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# The histopathology of tissue lead retention

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**Summary.** An experimental murine model of chronic lead retention is established. This model excludes joint communication and synovial fluid solubility of lead ions recognized in human gunshot injury. Degenerative changes in the visceral organs and bone appear in these mice 30 days after implantation of lead spheres in the subcutaneous tissues of the posterior body wall. Degeneration, necrosis and interstitial fibrosis of the lung, kidney and remodeling of femoral bone are noted during the course of the experiment (120 days). It is concluded that the systemic diffusion of lead ions from a central focus is related to the development of an absorptive circulatory mechanism around the retained peripheral lead mass.

**Key words:** Lead intoxication, Retained bullet.

## Introduction

Human lead toxicity constitutes an unwelcome harvest reaped by the injection of paint chips, the imbibing of water flowing through lead pipes; even the drinking of «moonshine» whiskey prepared in leaden vessels.

There is yet another source, albiet much rarer, the absorption of ions from missles, long embedded in human tissues. Lead bullets and shotgun pellets are the chief offenders.

The first discourse linking lead bullets to plumbism was made by Bronvin (1867). Ellis, 1874, described the case of a man who had received a gunshot wound to the knee joint 12 years before his death. Terminal clinical findings suggesting chronic lead intoxication were observed.

Machle (1940) cited 40 cases from the world literature up to that year, noting existing relationships between the lodgement of bullets in joint spaces and the synovial fluid bathing the missles with clinical lead toxicity. In 10 of these, data was available relevant to the increased burden of lead in the blood and urine accompanied by gastrointestinal, cerebral and neurologic findings compatible with plumbism in subsequent years. Additional cases have been added in later years (Von Hagen, 1942; McNallay, 1949; Rossen, 1965; Choie and Richter, 1972. Glakin, 1972; Switz et al., 1976; Dillman et al., 1979; Grogan and Bucholz, 1981; Beazley and Rosenthal, 1984; Viegas and Calhoun, 1986; Slavin et al., 1988).

The description by DiMaio et al. (1983) of a 54 year old woman who had received a gunshot wound to her knee 5 months prior to her death, is of special interest because it relates both the laboratory findings and the clinical symptoms of lead poisoning presented by this patient. The hematological studies revealed marked anemia and basophilic stippling of red blood cells together with serum lead levels approaching 5.1 mg/L shortly before her demise following a Gran mal seizure. The normal serum content of lead is less than 0.6 mg/L. At autopsy, cerebral edema, uncal herniation and brainstem hemorrhage were observed. Microscopically, the hepatocytes and proximal tubular cells were found to contain eosinophilic intranuclear occlusions. The cerebral perivascular spaces contained clumps of PAS-positive homogeneous material.

A case is reported by Less et al. (1988) of a policeman who suffered multiple shotgun wounds of the right arm and chest with the retention of a large number of lead pellets. In the years between the time of injury in 1972 until 1983, his mental condition markedly deteriorated with impairment of his intellectual functions, accompanied by insomnia and frequent emotional outbursts. Laboratory studies revealed a high serum lead content and elevated urinary excretion of coproporphyrin and porphobilinogen.

The present study is directed toward the establishment

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of an animal model that will display the morphology of long-standing tissue retention of this element.

The experimental design of this work explores the visceral consequences of tissue lead implantation in remote body areas. Small spheres of lead were inserted subcutaneously into the posterior lumbar region of mice, remote from major joint spaces, in order to evaluate organ alterations that may appear in the absence of contact with synovial fluid, considered to be a primary route for absorption into the body economy.

## Materials and methods

Forty five mature Swiss Webster mice were anesthetized with an aqueous solution of ketamine xylizine intramuscularly administered (30 mg/Kg per animal).

A 5 mm incision was made through the shaved, aseptically prepared skin over the posterior lumbar region of 40 mice. Into a small subcutaneous cavity formed by blunt dissection, was inserted a previously weighed 0.1 gm lead sphere, chemically sterilized by immersion into 70% alcohol (Fig. 1).

