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Biochemical Characterization of the Type C Retrovirus Associated with Lymphoproliferative Disease of Turkeys

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Turkeys inoculated with spleen extracts from lymphoproliferative disease (LPD)-affected birds developed viremia, followed by typical LPD lesions. Electron microscopy and biochemical characterization established that the virus present in the blood of infected turkeys is a type C retrovirus. The viral particles possess a buoyant density of 1.17 g/ml in sucrose gradients; they contain high-molecular-weight RNA and an RNA-instructed DNA polymerase with efficient exogenous and endogenous activity. The LPD virus polymerase is preferentially activated by magnesium ions. Cross nucleic acid hybridization assays revealed no sequence homology between the viral genome of LPD and avian myeloblastosis virus or reticuloendotheliosis virus, thus indicating that the LPD virus belongs to a distinct group unrelated to the avian leukosis-sarcoma virus complex or to the reticuloendotheliosis virus group.

Lymphoproliferative disease (LPD) is a leukotic condition occurring naturally in turkeys. When 10- to 12-week-old birds are affected, the mortality exceeds 20%, inflicting severe economic damage. The disease is clinically characterized by enlargement of the spleen and liver and occurrence of infiltrative lesions in other organs such as the pancreas, thymus, heart, lung, gonads, and peripheral nerves (1, 12; M. Ianconescu, K. Perk, A. Yaniv, and A. Zimber, Refu. Vet., in press). Microscopically, the lesions are composed of randomly distributed pleomorphic mononuclear cells of the lymphoid series (1; K. Perk, M. Ianconescu, A. Yaniv, and A. Zimber, J. Natl. Cancer Inst., in press). The viral etiology of this disease is suggested both from its reproducibility by the inoculation of a cell-free preparation from LPD-affected spleens and from the detection of viral particles in tissue sections of LPD tumors. Because of its morphological appearance, estimated size, density, and sensitivity to lipid solvents, the virus associated with LPD has been relegated to the Retroviridae (1). However, the fact that no antigenic relationship could be demonstrated between this virus and avian leukosis viruses or reticuloendotheliosis virus (REV) (1) suggests that the retrovirus associated with LPD might represent a distinct class of avian RNA tumor virus.

In a previous study (Perk et al., J. Natl. Cancer Inst., in press), we have shown that virus particles bud from the outer membranes of tumor cells in proliferating lesions of LPD. By the morphology of the bud, the LPD virus could be classified as a type C virus. This type C virus, having a diameter of 100 to 120 nm, resembles the viral particles of the REV group but differs in both diameter and electron density of the cores from the avian leukosis-sarcoma viruses. The present study is a first report on the biochemical features of the type C virus associated with LPD of turkeys. Employing nucleic acid hybridization, we were able to demonstrate that the LPD virus belongs neither to the avian leukosis-sarcoma virus complex nor to the REV group.

MATERIALS AND METHODS

Source and purification of viral particles. LPD virus was obtained from the plasma of turkeys inoculated with spleen extracts from LPD cases, kindly supplied by P. M. Biggs. REV strain T, obtained from the American Type Culture Collection, was grown in chicken embryo fibroblasts. The plasma and tissue culture media were clarified by successive centrifugation at 2,000 × *g* and 8,000 × *g* for 10 min. Viral particles were sedimented through a column of 20% (vol/vol) glycerol in TNE (0.01 M Tris-hydrochloride [pH 7.4], 0.1 M NaCl, 0.001 M diamine-EDTA) at 95,000 × *g* for 60 min. After resuspension in TNE, viral particles were layered over a density gradient of 20 to 55% (wt/wt) sucrose in TNE and centrifuged for 16 h at 95,000 × *g*. Fractions were collected and assayed for polymerase activity, as detailed below; those corresponding to the peak of DNA polymerase

activity were pooled, diluted in TNE, and pelleted by centrifugation at 95,000 × *g* for 60 min.

