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Further Observations on the Effects of Lead Implantation in Rat Brains

Asao Hirano and Joseph A. Kochen

Division of Neuropathology, Department of Pathology, and Department of Pediatrics, Montefiore Hospital and Medical Center, Bronx, New York 10467 U.S.A.

Summary. Lead pellets were implanted into the forebrains of adult rats. Various cytoplasmic inclusions were found in macrophages, astrocytes, pericytes and microglia. Certain macrophages and reactive astrocytes displayed intranuclear inclusions as well. The hypothalamus, in some of the animals, showed sponginess which proved to be the result of swollen axons.

Key words: Lead — Macrophages — Intranuclear inclusions — Axonal swelling.

INTRODUCTION

As part of a continuing effort to understand the effects of lead on the central nervous system, we have undertaken an investigation of the changes in morphology accompanying lead implantation into the cerebrum of adult rats (Hirano and Kochen, 1976). A previous publication has described the effects on the vasculature (Hirano and Kochen, 1976). In the present report, we shall describe other changes observed in macrophages and in neurons.

MATERIALS AND METHODS

Details of the techniques employed have been described elsewhere (Hirano and Kochen, 1976). Briefly, they consisted of implanting small pellets of lead acetate into the forebrains of adult rats by means of a trochar inserted through a burr hole. The animals were sacrificed at various intervals up to 6 months after implantation. After perfusion fixation with 5% glutaraldehyde in 1/15 M phosphate buffer, pH 7.4, the tissues near the implant, as well as from various other areas in the brain, including the opposite hemisphere, were dissected out and processed for electron microscopy by conventional means.

RESULTS

Our previous report dealt with the changes seen in the vasculature and included some observations concerning the presence of lead within macrophages. The latter deposits appeared as extremely dense, discretely granular inclusions that were

Fig. 1. An unstained section of a reactive astrocyte near the implant. Numerous dense cytoplasmic and extracellular lead deposits are seen. Less dense lipid droplets (*L*), some including dense granules (small arrows), are present. The nucleus contains an abnormal inclusion (large arrow) as well as a nucleolus. × 12000

identical to the extracellular fragments of the implant which were often seen in the areas studied. They were as dense in unstained sections as in those stained in lead and uranyl salts (Fig. 1).

At least two other kinds of inclusions were also noted. The first was only moderately dense and, in unstained sections, were relatively lucent (Fig. 1). These

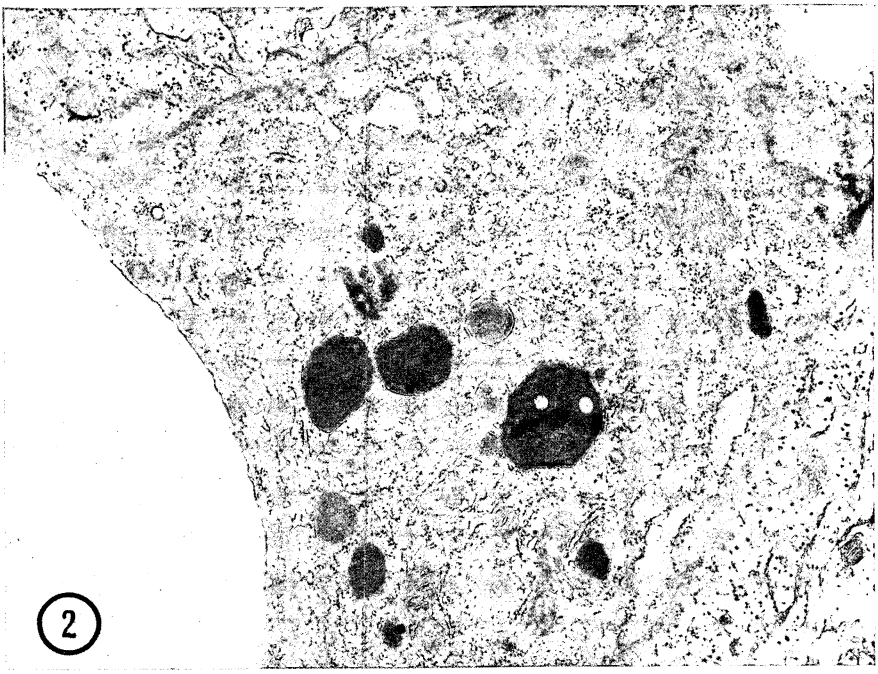

Fig. 2. A stained section of a perivascular cell containing dense cytoplasmic deposits in an area remote from the implant. × 15000

