BRAIN EDEMA

Proceedings of the Symposium.
September 11-13, 1965, Vienna.

Edited by

IGOR KLATZO and FRANZ SEITELBERGER

SPRINGER-VERLAG. WIEN. NEW YORK. 1967

in Brain Edema. Ed. I. Klatzo and F. Seitelberger - Springer Verlag, Wien

P. LAMPERT
F. GARRO
A. PENTSCHEW

LEAD ENCEPHALOPATHY IN SUCKLING RATS*

AN ELECTRON MICROSCOPIC STUDY

The morphology of lead encephalopathy in infants and children is well documented by light microscopy (Blackman, 1937; Pentschew, 1958; Smith, et al., 1960; Popoff, et al., 1963; Pentschew, 1965). Vascular damage and serous exudation followed by endothelial, microglial and astrocytic prolifereation are prominent features. The cerebellum is most frequently affected. Similar morphological alterations develop in the brains of suckling rats when their mothers are fed a diet containing lead carbonate (Pentschew, et al., 1966). The purpose of this paper is to present some electron microscopic observations on this lead encephalopathy in suckling rats together with an account on the vascular permeability to Trypan Blue and Thorotrast, an electron dense tracer.

MATERIAL AND METHODS

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed during this study. Long-Evans rats were mated and the dams were kept in individual cages. At delivery the dams were put on a diet of Purina laboratory chow containing four percent lead carbonate (Fisher Scientific Company). They remained in good health but their offspring, getting lead through lactation, grew less well than normal control

*This investigation was supported in part by Research Grant NB-02275 from the National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland, and the Veterans Administration Central Laboratory for Anatomic Pathology and Research, AFIP, Washington, D.C.

litters. About 20 to 30 days after birth the suckling rats, regardless of sex, developed a sudden onset of paraplegia of their hind legs. Most of them died two or three days thereafter. A few recovered even while on a lead diet. If put on a normal diet at the onset of the paraplegia, all animals recovered.

For light microscopic studies, groups of suckling rats were sacrificed by formalin perfusion before and at the onset of the paraplegia. At either one or 24 hours before sacrifice, some of these animals were given intravenous or intraperitoneal injection of 0.5 ml. of a 1:100 dilution of Trypan Blue. Control rats of the same age but of greater weight were given 1 ml. of the diluted Trypan Blue.

For electron microscopy, eight suckling rats from different litters were sacrificed by osmium perfusion at the onset of the paraplegia, i.e., 19 to 31 days after birth. Five of these rats were given an intravenous injection of 0.25 or 0.5 cc. of Thorotrast (colloidal thorium dioxide, Fellows Testagar, Detroit) one hour before sacrifice. One paraplegic rat received 0.5 cc. of Thorotrast 20 hours before osmium perfusion. One normal control rat, 25 days old, received 0.5 ml. of Thorotrast one hour before sacrifice and two normal controls, 26 and 30 days old, were killed 24 hours after the intravenous injection of Thorotrast.

The animals were anesthetized with Nembutal. Their chest was opened, the right atrium incised, the left ventricle cut, and a cannula inserted into the aorta. The blood was first flushed for 30 seconds with Palay's balanced salt solution which was followed for about 30 minutes by Dalton's osmium fixative. A flow of about three drops per second, as observed in a drip chamber three feet above the animal, was maintained throughout the perfusion. The black, firm brains were removed and dissected with the aid of a dissecting microscope. Small blocks were cut from the striatum and cerebellum, dehydrated and embedded in epon according to Luft. Thin sections were stained with uranyl acetate or lead tartrate and examined with an RCA EMU 3G electron microscope operating at 50 kv.

RESULTS

The cerebellum of paraplegic animals presented a reddish brown discoloration due to the presence of abundant petechial haemorrhages (Fig. 1b, c) One hour after the intravenous injection of Trypan Blue, staining of the brain was visible in certain areas, most strikingly in the striatum, the occipital lobes, the cerebellum, and the lower spinal cord (Fig. 1b). Twenty-four hours after the intraperitoneal injection of Trypan Blue staining of the brain was more widespread, but was still most intense in the above mentioned sites

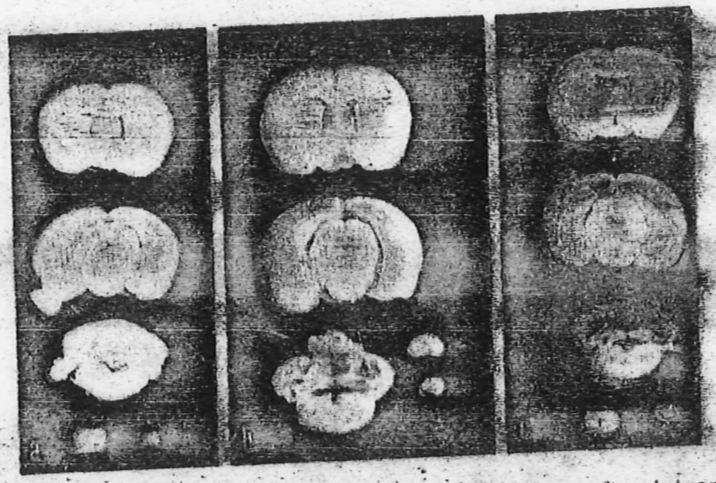

Fig. 1. a) Normal brain of 25-day-old rat 24 hours after intraperitoneal injection of Trypan Blue. No staining of the brain occurred. b) Brain of lead poisoned, 25-day-old rat one hour after the intraperitoneal injection of Trypan Blue. Some staining is visible in the striatum, occipital lobes, cerebellum, and spinal cord. c) Brain of lead poisoned, 26-day-old rat 24 hours after intraperitoneal injection of Trypan Blue. Staining of the brain is more intense and widespread.