RNA-instructed DNA polymerase tests. (i) Exogenous polymerase assay. Reaction mixtures for homopolymer-templated reactions contained the following: 50 mM Tris-hydrochloride, pH 8.2; 48 mM potassium chloride; 6 mM magnesium chloride; 0.4 mM dithiothreitol; 0.2 mM dATP and 0.1 mM TTP (P-L Biochemicals); [^{3}H]TTP (40 to 50 Ci/mmol; New England Nuclear Corp.), yielding 1,000 cpm/pmol; and 0.4 μg of synthetic oligo(dT)$_{12-18}$·poly(rA) template (P-L Biochemicals). The reaction mixture was added to Nonidet P-40-disrupted viruses and then incubated at 37°C. Acid-precipitable radioactivity was tested after precipitation with cold trichloroacetic acid at a final concentration of 10% and collection on nitrocellulose filters. After exhaustive washings with cold 5% trichloroacetic acid, filters were dried, and the radioactivity was counted in 0.4% 2,5-bis-2-(5-tert-butylbenzoaxozolyl)thiophene in toluene.

(ii) Endogenous polymerase assay. Unless otherwise stated, the reaction mixtures contained the following: 100 μg of actinomycin D per ml; 50 mM Tris-hydrochloride (pH 8.2); 10 mM NaCl; 1.2 mM each dGTP, dCTP, and dATP; and 400 μCi of [^{3}H]TTP (40 to 50 Ci/mmol; New England Nuclear Corp.) per ml. The concentrations of $MgCl_2$ and $MnCl_2$ are given below. After virus disruption at 0°C for 10 min in the presence of 0.05% Nonidet P-40, the appropriate reaction mixture was added, and the whole then incubated at 37°C.

Synthesis and purification of [^{3}H]DNA. [^{3}H]-dTMP-labeled complementary DNA (cDNA) was synthesized in an endogenous reverse transcriptase reaction with detergent-disrupted viral particles in the presence of actinomycin D. The reaction was carried out at 37°C until the kinetics of [^{3}H]dTMP incorporation reached a plateau. The reaction was terminated by the addition of NaCl and sodium dodecyl sulfate to final concentrations of 0.4 M and 1%, respectively. The [^{3}H]DNA product was deproteinized and further purified by chromatography on a Sephadex G-50 column equilibrated with 1 mM EDTA containing 0.02 M sodium bicarbonate. The peak radioactive fractions in the excluded volume were pooled and lyophilized, and the RNA template was hydrolyzed by alkali.

Isolation of viral RNA. The procedure used for the isolation of 70S viral RNA was as detailed previously (20). Purified viral particles were lysed by the addition of sodium dodecyl sulfate and deproteinized with phenol-cresol containing 8-hydroxyquinoline, and RNA was purified by centrifugation on a linear glycerol gradient (10 to 30% in TNE) at 234,000 × *g* for 90 min. The RNA-containing fractions were pooled, and the nucleic acid was precipitated by the addition of 2 volumes of ethanol in the presence of 0.2 M NaCl.

Molecular hybridization. DNA-RNA hybridizations between [^{3}H]DNA (1,000 cpm) and 70S viral RNA were performed in a 10-μl reaction volume containing 0.06 M NaCl, 0.04 M Tris-hydrochloride (pH 7.2), 0.002 M EDTA, and 0.1% sodium dodecyl sulfate. The reaction mixtures were sealed in siliconized capillaries, denatured at 100°C for 1 min, chilled on ice, and then incubated at 68°C to attain the desired C_rt values. Hybrid formation was assayed by resistance to S1 nuclease (A. Yaniv and A. Gazit, Virology, in press).

Electron microscopy. Virus pellets were prepared by centrifugation of 25 ml of plasma through 13 ml of 20% glycerol in TNE at 95,000 × *g* for 60 min. After fixation in 2.5% glutaraldehyde, the pellets were post-fixed with 10% osmium, dehydrated in ethanol and propylene oxide, and embedded in Epon Araldite. Ultrathin sections were cut on an LKB ultratome, stained with uranyl acetate and lead citrate (2), and then examined with JEM-7 and 101 electron microscopes.