were judged to be lipid-like in nature. Interestingly, while they were more common in macrophages and reactive astrocytes, pericytes, microglia and meningeal cells near the implant, similar inclusions were also found in remote areas (Fig. 2), even the opposite hemisphere. While we consider them essentially lipid on the basis of their smooth contour and general morphology, occasional small dense granules, apparently lead, were seen within the lipid-like inclusions (Fig. 1).

The second inclusion to be described was found only in areas within the brain near the implant. These were seen within macrophages and reactive astrocytes. Their distribution was intranuclear, intracytoplasmic and in the extracellular spaces of necrotic areas where they were mixed with cellular debris. They were irregular, moderately dense inclusions up to 2 μ in diameter. They were devoid of a limiting membrane and often displayed radiating, spicule-like extensions. They possessed either a homogeneous or vaguely fibrillar or granular consistency. In unstained sections, the intracellular bodies appeared relatively lucent while extracellular inclusions of this type were sometimes quite dense.

Except for the presence of macrophages with lipid-like inclusions, most of the brain appeared normal. In 2 out of 4 animals examined, however, the hypothalamus showed striking changes even in the light microscope. In these rats, which were sacrificed 1 month and 3 months, respectively, after implantation, certain areas of the hypothalamus showed sponginess in the neuropil (Fig. 3).

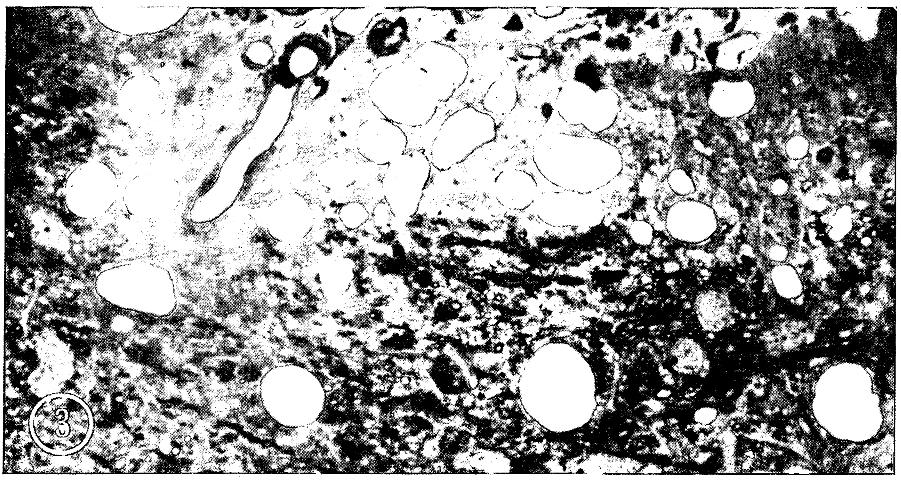

Fig. 3. A light micrograph of a section through the hypothalamus of a rat sacrificed 3 months after implantation. Distinct spongy changes are seen. × 250

