## CASE REPORT

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# Neuropathological Findings Associated with Retained Lead Shot Pellets in a Man Surviving Two Months After a Suicide Attempt

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**ABSTRACT:** We describe the neuropathological findings in a 30-year-old man who died two months after attempting suicide with a shotgun. We focused our study on lesions associated with retained lead shot pellets and distant therefrom, as well as lesions distant from the principal site of injury. At the sites of the retained lead shot pellets, we found macrophage proliferation and astrocyte activation, together with axonal spheroids and signs of neuronal damage. In the remaining white matter we observed axonal swellings, astrocyte activation and rarefaction of the neuropil; regressive phenomena of the neurons were also present. All axonal spheroids immunoreacted with antibodies against APP, αB-crystallin, NF subunits and ubiquitin. Most reactive astrocytes were positive for GFAP and αB-crystallin immunostaining. Some neurons immunoreacting with αB-crystallin were also found. These data indicated that an important local reaction developed at the sites of lead shot retention, and mild signs of diffuse axonal damage were found throughout the brain.

**KEYWORDS:** forensic science, forensic pathology, shotgun, wound, death, autopsy, lead shot pellets, neuropathology, axonal damage

In cases of death caused by shotgun wounds, exitus usually occurs immediately or a few days after the destructive impact of the burst pattern in the brain (1). Many lead shot pellets penetrate the brain and localize diffusely to the gray and deep white matter (2). In surviving subjects, retained lead shot pellets are visible by CT scan and MRI (2). Little is understood about the neuropathological alterations due to the presence of these foreign bodies in the brain. We describe the neuropathological findings of a 30-year-old man who died two months after a suicide attempt. We focused our study on the histological changes produced by several retained lead shots, including those that were distant from the principal site of injury.

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### Case History

The subject self-inflicted a shotgun wound to his right temple, provoking extensive injury with fracture of the brain-pan and loss of cerebral matter. When the patient arrived at the emergency room, neurological examination showed coma state with signs of decerebration. Brain CT scan showed confluent hemorrhagic areas of contusion with edema in the frontal lobes, which were more accentuated on the right side, where the temporal lobe was also affected. Several retained lead shots were observed, most of them in the brain-pan (Fig. 1). The patient underwent surgical removal of malacic and hemorrhagic tissue from the right frontal lobe. He was treated with desametazone, glycerol, and antibiotics. After two weeks, neurological examination showed coma (GCS = 9), arm hypertonia, and midriasis on the left side. The neurological and general conditions remained unchanged until the 60th day, when he died.

### Material and Methods

At autopsy, a precise characterization of the gunshot wound was not possible because of surgical manipulation and biological repair processes. Nevertheless, the forensic pathologist concluded that it was a contact wound. In the right frontoparietal region, we observed a roundish cavity having a diameter of about 4 cm, sharp edges, and an advanced state of fibrosis. The dura mater was replaced with a patch of fibrous material. Since no lead shot reached the contralateral region of the skull, it was impossible to establish a precise trajectory. The firearm used was a monobarrel shotgun (brand Gamma, caliber N° 20). The ammunition used was a caliber 20 cartridge (brand Fiocchi); each lead shot pellet weighed 0.0995 gm (N° 7, according to the Italian metric number system).

The brain was fixed in 10% formalin for 30 days. Coronal sections of representative areas were embedded in paraffin according to standard procedures and stained with the following techniques: Hematoxylin and Eosin, Woelcke for myelin, Nissl and silver impregnation (Bodian staining).

Immunohistochemical analysis was performed using the avidin-biotin-complex; color was developed using 3,3′-diaminobenzidine as chromogen. The sections were incubated with antibodies for ubiquitin (Dakopatts): glial fibrillary acid protein (Dakopatts): 68,

160, and 200 NF subunits (Sigma): APP (Roche Diagnostics); and αB-crystallin (Novocastra).

## Results

Macroscopic examination showed that a portion of the right frontal lobe was missing due to the shot and subsequent surgical removal. The right temporal and left frontal lobes, cerebellum, midbrain and pons, including the superior and middle cerebellar peduncles, showed a malacic face as a sign of a previous hemorrhage and contusion. The examination of coronal sections showed the presence of several lead shot pellets, both in the deep white matter and gray matter (Fig. 2). There were no apparent changes in the surrounding cerebral matter. The path of some shots was visible through the white matter.

