determine whether the effects were due to chemical toxicity or to viral infection. Both the bottle cultures and the overlaid cell cultures were observed daily for 14 to 21 days.

The number of viruses detected in the sewage effluents ranged from 158 to 7475 per 100 liters and averaged 2118 per 100 liters during 1974 (Table 1). The largest number of viruses was detected during August and September, and the smallest during June and July. Generally, the greatest enteric virus concentrations in sewage occur during the fall of the year, which agrees with our data. The viruses identified in the sewage effluent included seven types, which varied in occurrence with the time of year.

No viruses were detected in any of the well samples, regardless of sampling date during the 14-day flood period (Table 1). Some eluates had nonspecific cytotoxicity, which usually meant that several blind passages in cell cultures were necessary to confirm the absence of viruses. The absence of any detectable viruses in the well water concentrates indicated that the virus count was reduced by a factor of at least 10^4 (99.99 percent) during percolation of the wastewater through 3 to 9 m of the basin soil.

Our results indicate that human viral pathogens do not move through soil into the groundwater, which is significant, especially since the Flushing Meadows Project had renovated wastewater continually for 7 years at infiltration rates of about 100 m per year.

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4. Four wells at the Flushing Meadows test site were sampled: (i) East Center Well, depth 9.14 m; (ii) Well 1–2, depth 6.10 m; (iii) East Well, depth 76.2 m, with casing perforated from 3.05 to 9.14 m and a lateral flow of 22.5 m from basins 3 and 4; and (iv) Well 7, depth 6.10 m, with lateral flow from basin 6 of 33.0 m.
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6. Modifications of virus concentration methods (*5, 7*): (i) conditions for virus adsorption were *p*H 3.5 with 0.0005*M* $AlCl_3$; (ii) no clarifiers were used; (iii) the virus adsorber in the first stage of the concentration procedure consisted of a fiber glass cartridge filter (model K27R10S, Commercial Filter Division) followed by a series of Cox filters 142 mm in diameter with 5-, 1-, and 0.65-μm pore sizes; and (iv) viruses in the first eluate were reconcentrated on a series of Cox filters 90 mm in diameter with 5-, 1-, and 0.65-μm pore sizes, yielding a final, neutralized eluate volume of about 20 ml. Before freezing, 0.25 ml of fetal calf serum and penicillin and streptomycin were added to the final eluate.
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Bovine Leukemia Virus Genes in the DNA of Leukemic Cattle

Abstract. *Reverse transcripts of the RNA genome of the bovine leukemia virus (BLV) as well as ^{125}I-labeled BLV RNA hybridize to the DNA of tissues from leukemic cattle with the adult form of the disease but not to bovine thymic lymphoma or normal bovine tissues.*

Epidemiological and pedigree studies suggest that leukemia in adult cattle is an infectious disease transmitted from animal to animal (*1*). Viral particles produced by short-term cultures of buffy coat cells from leukemic cattle (*2*) have been implicated, by serological and epidemiological studies (*3, 4*), in the etiology of the adult form of leukemia in this species and have been referred to as the bovine leukemia virus (BLV). Experimental transmission of BLV to sheep has also resulted in the development of leukemia in the recipient species (*5*). BLV is an enveloped virus with many of the distinguishing properties of other mammalian and avian leukemia viruses—maturation by budding from the plasma membrane (*2*, 3), RNA genome (*6*), and viral reverse transcriptase activity (*6, 7*)—and is therefore a member of the family Retraviridae (*8*). In order to test whether the genes of BLV can be directly shown in the DNA of bovine leukemia cells and to de-

Table 1. BLV-related sequences in infected and uninfected tissues.

Source of DNA*	$C_0t_{1/2}$†	Hybrid acceleration factor‡	Relative copy number§
Bat lung cell line CCL 88			
Cell line BLV-TblLu	4×10^{-4}	12.5	1
Cell line TblLu	5×10^{-3}	1.0	<0.1
Bovine tissue and a sheep tumor			
BI434‖, leukemia-free herd, buffy coat cells	5×10^{-3}	1.0	<0.1
BH116‖, leukemia-free herd, buffy coat cells	5×10^{-3}	1.0	<0.1
BI196‖, leukemia-free herd, buffy coat cells	4×10^{-3}	0.8	<0.1
Commercial calf thymus	5×10^{-3}	1.0	<0.1
Thymic lymphoma¶	5×10^{-3}	1.0	<0.1
NBC13**, bovine lymphoid cell line	5×10^{-4}	10.0	0.8
BF239‖, multiple-case herd, buffy coat cells	1.7×10^{-4}	29.5	2.4
27-264, leukemic cow, lymph node#	4×10^{-4}	12.5	1.0
27-252, leukemic cow, lymph node#	1.7×10^{-4}	29.5	2.4
Tumor from sheep experimentally infected with BLV¶	1.7×10^{-4}	29.5	2.4
Other species			
Rat, cell line NRK (*20*)	5×10^{-3}	1.0	<0.1
Mouse, BALB/c liver	4×10^{-3}	0.8	<0.1
Guinea pig liver	5×10^{-3}	1.0	<0.1
Domestic cat liver	5×10^{-3}	1.0	<0.1
Dog thymus, cell line FCf2Th (*21*)	4×10^{-3}	0.8	<0.1
Pig kidney, ATCC (CCL 33)	5×10^{-3}	1.0	<0.1
Sheep liver	5×10^{-3}	1.0	<0.1
Human tumor, cell line A204 (*22*)	5×10^{-3}	1.0	<0.1
Rhesus, cell line DBS-FRhL-1 (*23*)	5×10^{-3}	1.0	<0.1

