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Evidence for Retrovirus in Miniature Swine with Radiation-Induced Leukemia or Metaplasia*

By

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With 5 Figures

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Summary

Biochemical and morphological evidence indicates that a type-C retrovirus is present in the blood of swine (both leukemic and nonleukemic) exposed to strontium-90 radiation. Nonexposed swine that were leukemic also had virus present. The virus was shown to contain an RNase-sensitive DNA polymerase activity with cation, detergent and template requirements similar to those of known viral reverse transcriptases. The buoyant density of the virus was 1.14 to 1.16 g/ml, which can be converted, by treatment with ether, to a virion core having a density of 1.20 to 1.23 g/ml.

Linear regression analysis indicated a correlation between virus-associated DNA polymerase activity and the number of blast cells in the peripheral blood.

Introduction

Strontium-90 is a component of fallout from nuclear weapons which is known to enter the food chain of man. This radionuclide, which competes with calcium for incorporation into bone, has a half-life of 27 years and emits 0.5 MeV beta irradiation. The bone-seeking nature of ^{90}Sr, as well as reports that exposure (by injection) of rats (10) and dogs (9) to ^{90}Sr-induced (primarily) bone sarcomas, led to the prediction that bone tumors would be the primary effect of exposure to ^{90}Sr. However, these animal experiments were performed with single or multiple injections of ^{90}Sr. Administering ^{90}Sr in this manner caused uneven deposition of the radionuclide in the

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bone, severe local damage and subsequent tumor development at the sites of heavy deposition and damage. In contrast, environmental exposure of humans to ^{90}Sr is likely to be chronic, via ingestion of low levels of radiation, resulting in a different temporal and spatial distribution of dose. Therefore, to simulate the most probable route of entry and distribution of ^{90}Sr in man, a study of the effects of chronic (daily) ingestion of ^{90}Sr in swine was performed at this laboratory.

Miniature swine were used in these studies because they are omnivores that have a mature weight, dietary requirement, gastrointestinal tract and bone mass similar to man. These physiological and anatomical similarities, in addition to their 14- to 15-year life span, make swine an excellent large-animal model for assessing the toxicity of ingested ^{90}Sr.

In the studies at this laboratory (described above), instead of developing large numbers of bone tumors, as expected, the swine were stricken by a variety of hematopoietic disorders. The spectrum of induced diseases included myeloid metaplasia, myeloblastic and granulocytic leukemia, and lymphoblastic leukemia. Only a few animals developed malignant osteosarcomas (6, 18, 19). The incidence of leukemia in various experimental groups ranged from a low of 4 percent in aged control animals to nearly 80 percent in animals exposed to ^{90}Sr in utero (the F_1 and F_2 generations) and to 125 μCi of ^{90}Sr per day throughout their lifetime.

Because viruses have been shown to cause the induction of leukemia in several animal species, and because murine leukemias induced by X-irradiation may be transmitted by cell-free filtrates (16, 24), the possibility exists that radiation "activates", in some manner, the expression of quiescent, endogenous retrovirus genomes. This idea is supported by the observation that Type-C retrovirus has also been reported in association with "radiation-induced" leukemias in several strains of mice (17) and rats (20). In addition, retrovirus has been implicated in the osteogenic sarcoma induced in mice following exposure to radiation (10, 11, 12). Our working hypothesis was that a retrovirus is an etiologic factor in ^{90}Sr-induced hematopoietic disorders of swine.

Materials and Methods

Animals

Three generations of female, Pitman-Moore miniature swine received feed containing ^{90}Sr levels ranging from 1 to 3100 μCi/day. The original dams were fed ^{90}Sr daily, starting at 9 months of age, and were bred to unexposed males. Strontium-90 levels in the feed of their female offspring were gradually raised, commensurate with growth and food intake, to reach the same ^{90}Sr level as the dams by 6 months of age. This F_1 generation was also bred (to unexposed males) to provide an F_2 generation, which was exposed to ^{90}Sr in the same way. Additional details of this study are provided elsewhere (6, 18, 19, 26). Since the virus study was initiated late in the animal experiments, many animals had already died, and we were unable to examine

all of the original animals for virus. The ^{90}Sr exposures were determined by skeletal and soft-tissue radioanalysis. These data were utilized to calculate average dose rates to bone for the exposed animals, using methods detailed elsewhere (26).

