Proc. Nat. Acad. Sci. USA
Vol. 73, No. 4, pp. 1014–1018, April 1976
Biochemistry

Bovine leukemia virus: An exogenous RNA oncogenic virus

(simultaneous detection/relatedness/DNA·DNA hybridizations)

R. KETTMANN*†, D. PORTETELLE*†, M. MAMMERICKX‡, Y. CLEUTER*, D. DEKEGEL§, M. GALOUX*†, J. GHYSDAEL*, A. BURNY*†, AND H. CHANTRENNE*

* Département de Biologie Moléculaire, Université Libre de Bruxelles, Belgium; †Faculté Agronomique, Gembloux, Belgium; ‡Institut National de Recherches Vétérinaires, Uccle, Bruxelles, Belgium; and §Vrije Universiteit Brussel and Institut Pasteur du Brabant, Bruxelles, Belgium

Communicated by S. Spiegelman, December 29, 1975

ABSTRACT **Short-term cultures of bovine leukemic lymphocytes release virus particles with biochemical properties of RNA oncogenic viruses. These particles, tentatively called bovine leukemia virus (BLV), have a high molecular weight RNA-reverse transcriptase complex and a density of 1.155 g/ml in sucrose solutions. Molecular hybridizations between BLV [^{3}H]cDNA and several viral RNAs show that BLV is not related to Mason-Pfizer monkey virus, simian sarcoma associated virus, feline leukemia virus, or avian myeloblastosis virus. These results were confirmed by hybridization between BLV 70S RNA and [^{3}H]cDNA synthesized in the various viruses tested. The high preference of BLV reverse transcriptase for Mg^{++} as the divalent cation suggests that BLV might be an atypical mammalian leukemogenic "type C" virus. DNA·DNA hybridization studies using BLV [^{3}H]cDNA as a probe strongly suggest that the DNA of bovine leukemic cells contains viral sequences that cannot be detected in normal bovine DNA.**

Bovine leukemia is a lymphoproliferative disease appearing in cattle herds under several forms (1). The following observations lead to the conclusion that viruses are the most probable etiological agents of the enzootic form of the disease:

(*a*) Bovine leukemia often appears in geographically localized foci. It spreads by horizontal as well as vertical transmission (mostly from mother to offspring) (2–4).

(*b*) Infected animals develop antibodies directed against an antigen present in the virus fraction of leukemic lymphocyte cultures. This antigen can be detected by immunofluorescence, immunodiffusion, or complement fixation (5–8).

(*c*) Virus particles are occasionally seen in milk and tissues of leukemic animals (9, 10).

(*d*) Cultures of bovine leukemic material produce virus particles generally considered as type C (10–14) although they are morphologically somewhat different from typical type C viruses (15, 16).

(*e*) Whole blood from leukemic animals transfers the disease with high frequency when fed to newborn calves (17, 3) or sheep (17–19). Successful infections are also obtained with the viral concentrate from short-term cultures (20, 21).

Considering all these observations, it seemed to us of basic interest to identify biochemically bovine leukemia virus (BLV), to determine by molecular hybridization to what extent it could be related to other known type C viruses, and, finally and most importantly, to characterize it as an exogenous (class 2) or endogenous (class 1) bovine virus (22).

MATERIALS AND METHODS

Animals and Cell Cultures. Our experimental herd was established from animals diagnosed as leukemic by hematological test (key of Göttingen). A sample of leukocytes from each animal was submitted to short-term culture and examined by electron microscopy for the presence of "C type" virus. Every culture derived from a leukemic animal produced virus particles, while cultures made of normal leukocytes remained negative (4). All the animals used in this study for production of BLV were tested and found negative for the bovine viruses other than BLV found in Belgium. Cells were cultured as described by Stock and Ferrer (23).

Virus Concentration from Short-Term Culture Supernatants. The medium was clarified by centrifugation at 1500 × *g* for 45 min at 3°, and the virus was purified according to Bishop *et al.* (24) except that TNE (0.01 M Tris·HCl, pH 8.3; 0.15 M NaCl; 0.001 M EDTA) was used instead of minimal essential medium or Tris·HCl, pH 7.5.