At histological examination, the sites where the lead shot pellets were retained had the appearance of cavities often rich in fibrin and mononuclear cells. These cavities were immediately surrounded by an area of spongiosis with many macrophages (Fig. 3), which in turn was surrounded by a layer of spongiosis rich in astrocytes (gemistocytes) (Fig. 4). Occasionally, a few perivascular mononuclear cells were found. Several axonal swellings could be observed adjacent to the lesion foci (Fig. 5). When the lead shot pellet was in proximity to the cerebral cortex, neuronal loss and damaged neurons were also present; in particular, we observed several neurons

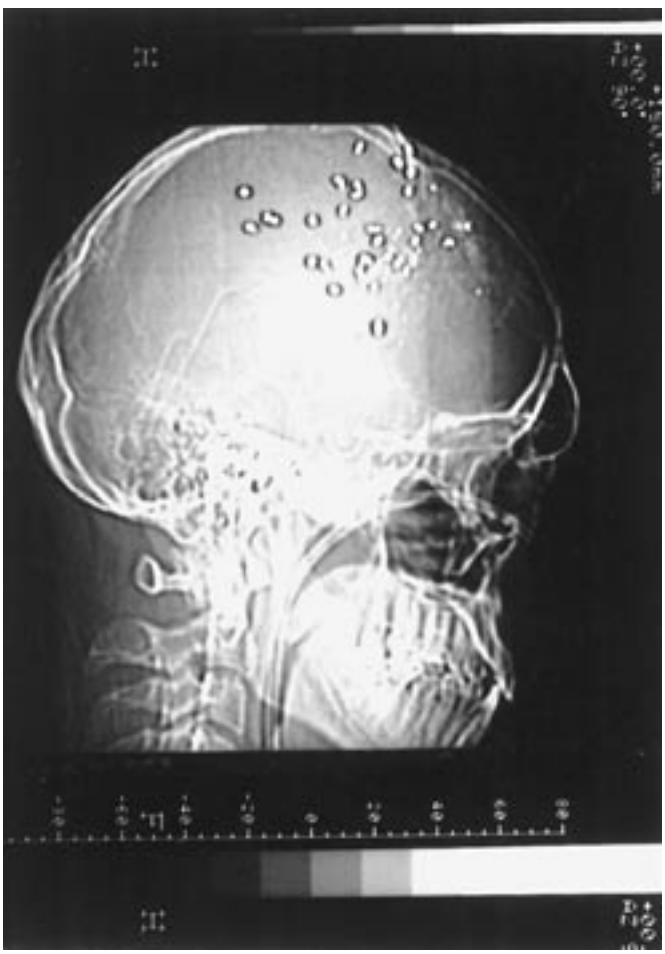

FIG. 1—*CT scan showing where several retained lead shots were observed, most of them in the brain-pan.*