Electron Microscopy

Virus was purified from plasma by low-speed ($4{,}000 \times g$) preclarification for 10 minutes, followed by pelleting ($100{,}000 \times g$) for 3 hours. The virus pellet was washed with TNE (10 mM Tris, 150 mM NaCl and 1 mM EDTA; pH 7.8) and resuspended in 2 percent glutaraldehyde in 0.1 M sodium cacodylate, which was adjusted to a pH of 7.5 with 0.1 M HCl. After 16 hours of fixation, the virus was washed with two changes of buffer and post-fixed for 40 minutes in 1 percent osmium tetroxide. The pellet was then washed three times with cacodylate buffer, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (25). After sectioning, the sample was placed on a grid and stained with uranyl acetate and lead citrate (27).

Virus Purification

Plasma was layered on 8 ml of 30 percent glycerol-TNE over a 6-ml cushion of 100 percent glycerol, and centrifuged. The 100 percent glycerol cushion was then removed, and the plasma was resuspended in TNE and layered onto a 25 to 60 percent sucrose-TNE linear gradient. After centrifuging at $98{,}000 \times g$ at 4° C for 16 hours, fractions of the gradient were collected, densities were determined and two 50-μl portions of the fraction were assayed for RNA-dependent DNA polymerase (RDDP) activity. The active fractions were pooled, diluted in TNE and centrifuged at $98{,}000 \times g$ for 2 hours. The resultant pellets were used as a source of material for RDDP assays.

RDDP Assays

The viral pellet was diluted in 0.01 M Tris (pH 8.3) to a protein concentration of approximately 1 mg/ml. Fifty μl of this solution were diluted sufficiently to contain 0.01 percent Triton X-100 and 25 mM dithiothreitol. The standard endogenous templated reaction mixture (100 μl) contained the solubilized virus and the following concentrations of reagents: 62.5 mM Tris HCl (pH 8.3); 80 mM NaCl; 10 mM $MgCl_2$; 500 μM each dGTP, dATP, dCTP; 50 μM ^{3}H-TTP (50 Ci/mM); 100 μg/ml actinomycin D; and 50 μg/ml distamycin A. The reaction was carried out at 37° C in a volume of 100 μl and was stopped by adding 1 percent SDS and 0.4 M NaCl.

The polymerase assays which utilize "activated" DNA as the template consisted of 200 mM Tris HCl (pH 8.3), 25 mM KCl, 1 mM dithiothreitol, 0.1 mM dATP, 0.1 mM dCTP, 0.1 mM dGTP, 15 mM Mg $(CH_3CO_2)_2$, 0.5 μg "nicked" calf thymus DNA, 5.7×10^6 cpm of ^{3}H-TTP or ^{3}H-dGTP (specific activity, 40 Ci/mM), 50 μg disrupted protein (virus) and H_2O to a final volume of 100 μl.

Assays for DNA polymerase activity which utilize Poly $(A)_n \cdot$ oligo $(dT)_{12-18}$, Poly $(Am)_n \cdot$ oligo $(dT)_{12-18}$, or Poly $(dA)_n \cdot$ oligo $(dT)_{12-18}$ as the exogenous synthetic template and primer consisted of 200 mM Tris HCl (pH 8.3), 2 mM Mn $(CH_3CO_2)_2$, 50 mM KCl, 5 μg of the desired synthetic template-primer [Poly $(A)_n \cdot$ oligo $(dT)_{12-18}$, Poly $(Am)_n \cdot$ oligo $(dT)_{12-18}$, or Poly $(dA)_n \cdot$ oligo $(dT)_{12-18}$], 1 mM dithiothreitol, 5.7×10^6 cpm of ^{3}H-TTP (specific activity, 40 Ci/mM), 50 μg disrupted protein (virus) and H_2O to a final volume of 100 μl.

DNA polymerase assays, in which Poly $(Cm)_n \cdot$ oligo $(dG)_{12-18}$, Poly $(C)_n \cdot$ oligo $(dG)_{12-18}$, or Poly $(dC)_n \cdot$ oligo $(dG)_{12-18}$ are used as the exogenous synthetic template-primer, contain 200 mM Tris HCl (pH 8.3), 2 mM Mn $(CH_3CO_2)_2$, 50 mM KCl, 5 μg of synthetic template-primer [Poly $(Cm)_n \cdot$ oligo $(dG)_{12-18}$, Poly $(C)_n \cdot$ oligo $(dT)_{12-18}$ or Poly $(dC)_n \cdot$ oligo $(dG)_{12-18}$], 1 mM dithiothreitol, 5.7×10^6 cpm of ^{3}H-dGTP (specific

activity, 40 Ci/mM), 50 μg disrupted protein (virus) and H_2O to a final volume of 100 μl.

