DNA III in pseudovirions. (*a*) CsCl density equilibrium gradient of a preparation of Py virus (TdR-H^3 2 μc/ml, 16–66 hr p.i.; 18 Petri dishes). Purification as in Fig. 1. Four drops per fraction. The difference between the activities of fractions *L* and *M* is not considered significant. (*b*, *c*, and *d*) Aliquots from *L*, *M*, and *N* (in *a*) subjected to a second cycle of CsCl density equilibrium centrifugation (*L* → 2, *b*; *M* → 2, *c*; *N* → 2, *d*). Density marker: Py virions ρ = 1.330 gm cm^{-3} (>95% Py DNA I; TdR-C^{14}). One drop per fraction. (*e*, *f*, and *g*) Sedimentation pattern of DNA extracted from aliquots of *L* (→ 2, *e*), *M* (→ 2, *f*), and *N* (→ 2, *g*). Sedimentation velocity analysis: 0.2 ml SDS extract layered on 3 ml CsCl solution (ρ = 1.505 gm cm^{-3}, pH 7.5). Centrifuged for 3.5 hr as in Fig. 1. Fractions (40 ± 1) of 2 drops each collected on filter papers. The sedimentation coefficients are relative to $s^0_{20,w}$ of Py DNA I taken as 20*S*.

ence of "light" pseudovirions, i.e., of viral capsids that contain fragments of mouse DNA of mol wt $< 3 \times 10^6$. Upon recentrifugation they retain their original buoyant densities (Fig. 2*b*, *c*, and *d*).

DNA extracted from *fractions within the virus band* ($\rho > 1.315$ gm cm^{-3}; *K* and *L* in Fig. 2*a*) contains the three components described earlier: In most viral preparations components I and II account for at least 80 per cent and III for 1–20 per cent of the total DNA. In 12 out of 42 viral preparations analyzed, however, III accounted for as much as 60–90 per cent. From this it follows that some viral preparations consist mainly of pseudovirions. The reasons for the variability in the relative amounts of pseudovirions remain unknown. In all preparations, however, the *fractions on the light side of the virus band* ($\rho < 1.315$ gm cm^{-3}) contained mainly III (Fig. 2*f* and *g*). Small amounts of Py DNA I present in these fractions are absent in preparations of light pseudovirions which were sedimented through sucrose gradients prior to DNA extraction.[18] Thus, pure preparations of (light) Py pseudovirions which contain only Py DNA III can be obtained by CsCl density gradient equilibrium centrifugation followed by sedimentation through sucrose gradients.

(2) *Evidence that Py DNA III is enclosed in Py viral capsids:* Py viral preparations (labeled with radioactive TdR) were centrifuged in CsCl density equilibrium gradients. Fractions (of 1, 2, or 4 drops each) were collected, dialyzed, and subjected to *sedimentation velocity analysis in sucrose gradients.* As velocity marker a preparation of TdR-C^{14}-labeled Py virions ($\rho = 1.330$ gm cm^{-3}) was used: Pseudovirions (i.e., viral capsids containing mouse DNA) derived from the virus band ($\rho > 1.315$ gm cm^{-3}) exhibit the same sedimentation pattern as Py virions (Fig. 3*a*)