The wounds were closed with surgical clips and the animals allowed to fully recover.

Glass beads of about the same dimension were similarly implanted into the backs of 5 control mice.

All of the animals were sacrificed by carbon dioxide at intervals of 1 to 20 weeks. At autopsy, the spheres were carefully removed and re-weighed after the implantation site had been grossly examined. Smears were prepared from tail vein blood immediately prior to death. These were conventionally stained by the Wright-Giemsa technique. The heart, lungs, liver, spleen, pancreas, kidneys, colon and portions of the right femur were removed and fixed in 10% neutral buffered formalin. The brain was removed intact and preserved in 10% neutral buffered formalin for a subsequent separate study.

Representative tissue blocks were dehydrated, paraffin-embedded and sectioned at 3μm. The histologic stains used included hematoxylin and eosin, a trichrome stain, the Sirius Red technique for the definition of collagen (Greenberg, 1986) and the Mallory method for tissue lead identification (Mallory and Parker, 1939).

## Results

After exposure to metalic lead, microscopic alterations appeared in the heart, lungs, liver, kidney and bone. At the site of implantation in the posterior lumbar region, thin encircling strands of connective tissue the lead spheres appeared by the fourth week (Fig. 2). These condensed into thick bundles that contained thin-walled vascular channels. The lead spheres remained relatively intact during the course of the experimental period (120 days). Only about 0.02 to 0.05 gram of the original weight was lost.

Focal areas of degeneration and necrosis of the hepatocytes and the proximal tubular cells were discernable by the end of the third week.


**Fig. 1.** Showing the metalic sphere in place in the posterior lumbar region of the mouse.


**Fig. 2.** Illustrating the implantation site at week 4. Note the in-growth of connectiver tissue (Arrow).

Areas of degeneration and necrosis soon appeared in the hepatocytes and in the tubular cells (Fig. 3). After the first month, there was a gradual destruction of the femoral osteocytes and an increased formation of osteoid accompanied by an overgrowth of collagen. These changes resulted in a remodeling of the bone in the affected regions (Fig. 4).

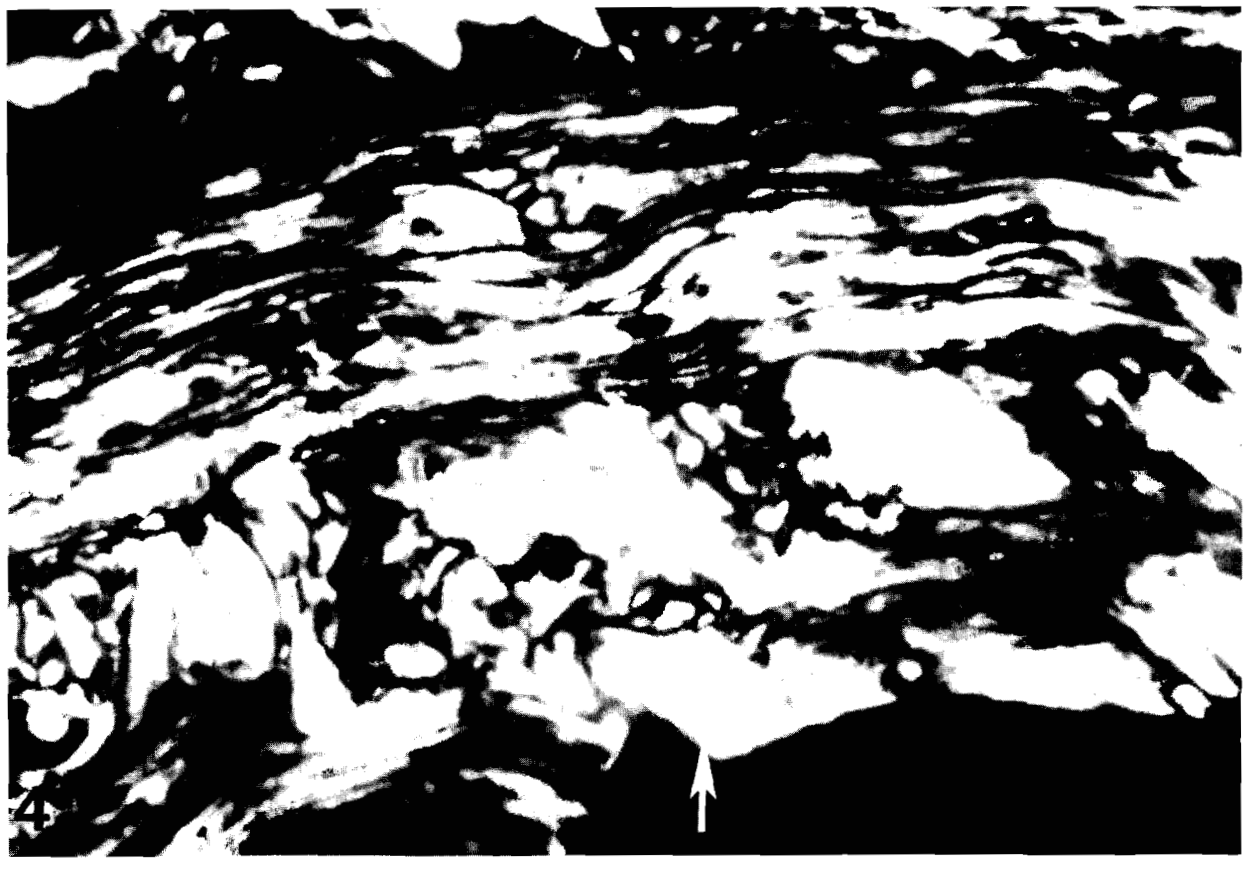
The myocardium is pale; the striations are indistinct, but necrosis is absent. The coronary vessels are morphologically unaltered.

