Lead Inclusion Bodies in Osteoclasts

Abstract. *Inclusion bodies occur frequently in the nuclei and rarely in the cytoplasm of osteoclasts in pigs with experimental lead poisoning. The light and electron microscope pictures of undemineralized sections are similar to those described for liver cord cells and renal tubular cells.*

Intranuclear inclusion bodies occur in liver cord cells and proximal tubular cells of the kidney with lead poisoning in man and animals (*1*, *2*). They are acid-fast, indicating a sulfhydryl-containing protein, presumably of the nonhistone type without DNA or RNA (*3*). They consist of a dense core and peripheral microfibrils (*1*, *3*). Lead in the inclusion body has been demonstrated by autoradiography (*4*), x-ray microanalysis (*5*), and chemical techniques (*6*). We report here inclusion bodies in osteoclasts in pigs with lead poisoning.

Six Yorkshire pigs, 4 weeks of age, were fed a conventional diet containing 0.7 percent Ca and 0.6 percent P. Lead was added as lead acetate to the food of three of the pigs; the lead concentration was 1000 parts per million (ppm). Six other pigs of the same breed and age were fed a diet containing 1.1 percent Ca and 1.0 percent P, with 1000 ppm of lead added to the food of three of the pigs. Two pigs of each of the four groups were killed and necropsied after 9 weeks, the remaining ones after 13 weeks. Blood samples taken every 2 weeks from the cranial vena cava were analyzed for lead by atomic absorption spectrophotometry, as were necropsy samples of kidney, liver, humerus, and femur. At necropsy, pieces of kidney and liver were fixed in Bouin's solution, embedded in paraffin, sectioned at thicknesses of 6 μm, and stained with hematoxylin and eosin and with the Ziehl-Neelsen and Kinyoung acid-fast stains. Slices 2 mm thick were cut midsagittally of the epiphysis-metaphysis of humerus, metacarpus, and femur; midsagittally of the costochondral junction of two ribs; and transversely of the mandible. They were fixed in 10 percent buffered formalin and embedded in paraffin, either as fixed or after demineralization under water-pump vacuum in 10 percent formic acid buffered to *p*H 4.5 with sodium citrate. Sectioning and staining were the same as for soft tissues. Formalin-fixed, undemineralized bone slices were refixed in 1 percent OsO_4 in Millonig's phosphate buffer at *p*H 7.3 for 1 hour, dehydrated in alcohol, embedded in Epon-Araldite (*7*), cut, and stained with uranyl acetate alone or in combination with lead citrate. Sections were viewed in an electron microscope at a peak voltage of 50 kv.

Ingestion of lead in the low-Ca diet resulted in an average lead concentration of 0.6 ppm in the blood after 2 weeks, and a final lead concentration of 1.1 ppm after 13 weeks. With ingestion of lead in the high-Ca diet, the lead concentration in the blood was 0.5 ppm after 2 weeks and there was no further elevation. The average lead concentrations in samples from the kidney, liver, humerus, and femur were 59, 159, 1800, and 2117 ppm, respectively, in pigs on the low-Ca diet with lead added, and 31, 27, 219, and 312 ppm, respectively, in pigs on the high-Ca diet with lead added.

Intranuclear inclusion bodies occurred in abundance in liver cord cells and in renal tubular epithelium in pigs with lead in their diets; none were observed in the controls.

In agreement with observations on the remodeling of the human metaphysis (*8*), the physiological resorption of metaphyseal trabeculae occurred mainly by osteocytic osteolysis. The initial bone lesion in lead-intoxicated pigs was inhibition of this osteocytic activity. The trabeculae thus became wider, which accounted for the increased radiographic density of the metaphysis. Necrosis of the osteocytes occurred eventually, and this dead bone tissue was then the object of osteoclasis (Fig. 1). These changes occurred in all bone sections from lead-treated pigs but were most prominent in metaphyseal trabeculae, the bone tissue with the greatest turnover rate.