(Fig. 1c). Animals sacrificed at 15 days after birth, i.e., before they became paraplegic, presented no hemorrhages in the cerebellum but permeability to Trypan Blue was already noted at this stage. Control animals of the same age showed no staining of the brain, except in areas known to be permeable, i.e., the area postrema, pineal gland, neurohypophysis and choroid plexus (Fig. 1a).

Light microscopy of the severely affected cerebellum demonstrated perivascular hemorrhages which were sprinkled throughout the molecular and granular layers as well as in the white matter. Subpial hemorrhages were particularly numerous. There were large cyst in the white matter. The margin of these cysts presented widely separated but intact myelinated fibers and glial cells. The neurons of the granular layer and the Purkinje cells were generally well preserved. Macrophages and a few reactive astrocytes were present. Proliferated capillaries with prominent endothelial cells were striking in the molecular layer. In the striatum smaller and less frequent perivascular hemorrhages occurred. A separation of individual myelinated axons within fiber bundles was noted. There were also a few proliferated microglial cells and astrocytes. The oligodendroglial cells were normal. Further light microscopic observations on lead encephalopathy in suckling rats are reported in greater detail elsewhere (Pentschew, et al., 1966).

Electron microscopy of the striatum and cerebellum of paraplegic rats revealed the following observations. The extracellular space was enlarged in the gray and white matter (Figs. 2, 4, 5). While this was widespread in the white matter it occurred only in foci around vessels or hemorrhages in the gray matter. In the latter, the presence of red cells squeezed within a compact neuropil suggested that normal, narrow intercellular spaces were restored (Fig. 3). Fine granular material, possibly precipitated plasma proteins, was found in widened extracellular spaces.

Nerve cells were well preserved. Axons occasionally presented spheroid enlargements filled with mitochondria, dense bodies and vesicular elements. Some axons presented a clumped, amorphous axoplasm and a myelin sheath with loosened myelin lamellae. The latter, degenerating axons were often engulfed by microglial cells. Myelin sheaths around normal axons were remarkably well preserved even in white matter exhibiting enormously widened extracellular spaces (Figs. 4, 5). Occasionally, however, wide splits within compact sheaths occurred. Large spaces between axons and surrounding myelin sheaths or "empty" loops of myelin were also noted.

Oligodendrocytes were well preserved (Fig. 4). They were identified by their location, i.e., arranged in rows in the white mat-

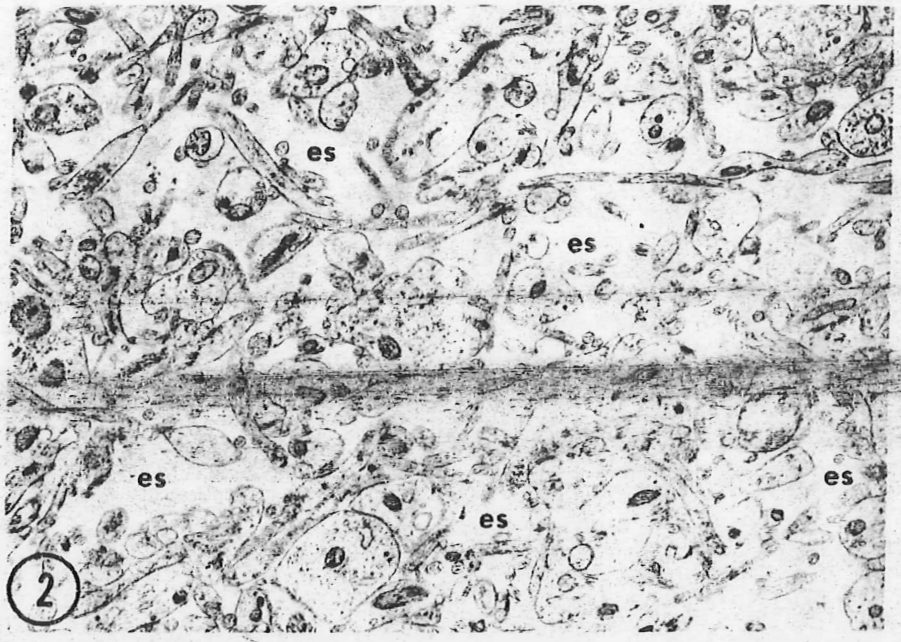

Fig. 2. Striatum of lead poisoned, 19-day-old rat. Note widened extracellular spaces (es). X10,000 (reduced).

ter and as satellites around nerve cells. They had a "dark" cytoplasm rich in ribosomes and devoid of filaments. They did not exhibit phagocytic properties.