RESULTS

Detection of type C particles possessing DNA polymerase activity in the plasma of LPD-infected turkeys. Initial assays had revealed the presence of particulate reverse transcriptase activity in the plasma of turkeys infected with spleen extracts from LPD cases. The appearance of virion-associated reverse transcriptase activity was therefore checked to establish the development of viremia associated with the disease. Plasma samples were collected at weekly intervals, and the high-speed pellets were tested for exogenous DNA polymerase activity; the sacrificed birds were examined for gross and microscopic lesions. Particles possessing DNA polymerase activity were detected in all infected turkeys between weeks 1 and 3 postinoculation. In week 3, gross changes, such as enlargement of the spleen and pancreas, were observed in 2 out of 30 turkeys, while microscopic lesions typical of LPD were identified in all birds. Electron microscopy of selected areas in high-speed pellets of viremic plasma revealed a high concentration of mature and several immature virus particles (Fig. 1). The immature particles possessed an outer coat, an intermediate layer, and a relatively electron-lucent nucleoid, whereas the mature ones had the centrally positioned nucleoid characteristic of mature type C particles. Plasma samples obtained from uninfected turkeys did not contain type C particles or polymerase activity, and tissue specimens from control birds did not evidence lymphoproliferative lesions.

Biochemical characteristics of the LPD virus. The buoyant density of the viral particles present in the plasma of infected turkeys was determined by assaying fractions of sucrose equilibrium density gradients. Figure 2 shows a single peak of DNA polymerase activity at a density of 1.17 g/ml.

To ensure that the sedimented DNA polymerase was in fact a viral RNA-instructed DNA polymerase and not a cellular DNA polymerase, the virion-associated enzymatic activity was examined for its synthetic template preference. The virus polymerase responded to the synthetic template oligo(dT)$_{12-18}$·poly(rA), demonstrating a linear kinetics for more than 60 min. In con-

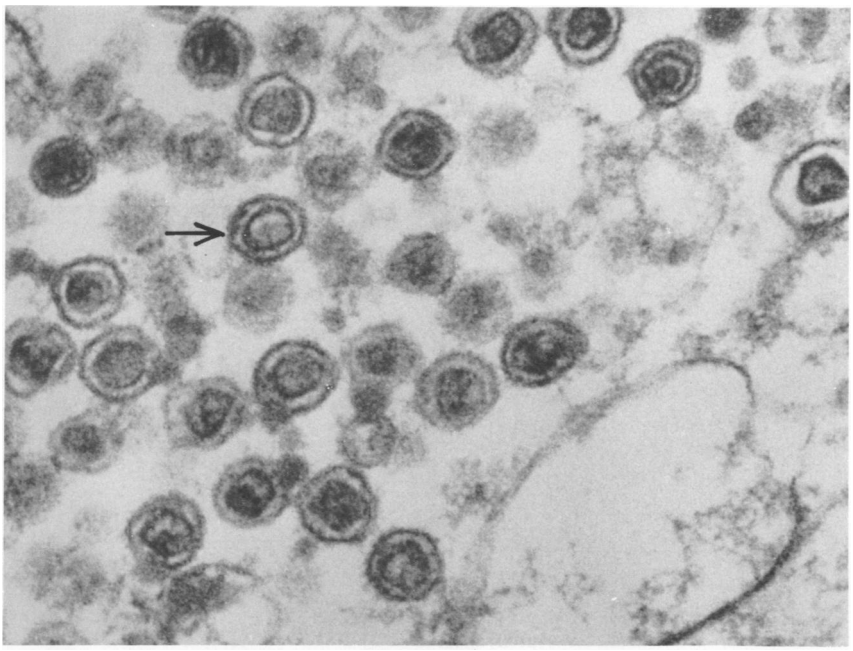

FIG. 1. *Electron micrograph of a high-speed pellet of plasma showing many typical mature type C particles and an immature particle (arrow). Approximately ×90,000.*

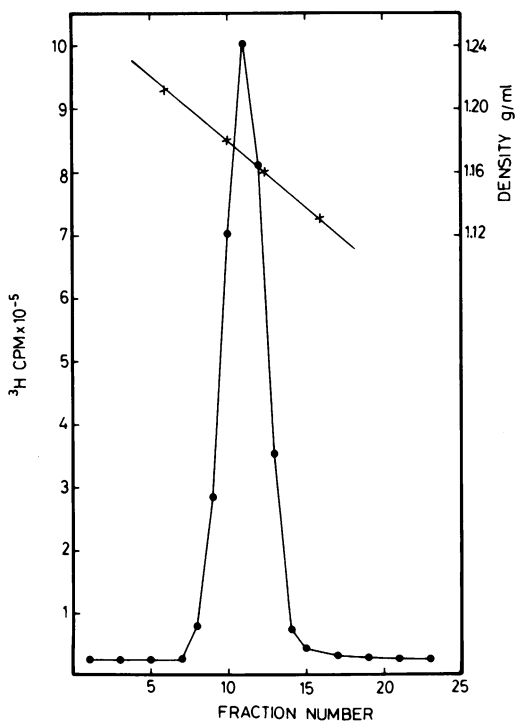

FIG. 2. *Isopycnic sedimentation profile of LPD virions in sucrose gradient. After centrifugation of concentrated viral particles as described in the text, 20-μl samples of gradient fractions were tested for DNA polymerase activity, using oligo(dT)$_{12-18}$·poly(rA) synthetic template. After incubation for 30 min at 37°C, acid-precipitable radioactivity was measured.*