By light microscopy the intranuclear inclusion bodies in osteoclasts were best demonstrated in undemineralized, Kinyoung-stained sections (Fig. 1, inset). Sections with very large osteoclasts contained up to 100 inclusion bodies, which were irregularly shaped, stained red, and in the size range from dust particles to large droplets. They were not related to the nucleolus. Nuclei containing inclusion bodies were large, with coarse and marginated chromatin.

Electron micrographs showed that the intranuclear inclusion bodies in osteoclasts consisted of a dense core surrounded by a fibrillar network (Fig. 2). Similar structures were rarely present in the cytoplasm, an observation made in a study of the renal tubular epithelium in lead poisoning in rats (*9*).

Lead inclusion bodies were not observed in osteoblasts or osteocytes. The histological bone changes, including

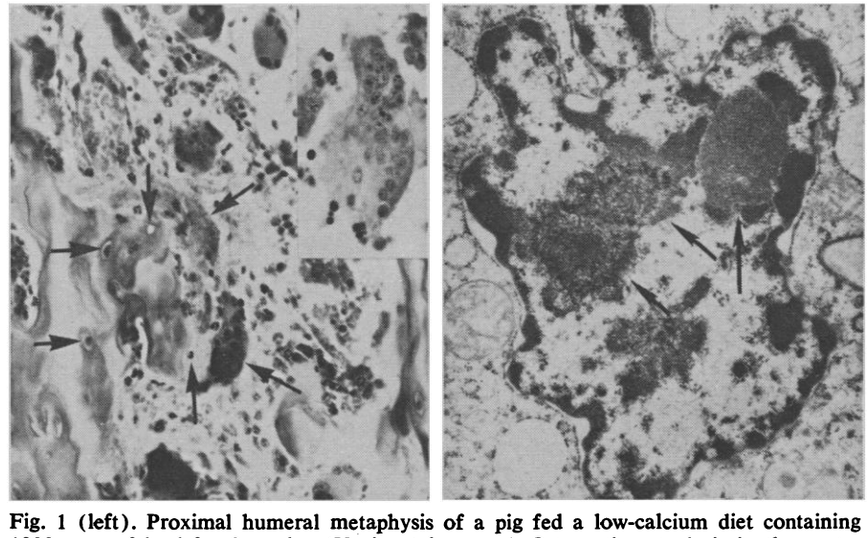

Fig. 1 (left). Proximal humeral metaphysis of a pig fed a low-calcium diet containing 1000 ppm of lead for 9 weeks. (Horizontal arrows) Osteocytic osteolysis in the center of a trabecula. (Vertical arrows) Necrosis of osteocytes with (oblique arrows) osteoclasts resorbing devitalized bone. Demineralized section, hematoxylin-eosin stain, × 255. (Inset) Osteoclasts with numerous intranuclear inclusion bodies. Undemineralized section, Kinyoung stain, × 410. Fig. 2 (right). Electron micrograph of nucleus of osteoclasts from the proximal humeral metaphysis of a pig fed low-calcium diet containing 1000 ppm of lead for 9 weeks. (Oblique arrows) Inclusion bodies; (vertical arrow) nucleolus. Undemineralized section, uranyl acetate and lead citrate stain, × 7800.

the prevalence of intranuclear inclusion bodies in osteoclasts, mirrored in degree the lead concentration in bone. Thus, they increased with increasing chronicity and were less pronounced in pigs with higher levels of dietary calcium.

Our interpretation of the sequence of events in lead intoxication is based on an application of Belanger's postulates on bone metabolism—namely, that osteocytic osteolysis is the primary mechanism in bone resorption in both physiological and pathological situations and that osteoclasts is a late phenomenon, concerned with the removal of already altered bone (*10*). During osteolysis, the osteocyte is metabolically very active and responds quickly to lead intoxication, and with greater chronicity it dies and the dead bone tissue is resorbed by osteoclasts. Lead is ingested by these cells of known phagocytic capacity. The pathogenesis of the lead-containing inclusion bodies is yet to be elucidated.