Virus Concentration from Cells. Cultured lymphocytes (3 g) were homogenized with an Ultra-Turrax homogenizer (Janke and Kunkel, type ZF) at full speed three times for 20 sec each time in four volumes of TNE at 4°. The homogenate was then processed as the virus suspension.

Assay of 60–70S RNA-Directed DNA Polymerase: Simultaneous Detection Test. Pellets obtained after equilibrium density gradient centrifugation of BLV were resuspended in 0.01 M Tris·HCl, pH 8.3 at a protein concentration of 3 mg/ml. Triton X-100 was adjusted to a final concentration of 0.03%, and a simultaneous detection test was performed (25).

Preparation of BLV [^{3}H]cDNA. The 60–70S RNA·[^{3}H]DNA complex of a BLV simultaneous detection test was recovered by alcohol precipitation, treated with alkali to destroy RNA, and chromatographed on hydroxyapatite to purify single-stranded [^{3}H]cDNA molecules (26, 27).

Preparation of [^{3}H]cDNA Probes of Avian Myeloblastosis Virus (AMV), Simian Sarcoma Associated Virus (SSV-1), Mason-Pfizer Monkey Virus (MPMV), and Feline Leukemia Virus (FeLV). The reaction conditions were those previously described (28, 36) except for FeLV where Mg^{++} was replaced by Mn^{++} at a final concentration of 0.001 M.

Preparation of Viral RNAs. Viral proteins were solubilized and digested by a mixture of sodium dodecyl sulfate and proteinase K (Merck, Darmstadt) at final concentrations of 0.5% and 0.2 mg/ml, respectively. The digested mixture was extracted twice at room temperature with phenol–cresol–chloroform (6:1:7). RNA (60–70S) was recovered from the aqueous phase by ethanol precipitation and purified by sedimentation in sucrose gradient.

Preparation of DNAs. DNA from leukemic and normal cells was isolated according to Sweet *et al.* (29). The sedimentation constant of all DNAs was reduced to a value of 6–8 S by ultrasonic vibrations. DNAs extracted by this method had an $A_{260}/A_{280\,nm}$ ratio of 1.85–1.95.

Hybridization Reactions. [^{3}H]cDNA (2000 cpm) was added to indicated amounts of RNA (or DNA) in a final vol-

Abbreviations: BLV, bovine leukemia virus; C_rt, product of concentration of viral RNA (moles/liter) and time (sec); AMV, avian myeloblastosis virus; SSV-1, simian sarcoma associated virus; MPMV, Mason-Pfizer monkey virus; FeLV, feline leukemia virus.

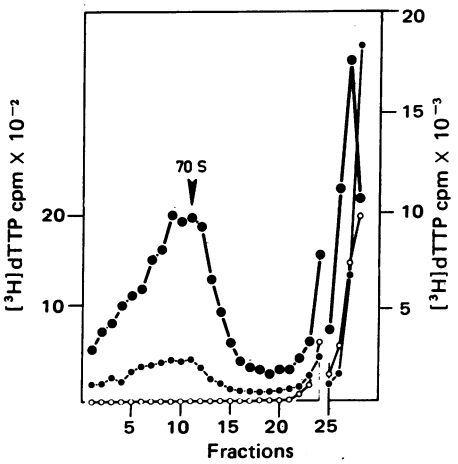

FIG. 1. Detection of the 60–70S RNA · [^{3}H]cDNA complex of BLV from culture supernatants. Twelve hundred milliliters of bovine lymphocyte culture supernatant were processed as described in *Materials and Methods*. The virus concentrate was divided into three equal parts. A standard RNA-directed DNA polymerase reaction was performed on one part. The size of the RNA · [^{3}H]cDNA product was determined on sucrose gradient (●——●). A second part of the virus concentrate was used in a reaction mixture lacking dATP (•——•). The third part of the virus concentrate was incubated in the complete reaction mixture supplemented with 100 μg/ml of RNase A (O——O).

ume of 56 μl of 0.4 M NaCl, 0.001 M EDTA, 0.1% sodium dodecyl sulfate, and 0.01 M Tris · HCl, pH = 7.7. The mixture was incubated at 68° for various periods of time. The extent of hybrid formation was estimated by S_1 nuclease digestion (30).

