JOURNAL OF VIROLOGY, Oct. 1973, p. 841-846
Vol. 12, No. 4

Deoxyribonucleic Acid of Marek's Disease Virus in Virus-Induced Tumors

K. NAZERIAN,[1] T. LINDAHL, G. KLEIN, AND L. F. LEE[1]

Departments of Tumor Biology and Chemistry, Karolinska Institutet, Stockholm 60, Sweden

Received for publication 29 May 1973

DNA was extracted from [^{3}H]thymidine-labeled Marek's disease virus (MDV) and purified by two cycles of CsCl gradient centrifugation in a fixed-angle rotor. The DNA was transcribed in vitro into ^{32}P-labeled complementary RNA (cRNA). MDV cRNA did not hybridize with DNA from chicken embryo fibroblast cultures or from chicken spleen, but hybridized efficiently with DNA from MDV particles or MDV-infected cell cultures. Five Marek's disease tumors from different chickens and different organs (ovary, liver, testis) were all found to contain MDV DNA sequences. The relative amount of MDV DNA varied from tumor to tumor and was between 3 and 15 virus genome equivalents per cell. The content of virus DNA per cell in spleens from tumor-bearing chickens was much lower than in tumors from the same animals. MDV-infected cell cultures contained a large proportion (28–59%) of virus antigen-positive cells, as measured by immunofluorescence, but tumor cells were negative in this respect (<0.02% positive cells). These data indicate that MDV is present in a provirus form in tumor cells.

Marek's disease (MD) is a neoplastic disorder of chickens (2) and is caused by a herpesvirus (4, 18). However, many investigators have failed to detect virus-specific antigens or virus particles in hundreds of tumors examined. The mechanism by which MD tumors develop, therefore, has remained unclear. It has been proposed (22) that MD tumors could be caused by an "intrinsic" mechanism, in which abortively infected lymphoid cells become transformed, resulting in a massive proliferation. As no direct proof of the presence of virus footprints has been demonstrated in tumor cells, it has also been suggested (22) that the lymphoid proliferation in MD may be a secondary event mediated by the host response to an "extrinsic" factor such as virus-infected or antigen-bearing cells. Recently, it was shown (12) that lymphoid tissues from tumor-bearing chickens had a significantly higher rate of DNA synthesis than lymphoid cells from healthy or nontumor-bearing MD virus (MDV)-inoculated chickens.

Cells transformed by several DNA viruses, i.e., SV40, adenoviruses, and Epstein-Barr virus (EBV), retain virus DNA sequences as shown by nucleic acid hybridization (5, 28, 31). In the present work, we have used this method to search for the presence of MDV DNA in virus-induced tumors in chickens.

MATERIALS AND METHODS

Reagents. Nucleoside triphosphates were obtained from Schwarz Mann. [α-^{32}P]CTP (20 Ci/mmol) was a gift from P. Reichard, [^{3}H]thymidine (14 Ci/mmol) was purchased from NEN Chemicals, and $^{32}P_i$ from AB Atomenergi, Sweden. Calf thymus DNA was obtained from Worthington Biochemical Corp., and highly purified yeast tRNA was made as described (15). *E. coli* RNA polymerase (1,000 U/mg) was obtained from General Biochemicals, pancreatic DNase (RNase-free) from Worthington, and Pronase from Calbiochem. The Pronase was preincubated as a 0.5% solution in 0.05 M Tris-hydrochloride, pH 7.5, for 2 h at 37 C before use. Membrane filters (Selectron-Filter BA 85) were purchased from Schleicher and Schuell. SSC is 0.15 M NaCl, 0.015 M sodium citrate.

Viruses. The GA strain of MDV in its 22nd cell culture passage and the FC-126 isolate of herpesvirus of turkeys (HVT) were used. This MDV strain was originally isolated (6) from an acute case of MD and has been clone purified in cell culture (26). It has retained its oncogenicity to this passage level. HVT was originally isolated from turkeys (29) and was found apathogenic for both turkeys and chickens.