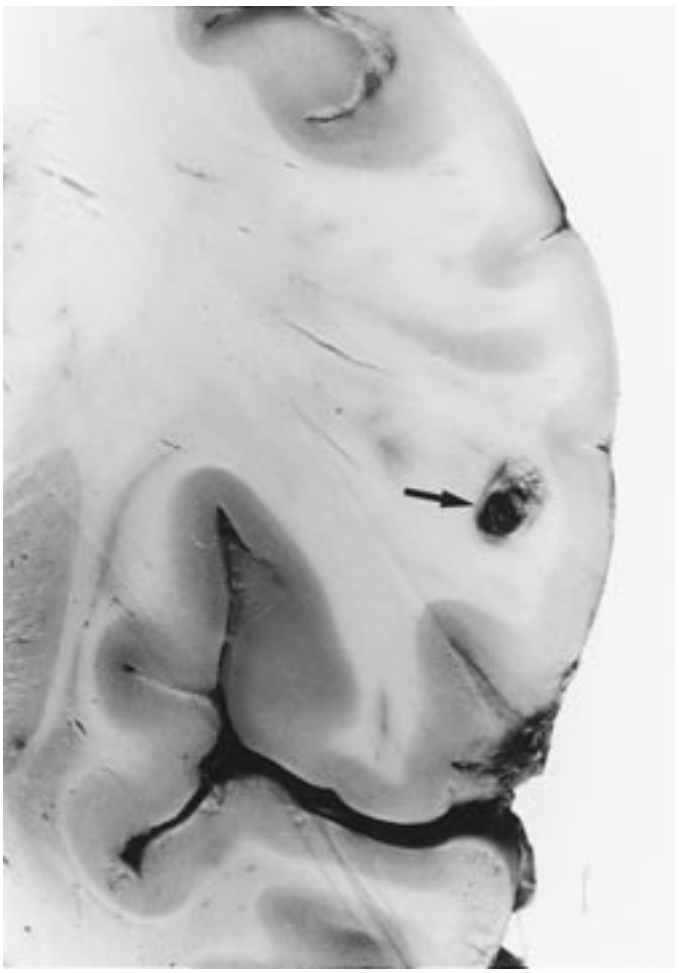

FIG. 2—*Macroscopic cerebral section showing a retained lead shot* (arrow) *in the subcortical region.*

with deposits of dark, granular mineral salts (ferrugination) (Fig. 6).

Outside these lesions, there was slight demyelination and spongiosis of the white matter, with some axonal swellings; occasionally, foci of rarefaction of the neuropil were observed. Such lesions also involved the corpus callosum. Some giant neurons with eosinophilic cytoplasm were observed in the cortex and basal ganglia.

Immunocytochemical analysis showed that axonal spheroids immunoreacted with anti-NF subunits, αB-crystallin, APP (Fig. 7*A*), and ubiquitin antibodies. Many GFAP-positive cells (reactive astrocytes) were found sporadically in the above-described lesion zones, but also distant from the sites of retained lead shots. In fact, marked astrocytosis was observed in the subcortical region. Glial cells immunoreacting to αB-crystallin were sparsely present in the white matter; occasionally, some neurons that were positive to αB-crystallin were also observed in apparently nondamaged areas (Fig. 7*B*).

## Discussion

To our knowledge, no studies have been performed to date regarding the pathological changes associated with the presence of lead shot pellets in the brain. Many reports have dealt essentially with le-

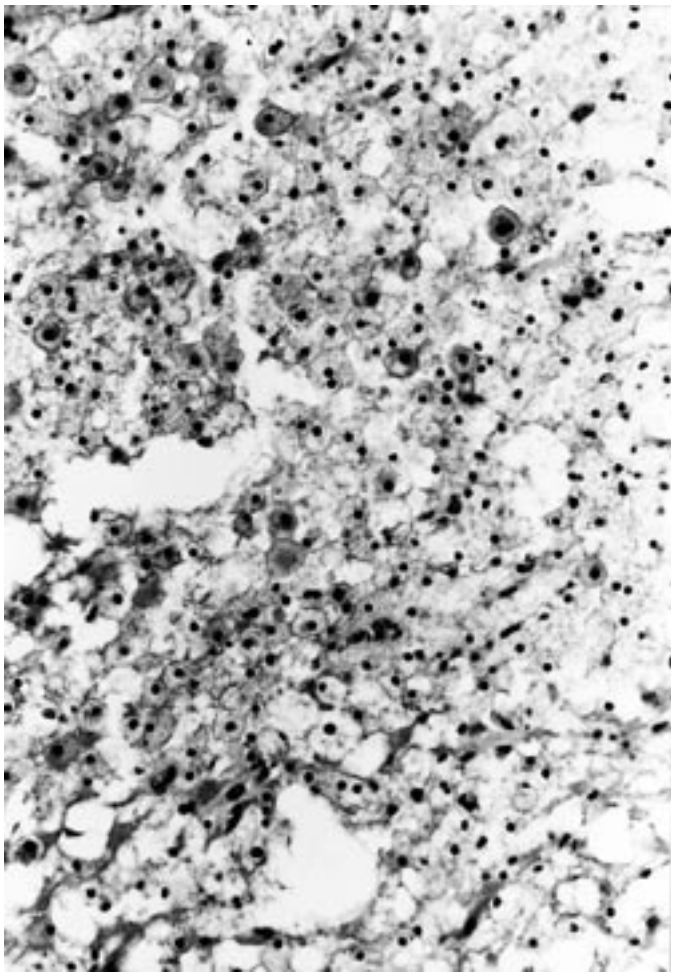

FIG. 3—*(H&E stain, × 130). Many macrophages immediately surrounding a focus of lead shot retention.*