*DNA was extracted from the indicated source (see legend to Fig. 1). †The $C_0t_{1/2}$ is the C_0t value at which 50 percent of the [^{3}H]DNA probe has reassociated. The conditions for hybridization and S_1 analysis are described in the legend to Fig. 1. ‡The hybridization acceleration factor is the $C_0t_{1/2}$ of BLV [^{3}H]DNA with TblLu DNA/$C_0t_{1/2}$ with the indicated DNA. §The relative copy number refers to the frequency of BLV-related sequences per cellular genome normalized to that found in cell line TblLu infected with BLV. ‖The herd and cow designation of the University of Pennsylvania School of Veterinary Medicine at New Bolton Center. ¶Provided by Dr. M. J. Van Der Maaten, National Animal Disease Center, North Central Region, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa. #Leukemic cattle referred to the University of Pennsylvania Clinic, New Bolton Center, by a veterinary practitioner. **Cell line established from peripheral lymphocytes of a leukemic cow.

termine the relationship of the virus to other mammalian leukemia viruses, we have utilized the techniques of nucleic acid hybridization.

To obtain large quantities of virus, BLV was successfully transmitted to a cell line derived from bat lung, TblLu (American Type Culture Collection, CCL 88), that has been shown to be permissive for the replication of various mammalian leukemic viruses (*9, 10*). The infection was initiated by cocultivation of the bat lung cell line with fetal lamb spleen cells producing BLV (*11*).

We have found that relatively little single-stranded DNA capable of hybridizing to viral RNA is produced by the endogenous reverse transcriptase reaction in the presence of actinomycin D. Similar observations have been made with the Mason-Pfizer monkey virus (*12*) and the guinea pig leukemia virus (*13*). Consequently, we have purified the double-stranded viral DNA synthesized in this reaction, in the absence of actinomycin D, and have used it as a probe for the hybridization studies. Double-stranded DNA probes can be used to detect viral-related sequences in the cellular genome, as previously shown for the DNA virus SV40 (*14*) as well as for the mammalian (*15*) and avian leukemia viruses (*16*). In such studies the presence of viral-related DNA sequences is detected by measuring the effect of unlabeled cellular DNA on the kinetics of self-annealing of the labeled DNA probe. Cellular DNA's that contain viral-related sequences increase the rate of self-annealing of the probe, and thereby decrease the $C_0t_{1/2}$ of the reaction (see Table 1, footnote †). The DNA from BLV-TblLu, but not from the uninfected bat cell line, decreases the $C_0t_{1/2}$ of the BLV [^{3}H]DNA self-annealing reaction by a factor of 12.5, from 5×10^{-3} to 4×10^{-4}. For the purposes of comparison (Table 1), we have normalized the frequency of viral-related sequences in the cellular DNA's tested to that of the BLV-infected bat lung cells. Buffy coat cell DNA from leukemic cattle of different multiple case herds or tumor tissue from leukemic cattle referred to the University of Pennsylvania clinic contained from 0.8 to 2.5 times the number of copies of BLV genome per cellular genome as compared to the tissue culture infected cells (Fig. 1 and Table 1). Further, DNA from a tumor in a sheep experimentally infected with BLV also contains nucleic acid sequences related to the BLV genome. In contrast, buffy coat cell DNA from cows in leukemia-free herds or commercial calf thymus DNA had no effect on the self-annealing of BLV [^{3}H]DNA. Thymic lymphoma in cattle, unlike bovine leukemia, has not been associated with BLV infection by serological and epidemiological studies (*17*); our results support this conclusion in that the DNA from a bovine thymic lymphoma lacked any detectable sequences related to the BLV genome.