Cell culture. Duck embryo fibroblast (DEF) and chicken embryo fibroblast (CEF) cultures were used for propagation of the viruses and preparation of DNA. Procedures and media were previously described (16).

[1] Present address: U.S. Dept. of Agriculture, Regional Poultry Research Laboratory, East Lansing, Mich. 48823.

Tumors. One-day-old White Leghorn chicks were intraabdominally inoculated with 2.5×10^5 MDV-infected cells in 0.2 ml of phosphate buffered saline, pH 7.4 (PBS). At 4 to 6 weeks postinoculation, chickens with clinical symptoms of the disease were sacrificed and tumors and spleens were removed. Tumors were lymphoid in appearance and were well isolated from the adjacent tissues. Spleens were normal in appearance and had no detectable lymphoid infiltrations. Spleens were also removed from control uninoculated chickens of the same age. These were also normal in appearance and size.

Propagation and purification of the viruses. The viruses were propagated in roller bottle cultures of DEF or CEF as described previously (13).

The culture medium was decanted 2 days after infection and was replaced with fresh thymine-free medium 199 with 1% dialyzed calf serum, supplemented with 5 μCi of [^{3}H]thymidine per ml. Medium from 12 infected roller bottle cultures (total of 600 ml) was harvested 72 h after infection, chilled to 4 C, and centrifuged at 7,000 rpm for 10 min in a Sorvall GSA rotor in order to remove the cellular debris. The supernatant fluid was further centrifuged at 21,000 rpm for 1 h in a Spinco 21 rotor. Pellets were suspended in 10 ml of fresh medium without calf serum and centrifuged at low speed (2,000 rpm, 10 min). This was followed by two cycles of centrifugation at 25,000 rpm in a Spinco SW27 rotor, with a low-speed centrifugation between each run. The final pellet was suspended in 1 ml of PBS.

Extraction and purification of DNA. The MDV concentrate (1.2 ml) was lysed by addition of 60 μliters of 1 M Tris-hydrochloride, 0.2 M EDTA (pH 8.5), and 25 μliters of 25% sodium dodecyl sulfate, followed by heating at 55 C for 5 min. The solution was then incubated at 37 C with 1 mg of Pronase per ml for 2 h. After cooling to 20 C, an equal volume of redistilled, buffer-saturated phenol was added, and the phases were gently mixed. The material was chilled and centrifuged at 10,000 $\times$ *g* for 15 min at 4 C. The aqueous phase was recovered and dialyzed against several changes of 1 M NaCl, 0.01 M Tris-hydrochloride, and 0.001 M EDTA (pH 8.0), for 4 days at 4 C, followed by dialysis overnight against the same buffer without NaCl. The DNA solution was adjusted to a volume of 4.5 ml and a density of 1.710 g/cm^3 by the addition of dialysis buffer and solid CsCl. Two nanograms of ^{32}P-labeled *E. coli* DNA was added as a marker, and the solution was centrifuged at 32,000 rpm for 65 h at 20 C in a Spinco type 40 fixed-angle head rotor. Centrifuge tubes contained paraffin oil on top of the CsCl solutions. At the end of centrifugation, fractions were collected from the bottom of the tube and assayed for radioactivity. Fractions of density 1.706 to 1.711 g/cm^3 were pooled, recentrifuged in CsCl, and recovered in the same fashion. The ^{3}H-labeled MDV DNA was dialyzed against 0.2 M KCl, 0.01 M Tris-hydrochloride and 10^{-4} M EDTA (pH 8.0), for 2 days at 4 C.

DNA preparations from chicken cells, tissues, and tumors were made by standard procedures employing Pronase treatment and phenol extraction (Pettersson and Sambrook, 1973). ^{32}P-labeled *E. coli* DNA (10^6) was prepared according to Grossman (9).