trast, the enzyme utilized very poorly the synthetic homopolymer-oligomer template poly(dA)·oligo(dT)$_{10}$—a template preferred by DNA-dependent DNA polymerase.

The virion-associated DNA polymerase was also found to be efficiently active in an endogenous reaction. As shown in Fig. 3, the synthesis of polymeric DNA in the endogenous enzymatic reaction occurred at a high initial rate which diminished by about 60 min. The divalent cation preference of LPD virus was compared with that of REV virions. It was found that the LPD virus polymerase accepted either magnesium or manganese cations and that the maximum rate attainable in the endogenous reaction with magnesium as cofactor was greater than that achieved with manganese, occurring at a concentration of about 4 mM. In contrast, REV endogenous DNA polymerase could utilize only manganese, at a 6 mM optimum concentration (Fig. 4), as reported elsewhere (5). Omission of any of the nucleotide triphosphate from the reaction mixture or RNase treatment of the detergent-disrupted virions resulted in 75 to 90% inhibition of [^{3}H]dTMP incorporation (Table 1). This finding suggests that a relevant RNA-instructed DNA polymerase reaction had taken place, thus excluding the likelihood of deoxyribonucleotidyl terminal transferase activity in the endogenous DNA polymerase reaction.

Further evidence that an RNA molecule was being transcribed by LPD polymerase and on the sedimentation characteristic of this RNA

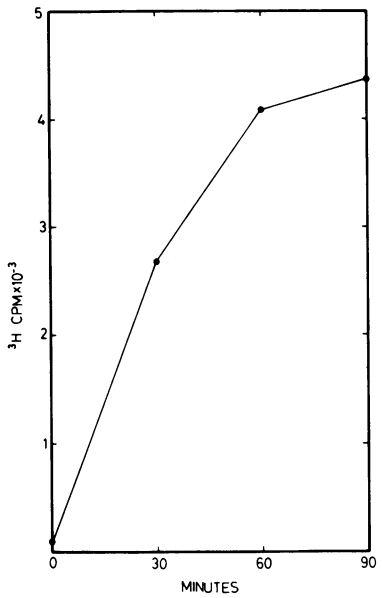

FIG. 3. *Kinetics of [^{3}H]dTMP incorporation in an endogenous reaction of LPD virus-associated DNA polymerase. Purified virus protein (37 μg) was added to a reaction mixture of RNA-instructed DNA polymerase (125-μl final volume) and incubated at 37°C. Samples (10 μl) were removed at the indicated times and assayed for acid-precipitable radioactivity.*

was obtained by analysis of the 10-min endogenous reaction product on linear glycerol gradients. The data (Fig. 5) showed that part of the radioactive DNA was traveling with a sedimentation coefficient of 70S, indicating that the rapidly sedimenting [^{3}H]DNA molecules were complexed to a high-molecular-weight structure. When the 10-min product was treated with RNase, there was no evidence of [^{3}H]DNA in the 70S region of the gradient, suggesting that the high-molecular-weight carrier structure was an RNA molecule possessing a sedimentation coefficient of 70S. The data thus described, coupled with the additional finding that the [^{3}H]-DNA synthesized in an endogenous reaction specifically hybridizes with the viral RNA template, provide adequate evidence for the association of a potent reverse transcriptase with the LPD virions.