To explore the possibility that BLV nucleic acid sequences not represented in the BLV [^{3}H]DNA probe are present in the normal bovine genome, BLV [^{125}I]RNA was also used as a probe. The hybridization kinetics of this probe with buffy coat cell DNA of a leukemic and a normal cow, as well as from the BLV-infected and uninfected cell line, TblLu, are shown in Fig. 2. Although hybridization did not reach 100 percent because of the more rapid rate of DNA:DNA reassociation as compared to RNA:DNA

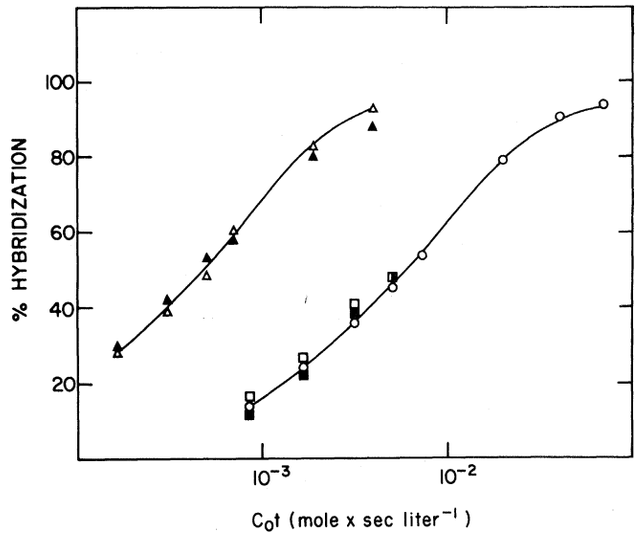

Fig. 1. The reassociation kinetics of BLV [^{3}H]DNA in the presence of DNA from BLV-producing and nonproducing cells. [^{3}H] Cytidine - labeled DNA was synthesized in the endogenous reverse transcriptase reaction with detergent-disrupted virus. The BLV used in the reaction had been banded on a sucrose gradient prior to use. The reaction mixture (*24*) contained two modifications: the magnesium acetate concentration was 20 m*M*, and no actinomycin D was added. After incubation at 37°C for 2 hours the product was deproteinized and further purified by fractionation on hydroxyapatite and chromatography on Sephadex G-50 (*24*). The specific activity of the [^{3}H]DNA was 1×10^7 count/min per microgram. The BLV [^{3}H]DNA had a sedimentation coefficient of 8*S* on an alkaline sucrose gradient. The amount of the viral genome represented in the [^{3}H]DNA as measured by its ability to protect BLV [^{125}I]RNA from ribonuclease digestion is 25 percent when the ratio of DNA to RNA was 40. Higher ratios of DNA to RNA did not appreciably increase this value. Nuclear DNA was extracted from cells as described (*25*). The hybridization reaction mixture contained 0.01*M* tris (*p*H 7.4), 0.4*M* to 0.7*M* NaCl, 2×10^{-3}*M* EDTA, 0.05 percent sodium dodecyl sulfate, 4 mg of nuclear DNA per milliliter, and 2 or 12 ng of BLV [^{3}H]DNA per milliliter. Hybridizations were initiated by heating the reaction mixtures to 98°C for 10 minutes, cooling on ice, and incubating at 65°C. Samples (0.05 ml) were taken at various times and were analyzed with the single-strand nuclease S_1 (*25*). C_0t values (C_0 is the concentration of BLV [^{3}H]DNA in moles of nucleotide per liter and t is the time in seconds) were calculated (*26*) and corrected to a monovalent cation concentration of 0.18*M* (*27*). BLV [^{3}H]DNA at either 2 ng/ml (□) or 12 ng/ml (○) was reannealed with DNA from cell line TblLu. DNA from NBC13, bovine leukemic lymphoid cell line (△), BI434 normal buffy coat cells (■), and cell line BLV-TblLu (▲) were tested for their effect on the self-annealing reaction of BLV [^{3}H]DNA at 2 ng/ml.