Astrocytes were identified by their "clear" cytoplasm and by the attachment of their processes to vessels and to neurons. A few glycogen granules and bundles of filaments were characteristic components of the astrocytic cytoplasm. Filaments were scanty in astrocytes of normal gray matter. In the paraplegic rats some astrocytes, particularly in the white matter, contained an increased number of filaments, mitochondria and ribosomes in their cytoplasm (Fig. 5). Cytoplasmic swelling was not observed. Foot processes alongside abnormal vessels sometimes contained vacuoles filled with amorphous material. An increase of glycogen granules, some lipid droplets and occasional myelin figures were observed in a few astrocytic processes.

Microglial cells were numerous. They had a "dark" cytoplasm rich in ribosomes. They usually contained abundant phagocytosed de-

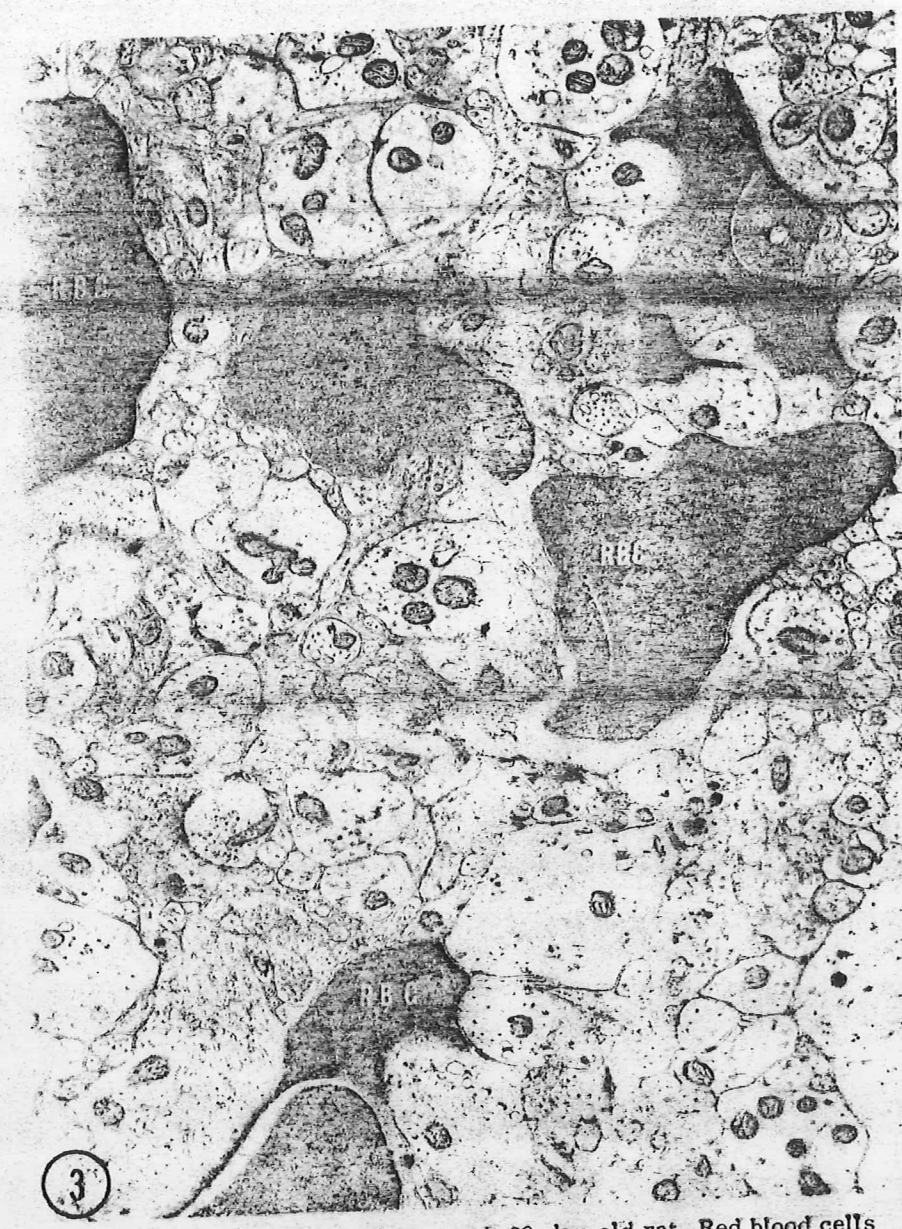

Fig. 3. Striatum of lead poisoned, 28-day-old rat. Red blood cells (RBC) are squeezed within compact neuropil. There are normal, narrow intercellular spaces between neuronal and glial processes. X11,500 (reduced).