Trichloroacetic acid was added to the samples to give a concentration of 10 percent. Ten μg yeast RNA were also added, and acid-precipitable radioactivity was assayed as described by SPIEGELMAN *et al.* (30). In kinetics experiments, 10-μl aliquots were removed from the reaction mixture at specified time intervals and assayed as above.

Analysis of RDDP Product

Prior to assay by simultaneous detection (28), the samples resulting from a 15-minute incubation of the endogenous RDDP assay mixture were cleared of protein by three phenol-creosol (1:1) extractions. One aliquot of the ^{3}H-DNA product was then treated with RNase A and RNase T_1 (20 μg/ml each), and another aliquot was treated with an equivalent amount of water for 30 minutes, at low-salt concentrations, at 37° C. Following a second extraction to remove RNase, the samples were layered on a 10 to 30 percent glycerol-TNE gradient and centrifuged at 200,000 × *g* for 3 hours at 4° C. Acid-precipitable radioactivity was then assayed from fractions of the glycerol gradient.

Cesium sulfate gradient analysis of the ^{3}H-DNA product was also carried out, using a portion of the 15-minute RDDP reaction mixture (described above) that had been cleared of excess protein. Unincorporated, labeled triphosphates were removed by passing the sample through a G-50 Sephadex column. The ^{3}H-DNA material was pooled, alcohol-precipitated at −20° C for 16 hours and centrifuged at 25,000 × *g* for 45 minutes at −20° C. Approximately 1000 cpm of the pelleted ^{3}H-DNA material were analyzed in each cesium sulfate gradient (21). Separate samples of the ^{3}H-DNA were alkali-digested in 0.4 M NaOH for 3 hours at 37° C, heated to 68° C in formamide, or treated with RNase A and RNase T_1, to destroy RNA:DNA hybrids. These samples were also analyzed using cesium sulfate gradients.

Ether Treatment of Viral Pellets

Previous studies have shown that treatment of retroviruses with ether will generate virus cores (8, 22, 29). The viral cores (containing RDDP) migrate to a denser region of a linear sucrose gradient, thus allowing them to be differentiated from membrane-associated cellular DNA polymerase.

A viral pellet was obtained, as described above, and divided into two samples. One portion was diluted with 2 ml ether in 1 ml TNE; the other portion was diluted in 1 ml TNE. The two mixtures were agitated for 30 minutes at 4° C. The ether phase was removed and the TNE virus fractions were bubbled gently at 4° C for 30 minutes with nitrogen. The samples were then centrifuged at 1.000,000 × *g* for 60 minutes. The resulting pellets were resuspended in TNE to a volume of 1 ml. These samples were then layered on separate 25 to 60 percent sucrose (TNE) gradients and centrifuged at 98,000 × *g* for 12 hours at 4° C. Fractions were collected from the bottom of the tube and assayed for RDDP activity.

Results

Examination of plasma from leukemic swine revealed viruses with typical C-type morphology (Fig. 1). The particles were about 100 nm in diameter, appeared to be enveloped, and had a centrally located, dense nucleoid region. Virus particles were readily observed in the electron micrographs of many plasma samples. Purification and concentration of

virus using sucrose gradients aided in detecting low concentrations of retrovirus and also provided an estimation of the buoyant density of these viruses.