RESULTS

Characterization of a 60–70S RNA and Reverse Transcriptase. BLV particles released in the culture supernatant were concentrated as described in *Materials and Methods* and used as a source of template-primer and reverse transcriptase in an RNA-dependent DNA synthesis reaction. The reaction proceeded linearly for at least 30 min. In some cases, linear incorporation lasted for as long as 2 hr. As a rule, reactions were stopped after 30 min and analyzed by the simultaneous detection technique (25). We systematically searched for optimum conditions for cDNA recovery. Incubation of the reaction mixture with proteinase K (Merck, Darmstadt) before phenol-chloroform extraction improved the cDNA yield by at least 70%.

Fig. 1 shows the outcome of a simultaneous detection test. Fractions 5 to 15 represent the region where 60–70S viral RNA · [^{3}H]cDNA complexes sediment. The presence of these complexes *per se* is a strong indication that BLV contains a high molecular weight RNA and reverse transcriptase, molecules characteristic of RNA oncogenic viruses. The synthesis of 60–70S RNA · [^{3}H]cDNA hybrids is dependent on the presence of the four deoxytriphosphates in the incubation medium. Leaving out dATP reduced the [^{3}H]dTMP incorporation to about 20% of the control value. In the presence of RNase A, [^{3}H]dTMP incorporation is reduced to a background level.

The same experimental technology was applied to virus detection in cultured leukemic lymphocytes. The same positive outcome of the simultaneous detection test was obtained. Similar results were also obtained when leukemic lymphocytes from sheep infected by bovine leukemic blood were examined (data not shown). This indicates that the observed endogenous DNA polymerase activity is both RNA dependent and not due to an end addition enzyme activity.

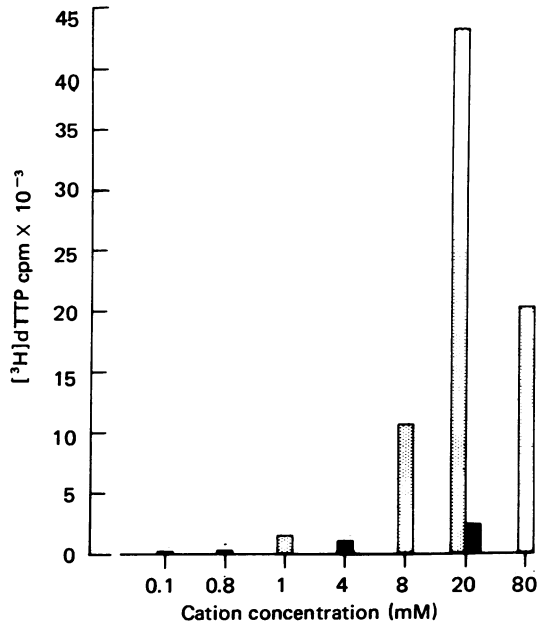

FIG. 2. BLV DNA polymerase activity as a function of Mg^{++} (*stippled bars*) or Mn^{++} (*solid bars*) concentration in the reaction mixture. [^{3}H]cDNA syntheses were run in the presence of the indicated concentrations of Mg^{++} or Mn^{++} and analyzed by sedimentation in sucrose gradients. Results are expressed as trichloroacetic acid precipitable radioactivity in the 60–70S region of the gradient against indicated cation concentration.

Requirements of the BLV-Reverse Transcriptase Reaction. *Nonionic Detergent.* In contrast to avian RNA tumor viruses (28, 31, 32), mammalian oncornaviruses (25, 26) require no detergent (33) or very limited concentrations of detergents for optimal rate of DNA synthesis. BLV endogenous synthesis of DNA was performed in the presence of various concentrations of Nonidet P-40 or Triton X-100. At the protein concentration used (3 mg/ml) maximum synthesis of 60–70S [^{3}H]cDNA occurred at 0.01% of Nonidet P-40 or 0.03% of Triton X-100 in the reaction mixture. Higher detergent concentrations practically abolish the reaction.

Divalent Cation. The preference of viral polymerase for Mg^{++} or Mn^{++} depends on the template-primer that is being used (34, 35). It has been shown also (36) that more similarity exists between the DNA polymerases from viruses of the same type than between the polymerases from viruses of different types but from closely related species. Divalent cation requirement may therefore be informative for the biochemical characterization of new oncornaviruses.