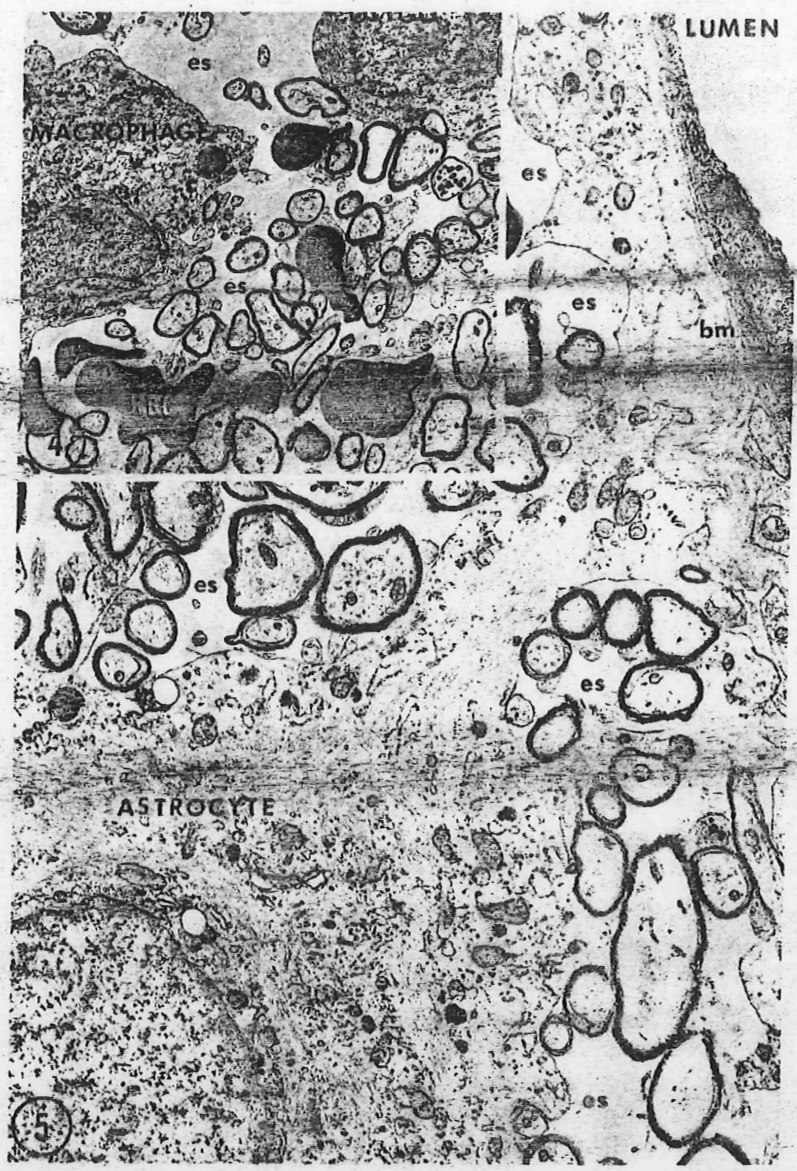

Fig. 4 (Top). Large extracellular spaces (es) are present in the white matter. Note well-preserved oligodendrocyte, myelin sheaths, red blood cells (RBC), and a macrophage filled with debris. Cerebellar white matter, lead poisoned, 19-day-old rat. X3,000 (reduced).

Fig. 5 (Bottom). Fibrous astrocyte filled with glial filaments in white matter with abundant extracellular spaces (es). Note the attachment of the astrocytic foot process to a vessel with loosened basement membrane (bm). Myelin sheaths are well preserved. Cerebellar white matter, lead poisoned, 19-day-old rat. X7,000 (reduced).

bris including breakdown products of red blood cells. Their shape varied. In regions showing abundant extracellular space these stuffed microglial cells or macrophages were round with a peripheral nucleus. In other areas where narrow intercellular spaces prevailed, e.g., the gray matter, these cells were slender with elongated nuclei. Dense bodies or lysosomes were scanty in these cells prior to phagocytosis but numerous thereafter.

The appearance of vessels varied considerably. The majority showed no visible changes at all. Some, particularly those located in tissue with abundant extracellular space, presented numerous, large vacuoles in their endothelial cells (Fig. 6). Overlapping endothelial processes were sometimes noted beneath or above the vacuolated endothelium. The basement membrane of vessels altered in this fashion appeared loosened and astrocytic foot processes were sometimes missing (Fig. 6). There were also capillaries which, on cross section, presented several big endothelial cells and a prominent pericyte (Fig. 7). Such proliferating vessels were generally found in compact neuropil, particularly in the molecular and granular layer of the cerebellum.

In normal control rats, no permeability to Thorotrast was demonstrated one or 24 hours after its intravenous injection. There was no uptake of Thorotrast particles by endothelial cells. In paraplegic rats Thorotrast traversed the wall of small vessels by passing through gaps between adjacent endothelial cells (Fig. 8). The electron dense particles then spread throughout the basement membrane and penetrated into the surrounding neuropil by migrating between and through glial cells. While passing through glial cells the particles were generally found within membrane bound compartments (Fig. 8). Amorphous material, possibly plasma proteins, escaped from the vessel in a similar fashion. The Thorotrast particles diffused readily throughout intercellular spaces and often collected in areas where intercellular clefts converge (Fig. 9). In the animal sacrificed 20 hours after the intravenous injection of Thorotrast, most of the particles were found within microglial cells (macrophages) (Fig. 10) but some particles were still encountered in intercellular spaces. It is of interest to note that so far we have not been able to demonstrate a permeability to Thorotrast in vessels with vacuolated endothelial cells.