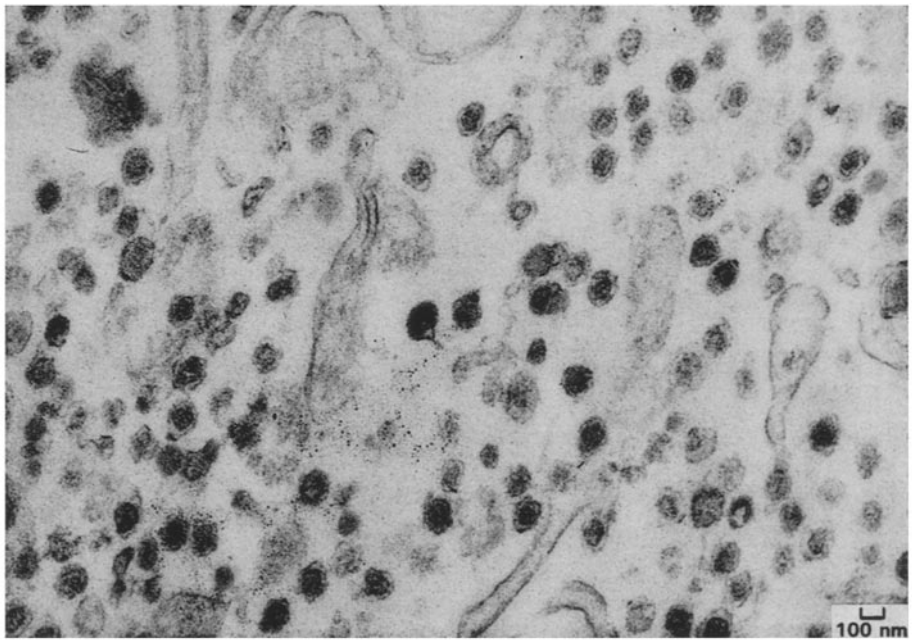

Fig. 1. Electron micrograph of virus particles in a pellet (centrifuged for 3 hours at 100,000 × *g*) of plasma from a leukemic pig. Marker bar = 100 nm

Fig. 2A shows actinomycin-D-resistant, DNA polymerase activity in fractions from an isopyknic sucrose gradient of a plasma pellet from a pig (#1850) with myelogenous leukemia. A peak of RNase-sensitive DNA polymerase activity is apparent in the fractions having a density of 1.12 to 1.17 g/ml. This activity is in the same fractions as the virus-like particles observed in the plasma. In order to further differentiate the virus-associated DNA polymerase from membrane-bound cellular DNA polymerase, a portion of the original pellet was treated with ether to remove the lipid component, and the resultant material was examined on another sucrose gradient. Following ether treatment, the DNA polymerase activity sedimented in a denser (1.20 to 1.23 g/ml) region of the gradient (Fig. 2B), providing evidence that the observed polymerase activity was associated with a virus core structure. We came to this conclusion because cellular DNA-dependent, DNA polymerases are solubilized by detergent treatment and remain at the top of the gradient (7), rather than at a more dense region (as do core-associated DNA polymerases).

In an attempt to further differentiate our virus-associated DNA polymerase activity from cellular polymerases, we varied several parameters

of the reaction mixture in order to determine the requirements of this enzyme. For each of the various parameters, ^{3}H-TTP incorporation was measured at 0, 15, 30, 45 and 60 minutes. The rate of incorporation of ^{3}H-TTP into an acid-precipitable product was maximal and linear between 15 and 45 minutes. The incorporation of ^{3}H-TTP was ~90 percent RNase-sensitive. The omission of one of the nucleotide triphosphates markedly decreased the rate of the reaction, indicating that a heteropolymeric nucleic

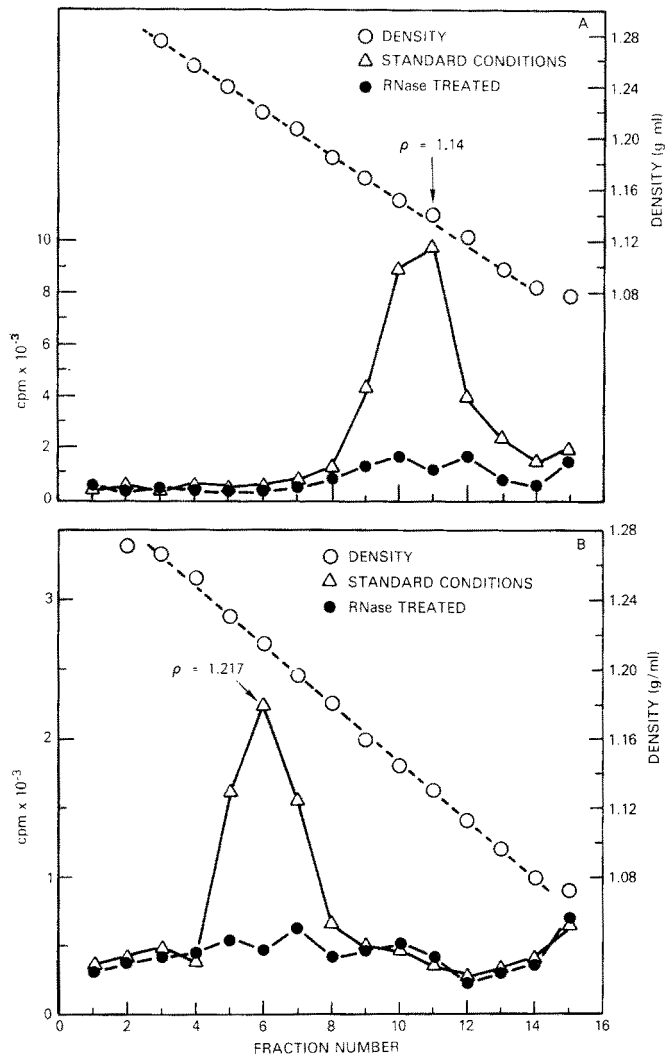

Fig. 2. Effect of ether treatment of endogenous DNA polymerase activity of particulate fractions obtained from plasma from a leukemic pig. Control *(A)* and ether-treated *(B)* samples were subjected to isopyknic sucrose gradient analysis. The DNA polymerase activity in 50-μl samples was assayed following incubation of the solubilized (Triton-treated) gradient fraction with RNase (○) or buffer (△)