Within the areas of injury in the liver, kidney, and both clumps of amorphous blue-staining material revealed by the Mallory technique in the cytoplasm of the degenerating cells, confirms the presence of lead. Inclusions bodies were observed within the nuclei of about 20% of the injured hepatocytes and proximal convoluted lining cells in the kidney. Occasionally these were noted in the cytoplasms. The inclusion bodies appeared within about 20 days and persisted.

The lungs exhibited focal thickening of the interstitial connective tissue which, over a period of several weeks, became more extensive, encroaching upon the alveolar walls. Some of the alveolar vessels were trapped in the

**Fig. 3.** Illustrating focal hepatic degeneration and necrosis at the end of week 4 (arrow). H & E × 160

**Fig. 4.** Illustrating the remodeling of femoral bone 6 weeks after the implantation of lead in the mouse. Note the increased presence of collagen (arrow). Sirus red stain × 100, polarized light.



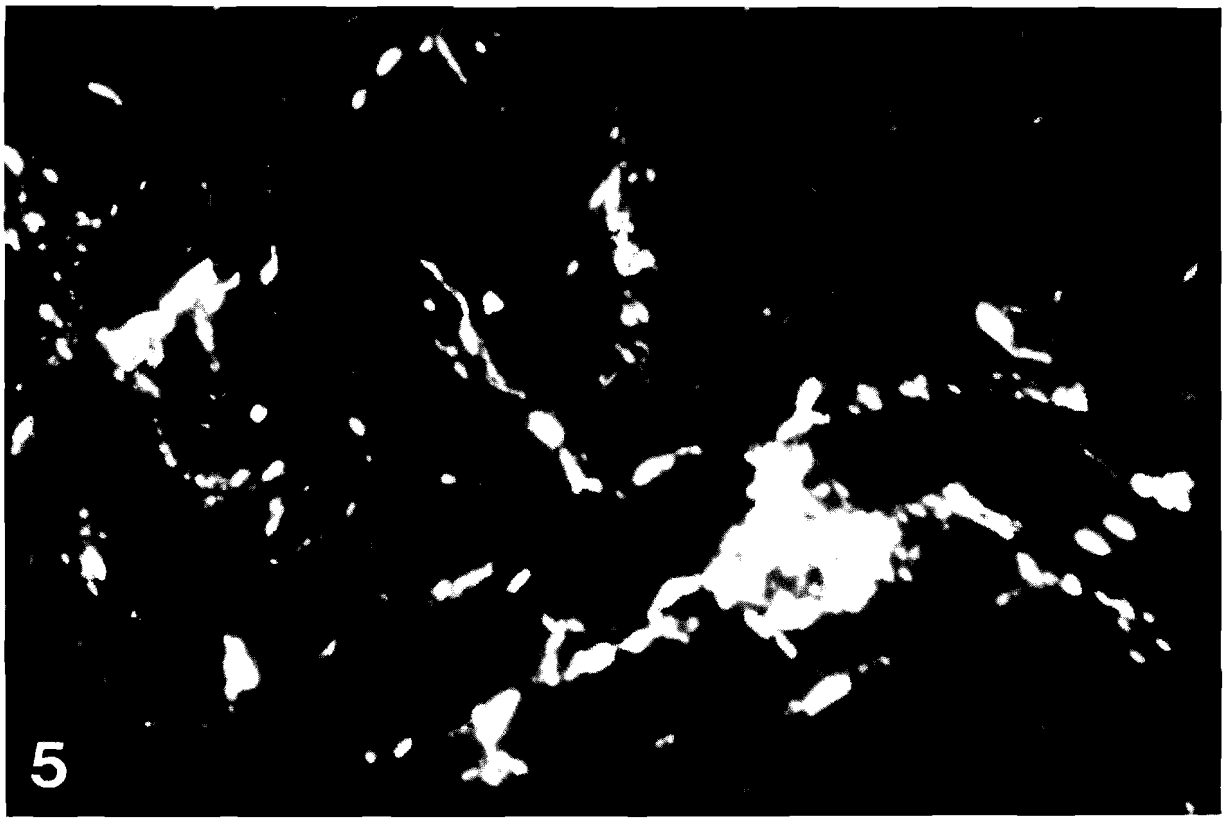



**Fig. 5.** Illustrating the increased collagen formation in the renal parenchyma at day 40. Sirus red stain × 100, polarized light.

advancing connective tissue, but pulmonary congestion was not a prominent feature, nor was there morphologic evidence morphologic of severe circulatory impairment, even at the terminus of the experiments.

Within the kidney, the interstitial connective tissue progressively increases in the cortex, then extends downward into the medulla (Fig. 5). By about the fortieth day, focal interstitial thickening, extends to involve most of the cortex and medulla. Some glomeruli are destroyed. The collecting tubules are focally engulfed; the lumens of others are reduced in size.