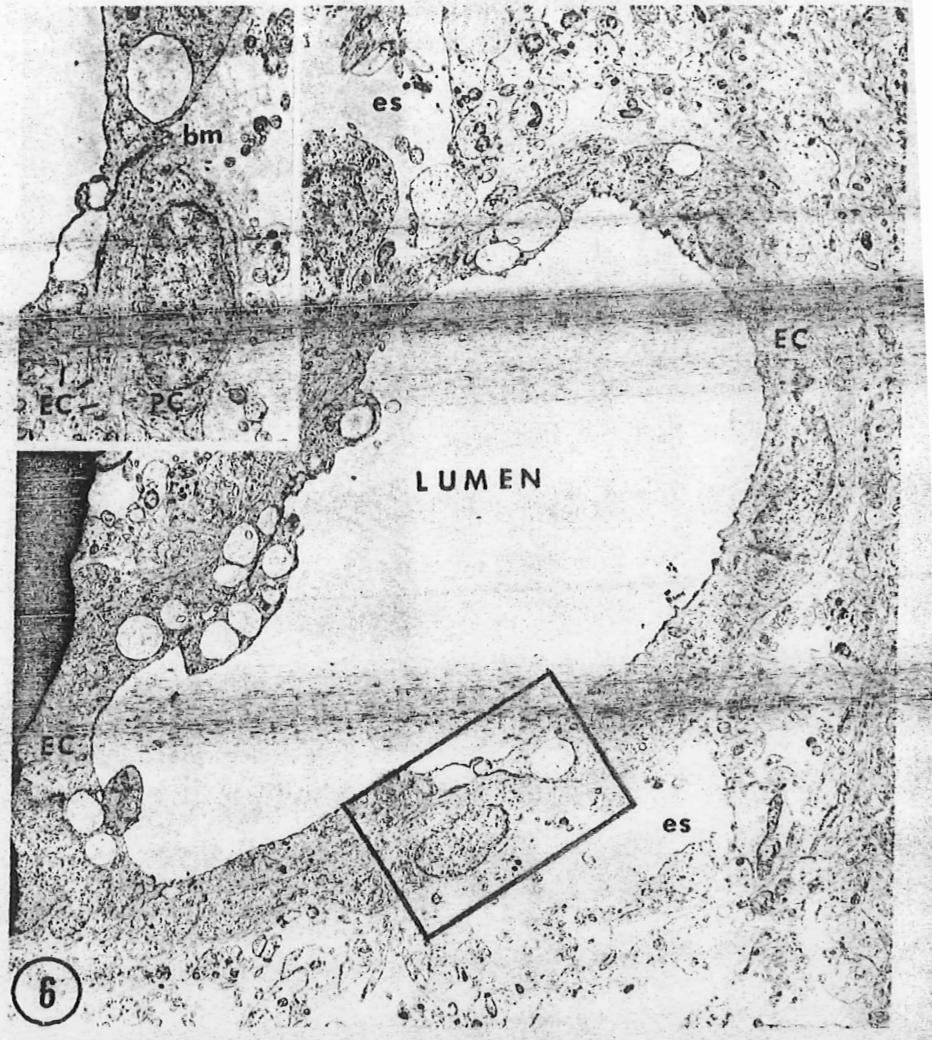

Fig. 6. Vessel with vacuolated endothelial cells within tissue showing abundant extracellular spaces (es). X3,000. Insert shows overlapping endothelial cells (EC), a well-preserved pericyte (PC), and a loosened basement membrane (bm). Astrocytic foot processes are missing. Striatum, lead poisoned, 19-day-old rat. X6,000 (reduced).