Endogenous [^{3}H]cDNA synthesis was performed under the conditions described in *Materials and Methods* except for divalent cation concentration. [^{3}H]cDNA radioactivity associated with high molecular weight RNA after sucrose gradient sedimentation was recorded in Fig. 2 as a function of divalent cation concentration used. As can be seen, no notable incorporation of [^{3}H]dTMP into DNA occurred at any of the Mn^{++} cation concentrations tested. Mg^{++}, on the other hand, clearly stimulated BLV reverse transcriptase. The optimum concentration for the reverse transcriptase activity in this assay was 20 mM Mg^{++}.

Buoyant Density in Sucrose Gradients. The buoyant density values of oncornaviruses in sucrose solutions vary according to the virus type (37). The B-type mouse mammary

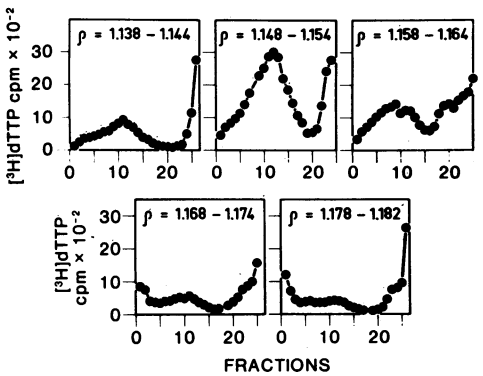

FIG. 3. Determination of BLV equilibrium density in sucrose solutions. A BLV concentrate, prepared from short-term culture supernatant (1200 ml) as described in *Materials and Methods*, was suspended in TNE buffer, layered on a linear gradient (20–50% sucrose in TNE buffer), and centrifuged overnight at 25,000 rpm in a Spinco SW 27 rotor at 4°. Fractions within regions of the indicated densities were pooled and assayed by the simultaneous detection test. (See *Materials and Methods.*)

tumor virus equilibrates at a density of 1.18 g/ml in sucrose, while type C viruses have a density of 1.16 g/ml in sucrose.

In order to determine the density values of BLV, the virus released in the supernatant of 1200 ml of culture was processed as described in the legend to Fig. 3. The simultaneous detection profiles obtained reveal that BLV equilibrates in these conditions between 1.148 and 1.164 g/ml, the density region of C type viruses.

Relatedness of BLV to Other RNA Oncogenic Viruses. A constant amount (2000 cpm; specific activity = 2×10^7 cpm/μg) of single-stranded molecules was annealed to increasing amounts of BLV RNA. Percentages of hybridization were calculated from the ratios of S_1-resistant counts over total [^{3}H]cDNA counts in the controls. The values obtained were recorded (Fig. 4) as functions of C_rt (concentration of viral RNA in moles/liter times time in seconds (38). $C_rt_{1/2}$ equals 7×10^{-2} moles × sec/liter. In similar experiments with AMV and Rauscher leukemia virus (RLV), values such as 3×10^{-2} (J. Ghysdael, in preparation) and 1.5×10^{-2} (39) were reported. The somewhat higher value obtained here in the BLV system is probably due to some contamination of BLV 60–70S RNA. Since BLV is produced by degenerating cells, contamination of the 60–70S region of sucrose gradients by cellular nucleic acid cannot be ruled out. Such a C_rt curve also shows that 90% of the cDNA engaged in the reaction was hybridized at C_rt values of 1 and above. Annealing experiments tending to detect an hypothetical relatedness between BLV and other known RNA oncogenic viruses must be carried out up to, at least, C_rt values of 1. Such experiments were performed with globin mRNA as control and MPMV, FeLV, AMV, and SSV-1 RNAs. Within the limits of our experiments, we can conclude that the four viruses tested do not share common RNA sequences with BLV. A control experiment was then performed where BLV 60–70S RNA was annealed to [^{3}H]DNA synthesized in the above four viruses tested (Table 1). Again, MPMV, AMV, SSV-1, and FeLV appeared to be unrelated to BLV.