## Discussion

Of special interest, is a possible relationship between the implanted lead spheres and the observed alterations in the visceral organs of the mouse. This is given credence by the positive histological staining for lead. Further support is given by the presence of inclusion bodies in the nuclei of the hepatocytes and renal tubular cells. These data supports prior observations that these inclusions frequently persist in areas of lead intoxication (Richter, et. al. 1968; Hsu, et. al. 1973). Within the kidney, they are limited to the cells of the proximal tubules; in other organs they have a wider parenchymal distribution. Although primarily within in the cell nuclei, they may occasionally apear also in the cytoplasm, of some cells, notably the hepatocytes and osteoclasts (Hsu et al., 1973).

The intranuclear inclusions do not contain DNA or RNA, but do possess proteins of nonhistone character (Richter et al., 1968). Chemical analysis of separated inclusion bodies confirms the presence of lead as a major constituent (Goyer et al., 1970; Underwood, 1979; Windler et al., 1978). Barltrop demonstrated that even a normal intake of lead results in a subcellular distribution of this element in both the liver and kidney. He believes the mitochondria are the focus for lead accumulation (Barltrop et al., 1971). Others have expressed the opinion that inclusion bodies function as intracellular depots for non-diffusable lead (Moore and Goyer, 1974).

Just how lead ions may be transported from joint regions into the viscera may be presumed by the absorption of lead ions dissolved from missles bathed in synovial fluid.