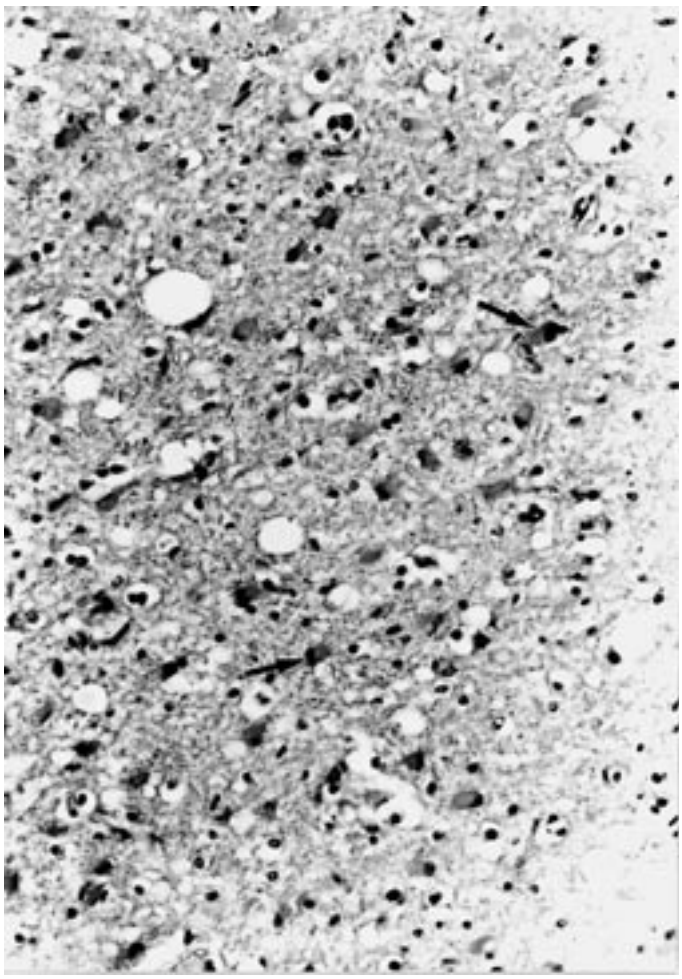

FIG. 4—*(H&E stain, × 100). Many reactive astrocytes (gemistocytes)* (arrows) *in a spongiotic area.*

sions produced by penetrating bullets following gun or rifle shots. Most shotgun wounds are the consequence of accidents. Sometimes, the shotgun wound is not fatal (3–7), and a subject will live with retained lead shot pellets inside the body. Previous reports have demonstrated that, besides the complications due to lead shot retention in particular sites (eye injuries (8), cardiac embolism (9), myocardial infarction (10), etc.), chronic lead poisoning caused by slow absorption from the retained foreign bodies occurs more frequently (11–13). In one case, a polyneuropathy was even described (14). This pathogenetic mechanism is confirmed by high lead levels found in the blood of these subjects (12,15). In the present case, the length of survival of the patient did not allow us to evaluate this problem but permitted us to study extensively the neuropathological lesions.

Our study indicates that lead shot retention in the brain produces two types of lesions:

1. A local reaction, consisting in macrophage activation to eliminate the degraded material due to focal necrosis. Around this zone, we underline the presence of gemistocytes, some of which were GFAP-positive, and in certain cases formed a true wall delimiting the lesion, which might be destined to spread progressively. The nature of involvement of the cerebral cortex in proximity to sites of lead shot retention was somewhat surprising, as calcified neurons were usually seen in residual lesions from conditions such as hemorrhage, infarction, and viral encephalitis. Furthermore, we noted the presence of axonal swellings as a marker of axonal injury. All of these lesions are, for the most part, due to the direct damage of the penetrated foreign body. This local reaction, in the case of prolonged survival, may lead to a relatively wide area of demyelination and reactive fibrosis.