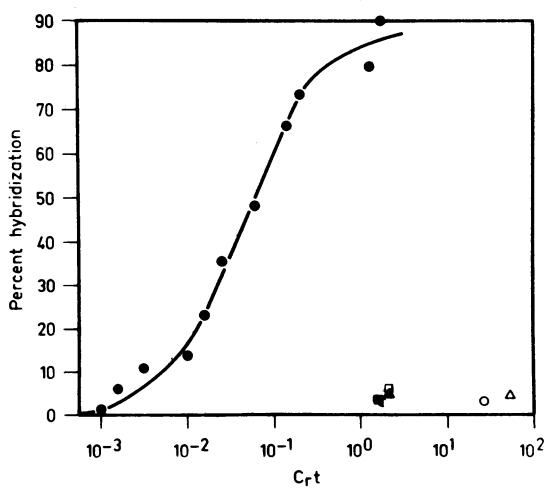

FIG. 4. Kinetics of annealing of BLV cDNA to 60–70S BLV RNA. C_rt is the product of nucleic acid concentration (in A_{260}) and hybridization time (in hours/2). No corrections were made for salt concentrations. The hybridization mixtures contained from 0.078 ng to 160 ng of BLV 70S RNA and were all incubated at 68° for 3 days. The extent of hybridization was determined by S_1 nuclease treatment (●——●). Hybridizations with hemoglobin mRNA as a control (△) and with 70S RNA of RLV (□), MPMV (■), AMV (○), SSV-1 (▲), and FeLV (◐) were run up to indicated C_rt values.

Table 1. Percent hybridization of [^{3}H]cDNA probes synthesized in various RNA viruses with globin mRNA (as control) and various viral 60–70S RNAs*

RNAs	[^{3}H]cDNA probes			
	BLV	SSV-1	AMV	FeLV
Globin mRNA	4.3	0.0	0.0	0.0
AMV	3.1	—	90.2	—
SSV-1	4.9	90.0	—	—
MPMV	3.6	—	—	—
FeLV	—	—	—	63.1
BLV	85.1	0.3	2.6	0.8

* Hybridizations were run at C_rt values ≥ 2.

BLV Genome Sequences in DNA from Normal Bovine Cells and Bovine Lymphosarcoma Cells. A constant amount (2000 cpm; specific activity = 2×10^7 cpm/μg) of single-stranded BLV [^{3}H]cDNA was annealed to increasing amounts of normal and leukemic bovine DNA. After about 20 days, the percentages of hybridization were determined and recorded as functions of C_0t. Fig. 5 shows the kinetics of hybridization of BLV [^{3}H]cDNA with normal and leukemic bovine DNA. About 60% of the radioactive probe enters S_1 nuclease-resistant hybrids after annealing with bovine leukemic DNA. The same probe formed hybrids with normal bovine DNA at a much slower rate, reaching only 15% at log C_0t values of about 4.5.

These observations are consistent with the proposition that BLV contributes genome sequences to the leukemic cell which are not detectable by this technique in normal bovine DNA. Definite proof that this proposition is indeed correct must await further experimental evidence. Recycling experiments (29, 40, 41) and thermal stability analysis of the hybrids are presently under way.

DISCUSSION

Bovine leukemia is by far the best known natural model system for epidemiological studies. As outlined in the introduction of this report, the disease spreads by horizontal and vertical transmission. In the latter case, however, experiments strongly suggest that transmission of the disease is most probably due to perinatal infection (3). It was therefore of obvious interest to try to identify the putative agent, and characterize it biologically and biochemically. A major step