In the present study, the lead spheres were not in contact with this fluid since implantation was at a distance from any joint surface, but the encroachment of blood vessels around the implantation site could permit the entrance of lead ions into the circulation, as it also, might in human gunshot victims who sustained soft tissue wounds (Lees et al., 1988). The ions, dissolved in the interstitial fluid, gain access to the circulatory system through these proximal vessels. The majority of the lead mass may persist, slowly losing ions from its surface. The observed injurious toxic effects may follow the absorption of only small amounts of lead (Leonard, 1969).

In the mouse, the prolonged exposure to lead ions is implicated in the collagenous remodeling of bone. It has been shown by Beazley and Rosenthal (1984) that there is much proliferation of connective tissue around the synovial membranes as well as external to the joint cavities. This has been attributed to the mechanical inflammation created by the lead missle itself (Slavin et al., 1988).

In the present study, no evidence of inflammation is noted because of the careful attention paid to aseptic surgical technique. The absorbed lead ions appear to account for the widespread organ injury. The gradual encroachment of interstitial connective tissue upon the alveolar walls decreases the pulmonary reserve, adding anoxia as a further potentiating factor of tissue injury. The observed changes in the murine lung and heart have not yet been described in man. The changes in the human central nervous system in relation to lead absorption have been described by Repko (1979).

A comprehensive examination of this system will be the subject of a subsequent report.

The disappearance of lead ions from a primary lead focus is a significant part of this study. The lead intoxification in human retained gunshot wounds, suggests that at least part of the original lead mass must migrate to distant body regions. The dissolution of solid lead into a soluble form which may enter the circulation, is related, in part, to the surrounding chemical mileau (Richter et al., 1968).

Two liquids are implicated. One is the synovial fluid; the other is the interstitial fluid. It has been shown that lead is more readily soluble in body fluids than it is in water, its solubility is further augmented when nacent carbon dioxide tension is raised or when the pH is increased in anoxic states (pulmonary fibrosis).

Interstitial fluid also may possess this ability and may dissolve lead in distant foci such as the subcutaneous tissue of mice. The lead may then enter the circulation through local vasculature.

There are additional factors to be considered in the induction of systemic lead intoxification. First, is the relationship of the body fluid mass to that of the lead sphere (DiMaio et al., 1983). The higher this ratio, the more lead ions will be liberated from the parent mass.

Secondly, is the presence of increased vascularity after tissue injury. This provides a pathway for the continuous escape of lead ions into the blood stream (Leonard, 1969).

A local chemical medium bringing together body fluids under optimal pH combined with a dialysing membrane surface, such as a synovial membrane or vascular endothelium, will permit the systemic diffusion of lead ions, even from a small initial mass; to induce parenchymal lead injury in distant organs.

Single lead masses may not totally disappear, even over many years (28), but lead ions stemming from absorbed lead masses or from fragments derived from them, may prove to be as toxic in producing lead intoxication as those stemming from intact missles (Barltrop et al., 1971).

The encapsulation of foreign bodies by fibrous tissue excludes them from contact with body fluids and the consequence of systemic spread. When the missle enters a joint space, the synovial bath it receives contributes a solvent effect that permits the entrance of lead ions into the systemic circulation. Data derived from this investigation indicates that a factor of significance in the systemic diffusion of lead ions from a central focus, is the development of an absorptive circulatory mechanism around the retained lead mass; in this instance, the formation of new vessels around the site of implantation.

### *Conclusions*

1.–Lead ions can be systemically absorbed when the metal is implanted superficially in remote regions of the mammalian body.

2.–Absorption is initiated soon after implantation.

3.–Absorption is facilitated by the solution of lead ions by either synovial or interstitial body fluid. The bulk of the initial lead mass persists.

4.–The systemic absorption is expressed by parenchymal injury in the heart, lung, liver, and kidney.

5.–The ionic abosorption of lead from bullets left in the tissues of human gunshot victims have the potential for inducing morphologic alterations.

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