Preparation of complementary RNA. The reaction mixture (0.79 ml) contained [^{3}H]MDV DNA [0.21 μg), 0.15 M KCl, 0.04 M Tris-hydrochloride (pH 8.0), 0.01 M $MgCl_2$, 5×10^{-4} M dithiothreitol, 10^{-4} M EDTA, 6×10^{-4} M ATP, 2×10^{-4} M GTP, 2×10^{-4} M UTP, 4×10^{-5} M [α-^{32}P]CTP (20 Ci/mmol), 10% glycerol, and 8 μg of RNA polymerase. After incubation at 35 C for 3 h, the solution was chilled to 20 C, and 10 μliters of 0.5% yeast tRNA, 5 μliters of 0.02 M $CaCl_2$, and 10 μliters of 0.1% pancreatic DNase were added. This reaction mixture was incubated for 30 min at 20 C to degrade the DNA, and then 60 μliters of 0.2 M EDTA (pH 7.0), 10 μliters of 10% sarcosyl, and 0.8 ml of buffer-saturated phenol were added. After two phenol extractions, the solution was extracted four times with ether and freed from ether by bubbling through nitrogen. The complementary RNA (cRNA) was subsequently purified by gel filtration on Sephadex G 75 (1 by 20 cm) in one-tenth-strength SSC and stored at −70 C.

Nonradioactive cRNA from *E. coli* was prepared in the same fashion, except that the reaction mixture contained 30 μg of *E. coli* DNA per ml, instead of the MDV DNA, and 2×10^{-4} M nonradioactive CTP.

DNA-cRNA hybridization. The procedures were essentially those described by Birnstiel et al. (3). A 10-μg amount of denatured DNA, fixed to a 13-mm filter, was incubated with 0.4 ng of [^{32}P]MDV cRNA and 300 ng of *E. coli* cRNA in 0.3 ml of 6 $\times$ SSC and 50% formamide for 72 h at 42 C. All data were corrected for [^{32}P]decay, and all hybridization experiments were completed within 3 weeks after the preparation of [^{32}P]MDV cRNA.

Immunofluorescence technique. MDV-infected and control CEF cultures, MD tumors, and spleen tissues from tumor-bearing and control chickens were suspended in PBS at a concentration of approximately 5×10^5 cells per ml. Individual smears were made on clean slides by using a 20-μliter pipette. Smears were allowed to dry at room temperature and were then fixed in chilled acetone for 10 min. These were then indirectly stained (25) with a chicken serum known to be positive for MDV-specific antibodies. Slides were examined for the presence of virus specific immunofluorescent (IF) antigens.

Electron microscopy. Negatively stained preparations of virus concentrates were made in 2% potassium phosphotungstate and were examined as previously described (17).

RESULTS

Purification of MDV and HVT. In previous experiments on MDV DNA, MDV nucleocapsids from the cytoplasm of lysed, virus-infected cells were used as starting material (14). Because we encountered difficulties in freeing such preparations from cellular DNA, virus-containing medium from highly infected cultures was instead used as starting material in the present work. Such media contain relatively large numbers of virus particles (13). Both viruses were concentrated and partially purified by three cycles of high- and low-speed centrifugation. This procedure resulted in relatively clean virus

concentrates, as determined by electron microscopy. Large numbers of predominantly naked nucleocapsids were observed, with very little contamination of amorphous cellular material.

Purification of virus DNA. In view of the small amount of virus available, it was expected to be difficult to localize virus DNA by standard spectral methods. We have, therefore, used ^{3}H-labeled virus and performed the hybridization experiments with ^{32}P-labeled cRNA.

In comparison to chicken DNA, the DNA of MDV and HVT contain a slightly higher proportion of guanine and cytosine base pairs (13, 14). As very high resolution can be obtained by CsCl density gradient equilibrium centrifugation in fixed-angle head rotors (7), a partial separation of MDV DNA from host DNA is possible by this technique. The properties of chicken DNA in such a gradient are shown in Fig. 1. The DNA bands at a buoyant density of 1.702 g/cm^3, at a position clearly different from that of the marker DNA, *E. coli* DNA of 1.710 g/cm^3, although some overlapping of the bands occurs. When the [^{3}H]DNA from lysed MDV concentrates was centrifuged, 70% of the material banded as a peak at a buoyant density of 1.708 g/cm^3, and this DNA was further purified by recentrifugation (Fig. 2). The densities observed here for chicken DNA and for MDV DNA are close to the previously reported values of 1.701 and 1.706 g/cm^3, respectively (14, 27). No DNA directly detectable by UV absorption was found in the position of cellular DNA in either of the centrifugation experiments on virus DNA, precluding heavy contamination of the