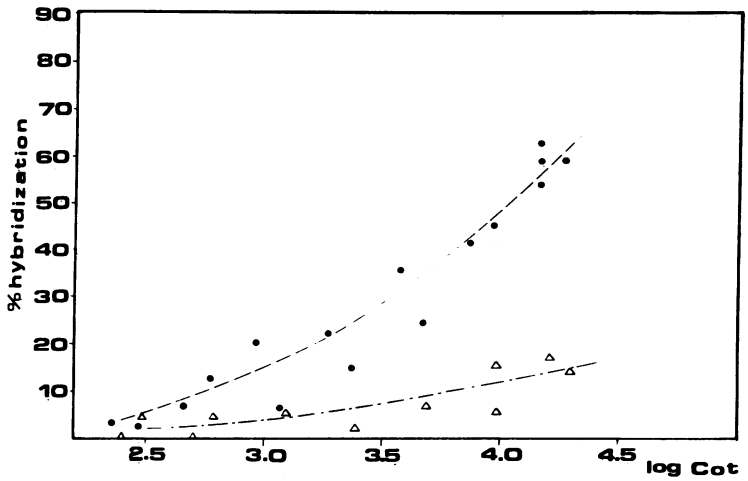

FIG. 5. Hybridization kinetics of BLV [^{3}H]cDNA with normal (△) and leukemic (●) bovine DNA. Hybridization reactions were carried out with 2000 input cpm of BLV [^{3}H]cDNA at a specific activity of 2×10^7 cpm/μg, at 68° in Tris · HCl, 0.01 M, pH = 7.7; NaCl, 0.4 M; EDTA, 0.001 M; sodium dodecyl sulfate, 0.1%. The dashed lines are not mathematically derived, but simply fit to the data for illustrative purposes.

towards this goal was accomplished when virus production was achieved in short-term cultures of leukemic lymphocytes. We report here on our attempt to study biochemical features of the virus. The positive outcome of simultaneous detection tests, the sensitivity of the reaction to ribonuclease treatment, and the strict requirement for the four deoxyribonucleoside triphosphates (Fig. 1) practically identifies BLV as an RNA tumor virus. The definite proof, however, that BLV possesses a high molecular weight RNA-reverse transcriptase complex could only be obtained through back hybridization of the DNA synthesized *in vitro* with the viral 60–70S RNA. Fig. 4 shows that this is, indeed, the case.

Parameters of the endogenous reverse transcriptase reaction were then determined. As a rule, reverse transcriptase from mammalian leukemogenic viruses are extremely sensitive to nonionic detergent concentrations (32). According to our titration experiments, BLV reverse transcriptase exhibits maximum activity when Triton X-100 reaches a concentration of 0.03% in the solution where protein concentration averages 1.5 mg/ml. Under the same conditions of protein concentrations, the optimal Nonidet P-40 concentration is 0.01%.

It could also be informative to investigate the metal requirements of BLV endogenous DNA synthesis. The optimum Mg^{++} concentration averages 20 mM, while Mn^{++} is ineffective at any of the concentrations tested (Fig. 2). As pointed out by Waters and Yang (35), divalent metal requirements of a given reverse transcriptase reaction primarily depends on the template-primers used. If, however, the endogenous synthesis of DNA on an RNA template is considered, the following rules seem to obtain.

(*i*) Mammalian type C viruses: DNA synthesis proceeds equally well in the presence of Mg^{++} or Mn^{++} provided optimal concentrations are used. The reaction may even be stimulated if both cations are present at a given concentration (36).

(*ii*) Type B viruses: Mg^{++} is a mandatory requirement for DNA synthesis (42).

(*iii*) MPMV (36, 42), guinea pig virus (GPV) (42), and BLV: they are morphologically different from type B viruses but share their divalent cation requirements.

Further characterization of BLV included an equilibrium density gradient centrifugation in sucrose gradients. The simultaneous detection tests performed on material banding at the indicated densities (Fig. 3) show that BLV equilibrates between 1.148 and 1.164 g/ml, a density region characteristic of type C virus particles.

The potential relatedness of BLV to other known RNA oncogenic viruses was screened by hybridization of the various viral RNAs to BLV [^{3}H]cDNA (Fig. 4) and, *mutatis mutandis*, of bovine viral RNA to the various viral [^{3}H]cDNAs. The results of this double check are quite clear-cut: AMV, MPMV, FeLV, and SSV-1 are unrelated to BLV.

Epidemiological and experimental evidence strongly suggests bovine leukemia to be an infectious disease. It was therefore of crucial interest to try to classify BLV as an exogenous or an endogenous virus. The biochemical strategy of such experiments has been designed (40) and includes a recycling step. The recycling step is a mandatory prerequisite before classification of a virus if the normal cell DNA of the species studied contains at least one copy equivalent of the viral genome. In this case, indeed one could interpret leukemogenesis as an amplification of existing DNA sequences.