Although LPD was not found to be immunologically related to the REV group or to the avian leukosis-sarcoma virus complex (7), we deemed it interesting to search for possible nucleic acid sequence homology between the LPD viral genome and viruses belonging to the mentioned groups. To assess this possibility, we chose two prototypes, REV and avian myeloblastosis virus (AMV), and attempted nucleic acid hybridization between these viruses and LPD virions. We first prepared [^{3}H]DNA products from a 60-min endogenous RNA-instructed DNA polymerase reaction of purified LPD and REV virions in the presence of 100 μg of actinomycin D per ml. Before carrying out cross-hybridization experiments, we measured the

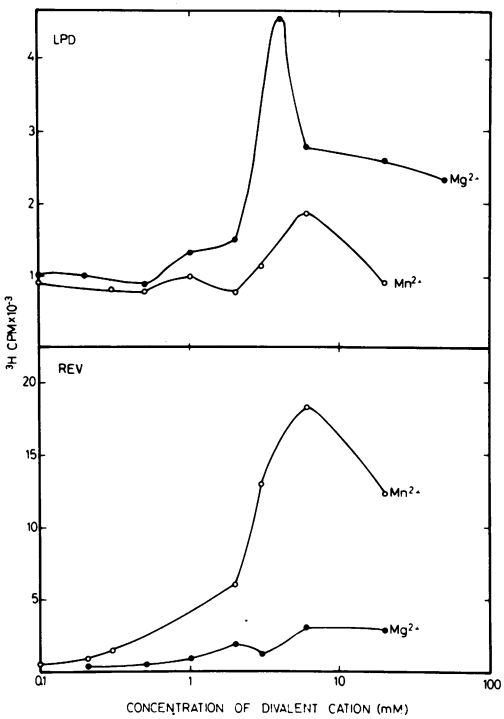

FIG. 4. *Determination of optimal cation requirements of endogenous viral DNA polymerase activity of LPD virus or REV. Endogenous DNA polymerase reactions were carried out with approximately 5 μg of protein of each disrupted virus in 50 μl of reaction mixture containing the indicated amount of magnesium (●) or manganese (○) ions for each point. At the end of 30 min of incubation at 37°C, acid-precipitable radioactivity was measured.*

TABLE 1. *Requirements of LPD virus DNA polymerase*[a]

Reaction mixture	[^{3}H]dTMP incorporated (cpm)
Complete	6,327
dATP omitted	1,675
dCTP omitted	824
dGTP omitted	840
Preincubation with RNase	615

[a] Endogenous DNA polymerase assays were performed for 30 min at 37°C as described in the text, with omissions and additions as indicated. RNase A at a concentration of 50 μg/ml was added to detergent-disrupted viruses, then incubated for 10 min at 37°C, and added to a complete reaction mixture.

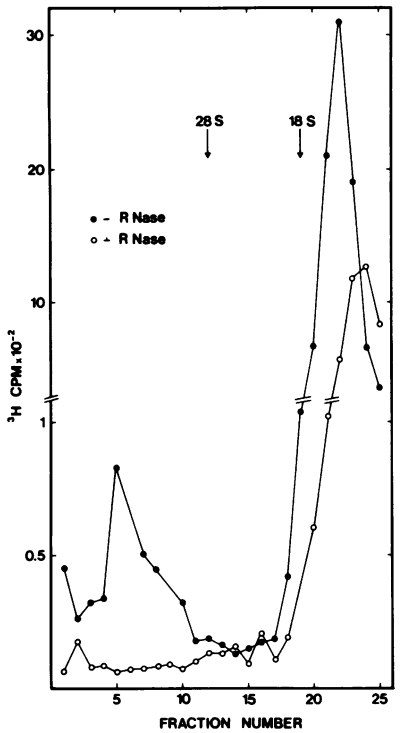

FIG. 5. *Sedimentation analysis of the 10-min endogenous reaction product of LPD virus DNA polymerase. A sample of 36 μg of virus protein was subjected to an endogenous RNA-instructed DNA polymerase reaction. The product was extracted by phenol and divided, and one half was treated with boiled RNases A and T1 (50 μg/ml each) for 15 min at 37°C. The nucleic acids of the two samples were then layered separately on linear glycerol gradients (10 to 30% in TNE) and centrifuged at 234,000 × g for 90 min at 4°C. External markers were 18 and 28S ^{3}H-labeled rRNA. Fractions were collected from below and assayed for acid-precipitable radioactivity.*

melting temperatures (T_m) of homologous RNA-DNA hybrids to determine the fidelity of hybridization of the cDNA with their homologous RNA templates. The steep melting curves with T_m values of 86°C (Fig. 6) bear testimony that extensive mismatching did not occur in the homologous systems. Next, kinetics of association were performed between the [^{3}H]DNA product of LPD virus and RNAs of LPD virus, REV, and AMV (Fig. 7) and between [^{3}H]DNA of REV and RNAs extracted from REV or LPD virions (Fig. 8). Viral RNAs annealed with their homologous cDNA products at low C_rt values for 50% hybridization (8×10^{-2} mol·s/liter), but no hybridization occurred between the cDNA products and the heterologous viral RNAs.