2. In the brain regions that were distant from the site of impact, we observed focal lesions indicative of axonal damage. In particular, the immunoreactivity of axonal enlargements to APP, αB-crystallin, NF subunits, and ubiquitin antibodies, suggests a mild form of diffuse axonal damage, probably caused by a focal accumulation of cytoskeletal elements due to an impairment of axonal transport. This hypothesis is supported by the expression of αB-crystallin in axonal spheroids, glial cells, and some neurons. This protein, which belongs to the family of small heat shock proteins, is, in fact, upregulated in response to pathological accumulation of neurofilaments in the axon or cellular body. Also, the finding of focal zones of rarefaction of the neuropil and many reactive GFAP-positive astrocytes, mostly in subcortical regions, is in line with the above considerations.

Despite the involvement of the corpus callosum, the absence of diffuse macroscopic hemorrhagic lesions in the present case does not allow us to use the term of Diffuse Axonal Injury (DAI). However, the above-described lesions as a whole surely are able to provoke functional damage to the brain and may explain the severe

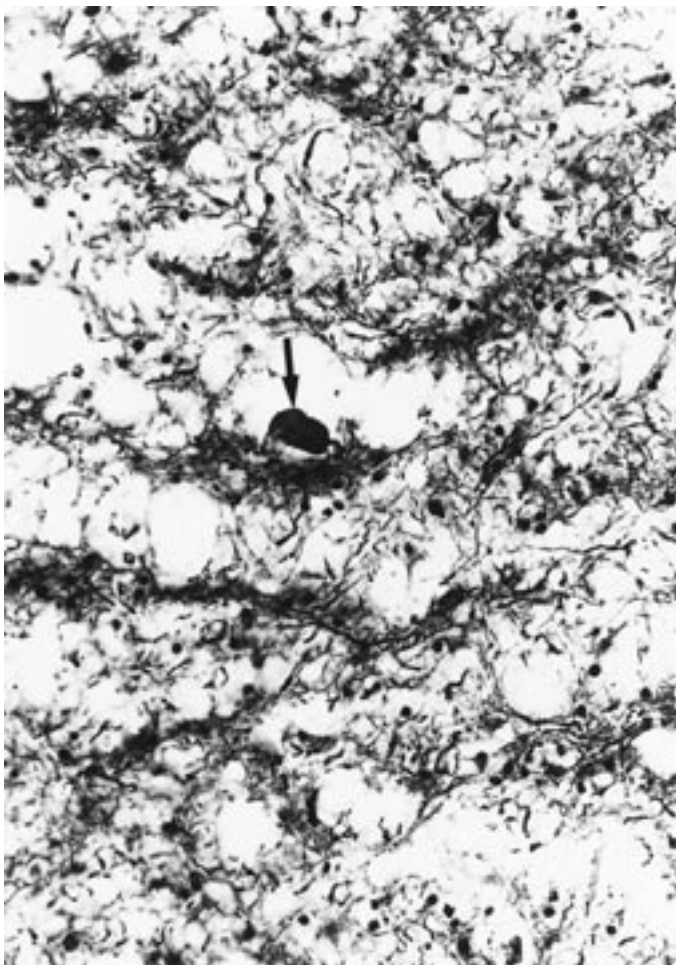

FIG. 5—*(Silver impregnation, × 250). An axonal spheroid* (arrow) *in a zone with rarefaction of the neuropil.*