That viral transformation implies *de novo* insertion of viral DNA sequences into the genome of the infected host is easy to demonstrate in cells transformed by a nonindigenous virus (43–45). That the same phenomenon holds true for cells infected by an indigenous virus, was clearly demonstrated by Baxt and Spiegelman (40) in the case of human leukemias. From studies on the leukemic member of identical twins (41), it was further concluded that leukemia specific information must have been inserted subsequent to fertilization. These facts observed in human systems have been extended to avian lymphoma (46) and leukemia (47) and to murine leukemia (29). The data we present here (Fig. 5) about bovine leukemia, a "field" leukemia, strongly suggest that leukemia specific sequences are present in the DNA of the leukemic cell.

What is the information carried by the extra sequences? From studies with a transformation defective mutant of Prague Rous sarcoma virus-C, it has been suggested (48) that the sarcoma virus adds transformation specific sequences to the DNA of normal cells. The clear-cut identification of the viral sequences responsible for each viral function should help answer the question.

In conclusion, the data presently available strongly suggest that bovine leukemia is an infectious disease both on epidemiological as well as on biochemical grounds. It seems also highly probable that it will be the first natural system in which Koch's postulate will be fulfilled.

We thank Dr. J. Gruber of the National Cancer Institute for providing viruses and Dr. M. Janowski for a gift of purified S_1 nuclease. The skillful technical assistance of P. Ridremont, G. Vandendaele, J. Severs, and L. Vanheule is also gratefully acknowledged. This work was made possible through the generous financial support of the Belgian Ministry of Agriculture, the "Caisse Générale d'Epargne et de Retraite" and the State Contract "Actions concertées". R.K. is "Aspirant FNRS"; D.P. is "Assistant C.G.E.R."; J.G. holds a fellowship from IRSIA.