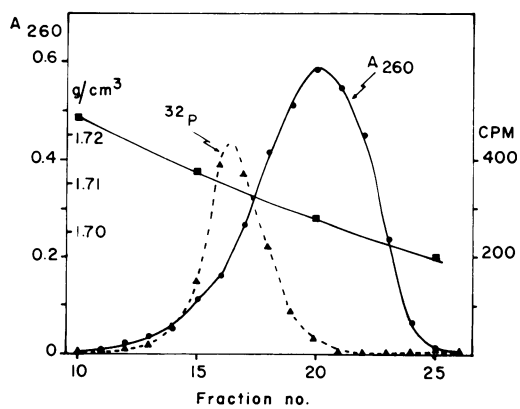

FIG. 1. *CsCl density gradient equilibrium centrifugation of a mixture of 80 μg of chicken spleen DNA and 3 ng of ^{32}P-labeled E. coli DNA in a fixed-angle head rotor (Spinco Type 40). Fractions were collected from the bottom of the tube, and the refractive index of every fifth fraction was measured. All fractions were diluted with 2 vol of 0.01 M Tris-hydrochloride (pH 7.5) and analyzed for UV absorption and radioactivity.*

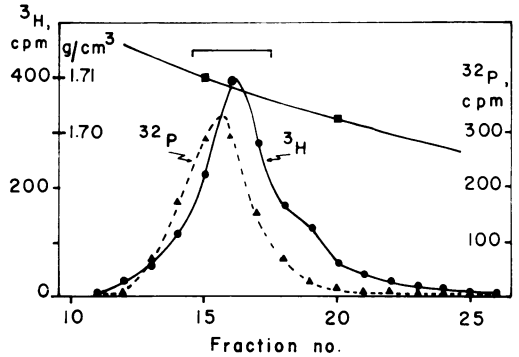

FIG. 2. *CsCl density gradient equilibrium centrifugation of ^{3}H-labeled MDV DNA and ^{32}P-labeled E. coli DNA in a fixed-angle head rotor. Conditions were as in Fig. 1. Fractions 15 to 17 were pooled and used for preparation of cRNA.*

[^{3}H]MDV-DNA with nonradioactive host DNA.

The UV absorption of the purified MDV DNA was measured against an appropriate blank, a CsCl solution dialyzed against the same buffer as the MDV DNA. The absorbancy measured at 260 nm (A_{260}) of the [^{3}H]MDV DNA was 0.012, corresponding to ~0.6 μg of DNA per ml. In view of the large potential error of concentration estimates from such low absorption values, an attempt was made to obtain more accurate data by studying the ability of the DNA preparation to serve as a template for *E. coli* RNA polymerase. Under the conditions used for preparation of cRNA, a reaction mixture containing the MDV DNA solution yielded 149 pmol of ^{32}P per ml in acid-insoluble form. When calf thymus DNA was instead transcribed in the same fashion, 0, 0.1, 0.2, 0.4, 0.8, and 20 μg of DNA per ml gave 22, 45, 84, 123, 262, and 3,060 pmol of ^{32}P per ml in acid-insoluble form, respectively. With the assumption that MDV DNA and calf thymus DNA are transcribed equally effectively by the heterologous RNA polymerase under the conditions used here, a concentration of 0.42 μg/ml was obtained for the MDV DNA solution, in fair agreement with the spectral data, and this value was used.