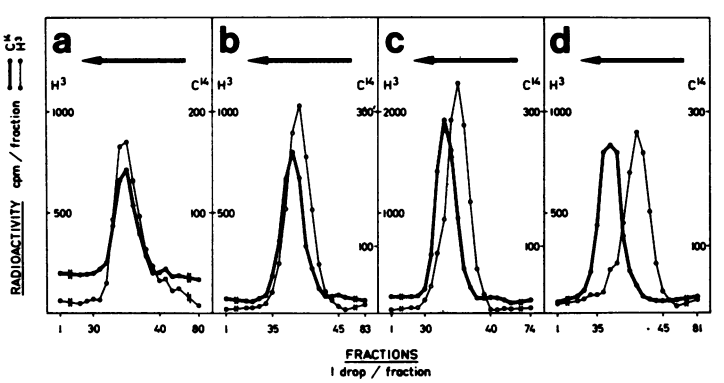

FIG. 3.—Sedimentation pattern of Py pseudovirions in sucrose gradients. In this experiment a viral preparation was used where >90% of total DNA was present in the form of Py DNA III. Thus, most of the viral particles in the virus band are pseudovirions. The results in this figure show that pseudovirions from the virus band exhibit essentially the same sedimentation pattern as Py virions while light pseudovirions sediment slightly slower (maximal difference: ~10%, *d*). *Velocity marker:* Py virions (TdR-C^{14}; ρ 1.330 gm cm^{-3}) as used in Fig. 2. *Py pseudovirions:* (*a*) $\rho = 1.330 \pm 0.005$ gm cm^{-3}; (*b*) $\rho = 1.320 \pm 0.005$ gm cm^{-3}; (*c*) $\rho = 1.307 \pm 0.005$ gm cm^{-3}; (*d*) $\rho = 1.297 \pm 0.005$ gm cm^{-3}. Sedimentation velocity analysis: 0.2 ml of pseudovirions (H^3) and Py virions (C^{14}) layered on a sucrose gradient (4.4 ml; 0.15 *M* NaCl–0.001 *M* Na phosphate, pH 7.0; 10–40% sucrose). Centrifuged for 25 min under the conditions described in Fig. 1. Single drops collected on filter papers.

while pseudovirions from the light side of the virus band sediment slightly slower (Fig. 3*b*, *c*, and *d*).

These results and the fact that pseudovirions exhibit hemagglutinating activity as do Py virions suggested that Py DNA III is present in Py viral capsids. This assumption is supported by *electron microscopy* of (light) pseudovirions (Fig. 4*b*). Since hemagglutination inhibition tests with mouse antisera directed against Py virus do not reveal differences between Py virions and pseudovirions,[18] it is likely that the capsid protein of the two types of particles is closely similar or identical.

(3) *Evidence that component III consists of linear DNA molecules of heterogeneous lengths: Sedimentation velocity analyses:* III extracted from pseudovirions within the virus band sediments with ~14*S* (Fig. 2*e*). In contrast, III derived from pseudovirions from the light side of the virus band ($\rho < 1.315$ gm cm^{-3}) exhibits sedimentation coefficients <14*S* (Fig. 2*f* and *g* and Fig. 5). Sedimentation velocity analyses of III under alkaline conditions (pH > 12) lead to similar results: Again, III derived from light pseudovirions sediments slower than III from pseudovirions within the virus band. Under the conditions used (pH > 12) Py DNA III was fully denatured and thus was present in the form of random coils. The observed differences in sedimentation coefficients are therefore the expression of differences in molecular weights ($S = kM^{0.4}$).[21]

Electron microscopy of Py DNA: A viral preparation was subjected to CsCl density gradient equilibrium centrifugation. The fractions of the virus band were pooled, dialyzed, and then sedimented through a sucrose gradient. Sedimentation velocity analysis by band centrifugation of the DNA extracted from this preparation revealed a fast (20*S*) and a slow (~14*S*) band containing 25 and 75 per cent, respectively, of the total DNA. Electron microscopy of individual fractions shows