Table 1. *DNA polymerase activity of purified porcine virus*

Condition	Substrate	^{3}H Incorporated, pmole[a]
Endogenous reaction		
Complete reaction mixture[b]		3.1
Minus divalent cation (Mg^+ or Mn^+)		0.06
Minus dATP		>0.01
Minus dGTP		>0.01
Minus oligo $(dT)_{12-18}$		0.97
Plus RNase A and T_1		0.2
Minus actinomycin D and distamycin A		6.3
Minus Triton X-100		0.5
Exogenous templates and primers[c]		
Poly $(A)_n$ · oligo $(dT)_{12-18}$	[^{3}H]dTTP	27.8
Poly $(Am)_n$ · oligo $(dT)_{12-18}$	[^{3}H]dTTP	2.64
Poly $(dA)_n$ · oligo $(dT)_{12-18}$	[^{3}H]dTTP	0.21
Poly $(Cm)_n$ · oligo $(dG)_{12-18}$	[^{3}H]dTGP	3.06
Poly $(C)_n$ · oligo $(dG)_{12-18}$	[^{3}H]dTGP	2.5
Poly $(dC)_n$ · oligo $(dG)_{12-18}$	[^{3}H]dTGP	7.16
Activated DNA	[^{3}H]dTGP	0.003
Activated DNA	[^{3}H]dTTP	0.08
mRNA · oligo $(dT)_{12-18}$	[^{3}H]dTTP	0.26

[a] Enzyme activity is expressed as pmole of ^{3}H-labeled TTP incorporated per 10 µl of reaction mixture at 37° C in a 60-minute reaction

[b] Standard reaction conditions are described in Materials and Methods

[c] RDDP assays using exogenous templates are described in Materials and Methods

acid was being synthesized, thus ruling out the possibility that the enzyme is a terminal deoxynucleotidyl transferase (5). These data, combined with the information in Table 1, elucidate the requirements and template preferences of the DNA polymerase. The virus-associated enzyme activity also required a divalent cation for optimal synthesis. Furthermore, the DNA polymerase did not initiate *de novo* DNA synthesis without the presence of an oligodeoxynucleotide primer to provide a 3′OH terminus. Finally, polymerase activity was greatest only when solubilized by low concentrations (0.01 percent) of Triton X-100, suggesting that the virus must be disrupted for maximal activity.

Optimal incorporation of ^{3}H-TTP was obtained by excluding from the reaction mixture actinomycin D and distamycin A, which inhibit DNA-dependent DNA synthesis. However, even though the inclusion of these antibiotics results in about 50 percent less ^{3}H-TTP being incorporated, they facilitate the detection of RNA:DNA hybrids and were therefore used in other procedures in this experiment.

In addition to endogenous RNA, several synthetic polyribonucleotide homopolymers have been shown to be adequate template-primer combi-

nations for an RDDP reaction (Table 1). Although these templates are considered to be the most discriminating in distinguishing between RDDP and normal, cellular DNA polymerases, they are not (by themselves) considered diagnostic. Nonetheless, our virus-associated enzyme preferred these synthetic RNA-DNA hybrids. Furthermore, the capacity of this enzyme to copy a natural (primed) RNA template, even at 10 percent of the level of the endogenous reaction, is also proof that the enzyme is a viral reverse transcriptase (RDDP).