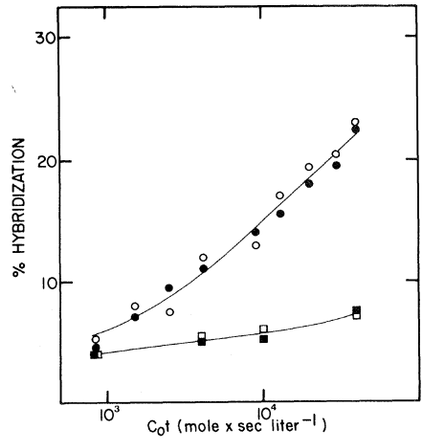

Fig. 2. Hybridization of BLV 70*S* [^{125}I]RNA to DNA extracted from normal and leukemia buffy coat cells and from normal TblLu cells and cells infected with BLV. The 70*S* RNA from banded virus was iodinated by a modification of the Commerford method (*28*) as described by Colcher *et al.* (*29*). The specific activity of the [^{125}I]RNA was 1×10^7 count/min per microgram. The hybridization reaction mixture contained 0.01*M* tris (*p*H 7.4), 2×10^{-3}*M* EDTA, 0.05 percent sodium dodecyl sulfate, 10 μg of yeast tRNA per milliliter, 2 ng of [^{125}I]RNA per milliliter, 5 mg of DNA per milliliter, and 1.0*M* NaCl. Hybridizations were initiated by heating the reaction mixture to 98°C for 10 minutes, cooling on ice, and incubating at 65°C. Duplicate samples (0.05 ml) were removed at intervals; the extent of hybridization was determined by digestion with ribonucleases A and T_1 (*29*). The BLV [^{125}I]RNA was hybridized with nuclear DNA extracted from: BLV-infected cell line TblLu (●); cell line TblLu (□); NBC13, bovine leukemic lymphoid cell line (○); and BI434, normal buffy coat cells (■).

hybridization (*18*), the BLV [^{125}I]RNA hybridized only with the DNA's from the tissues of the leukemic cows and from infected cell culture lines. We conclude from these results that the BLV genome is not endogenous to the bovine cellular genome.

These results are consistent with the acquisition of the BLV genome by leukemic cattle as a result of the horizontal transmission of the virus from some other species. The BLV [^{3}H]DNA probe was therefore used to examine the cellular DNA of other species for the presence of BLV-related sequences. Some of these species live side by side with cattle and might thus be the source of BLV. None of the species tested contain nucleic acid sequences related to the BLV genome (Table 1). We conclude that the BLV genome is not highly related to any of the endogenous type C viral genomes of the species tested. Similar results have been obtained by Kettman *et al.* (*6*), and are consistent with immunological studies showing a lack of relationship of the major BLV structural protein to that of other mammalian type C viruses (*7*, *19*).

In summary, we have shown by nucleic acid hybridization that the BLV genome is not endogenous to the cellular genome of normal cattle. The etiologic agent causing lymphosarcomas or leukemia in this species is an infectious virus derived from another as yet unknown species. This is, therefore, one species where eradication of the vectors for virus spread should lead to prevention of the disease.

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α-Methylphenylalanine, a New Inducer of Chronic Hyperphenylalaninemia in Suckling Rats

Abstract. *α-Methylphenylalanine reduces the phenylalanine hydroxylase activity of rat liver by 75 percent. Daily injections of this substance (plus phenylalanine) into rats from the 3rd to 15th day of age had no obvious toxic effects, and maintained a plasma concentration of phenylalanine comparable to that of phenylketonuric subjects.*

The first report of an agent, *p*-chlorophenylalanine, which could effectively diminish the rate of clearance of ingested phenylalanine, appeared in 1966 (*1*). Since then rats treated with *p*-chlorophenylalanine provided the most promising animal models (*2*) for phenylketonuria, the condition of mental deficiency associated with the absence (from genetic causes) of phenylalanine hydroxylase (*3*). A general problem in imitating the consequences of this absence of an enzyme in experimental animals is that the agents used to inhibit that enzyme are not completely specific. *p*-Chlorophenylalanine, for example, decreases the activity of tryptophan hydroxylase in the brain (*4*) as well as that of the phenylalanine hydroxylase in the livers of rats, and thus one might question whether the various cerebral abnormalities observed were caused by the hyperphenylalaninemia itself. The new inducer of hyperphenylalaninemia described here, α-methylphenylalanine, has a different chemical structure, and its side effects, if any, are unlikely to be identical to those of *p*-chlorophenylalanine. The study of rats treated with α-methylphenylalanine is thus important because abnormalities in common with those in rats treated with *p*-chlorophenylalanine are the ones most likely to be specifically associated with hyperphenylalaninemia, the common metabolic effect of the two agents.

The hepatic phenylalanine hydroxylase activity was determined (*5*) in an assay system supplemented with substrate, pteridine cofactor, and dithiothreitol. The activity of liver extracts was not inhibited by the addition of 0.8 to 8 m*M* α-methylphenylalanine in vitro (nor did the extracts catalyze the conversion of this analog to α-methyltyrosine). However, the phenylalanine hydroxylase activity in the livers of adult or immature rats injected with α-methylphenylalanine decreased. Since the purpose was to maintain hyperphenylalaninemia throughout early postnatal life (in the rat the first 15 days), we determined the response to various doses of α-methylphenylalanine on 6-day-old rats—an age when the phenylalanine hydroxylase content is about 60 percent of that in adult liver (*5*); maximal inhibition occurred with 24 μmole/10 g (Fig. 1). Additional experiments indicated that at least 20 hours were required to attain this minimal level. The content of cerebral serotonin (254 and 205 ng/g) and that of 5-hydroxyindoleacetic acid (303 and 324 ng/g) in two of these rats were not different from those in two control rats (249 and 243, and 318 and 342, respectively) (*6*). [In contrast, the