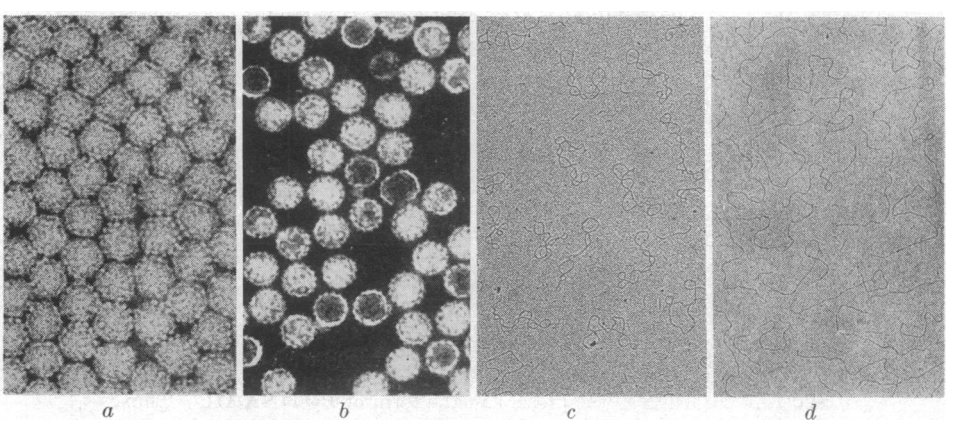

FIG. 4.—Electron microscopy of (*a*) Py virions, (*b*) pseudovirions, (*c*) Py DNA I, and (*d*) Py DNA III. (*a*) Preparation containing mainly Py virions [$\rho = 1.330 \pm 0.005$ gm cm^{-3}; 80% Py DNA I + II and 20% III (~14*S*)]. Negative staining with Na phosphotungstate.[20] Magnification 10^5 (Hitachi HU 11 A). (*b*) Preparation of light pseudovirions [$\rho = 1.305 \pm 0.005$ gm cm^{-3}; contains only Py DNA III (12–13*S*)]. Negative staining as in (*a*). The diameter of pseudovirions is closely similar to that of Py virions (*ca.* 450 Å). Apparently "full," "empty," and "half-empty" viral particles can be distinguished. Magnification 10^5. (*c*) Twisted circular Py DNA I (20*S*). Magnification 10^4. (*d*) Linear Py DNA III (~14*S*). Magnification 10^4. The pictures were taken at the Center for Electron Microscopy of the University of Lausanne.

that the fast band consists of twisted circular DNA molecules (Py DNA I; 20S, Fig. 4c) with a mean length of 1.75 μ and a narrow length distribution (Fig. 6). In contrast, 95 per cent of the DNA molecules in the slow band are linear and correspond to Py DNA III (Fig. 4d). Their length distribution is very broad (Fig. 6). In the leading fractions of the slow band, circular DNA molecules (approximately 5% of the total DNA) are found which correspond to Py DNA II (16S).

DNA extracted from purified light pseudovirions contains only linear molecules. Their mean lengths become progressively smaller with decreasing buoyant densities of the pseudovirions from which they are extracted: Pseudovirions from the region of the capsid band contain linear helices of one fifth or less of the length of circular Py DNA.

In all instances tested, the length distributions of linear molecules were very broad and no molecules were found longer than circular Py DNA. These findings lend further support to the assumption that Py DNA III is enclosed in Py capsids. This suggests that the maximal amount of DNA which a Py capsid can contain is equivalent to one molecule of (circular) Py DNA. From this we conclude that those viral particles within the virus band that contain Py DNA III ($\sim$14S; mol wt $\sim 3 \times 10^6$) do not contain circular Py DNA.

(4) *The buoyant density in CsCl equilibrium gradients and the melting behavior of Py DNA III:* In CsCl density equilibrium gradients, I and II form bands at 1.709 gm cm^{-3} (Fig. 7a and b). In contrast, III has a lower buoyant density (1.702 gm cm^{-3}; Fig. 7a) corresponding to that of mouse cellular DNA (Fig. 7c). Conversion of circular Py DNA (by ultrasonication) into linear fragments (7–10S) does not detectably alter its buoyant density.

Py DNA I, after boiling followed by rapid cooling, exhibits spontaneous monomolecular renaturation. Py DNA II, however, shows a melting behavior similar to that of linear DNA derived from other viruses.[1] Likewise, Py DNA III can be denatured by short boiling (3 min) followed by rapid cooling. In CsCl equilibrium gradients denatured III exhibits an increase in density which is comparable to that observed for denatured mouse DNA. In some experiments, however, a fraction of the helices in III renatured spontaneously.[18]

(5) *Evidence for the presence in Py viral capsids of mouse cellular DNA synthesized prior to and during infection:* In several experiments Py virus was grown on MK

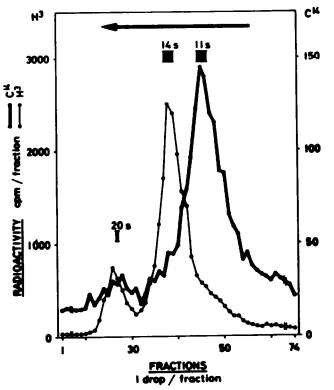

FIG. 5.—Py DNA III from light pseudovirions exhibits sedimentation coefficients <14 S. DNA from *light pseudovirions* (*TdR-C*^{14}; ρ = 1.295 ± 0.002 gm cm^{-3}) and from *pseudovirions from the virus band* (*TdR-H*^{3}; ρ = 1.322 ± 0.002 gm cm^{-3}) mixed and sedimented as in Fig. 2 (*e*, *f*, and *g*). Single drops collected on filter papers