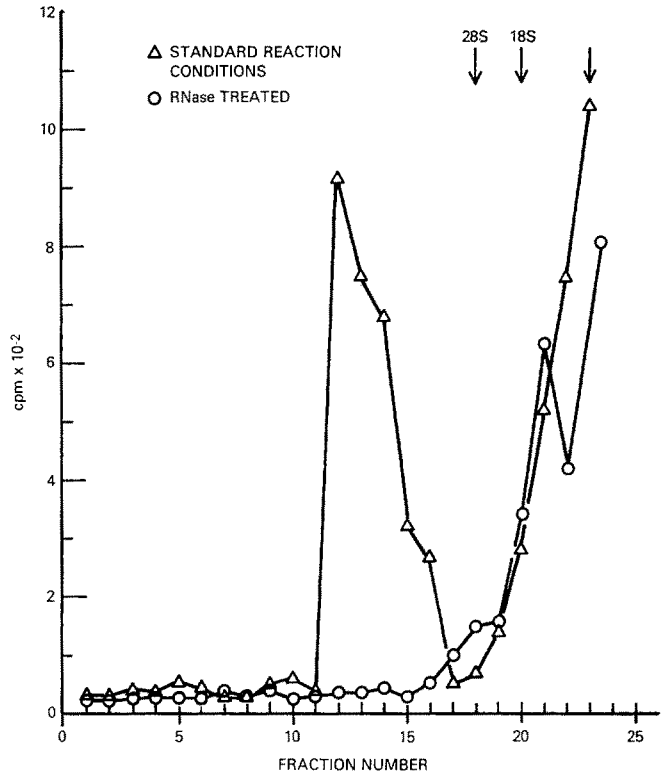

Fig. 3. Sedimentation velocity analysis of porcine virus endogenous DNA polymerase product in a 10 to 30 percent glycerol gradient. External markers (^{3}H-labeled 28S, 18S and 4S RNA from NC-37 cells) were used to estimate the sedimentation coefficient

We examined the early (15-minute) product from endogenous RDDP reactions to determine whether it was a result of an RNA-templated DNA polymerase or an RNA-primed DNA polymerase reaction.

Results of the first method used to examine the reaction products from endogenous RDDP reactions in sucrose velocity gradients (28) are shown in Fig. 3. The newly incorporated ^{3}H-TTP was found in a region of the gradient corresponding to 60 to 70S particles, a sedimentation coefficient similar to that expected from the viral RNA template. Following treatment

with RNase, the acid-precipitable radioactivity, representing small (5—7 S) ^{3}H-DNA, shifted to the top of the gradient, indicating that the newly synthesized DNA had indeed been associated with a high-molecular-weight RNA.

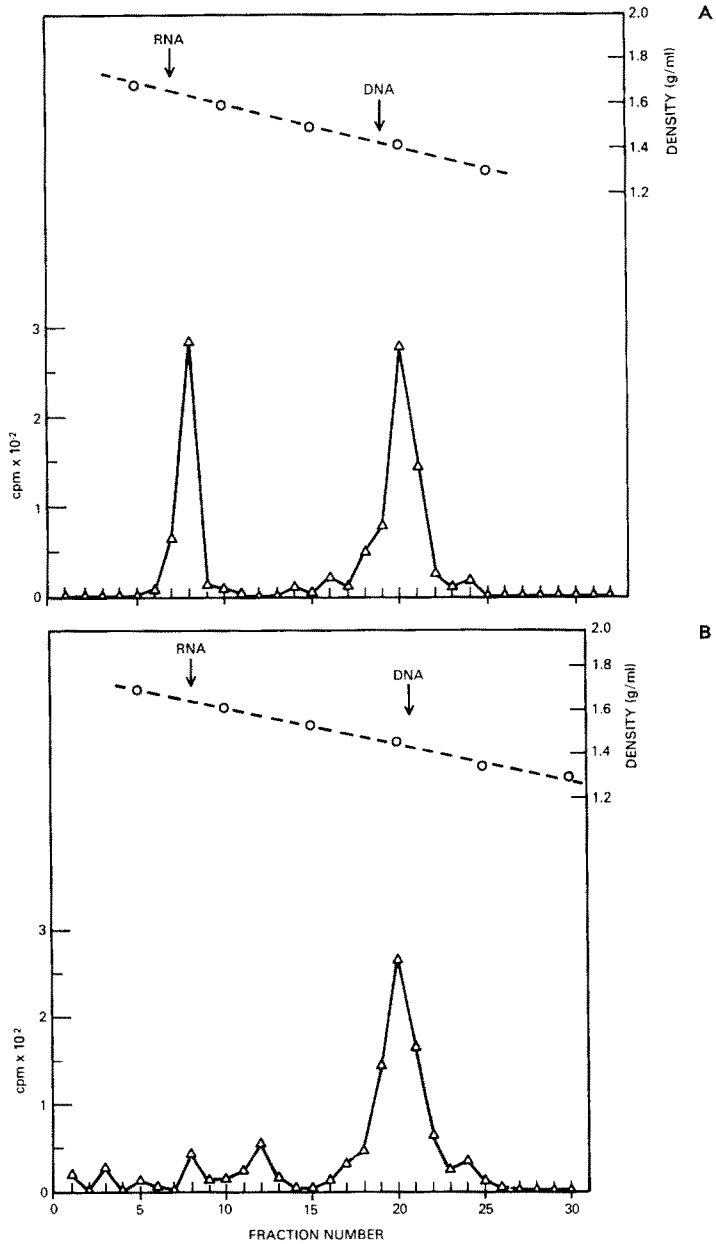

Fig. 4. Cesium sulfate equilibrium density gradient analysis of porcine virus endogenous DNA polymerase product *(A)*. Equivalent amounts of product were treated with heat *(B)*