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Virus-Free Adenocarcinoma of the Frog (Summer Phase Tumor) Transcribes Lucké Tumor Herpesvirus-Specific RNA

Abstract. *[^{3}H]RNA isolated from "virus-free," summer phase, renal adenocarcinomas of* Rana pipiens *labeled with [^{3}H]uridine hybridizes with DNA of herpesvirus particles isolated from the winter phase Lucké tumor. Transcription of the herpesvirus genome in virus-free tumors provides additional evidence for the viral etiology of this tumor.*

Herpesviruses are associated with several vertebrate neoplasms including Burkitt's lymphoma (human), Marek's disease (chicken), the Lucké renal adenocarcinoma (frog), and possibly human nasopharyngeal carcinoma and cervical carcinoma (*1*, *2*). However, evidence for the herpesvirus etiology of human cancer, although compelling in some instances, is mainly circumstantial particularly in view of the widespread occurrence of herpesviruses in nature and their possible occurrence as passenger viruses. The Lucké renal adenocarcinoma is a naturally occurring neoplasm of the leopard frog which exists in nature in two temperature-related states; winter phase tumors contain herpesvirus particles whereas summer phase tumors are free of detectable virus (*2*). Prolonged exposure to lowered temperature results in the change from the "virus-free" summer state to the overtly virus-containing winter state. These tumors provide a favorable system for determining unequivocally the relation between a herpes-type virus and cancer. If viral genetic material was transcribed in the "virus-free" summer tumor, the herpesvirus etiology of the Lucké adenocarcinoma would be established more firmly. We show in this report that RNA from the summer adenocarcinoma hybridizes with viral DNA isolated from Lucké tumor herpesvirus (LTHV).

LTHV was isolated and purified from winter tumors by a modification of the method of Wagner *et al.* (*3*). The mitochondrial supernatant was concentrated by centrifugation onto a cushion of 65 percent sucrose at 100,000*g* for 45 minutes in the Spinco SW41 rotor. Viral DNA was extracted (*4*) from purified virions banding at a density of 1.20 to 1.26 g/cm^3 in su-

Table 1. Detection by molecular hybridization of virus-specific RNA in the virus-free Lucké renal adenocarcinoma (summer phase tumor) labeled with [^{3}H]uridine. Hybridization was performed with duplicate 6.5-mm DNA-containing nitrocellulose filters incubated with 200 μl of [^{3}H]RNA in 4 × SSC (SSC is 0.15*M* sodium chloride and 0.015*M* sodium citrate) plus 0.1 percent sodium dodecyl sulfate for 18 to 24 hours at 66°C. Filters were washed in 2 × SSC before and after incubation at 25°C for 1 hour with pancreatic ribonuclease (20 μg/ml), then dried and counted by liquid scintillation. Background counts bound to an empty filter were not subtracted.

Experiment	[^{3}H]RNA (count/min)	Source of DNA (1 μg per filter)	RNA bound (count/min)	
			Filter 1	Filter 2
		Lucké renal adenocarcinoma		
1 (VR11-69M)	5×10^5	Lucké virus	1613	1614
		Normal frog	422	414
		Empty filter	34	31
2 (VR10-71K)	1.2×10^5	Lucké virus	1515	1451
		Normal frog	324	318
		Empty filter	31	31
3 (VR10-71F)	1.1×10^5	Lucké virus	1600	1608
		Normal frog	896	896
		Empty filter	30	31
4 (VR11-70F)*	6.8×10^5	Lucké virus	1637	1632
		Normal frog	405	406
		Empty filter	33	38
Controls 1–4		Adenovirus 2	67 to 68	
		E. coli	28 to 34	
		Normal frog kidney		
5	8.4×10^5	Lucké virus	121	132
		Normal kidney	428	452
		Empty filter	29	35
6	7.5×10^5	Lucké virus	39	49
		Empty filter	0	0

* Additional experiments in which 5 μg of Lucké virus DNA was used did not increase the fraction of RNA bound.