Properties of MDV cRNA. Most of the purified MDV DNA was used for the preparation of cRNA. Because of the small amount of DNA in the reaction mixture, only 0.3% polymerization of the radioactive precursor was obtained, and the total yield after purification by gel filtration was 0.03 μg of [^{32}P]cRNA, as derived from the total acid-insoluble radioactivity and the calculated specific activity of the cRNA of 7×10^7 counts per min per μg.

When 0.4-ng samples of the cRNA were added to a series of filters containing DNA from various sources, no significant hybridization

(<0.1%) was observed with CEF DNA above that obtained with heterologous DNA (Table 1). In contrast, a large proportion of the cRNA (up to 28% during the 72-h incubation period) hybridized to the filter when DNA preparations from several different batches of virus-infected cells were employed (Table 1). It is evident from these results that the cRNA preparation was satisfactory for the detection of MDV DNA sequences in the presence of a large excess of host cell DNA.

Filters containing different amounts of MDV DNA were also incubated with the cRNA preparation. As expected, the amount of hybridization was directly proportional to the amount of MDV DNA on the filter (Fig. 3). The MDV DNA preparation used in this work contained a trace (0.4%) of *E. coli* DNA, which had been added as a marker in the centrifugation experiments. A large excess of nonradioactive *E. coli* cRNA (1 μg/ml) was, therefore, added to the hybridization mixtures as a safety measure against binding of a minor proportion of the [^{32}P]cRNA to non-MDV DNA in this calibration experiment.

Properties of HVT cRNA. We prepared HVT DNA from approximately the same number of infected cells as used for the preparation of MDV by identical procedures. The yield was only 0.11 μg of DNA, as determined from the amount of cRNA obtained after transcription. This cRNA showed little or no preferential binding to host cell DNA over that observed with heterologous DNA, but hybridized well with DNA from HVT-infected cultures (Table 2). The HVT cRNA also hybridized with DNA from MDV-infected cultures, which qualitatively demonstrates the presence of homologous DNA sequences in these two viruses. It is noteworthy, in this connection, that HVT has been found to be antigenically related to MDV and protective against exposure to oncogenic MDV (21). The amount of HVT cRNA was insufficient for further work, however, and the point we wish to make here is that, of 0.11 μg of HVT DNA obtained in this preparation, a large proportion was clearly due to virus DNA and

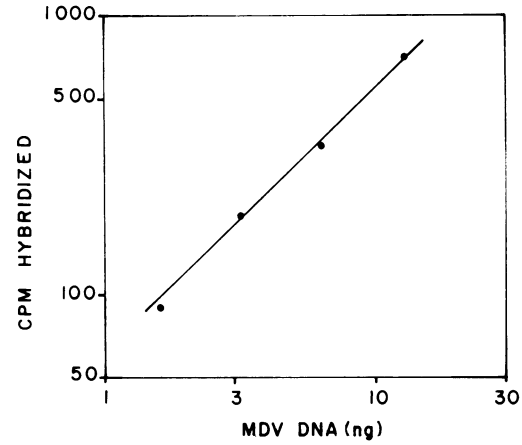

FIG. 3. *Hybridization calibration of ^{32}P-labeled MDV cRNA with homologous DNA. Known quantities of MDV DNA were mixed with 10 μg of salmon sperm DNA, denatured, and fixed to nitrocellulose membranes. Filters were then incubated with MDV cRNA as described in the text. The amount of ^{32}P bound to filters containing only salmon DNA (140 counts/min) has been subtracted from all values.*

TABLE 1. *Hybridization of [^{32}P]MDV cRNA[a] with DNA from MD tumors, MDV-infected cells, and control cells*

DNA source	IF antigen (% positive cells)	[^{32}P]MDV cRNA bound per 10 μg of DNA (counts/min)	MDV genome equivalents per diploid cell
Salmon sperm		126, 144[b]	
CEF	<0.02	138, 148	
MDV-infected CEF (1)	28	2907	
MDV-infected CEF (2)	59	7894	
MDV-infected CEF (3)	56	5648	
Normal chicken spleen	<0.02	156, 154	
Ovary tumor (bird no. 1)	<0.02	517, 505	11
Spleen (bird no. 1)	<0.02	170, 196	0-2
Liver tumor (bird no. 2)	<0.02	299, 333	5
Spleen (bird no. 2)	<0.02	160, 170	0-2
Testis tumor (bird no. 3)	<0.02	234, 250	3
Liver tumor (bird no. 4)	<0.02	606, 639	15
Ovary tumor (bird no. 5)	<0.02	486, 546	11

[a] 28,000 counts per min per 0.3 ml of incubation mixture.