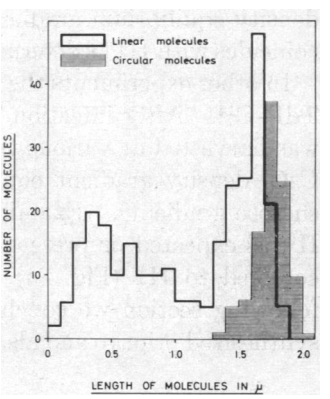

FIG. 6.—Histogram of the lengths of circular and linear Py DNA molecules. DNA from a viral preparation containing 25% I, 5% II, and 70% III (see *Results* and also electron micrographs of Fig. 4c and d).

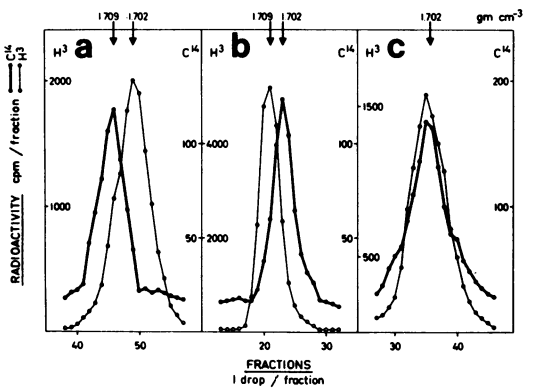

FIG. 7.—Buoyant density in CsCl of Py DNA I, III, and of mouse cellular DNA. (*a*) *Circular Py DNA* (C^{14}; 95% I and 5% II) centrifuged together with *Py DNA III* (H^3; 12–14*S*). (*b*) *Circular Py DNA* (H^3; 95% I and 5% II) centrifuged with *mouse cellular DNA* (C^{14}). Mitotically active MK cultures were labeled with TdR-C^{14} for 2 days; cellular DNA was extracted with SDS.[4] (*c*) *Py DNA III* (H^3; as in *a*) centrifuged together with *mouse cellular DNA* (C^{14}). Py DNA III and mouse DNA used in (*c*) were fragmented (7–10*S*) by ultrasonication. Density gradient: CsCl solution, ρ = 1.70 gm cm^{-3}; pH 8.0. Centrifuged for 48 hr under the conditions described in Fig. 1. Single drops collected on filter papers (*a* = 94, *b* = 64, *c* = 60 fractions).

cultures the DNA of which had been labeled prior to infection with radioactive TdR. In CsCl density equilibrium gradients radioactive label is mainly associated with the virus band.[18] In sucrose gradients the radioactive viral particles exhibit essentially the same sedimentation pattern as Py virions (or pseudovirions).[18] Sedimentation velocity analyses of the DNA extracted from these viral preparations show that radioactivity is mainly or entirely confined to III (Fig. 8*b*).[4] In CsCl density equilibrium gradients radioactivity forms a band at 1.702 gm cm^{-3} which coincides with III as determined by optical absorbance (260 mμ).

In other experiments the DNA of MK cultures was labeled prior to infection with TdR-C^{14}. After infection TdR-H^3 was added to the culture medium. The virus was harvested at various times between 48 and 120 hours p.i. and was purified by CsCl density gradient equilibrium centrifugation and by sedimentation through sucrose gradients. Examination of the DNA from these preparations shows that H^3, as expected, is present both in I (II) and III, while C^{14} is mainly or entirely confined to III (Fig. 8*a*). From these results and from those reported in the following section we conclude that component III contains mouse cellular DNA synthesized prior to and also after infection.