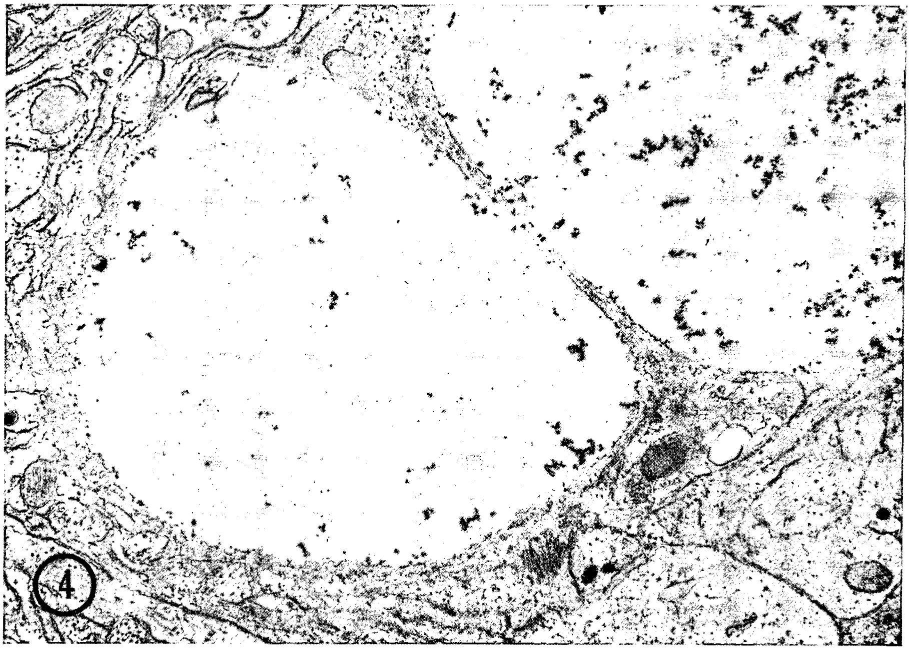

Fig. 4. An electron micrograph of unmyelinated processes from an area similar to that illustrated in Figure 3. Only dense granules and a fine flocculent material are present within the vacuole of the markedly distended processes. × 15000

In the electron microscope, these were shown to be large distensions of axons sometimes achieving a diameter of several microns. The involved fibers were either myelinated or unmyelinated and the axoplasm showed variable degrees of

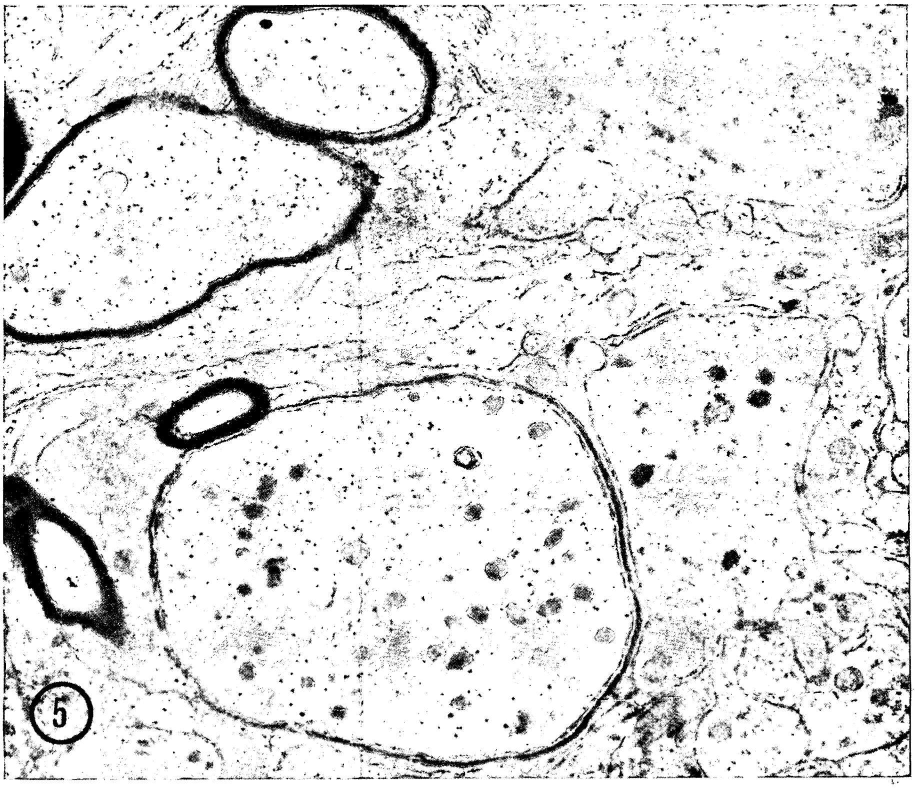

Fig. 5. Myelinated and unmyelinated moderately distended axons in an area adjacent to that illustrated in Figure 4. Secretory granules and other dense inclusions are seen within the axoplasm. × 15000