[b] Results of two independent experiments.

TABLE 2. *Hybridization of [^{32}P]HVT cRNA[a] with DNA from control, HVT-, and MDV-infected CEF*

DNA source	[^{32}P]HVT cRNA bound per 10 μg of DNA (counts/min), duplicate determinations
Salmon sperm	187, 209
Normal CEF	237, 255
HVT-infected CEF	3962, 3378
MDV-infected CEF[b]	5146, 5288

[a] 43,000 counts per min per 0.3 ml of incubation mixture.

[b] The slightly higher hybridization with the MDV-infected CEF than with the HVT-infected CEF is probably due to a more efficient infection of the cells in the former case.

not cellular DNA, in spite of the small amount of total DNA.

Hybridization with MD tumor DNA. DNA was isolated from five different MDV-induced lymphoid tumors, each from a single chicken. DNA preparations were also made from the spleens of two of these chickens and from the spleen of a healthy, uninoculated chicken. Samples (10 μg) of the different DNA preparations were denatured, fixed to membrane filters, and incubated with MDV cRNA. The DNA from all MD tumors clearly contained variable amounts of virus DNA but there was no obvious correlation between the size of the tumor and the amount of MDV DNA present. The spleen DNA from diseased chickens contained much less MDV DNA than the tumors. The present results are consistent with a small amount of virus DNA also being present in such spleens, but the sensitivity of the filter hybridization technique is hardly sufficient to prove this point. Using the molecular weights of 1.0×10^8 for MDV DNA (11) and 1.7×10^{12} for chicken cell DNA (1), the numbers of virus genome equivalents per tumor cell were estimated to range between 3 and 15.

All tumors and spleens from inoculated and control chickens were negative for MDV-specific immunofluorescent antigens (Table 1). The absence of such virus antigens in tumor cells is in agreement with previous findings by other investigators and is indicative of the absence of virus particles in the tumors.

DISCUSSION

The five MD tumors investigated here contained different amounts of virus DNA. Similar observations have been made in the analysis of EBV DNA in biopsies from Burkitt's lymphoma and nasopharyngeal carcinoma (19, 30). The significance of this apparent variation in virus DNA content between tumors is not known. The proportion of virus DNA in MD tumors, 0.01 to 0.1%, is of the same order as that observed in biopsies of EBV-associated tumors. However, as chicken cells contain less than half as much DNA as mammalian cells (1) whereas MDV DNA and EBV DNA are of very similar size (11, 14), the numbers of virus genome equivalents per cell are smaller in the present system.

The cRNA filter hybridization technique has been found to give the same results as the technique of DNA-DNA reassociation kinetics for herpesvirus DNA (20). Therefore, systematic errors in the calibration experiments, which have been troublesome in work with small DNA viruses (10), should not have been present here. However, the exact number of MDV genome equivalents per cell are somewhat difficult to evaluate. The estimate of 3 to 15 virus genome equivalents per cell in various tumors is thus based on the assumption that the MDV DNA used in the hybridization calibration experiment was completely free from contaminating cellular DNA, and that the concentration of this very diluted DNA solution was correctly evaluated. It seems quite possible that the potential error introduced by non-ideality in these two respects could be as large as 50%. Thus, our estimate of, e.g., 15 virus genome equivalents per cell in one of the tumors would indicate the presence of 10 to 22 MDV genome equivalents in this case.