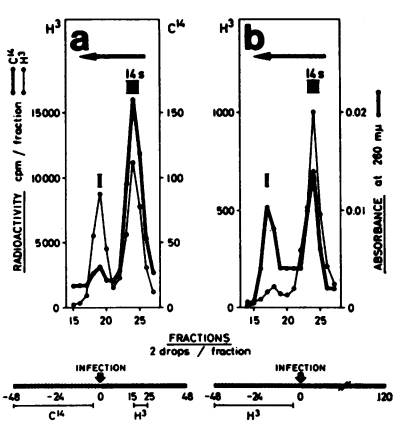

FIG. 8.—Evidence that component III contains DNA synthesized *prior to* and also *during* infection. (*a*) MK cultures grown in the presence of *TdR-C^{14}* (0.5 μc/ml) until confluent. Thereafter radioactive medium was replaced by nonradioactive medium and cultures were infected with Py virus (~10^9 PFU/ml). *TdR-H^3* (5 μc/ml) present from 15 to 25 hr p.i. Virus harvested 48 hr p.i. The fractions from the virus band were pooled and sedimented through a sucrose gradient (as in Fig. 3). The DNA was extracted and subjected to band centrifugation (as in Fig. 2). Fractions collected on filter papers. (*b*) MK cultures grown in the presence of *TdR-H^3* (1 μc/ml) until confluent. Virus harvested 120 hr p.i. and purified as in (*a*). The DNA was extracted and subjected to band centrifugation (as in Fig. 2). Fractions collected in 0.10 ml 0.001 *M* Na phosphate, pH 8.0. Optical absorbance refers to 10-mm path length.

(6) *Base-sequence homology between Py DNA III and mouse RNA: Hybridization with "synthetic" mouse RNA:* Mouse RNA was synthesized in a cell-free system with *E. coli* RNA polymerase, using mouse cellular DNA as a primer. Hybridization studies (Fig. 9) show that Py DNA III derived from pseudovirions within the virus band or from light pseudovirions[18] and mouse DNA exhibit a comparable degree of homology to synthetic mouse RNA. Similarly, Winocour reported homology of synthetic mouse RNA with "slow" Py DNA.[22]

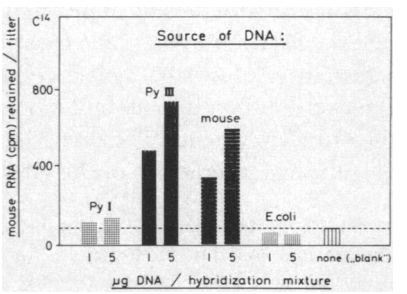

FIG. 9.—Base-sequence homology between Py DNA III and synthetic mouse RNA. *Synthetic mouse RNA-C*^{14} (3 µg; 20,000 cpm) hybridized in solution with either Py DNA I, III (derived from the virus band), mouse DNA or *E. coli* DNA. *Blank:* Mouse RNA-C^{14} "hybridized" in the absence of added DNA. DNA was fragmented (7–10*S*) by ultrasonication and then denatured by boiling (4 min; 0.001 *M* Na phosphate, pH 8.0) followed by cooling in ice water. Counting background deducted.

Hybridization with "natural" mouse RNA: Pulse-labeled RNA (uridine-H^3; 30 min) was extracted from uninfected MK cultures. Hybridization studies show that mouse DNA and Py DNA III exhibit a comparable degree of homology to natural mouse RNA. In contrast, essentially no homology can be detected between Py DNA I and natural mouse RNA, which is in accordance with the results reported by Benjamin[23] and by Diggelmann.[16]

(7) *Biological properties of Py pseudovirions:* The biological properties of highly purified preparations of pseudovirions from the region of the capsid band and from intermediate densities were compared with the properties of preparations of Py virions. From the results obtained (M. R. Michel and G. Pétursson, in preparation, and ref. 18) we conclude that pseudovirions are unable to direct the production of progeny virus, of viral hemagglutinin, or to induce cellular and viral DNA synthesis.

Discussion and Summary.—The results reported in this paper show that Py DNA III consists of linear molecules which are enclosed in Py viral capsids. Three lines of experimental evidence lead to the conclusion that most of the molecules in III are mouse DNA: (*a*) the existence of base-sequence homology between Py DNA III and mouse RNA, (*b*) the presence in III of mouse DNA synthesized *prior* to infection, and (*c*) the comparable buoyant density of III and mouse DNA in CsCl solutions. The results do not exclude, however, the possibility that some of the helices in III represent linear forms and fragments of Py viral DNA (genome). Py capsids that contain mouse cellular DNA instead of the viral genome (or fragments thereof) are defined as *Py pseudovirions.* As expected, they are not infective. The possibility remains open that they transduce genetic markers of the host cell. On the average, every infected MK cell produces 10^3 or more pseudovirions.