vacuolation. In its most extreme form, the axoplasm was watery and contained almost nothing but dense, fine granules and a faint reticulated material (Fig. 4). Sometimes these watery areas were surrounded by a narrow rim of denser material which may have been displaced axoplasm. In other cases, the swollen axoplasm contained various organelles including secretory granules which were still identifiable although they seemed to be losing structural integrity (Fig. 5). In addition, small dense granules of unknown origin were found scattered in the distended axons.

DISCUSSION

As pointed out previously (Hirano and Kochen, 1976), many of the changes observed might be considered to be nonspecific reactions to the implantation procedure itself. This was especially true of some of the alteration in the vasculature already described. The results reported here, however, are more likely to be related to the nature of the implant.

Dense, cytoplasmic inclusions in perivascular cells have been described in children with lead encephalopathy (see review by Clasen et al., 1974a) and in adult animals which had been fed lead since birth (Markov and Dimova, 1974). Our results seem to confirm these findings although these changes were much less prominent in our material except for regions close to the implant. Presumably, the relative paucity of these changes in remote areas is connected with the comparatively short period of exposure and the differences in the method of lead administration.

The nature and significance of the intranuclear inclusions seen in macrophages and astrocytes are unknown. They are presumably the same inclusions described in both human and experimental lead intoxication at the light (Blackman, 1936; Dalldorf and Williams, 1945; Wachstein, 1949; Landing and Nakai, 1959; Hass et al., 1964; Morgan et al., 1966; Horn, 1970, among many others) and electron microscopic levels (Beaver, 1961; Angevine et al., 1962; Richter et al., 1968; Choie and Richter, 1972, among others). Recent electron microscopic studies have reported similar deposits within the nuclei of both astrocytes (Goyer, 1973) and of proximal renal tubule epithelium (Goyer, 1971) in rats fed lead over a long period of time.

It is interesting to note that we have found apparently the same inclusion in cerebral macrophages as well. It should be pointed out, however, that both the intranuclear and cytoplasmic spicule-bearing inclusions were only moderately dense in unstained sections in contrast to fragments of the implanted pellet and certain of the highly granular intracytoplasmic deposits which remained dense even in unstained sections. Furthermore, the extracellular spicule-bearing inclusions also appeared dense in unstained sections. X-ray and biochemical analyses of similar deposits derived from renal tubule epithelium have indicated that the inclusions are composed of a lead-protein complex (Goyer, 1971, 1973; Waldron and Stöfen, 1974). Our results with unstained sections suggest that the lead content may vary depending on whether the inclusion is intra- or extracellular.

The similarity between the intranuclear inclusions and the moderately dense cytoplasmic and extracellular structures of similar configuration is striking. In our view, they are probably the same material. The different staining properties between the intra- and extracellular inclusions of this type presumably reflect different concentrations of lead.

In any event, it seems reasonable to assume that the intranuclear changes are the result of abnormal quantities of cytoplasmic lead. Renal epithelial cells and cerebral macrophages and astrocytes are capable of taking up extracellular, lead-containing material and apparently this tendency somehow influences the nucleus, resulting in the dense inclusions.

The significance and cause of the spongy changes in the hypothalamus are not understood. A previous light microscopic study of lead intoxication in several non human primates described vacuolation in the white matter of the central nervous system (Sauer et al., 1970). Clasen et al. (1974b) using the electron microscope, found axonal distension, similar to our findings in the cerebral cortex of lead intoxicated young monkeys, as well as areas of vacuolation in the gray matter of basal ganglia in rats (Clasen et al., 1974a). The latter were reported to be the result of astrocytic swelling. The difference in localization of the vacuolar changes between our animals and those of previous workers may, conceivably, be related to the different species or to mode of lead administration.

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Asao Hirano, M. D.
Div. of Neuropathology, Dept. of Pathology
Montefiore Hospital and Medical Center
111 East 210 Street
Bronx, N.Y. 10467, U.S.A.