For these estimates, it has been assumed that all MD tumor cells contain virus DNA sequences. MD tumors are almost exclusively composed of lymphoid cells of different sizes (23). In view of the fact that virus antigens were not detected in any of the tumors, the data indicate that MDV is present in tumor cells as a provirus, presumably in the form of uncoated virus DNA. This presence would correspond to the "intrinsic" model proposed by Payne (22). Of course, we can not exclude that most cells in the tumor may lack virus DNA, whereas a minority would replicate virus DNA without the production of detectable virus antigens; this situation would have to be postulated on the basis of the "extrinsic" model (22) that regards the tumor as a proliferative response to virus-producing cells. This appears to be an unlikely model, however, since the accumulation of virus antigen, detectable by immunofluorescence, precedes the replication of virus DNA in the other two lymphotropic herpesvirus systems, EBV and *Herpesvirus saimiri* (8; G. Klein, G. Pearson, A. Robson, D. V. Ablashi, L. A. Falk, L. Wolfe, F. Deinhardt, and H. Barin, Int. J. Cancer, in press). Application of the complement-fixing immunofluorescence technique for detection of a nuclear antigen analogous to the EBV determined nuclear antigen (B. M. Reedman and G. Klein, Int. J. Cancer, in press) and/or in situ nucleic acid hybridization could serve to further clarify this point. Further experiments are also needed to determine whether MDV DNA is present in the free or integrated state in MD tumor cells.

ACKNOWLEDGMENTS

We thank P. Reichard for a generous gift of [α-^{32}P]CTP. These studies were conducted under Public Health Service contract no. 1 CP 33316 within the Special Virus Cancer Program of the National Cancer Institute. Grants were also received from the Swedish Cancer Society (G.K. and T.L.) and the Swedish Natural Science Research Council (T.L.). K. N. was recipient of a research training program from USDA.