DISCUSSION

Leukosis of turkeys comprises at least two discrete diseases, namely, LPD and reticuloendotheliosis. Both conditions are characterized clinically by spleen and liver enlargement, with lesions in most organs and peripheral nerves (1, 11; Perk et al., J. Natl. Cancer Inst., in press). Microscopically, the LPD tumors are characterized by proliferation of pleomorphic cells of the lymphocytic series (7; Perk et al., J. Natl. Cancer Inst., in press), whereas the lesions of reticuloendotheliosis are mainly comprised of proliferating large lymphoreticular cells (11).

Implication of REV in the etiology of reticuloendotheliosis, its biochemical features, and its relatedness to other retroviruses have already been widely documented (4–6, 8–10, 13, 14). The recent findings of extracellular oncornavirus-like particles in thin sections of LPD lesions (1, 7), of type C virus particles budding from the surface of LPD tumor cells (Perk et al., J. Natl. Cancer Inst., in press), and of the induction of lymphoproliferative disease by cell-free spleen homogenates or by purified particles with a density of 1.16 to 1.18 g/ml (7) strongly suggest the involve-

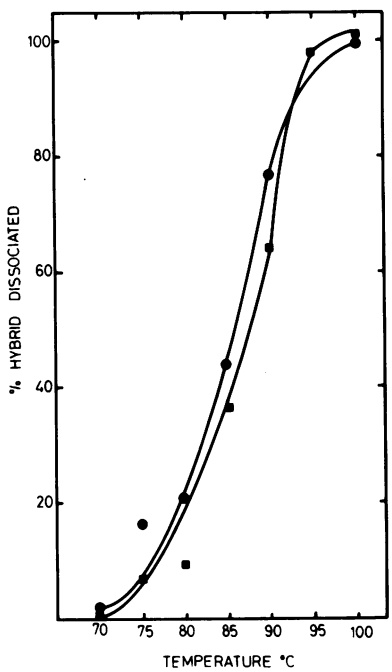

FIG. 6. *Thermal stability of hybrids formed between LPD viral ^{3}H-labeled cDNA and LPD virus 70S RNA (●) or between REV viral ^{3}H-labeled cDNA and 70S REV RNA (■). Hybridization reactions were carried out to a C_rt of 2 mol·s/liter at 68°C, as described in the text. Samples were diluted 10-fold in 0.4 M NaCl, heated at the indicated temperatures for 5 min, and then digested with S1 nuclease. The temperature at which 50% of the hybridized ^{3}H-labeled cDNA becomes dissociated (T_m) was determined from these melting profiles.*

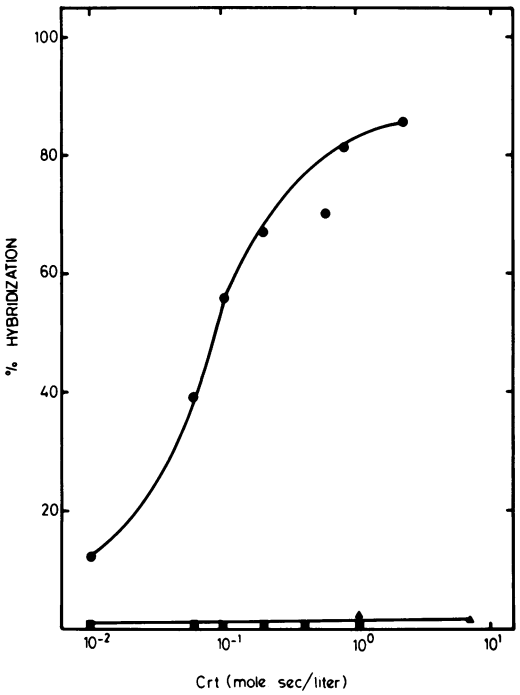

FIG. 7. *Association kinetics analysis of ^{3}H-labeled cDNA of LPD virus and 70S RNA extracted from purified LPD (●), REV (■), or AMV (▲) viral particles. LPD virus [^{3}H]DNA probe was synthesized in an endogenous reaction containing 4 mM $MgCl_2$. At the indicated C$_r$t values, the hybridization reaction was terminated and assayed for acquisition of S1 nuclease resistance as described in the text.*

ment of an RNA tumor virus in the etiology of LPD.