In a second analysis, when the density of the reaction product was examined in Cs_2SO_4 density gradients, a peak of acid-precipitable radioactivity was observed in regions of the gradient where RNA would normally be found (1.65 g/ml, Fig. 4A). The radioactivity could be shifted to the DNA region of the gradient (1.45 g/ml) by conditions that disrupt hydrogen bonds, e.g., heat (Fig. 4B) or alkali treatment, or by addition of RNase, which hydrolyzes RNA (data not shown). These are the results expected if

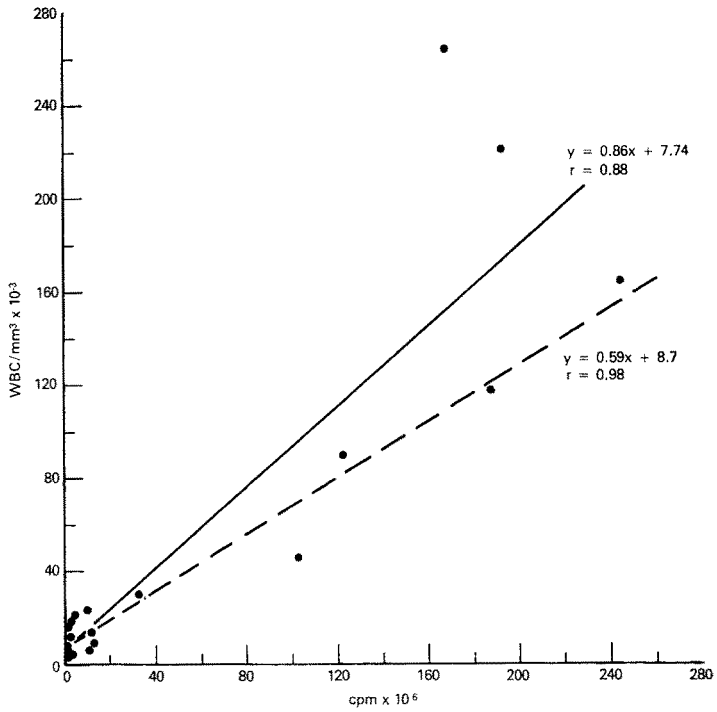

Fig. 5. White blood cell counts from 19 leukemic swine, plotted versus the virus-associated, DNA polymerase activity from the same animals. Linear regression analysis of these data indicated 0.88 correlation *(r)* between these variables. Removal of data for the two animals with the highest WBC counts increased the correlation to 0.98

the ^{3}H-DNA product is hydrogen bonded to an RNA molecule (presumably, its template). The hybrid bands in the RNA or hybrid region in these gradients because the density of the larger (70S RNA) molecule prevails.

Extracellular virus particles appeared to be present in the plasma in increasing numbers at the same time that peripheral white blood cell (WBC) levels were high. Independent confirmation of this association between virus and WBC count was found when we examined the levels of virus-particle-associated DNA polymerase relative to the number of blast cells in the peripheral blood of 19 leukemic animals. When examined by

Table 2. *Feeding regime, accumulated radiation dose and histopathological diagnosis of a selected group of leukemic swine*

Animal number	^{90}Sr feeding level (μCi/day)	Age at death (days)	Accumulated dosage (rad)[a]	Diagnosis[b]
77	25	3699	5,918	MPD
374	0	3194	NMD[c]	MPD
384	25	2789	5,032	LPD, UTL
401	25	3511	6,331	CML
489	0	3149	NMD	MPD, ML
612	1	2457	177	ML
695	25	4261	7,681	ML
883	5	4838	1,744	CLL
898	5	2456	886	ML, UTL, H
977	125	2055	16,440	MPD, UA, P
982	125	2046	16,368	MPD
1257	125	1726	19,043	MPD, P
1269	0	3649	NMD	MPD
1347	0	2979	NMD	MPD
1845	125	843	9,330	LPD, T
1850	125	1076	11,893	MPD, P
1857	125	1049	11,596	MPD
3128	313	1031	117	MPD
3499	—[d]	1034	NMD	MPD