LITERATURE CITED

1. Atkin, N. B., G. Mattinson, W. Becak, and S. Ohno. 1965. The comparative DNA content of 19 species of mammals, reptiles, and birds. Chromosoma **17**:1-10.
2. Biggs, P. M., A. E. Churchill, D. G. Rootes, and R. C. Chubb. 1968. The etiology of Marek's disease—an oncogenic herpes-type virus. Perspect. Virol. **6**:211-237.
3. Birnstiel, M. L., B. H. Sells, and I. F. Purdom. 1972. Kinetic complexity of RNA molecules. J. Mol. Biol. **63**:21-39.
4. Calnek, B. W., H. K. Adldinger, and D. E. Kohn, 1970. Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. Avian Dis. **14**:219-232.
5. Doerfler, W. 1970. Integration of the DNA of adenovirus type 12 into the DNA of baby hamster kidney cells. J. Virol. **6**:652-666.
6. Eidson, C. S., and S. C. Schmittle. 1968. Studies on acute Marek's disease. I. Characteristics of isolate GA in chickens. Avian Dis. **12**:467-476.
7. Flamm, W. G., H. E. Bond, and H. E. Burr. 1966. Density gradient centrifugation of DNA in a fixed angle rotor. A higher order of resolution. Biochim. Biophys. Acta **129**:310-319.
8. Gergely, L., G. Klein, and I. Ernberg. 1971. Host cell macromolecular synthesis in cells containing EBV-induced early antigens, studied by combined immunofluorescence and radioautography. Virology **45**:22-29.
9. Grossman, L. 1967. Preparation of phosphorus-32 labeled DNA p. 700-702. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 12A. Academic Press Inc., New York.
10. Hass, M., M. Vogt, and R. Dulbecco. 1972. Loss of simian virus 40 DNA-RNA hybrids from nitrocellulose membranes; implications for the study of virus host-DNA interactions. Proc. Nat. Acad. Sci. U.S.A. **69**:2160-2164.
11. Jehn, U., T. Lindahl, and G. Klein. 1972. Fate of virus DNA in the abortive infection of human lymphoid cell lines by Epstein-Barr virus. J. Gen. Virol. **16**:409-412.
12. Lee, L. F. 1972. Induction of deoxyribonucleic acid synthesis and the oncogenicity of Marek's disease virus. J. Virol. **10**:167-170.
13. Lee, L. F., R. L. Armstrong, and K. Nazerian. 1972. Comparative studies of six avian herpesviruses. Avian Dis. **16**:799-805.
14. Lee, L. F., E. D. Kieff, S. L. Bachenheimer, B. Roizman, P. G. Spear, B. R. Burmester, and K. Nazerian. 1971. Size and composition of Marek's disease virus deoxyribonucleic acid. J. Virol. **7**:289-294.
15. Lindahl, T., and J. R. Fresco. Preparation of highly purified tRNA from yeast, p. 601-607. *In* L. Grossman and K. Moldave (ed.), Methods of enzymology, vol. 12A. Academic Press Inc., New York.
16. Nazerian, K. 1970. Attenuation of Marek's disease virus and study of its properties in two different cell subcultures. J. Nat. Cancer Inst. **44**:1257-1267.
17. Nazerian, K., and B. R. Burmester. 1968. Electron microscopy of a herpesvirus associated with the agent of Marek's disease in cell culture. Cancer Res. **26**:2454-2462.
18. Nazerian, K., and R. L. Witter. 1970. Cell-free transmission and in vivo replication of Marek's disease virus. J. Virol. **5**:388-397.
19. Nonoyama, M., and J. S. Pagano. 1971. Detection of Epstein-Barr virus genome in nonproductive and productive cells. Nature N. Biol. **233**:103-106.
20. Nonoyama, M., and J. S. Pagano. 1973. Homology between Epstein-Barr virus DNA and viral DNA from Burkitt lymphoma and nasopharyngeal carcinoma determined by DNA-DNA reassociation kinetics. Nature (London) **242**:44-47.
21. Okazaki, W., H. G. Purchase, and B. R. Burmester. 1970. Protection against Marek's disease by vaccination with a herpesvirus of turkeys. Avian Dis. **14**:413-429.
22. Payne, L. N. 1972. Pathogenesis of Marek's disease—a review, p. 21-37. In P. M. Biggs, G. de-The, and L. N. Payne (ed.), Oncogenesis and herpesviruses. Int. Agency Res. Cancer, Lyon.
23. Payne, L. N., and P. M. Biggs. 1967. Studies on Marek's disease. II. Pathogenesis. J. Nat. Cancer Inst. **39**:281-302.
24. Pettersson, U., and J. Sambrook. 1973. Amount of viral DNA in the genome of cells transformed by adenovirus type 2. J. Mol. Biol. **73**:125-130.
25. Purchase, H. G. 1969. Immunofluorescence in the study of Marek's disease. I. Detection of antigens in cell cultures and an antigenic comparison of eight isolates. J. Virol. **3**:557-569.
26. Purchase, H. G., B. R. Burmester, and C. H. Cunningham. 1971. Pathogenicity and antigenicity of clones from strains of Marek's disease virus and the herpesvirus of turkeys. Infect. Immunity **3**:295-303.
27. Szybalski, W., and E. H. Szybalski. 1971. Equilibrium density gradient centrifugation p. 311-354. In G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research, vol. 2. Harper & Row, New York.
28. Westphal, H., and R. Dulbecco. 1968. Viral DNA in polyoma and SV40 transformed cell lines. Proc. Nat. Acad. Sci. U.S.A. **59**:1158-1165.
29. Witter, R. L., K. Nazerian, H. G. Purchase, and G. H. Burgoyne. 1970. Isolation from turkeys of a cell associated herpesvirus antigenically related to Marek's disease virus. Amer. J. Vet. Res. **31**:525-538.
30. zur Hausen, H., G. Klein, W. Henle, G. Henle, P. Clifford, and L. Santesson. 1970. EBV DNA in biopsies of Burkitt tumors and anaplastic carcinoma of the nasopharynx. Nature (London) **228**:1056-1058.
31. zur Hausen, H., and H. Schulte-Holthausen. 1970. Presence of EB virus nucleic acid homology in a virus free line of Burkitt tumor cells. Nature (London) **227**:245-248.