Our data demonstrate that the LPD gross lesions are preceded by viremia of type C viral particles. The early appearance of viruses in the blood of affected birds provides a sensitive and rapid means for detecting infection in flocks before heavy losses are incurred. The high level of viral particles present in the plasma of the affected birds has enabled us to isolate the LPD virus and, for the first time, describe its biochemical properties. The LPD virions possess a density of 1.17 g/ml in sucrose gradients—the density characteristic for the oncornaviruses. The particles contain an RNA-instructed DNA polymerase with properties similar to those of the analogous enzymes from the RNA tumor viruses (3). The enzyme in its exogenous reaction demonstrates a significant preference for ribohomopolymers over deoxyribohomopolymers as templates. The LPD virus polymerase, in the presence of Mg^{2+} as the divalent cation and of the four deoxyriboside triphosphates, efficiently synthesizes DNA molecules which are complementary to the intrinsic viral RNA. The sensitivity of the endogenous reaction to RNase, coupled with the detection of labeled nucleic acids which sediment at roughly 70S in the simultaneous assay procedure (16), indicates that the LPD virion contains high-molecular-weight RNA.

Although morphologically similar to REV, the LPD type C virus does not share any antigenic properties either with REV or with the avian leukosis-sarcoma virus complex (7). Furthermore, whereas REV replicates productively in vitro in a wide range of avian cells (15, 18), attempts to propagate the LPD virus in a variety of cell cultures have been unsuccessful so far (7; Ianconescu et al., Refu. Vet., in press). In addition, the two viruses also differ in their viral polymerase. Whereas REV enzyme requires only manganese (5, 19; our data [Fig. 4]), the LPD virus polymerase is preferentially activated by magnesium ions. Our cross nucleic acid hybridization experiments have revealed the absence of sequence homology between the viral genomes of LPD and AMV or REV. Inasmuch as AMV and REV are members of two nonrelated groups comprised of viruses that share extensive

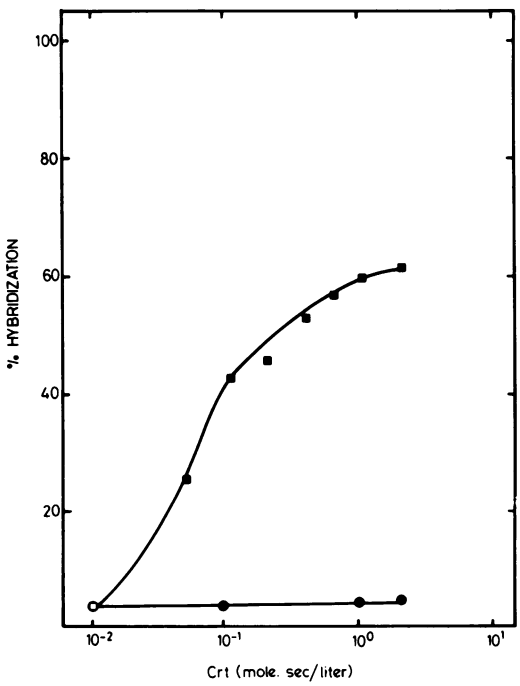

FIG. 8. *Association kinetics analysis of ^{3}H-labeled cDNA of REV and 70S RNA extracted from purified REV (■) or LPD (●) viral particles. REV ^{3}H-labeled cDNA was synthesized in an endogenous reaction containing 6 mM $MnCl_2$. The examination of hybrids was as detailed in the legend to Fig. 7.*

nucleic acid sequence homology (6, 17), our data suggest that the LPD virus belongs to a group distinct from the avian leukosis-sarcoma virus complex or from the REV group.