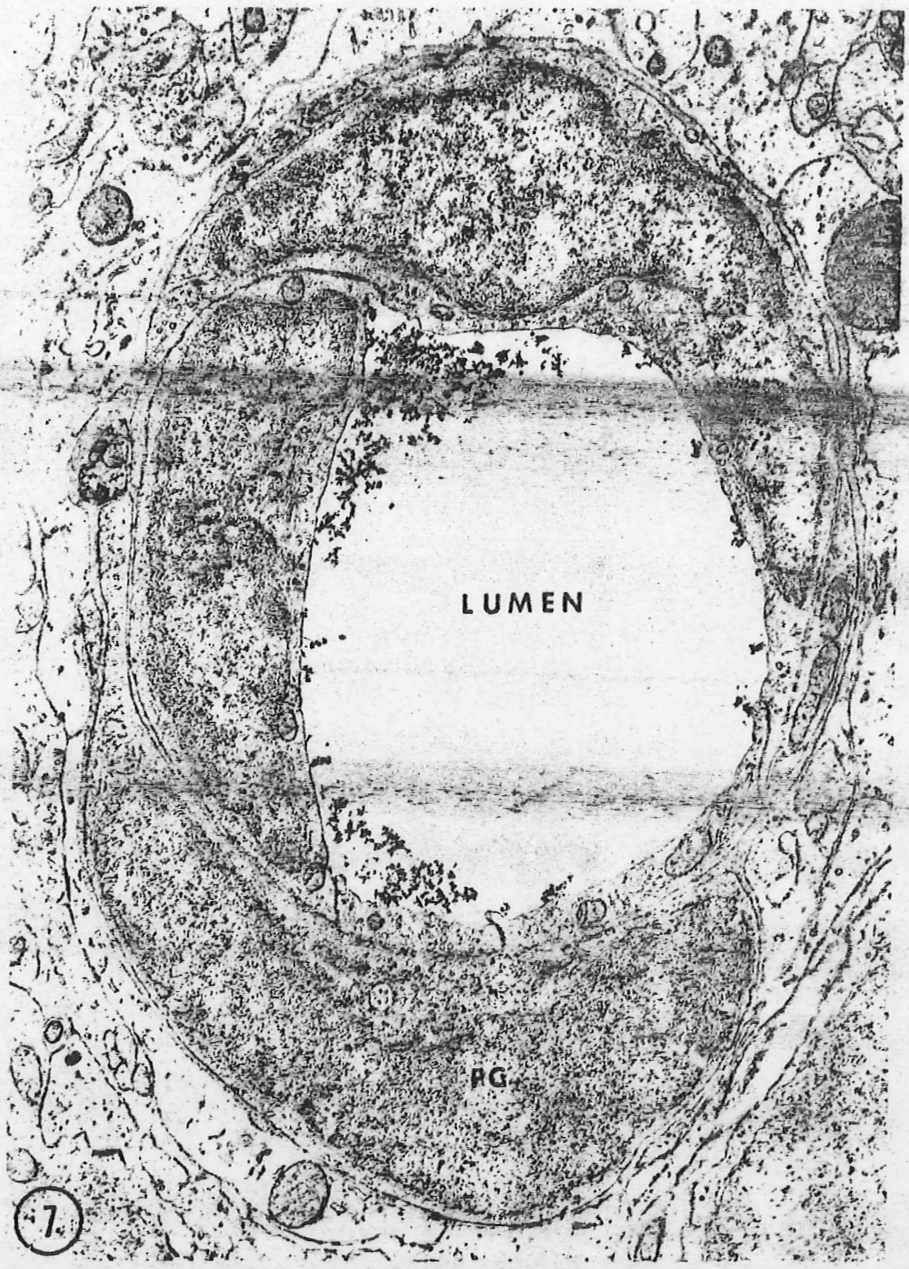

Fig. 7. Proliferated capillary in compact granular layer of the cerebellum. Several endothelial cells and a prominent pericyte (PC) are seen. Thorotrast particles are present within the lumen of the vessel but not between or within cells. Lead poisoned, 28-day-old rat. X10,000 (reduced).

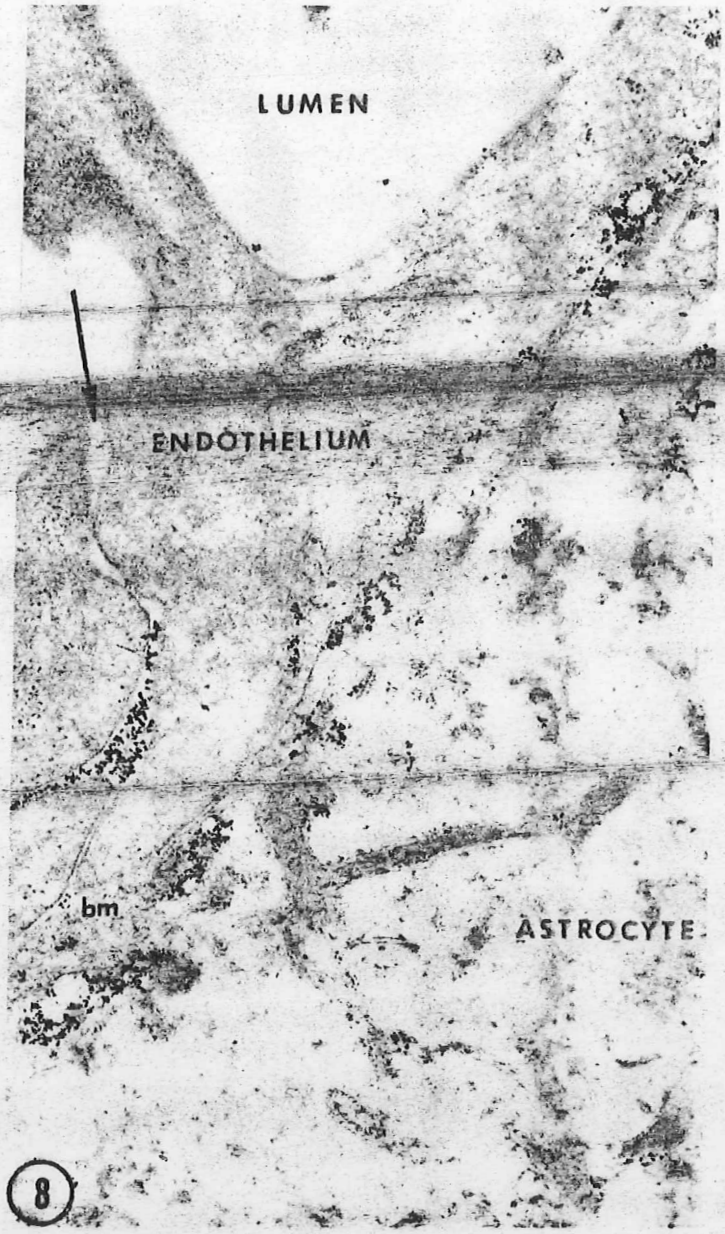

Fig. 8. One hour after intravenous injection of Thorotrast. The electron dense particles are found between endothelial cells (arrow), within the basement membrane (bm), and within vesicular structures inside an astrocytic process. Amorphous material, possibly plasma proteins, are also seen within membrane lined structures in the cytoplasm of the astrocyte. Cerebellar cortex, lead poisoned, 25-day-old rat. X28,000 (reduced).