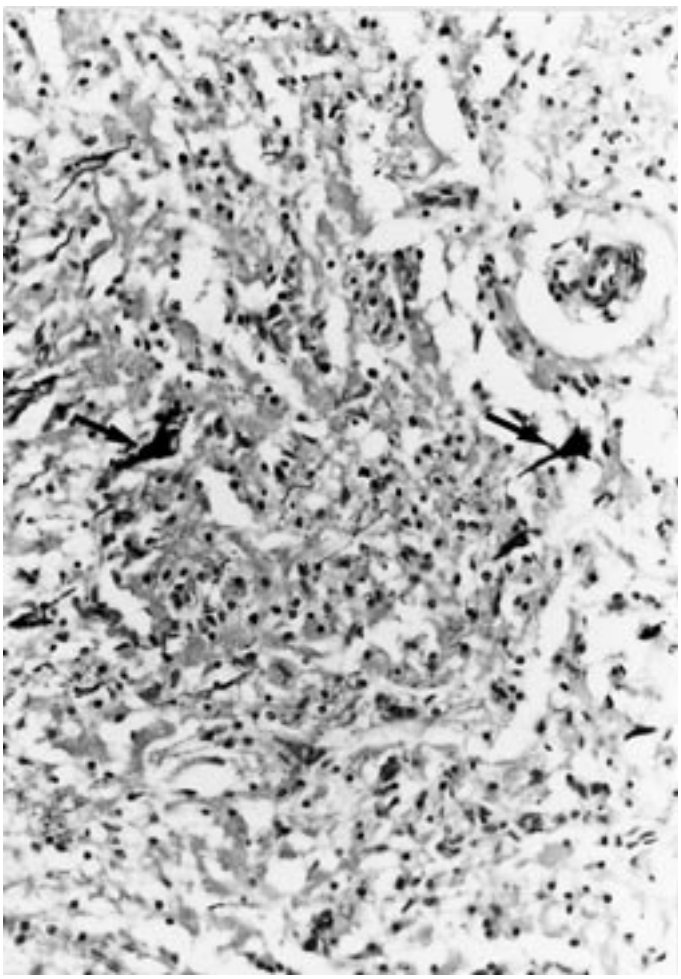

FIG. 6—*(H&E stain, × 180). Some pyramidal neurons showing ferrugination* (arrows) *in a damaged cortex, near a focus of lead shot retention.*

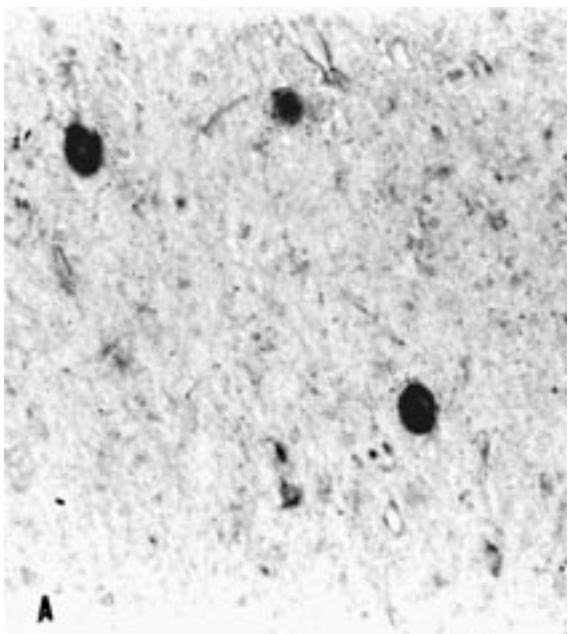


FIG. 7*a*—*(APP immunoreaction, × 200). Some axonal spheroids immunoreactive to the APP antibody.*

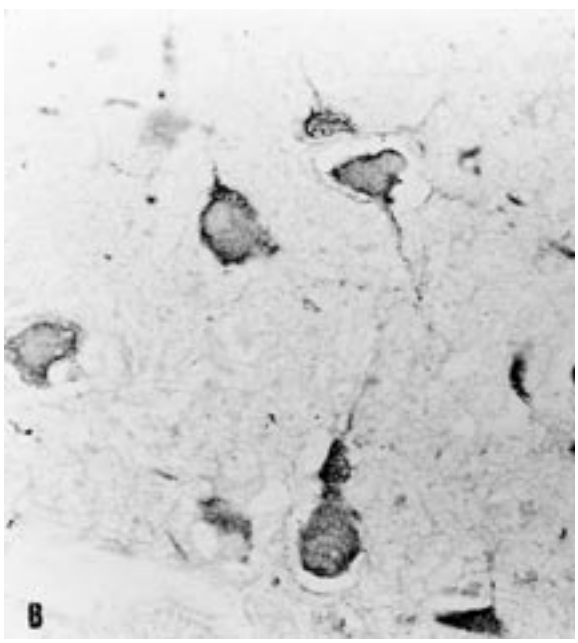


FIG. 7*b*—*(αB-crystallin immunoreaction, × 200). Some cortical neurons positive to the αB-crystallin antibody.*

cerebral involvement, even in cases in which the brain does not present apparently fatal lesions.

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