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LITERATURE CITED

1. **Biggs, P. M., J. S. McDougall, J. A. Frazier, and B. S. Milne.** 1978. Lymphoproliferative disease of turkeys. I. Clinical aspects. Avian Pathol. **7**:131-139.
2. **Gibbons, I. R., and J. R. Bradfield.** 1957. Experiments on staining thin sections for electron microscopy, p. 121-127. *In* Proceedings of the Stockholm Conference on Electron Microscopy. Academic Press Inc., New York.
3. **Green, M., and G. F. Gerard.** 1974. RNA-directed DNA polymerase—properties and functions in oncogenic RNA viruses and cells. Prog. Nucleic Acid Res. Mol. Biol. **14**:187-334.
4. **Halpern, M. S., E. Wade, E. Rucker, K. L. Baxter-Gabbard, A. S. Levine, and R. R. Friis.** 1973. A study of the relationship of reticuloendotheliosis virus to the avian leukosis-sarcoma complex of viruses. Virology **53**: 287-299.
5. **Kang, C.-Y.** 1975. Characterization of endogenous RNA-directed DNA polymerase activity of reticuloendotheliosis viruses. J. Virol. **16**:880-886.
6. **Kang, C.-Y., and H. M. Temin.** 1973. Lack of sequence homology among RNAs of avian leukosis-sarcoma viruses, reticuloendotheliosis viruses, and chicken RNA-directed DNA polymerase activity. J. Virol. **12**:1314-1324.
7. **McDougall, J. S., P. M. Biggs, R. W. Shilleto, and B. S. Milne.** 1978. Lymphoproliferative disease of turkeys. II. Experimental transmission and aetiology. Avian Pathol. **7**:141-155.
8. **Maldomado, R. L., and H. R. Bose.** 1973. Relationship of reticuloendotheliosis virus to the avian tumor viruses: nucleic acid and polypeptide composition. J. Virol. **11**: 741-747.
9. **Mizutani, S., and H. M. Temin.** 1974. Specific serological relationships among partially purified DNA polymerases of avian leukosis-sarcoma viruses, reticuloendotheliosis viruses, and avian cells. J. Virol. **13**:1020-1029.
10. **Moelling, K., H. Gelderblom, G. Pauli, R. R. Friis, and H. Bauer.** 1975. A comparative study of the avian reticuloendotheliosis virus: relationship to murine leukemia virus and viruses of the avian sarcoma-leukosis complex. Virology **65**:546-557.
11. **Paul, P. S., K. A. Pomeroy, P. S. Sarma, K. H. Johnson, D. M. Barnes, M. C. Kumar, and B. S. Pomeroy.** 1976. Naturally occurring reticuloendotheliosis in turkeys: transmission. J. Natl. Cancer Inst. **56**:419-421.
12. **Perk, K., M. Ianconescu, A. Yaniv, and A. Zimber.** 1978. Lymphoproliferative disease of turkeys—structure, ultrastructure and biochemistry. Refu. Vet. **35**:29.
13. **Peterson, D. A., K. L. Baxter-Gabbard, and A. J. Levine.** 1972. Avian reticuloendotheliosis virus (strain T). V. DNA polymerase. Virology **47**:251-254.
14. **Purchase, H. G., C. Ludford, K. Nazaerian, and H. W. Cox.** 1973. A new group of oncogenic viruses: reticuloendotheliosis, chick syncytial, duck infectious anemia and spleen necrosis viruses. J. Natl. Cancer Inst. **51**: 489-499.
15. **Purchase, H. G., and R. L. Witter.** 1975. The reticuloendotheliosis viruses. Curr. Top. Microbiol. Immunol. **71**:103-124.
16. **Schlom, J., and S. Spiegelman.** 1971. Simultaneous detection of reverse transcriptase and high molecular weight RNA unique to oncogenic RNA viruses. Science **174**:840-844.
17. **Stehelin, D., R. V. Guntaka, H. E. Varmus, and M. Bishop.** 1976. Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. J. Mol. Biol. **101**:349-365.
18. **Temin, H. M., and V. K. Kassner.** 1974. Replication of reticuloendotheliosis viruses in cell culture: acute infection. J. Virol. **13**:291-297.
19. **Waite, M. R. F., and P. T. Allen.** 1975. RNA-directed DNA polymerase activity of reticuloendotheliosis virus: characterization of endogenous and exogenous reactions. J. Virol. **16**:872-879.
20. **Yaniv, A., R. Kleinman, and E. Eylan.** 1976. RNA-instructed DNA polymerase associated with C-type particles produced in vivo by murine myeloma cells. J. Gen. Virol. **32**:301-309.