In CsCl density equilibrium gradients pseudovirions exhibit a broad spectrum of buoyant densities that extends from the virus band into the region of the capsid band. The DNA content of most pseudovirions is comparable to that of Py virions. Therefore, the bulk of pseudovirions is present in the virus band and could not be

separated from Py virions. A variable but minor fraction of pseudovirions is found on the light side of the virus band. Light pseudovirions contain less DNA than Py virions and their lower buoyant densities are mainly the expression of decreased ratios of DNA/capsid protein. Since they can be separated from Py virions, pure preparations of light pseudovirions can be obtained.

The biological significance of Py DNA III remains unknown. The experimental results favor the idea that the molecules in III are fragments of mouse cellular DNA excised at random. They make it appear unlikely that component III is derived from an extensively replicated minor species of mouse DNA. The extent to which the excision takes place must be limited since at no time after infection is a generalized breakdown of mouse cellular DNA observed. It may be speculated that the excision involves an endonuclease of the type described by Bernardi[24] and that the same enzyme may be involved in the replication of circular Py DNA or in a possible physical interaction of the viral genome with cellular DNA.

We thank Dr. G. Pétursson for useful discussions and for performing the electron microscopy of viral preparations and Dr. D. Pettijohn for the generous gift of RNA polymerase. We are indebted to Professors E. Kellenberger and R. Epstein for the critical reading of the manuscript.

Abbreviations: c, curie; cpm, counts per minute; DNase, deoxyribonuclease; EDTA, ethylenediaminetetraacetate; HA units, hemagglutination units; MK, mouse kidney; PFU, plaque-forming units; p.i., *post infectionem;* Py, polyoma; RNase, ribonuclease; rpm, revolutions per minute; *S,* Svedberg; SDS, sodium dodecylsulfate; TdR, deoxythymidine.

* This work was supported by grants 3901 and 4279 of the Swiss National Foundation for Scientific Research.

[1] Weil, R., and J. Vinograd, these PROCEEDINGS, **50,** 730 (1963).

[2] Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis, these PROCEEDINGS, **53,** 1104 (1965).

[3] Dulbecco, R., and M. Vogt, these PROCEEDINGS, **50,** 236 (1963).

[4] Weil, R., M. R. Michel, and G. K. Ruschmann, these PROCEEDINGS, **53,** 1468 (1965).

[5] Michel, M. R., cited in *The Molecular Biology of Viruses,* Symposium Edmonton, 1966, ed. J. S. Colter (New York: Academic Press, 1967), p. 595.

[6] Winocour, E., *Virology,* **19,** 158 (1963).

[7] Hirt, B., and U. Locher, in preparation.

[8] Dulbecco, R., and G. Freeman, *Virology,* **8,** 396 (1959).

[9] Eddy, B. E., W. P. Rowe, J. W. Hartley, S. E. Stewart, and R. J. Huebner, *Virology,* **6,** 290 (1958).

[10] Vinograd, J., R. Bruner, R. Kent, and J. Weigle, these PROCEEDINGS, **49,** 902 (1963).

[11] Meselson, M., F. W. Stahl, and J. Vinograd, these PROCEEDINGS, **43,** 581 (1957).

[12] Schildkraut, C. L., J. Marmur, and P. Doty, *J. Mol. Biol.,* **4,** 430 (1962).

[13] Kleinschmidt, A., and R. K. Zahn, *Z. Naturforsch.,* **14b,** 770 (1959).

[14] Kellenberger, E., O. Karamata, and D. Dussoix, private communication.

[15] Scherrer, K., and J. E. Darnell, *Biochem. Biophys. Res. Commun.,* **7,** 486 (1962).

[16] Diggelmann, H., in preparation.

[17] Pettijohn, D., and T. Kamiya, *J. Mol. Biol.,* in press.

[18] Michel, M. R., PhD thesis, University of Bern (1967), in preparation.

[19] Nygaard, A. P., and B. D. Hall, *J. Mol. Biol.,* **9,** 125 (1964).

[20] Huxley, H. E., and G. Zubay, *J. Mol. Biol.* **2,** 10 (1960).

[21] Studier, F. W., *J. Mol. Biol.,* **11,** 373 (1965).

[22] Winocour, E., *Virology,* **31,** 15 (1967).

[23] Benjamin, T. L., *J. Mol. Biol.,* **16,** 359 (1966).

[24] Bernardi, G., *J. Mol. Biol.,* **13,** 603 (1965).