Fig. 9 (Top). One hour after intravenous injection of Thorotrast. The electron dense particles are noted within enlarged extracellular spaces. At right, farther away from the leaking vessel, a few particles in intercellular spaces are indicated by arrows. Molecular layer of cerebellum, lead poisoned, 25-day-old rat. X22,000 (reduced).

Fig. 10 (Bottom). Twenty hours after intravenous injection of Thorotrast. The particles are collected within membrane bound compartments inside a macrophage. The neuropil presents normal intercellular spaces. Striatum, lead poisoned, 28-day-old rat. X10,000 (reduced).

DISCUSSION

Lead encephalopathy of suckling rats belongs to the so-called "dysoric encephalopathies," a group of diseases with similar histopathological features (Pentschew, et al., 1966). They are characterized by an abnormal vascular permeability, i.e., a dysfunction of the blood-brain barrier or "dysoria." This disturbance develops in selective areas of the brain which are not necessarily the same in each disease. The neurons remain relatively well preserved in spite of severe tissue changes. Astrocytes and microglial cells proliferate. The classical example of such a disease is Wernicke's encephalopathy where these alterations are observed in the mammillary bodies and certain nuclei of the brain stem.

The electron microscopic study reported here contributed direct morphological evidence of a vascular dysfunction in lead encephalopathy. Thorotrast (colloidal thorium dioxide), which does not penetrate through normal vessels, did so in the striatum and cerebellum of lead poisoned rats. The electron dense particles traversed the vessel wall by passing between endothelial cells and through the basement membrane (Fig. 8). The same separation of normally tightly joined endothelial cells and the escape of plasma and Thorotrast from vessels has also been described in early lesions of allergic encephalomyelitis (Lampert & Carpenter, 1965). Most likely this endothelial separation represents an unspecific reaction to injury similar to observations on increased vascular permeability in other organs after a variety of injuries (Majno & Palade, 1961; Movat & Fernando, 1963a, b; Casley-Smith, 1965). The significance of vacuolated endothelial cells is not clear at present. Vessels with vacuolated endothelium have been described in cerebral edema (Struck & Umbach, 1964; Herzog, et al., 1965). In the study reported here, vessels altered in this fashion, as well as capillaries with endothelial proliferation, did not show a permeability to Thorotrast.

The use of Thorotrast consisting of colloidal thorium dioxide suspended in dextrin as supplied by Fellows Testagar, Detroit, has been criticized as a means for testing vascular permeability. Rowley (1963) demonstrated that dextrin will induce a degranulation of mast cells with the subsequent release of histamine, an agent capable of producing a permeability change. According to Ashton and Cunha-Vaz (1965), histamine does not produce an increased permeability of cerebral and retinal vessels, however, in contrast to vessels in other organs. This would confirm our experience since we have not seen Thorotrast pass through vessels in normal young or adult rats.

Once past the endothelial and basement membrane, Thorotrast spreads between glial and neuronal processes. This finding suggested

that narrow intercellular spaces represent no barrier for the diffusion of colloidal substances. In allergic encephalomyelitis, a similar spread of plasma proteins and Thorotrast throughout intercellular spaces has been described (Lampert & Carpenter, 1965). There is evidence that the migration of particles is facilitated in areas where normally wider extracellular spaces exist, i.e., in the white matter (Levine, et al., 1963; Hirano, et al., 1964). For the same reason, a rapid spread of particles is anticipated in regions with abnormally widened extracellular spaces as observed in lead encephalopathy in the white and gray matter (Figs. 2, 4, 5). The fact that red cells are found squeezed within compact gray matter (Fig. 3) whereas they float freely within widened extracellular spaces in the white matter (Fig. 4), may be interpreted that in the gray matter narrow intercellular spaces are either more quickly restored or less easily created. We favor the former more dynamic interpretation.

Thorotrast particles and possibly also plasma proteins were not only observed in intercellular spaces in the affected regions of the brain but also within vesicles in astrocytic processes (Fig. 8). This finding is consistent with the observations by Klatzo and Miquel (1960) who showed that astrocytes incorporate proteins by pinocytosis. Similar observations have been described by Tani and Evans (1965) who observed the uptake of Ferritin by astrocytic processes in the edematous cat brain. Some astrocytes, particularly in the white matter, were further characterized by features known to be present in reactive, proliferating cells, i.e., they showed an increase of glial filaments, ribosomes and mitochondria.

Our study has not yet revealed any clues in regard to the origin of the numerous microglial cells or macrophages. The axonal changes, consisting of axoplasmic enlargements filled with mitochondria, dense bodies and vesicular elements, are interpreted as non-specific axonal reactions to injury. Such axonal enlargements have been described in a variety of conditions including trauma to axons (Lampert & Cressman, 1964). Since we also found degenerating axons, it must be assumed that axons were severed. This undoubtedly occurred in advanced cerebellar lesions in which the white matter was replaced by large cystic spaces. In this respect, it is even more remarkable that myelin sheaths around intact or altered axons at the margin of cysts remained well preserved.