1. Bendixen, H. J. (1963) *Leukosis Enzootica Bovis: Diagnostik, Epidemiologi: Bekaempelse,* Thesis Copenhagen, 164 pp.
2. Croshaw, J. E., Abt, D. A., Marshak, R. R., Hare, W. C. D., Switzer, J., Ipsen, I. & Dutcher, R. M. (1963) *Ann. N.Y. Acad. Sci.*, **108**, 1193–1202.
3. Mammerickx, M. (1972) *Ann. Med. Vet.* **116**, 647–659.
4. Mammerickx, M. & Dekegel, D. (1975) *Zentralbl. Veterinaer. med. Reihe B* **22**, 411–419.
5. Miller J. M. & Olson, C. (1972) *J. Nat. Cancer Inst.* **49**, 1459–1462.
6. Ferrer, J. F., Avila, M. & Stock, N. D. (1972) *Cancer Res.* **32**, 1864–1870.
7. Paulsen, J., Rudolph, R. & Miller, J. M. (1974) *Med. Microbiol. Immunol.* **159**, 105–114.
8. Miller, J. M. & Van der Maaten, M. J. (1974) *J. Nat. Cancer Inst.* **53**, 1699–1702.
9. Dutcher, R. M., Larkin, E. P. & Marshak, R. R. (1964) *J. Nat. Cancer Inst.* **33**, 1056–1064.
10. Wittmann, W. & Urbaneck, D. (1969) in *Leukose des Rindes: Handbuch des Virus. Infektionen bei Tieren Band V.* (Gustav Fischer, Jena), pp. 41–174.
11. Miller, J. M., Miller, L. D., Olson, C. & Gillette, K. G. (1969) *J. Nat. Cancer Inst.* **43**, 1297–1305.
12. Dutta, S. K., Larson, V. L., Sorensen, D. K., Perman, V., Weber, A. F., Hammer, R. F. & Shope, R. E. (1969) "Comparative leukemia research," *Bibl. Haematol. (Basel)* **36**, 548–554.
13. Kawakami, T. E., Moore, A. L., Theilen, G. H. & Munn, R. J. (1969) "Comparative leukemia research," *Bibl. Haematol. (Basel)* **36**, 471–475.
14. Olson, C., Hoss, H. E., Miller, J. M. & Baumgartner, L. E. (1973) *J. Am. Vet. Med. Assoc.* **163**, 355–360.
15. Van der Maaten, M. J., Miller, J. M. & Boothe, A. D. (1974) *J. Nat. Cancer Inst.* **52**, 491–497.
16. Calafat, J., Hageman, P. C. & Ressang, A. A. (1974) *J. Nat. Cancer Inst.* **52**, 1251–1257.
17. Wittmann, W. & Urbaneck, D. (1969) *Arch. Exp. Vet. Med.* **23**, 709–713.
18. Mammerickx, M. (1967–1973) *Rapports d'activité. Institut National de Recherches Vétérinaires* (Uccle, Bruxelles).
19. Mammerickx, M. (1970) *Experimenta. Anim.* **4**, 285–293.
20. Miller, L. D., Miller, J. M. & Olson, C. (1972) *J. Nat. Cancer Inst.* **48**, 423–426.
21. Olson, C., Miller, L. D., Miller, J. M. & Hoss, H. E. (1972) *J. Nat. Cancer Inst.* **49**, 1463–1467.
22. Gillespie, D. & Gallo, R. C. (1975) *Science* **188**, 802–811.
23. Stock, N. D. & Ferrer, J. F. (1972) *J. Nat. Cancer Inst.* **48**, 985–996.
24. Bishop, D. H. L., Ruprecht, R., Simpson, R. W. & Spiegelman, S. (1971) *J. Virol.* **8**, 730–741.
25. Schlom, J. & Spiegelman, S. (1971) *Science* **174**, 840–843.
26. Manly, K. F., Smoler, D. F., Bromfield, E. & Baltimore, D. (1971) *J. Virol.* **7**, 106–111.
27. Varmus, H. E., Levinson, W. E. & Bishop, J. M. (1971) *Nature New Biol.* **223**, 19–21.
28. Spiegelman, S., Burny, A., Das, M. R., Keydar, I., Schlom, J., Travnicek, M. & Watson, K. (1970) *Nature* **227**, 563–567.
29. Sweet, R. W., Goodman, N. C., Cho, J. R., Ruprecht, R. M., Redfield, R. R. & Spiegelman, S. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1705–1709.
30. Vogt, V. M. (1973) *Eur. J. Biochem.* **33**, 192–200.
31. Temin, H. M. & Mizutani, S. (1970) *Nature* **226**, 1211–1213.
32. Green, M. & Gerard, G. F. (1974) *Prog. Nucleic Acid Res. Mol. Biol.* **14**, 188–322.
33. Baltimore, D. (1970) *Nature* **226**, 1209–1211.
34. Scolnick, E., Rands, E., Aaronson, S. A. & Todaro, G. J. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1789–1796.
35. Waters, L. C. & Yang, W. K. (1974) *Cancer Res.* **34**, 2585–2593.
36. Abrell, J. N. & Gallo, R. C. (1973) *J. Virol.* **12**, 431–439.
37. Sarkar, N. H. & Moore, D. H. (1974) *J. Virol.* **13**, 1143–1147.
38. Britten, R. J. & Kohne, D. E. (1968) *Science* **161**, 529–540.
39. Fan, H. & Baltimore, D. (1973) *J. Mol. Biol.* **80**, 93–117.
40. Baxt, W. G. & Spiegelman, S. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3737–3741.
41. Baxt, W. G., Yates, J. W., Wallace, H. J., Jr., Holland, J. F. & Spiegelman, S. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2629–2632.
42. Michalides, R., Schlom, J., Dahlberg, J. & Perk, K. (1975) *J. Virol.* **16**, 1039–1050.
43. Baluda, M. A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 576–580.
44. Varmus, H. E., Vogt, P. K. & Bishop, J. M. (1973) *J. Mol. Biol.* **74**, 613–626.
45. Goodman, N. C., Ruprecht, R. M., Sweet, R. W., Massey, R., Deinhardt, F. & Spiegelman, S. (1973) *Int. J. Cancer* **12**, 752–760.
46. Neiman, P. E., Purchase, G. H. & Okazaki, W. (1975) *Cell* **4**, 311–319.
47. Shoyab, M., Evans, R. M. & Baluda, M. A. (1974) *J. Virol.* **14**, 47–49.
48. Neiman, P. E., Wright, S. E., McMillin, C. & MacDonnell, D. (1974) *J. Virol.* **13**, 837–846.