[a] Determined using method of PALMER *et al.* (1970)

[b] MPD = myeloproliferative disorder
LPD = lymphoproliferative disorder
UTL = uterine leiomyoma
CML = chronic myelogenous leukemia
CLL = chronic lymphocytic leukemia
ML = malignant lymphoma
UA = uterine adenocarcinoma
P = pneumonia
T = thymoma
H = hepatoma

[c] No measurable dose

[d] Animal exposed in utero

linear regression analysis, the data (Fig. 5) indicated a positive correlation ($r = 0.88$) between enzyme activity and WBC counts.

A further analysis of the data, from which counts of the two animals with the highest WBC levels were excluded, shows an even stronger correlation. This analysis indicates that the presence of those two high data points was not the only reason for the positive correlation.

These data indicate a relationship between disease state and the number of virus particles; however, there appears to be no clear correlation between the type of leukemia found and the radiation dose the animal received (Table 2).

A survey of 58 miniature swine (Table 3), 34 of which had been fed ^{90}Sr over an extended period, revealed that, of 23 animals with either malignant myeloproliferative or lymphoproliferative disorders, 22 had de-

Table 3. *Detection of retrovirus-like particles in plasma of irradiated and unirradiated miniature swine using the electron microscope and biochemical assays for RNA-instructed DNA polymerase*

	Diagnosis	Number of swine examined	Number of swine with detectable virus
^{90}Sr-exposed miniature swine	MPD[a]	12	11
	LPD[b]	7	7
	MM[c]	10	5
	Other tumors	5	2[d]
Unexposed miniature swine	MPD	4	4
	Other tumors	8	0
	NDP[e]	12	0

[a] Myeloproliferative disorders
[b] Lymphoproliferative disorders
[c] Myeloid metaplasia
[d] Bone tumors
[e] No detectable pathology

tectable virus in their plasma. The four animals with "spontaneously occurring" leukemia, which had not been exposed to ^{90}Sr, had virus particles and associated RDDP. The virus also appeared to be present in some animals (5 of 10 swine) with myeloid metaplasia, indicating that this may represent a premalignant condition (13). In contrast, using the same experimental techniques, no virus was detected in 12 control (unexposed) swine or in eight control swine with spontaneously developing uterine tumors. All ^{90}Sr-exposed control animals examined had pathological lesions.

Discussion

If, indeed, the virus causes leukemia in swine that did not receive radiation exposure, this system could serve as a large-animal model for studying myeloproliferative disorders. The ease with which sampling (blood and bone marrow) can be conducted would facilitate the study of drug therapy and of the development of drug resistance.

We have demonstrated that a virus is associated with ^{90}Sr-induced leukemia in miniature swine. As evidence that it is a retrovirus, we have demonstrated that it is morphologically similar to other known retroviruses; that the virus has a density characteristic of these agents; and, as in other retroviruses, that the virus density increases following gentle ether treatment to remove the lipid envelope. We have also demonstrated that the virus has a high-molecular-weight (60 to 70S) RNA genome and a virion-associated RDDP. This virus has not been found in "normal" swine, nor in nonirradiated swine with neoplasms (other than those that were

hematopoietic in origin), but it has been found in irradiated swine with myeloid metaplasia. These studies are of particular interest in view of the observation that retrovirus have been found in a number of stable porcine cell lines (1, 3, 4, 15, 23). In addition, studies have shown that Type-C viral genes are present in the family *Suidea* (31). These sequences apparently result from trans-species infection by an ancestral xerotropic murine virus and, as a result, porcine cellular DNA possess multiple copies of retrovirus gene sequences (2). This poses an interesting question regarding the relationship of this virus to the observed neoplasms: Is the virus an opportunistic agent, or is it an etiologic factor in the development of the observed malignancies?

The detection of similar virus from leukemic swine not exposed to radiation (Table 3) argues against radiation as the sole mechanism for "activating" this virus.

In addition, our prior observation that retrovirus from radiation-exposed swine with leukemia and bone tumors can transform primary pig kidney cells suggests that this virus may play a role in the oncogenic process (14). Finally, PK-15 virus, an endogenous virus from swine, was distinguishable from the retrovirus isolated from these leukemic animals in immunological and nucleic acid hybridization studies (manuscript in preparation). Whether the virus can play an etiologic role in the development of the observed malignancies in swine has not been directly investigated.

Acknowledgments

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