SUMMARY

1. Lead poisoning of suckling rats produces an abnormal vascular permeability in the brain particularly in the cerebellum, striatum, occipital lobes, and lower spinal cord.

2. The striatum and cerebellum were examined with the electron microscope. A considerable widening of the extracellular space was observed in both gray and white matter, but this was a transitory phenomenon in the gray matter.

3. Microglial, astrocytic, and endothelial proliferation occurred. Neurons, oligodendrocytes and myelin sheaths remained well preserved.

4. Intravenously injected Thorotrast (colloidal thorium dioxide), an electron dense tracer, traversed vessel walls by passing between but not through endothelial cells. After penetration of the basement membrane, the particles spread throughout intercellular spaces between glial and neuronal processes. Thorotrast was also noted within membrane-bound compartments of astrocytic processes. Twenty hours after intravenous injection of Thorotrast, most of the particles were found within macrophages but some were still in intercellular spaces.

5. These morphological features are characteristic of "dysoric encephalopathies."

REFERENCES

Ashton, N. and J. G. Cunha-Vaz: Effect of histamine on the permeability of the ocular vessels, Arch. Ophthal., 73, 211-223 (1965).

Blackman, S. S.: The lesions of lead encephalitis in children, Bull. Johns Hopkins Hosp., 61, 1-62 (1937).

Casley-Smith, J. R.: Endothelial permeability, II, The passage of particles through the lymphatic endothelium of normal and injured ears, British J. Exp. Path., 46, 35-49 (1965).

Dalton, A. J.: A chrome-osmium fixative for electron microscopy, Anat. Rec., 121, 281 (1955).

Herzog, I., W. A. Levy and L. C. Scheinberg: Biochemical and morphologic studies of cerebral edema associated with intracerebral tumors in rabbits, J. Neuropath. & Exp. Neurol., 24, 224-255 (1965).

Hirano, A., H. M. Zimmerman and S. Levine: The fine structure of cerebral fluid accumulation, III: Extracellular spread of cryptococcal polysaccharides in the acute stage, Am. J. Path., 45, 1-19 (1964).

Klatzo, I. and J. Miquel: Observations on pinocytosis in nervous tissue, J. Neuropath. & Exp. Neurol., 19, 475-487 (1960).

Lampert, P. and S. Carpenter: Electron microscopic studies on the vascular permeability and the mechanism of demyelination in

experimental allergic encephalomyelitis, J. Neuropath. & Exp. Neurol., 24, 11-24 (1965).

____ and M. Cressman: Axonal regeneration in the dorsal columns of the spinal cord of adult rats: an electron microscopic study, Lab. Invest., 13, 825-839 (1964).

Levine, S., H. M. Zimmerman, E. J. Wenk and N. K. Gonatas: Experimental leukoencephalopathies due to implantation of foreign substances, Am. J. Path., 42, 97-117 (1963).

Luft, J. M.: Improvement in epoxy resin embedding methods, J. Biophys. Biochem. Cytol., 9, 409-414 (1961).

Majno, G. and G. E. Palade: Studies on inflammation, I. The effect of histamine and serotonin on vascular permeability. An electron microscopic study, J. Biophys. Biochem. Cytol., 11, 571-605 (1961).

Movat, H. Z. and N. V. Fernando: Acute inflammation. The earliest fine structural changes at the blood-tissue barrier, Lab. Invest., 12, 895-910 (1963a).

____ and ____: Allergic inflammation, I: The earliest fine structural changes at the blood-tissue barrier during antigen- antibody interaction, Am. J. Path., 42, 41-50 (1963b).

Palay, S. L., S. M. McGee Russell, S. Gordon and M. A. Grillo: Fixation of neural tissue for electron microscopy by perfusion with solutions of osmium tetroxide, J. Cell Biol., 12, 385-419 (1962).

Pentschew, A.: Intoxikationen; in Handbuch der speziellen pathologischen Anatomie und Histologie (ed. by W. Scholz), Vol. XIII, 2, 1929-1971 (1958).

____: Morphology and morphogenesis of lead encephalopathy. Acta Neuropathologica, 5, 133-160 (1965).

____, F. Garro and P. Schweda: Systemized dysoric encephalopathy in the suckling rat produced by lead, Proceedings Vth Intern. Congress Neuropath., Excerpta Medica, 730-733 (1966).

Popoff, N., S. Weinberg and I. Feigin: Pathologic observations in lead encephalopathy with special reference to the vascular changes, Neurology, 13, 101-102 (1963).

Rowley, D. S.: Mast cell damage and vascular injury in the rat. An electron microscopic study of a reaction produced by Thorotrast, British J. Exp. Path., 44, 284-290 (1963).

Smith, J. F., R. L. Laurin, J. B. Nichols and A. Ashbury: Studies in cerebral edema and cerebral swelling. The changes in lead encephalopathy in children compared with those in alkyl tin poisoning in animals, Brain, 83, 411-429 (1960).

Struck, G. and W. Umbach: Vergleichende elektronenoptische Untersuchungen an der menschlichen Hirnrinde vor und nach Odemtherapie, Virch. Arch. Path. Anat., 337, 317-327 (1964).