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Electron Microscopic and Chemical Studies of the Vascular Changes and Edema of Lead Encephalopathy

A Comparative Study of the Human and Experimental Disease

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Lead encephalopathy was induced in suckling rats by administering lead to the mother. The brains were studied by light and electron microscopy, and the results were compared with observations in the human disease as well as in cases of cerebral ischemia in children. In their severe forms, both human and experimental lead encephalopathies are characterized by exudative extracellular edema and perivascular PAS-positive globules. The latter consist of osmiophilic non-membrane-limited cytoplasmic inclusions located, in the rat exclusively and in the human predominantly, in perivascular astrocytes. Intervascular strands are also found in both forms of the disease. In the rat these consist of basement membrane surrounding endothelial cytoplasm. Chemically, experimental lead encephalopathy with morphologically demonstrable edema is associated with an increase in brain water, sodium and serum albumin. Relative to the serum concentration, the increase in water is disproportionately greater than the sodium or albumin. There were no demonstrable changes in chloride or potassium (*Am J Pathol* 74:215-240, 1973).

THE STUDY OF LEAD ENCEPHALOPATHY in the experimental animal has been hampered by the repeated failure to reproduce the histologic changes characteristic of the human disease¹ by administering lead

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to adult animals.² In the many years that the effects of lead poisoning in adult rats, guinea pigs and rabbits have been studied in our laboratory, literally hundreds of brains have been examined histologically.^{3,4} In this period, we have not observed cerebral lesions comparable to the protein-rich edema characteristic of acute lead encephalopathy in man nor are we aware, with one possible exception, of any description of such lesions occurring in adult lead-poisoned experimental animals. The exception is a brief and unillustrated comment that "serous" exudates were found about capillaries in the molecular layer of the cerebellum in rabbits given subcutaneous injections of lead and subjected to hyperthermia.⁵

This situation has been entirely changed in recent years by the description of a technic for producing lead encephalopathy in suckling rats by poisoning the mother with lead.^{6,7} It is the purpose of this paper to further characterize this experimental disease morphologically, to compare it to the human condition and to provide quantitative chemical data on plasma uptake and tissue electrolyte changes.

Review

The classic description of lead encephalopathy in children was written by Blackman in 1937.¹ The most striking acute change described was the accumulation of a protein-rich edema fluid within the brain. Blackman also described "perivascular droplets" which were interpreted as old inspissated exudate. Subsequent workers have not added a great deal to Blackman's basic findings.⁸⁻¹¹ The periodic acid-Schiff (PAS) reactivity of the edema fluid was recognized⁹ along with that of the droplets,¹⁰ which were now viewed as products of degeneration. In a recent light microscopic study, the droplets were entirely ignored.¹¹ Electron microscopic studies of cerebral biopsies of children with lead encephalopathy treated by surgical decompression have been published.¹² Vascular alterations are restricted to the grey matter. The white matter showed an extracellular edema and destructive lesions of astrocytes.

The first description of experimental lead encephalopathy with histologic features characteristic of the human disease was that of Pentschew and Garro.⁶ The cerebellum showed multiple spotty hemorrhages with an increase in the number of capillaries in the molecular layer and the white matter serous transudates. Electron microscopic studies showed enlargement of the extracellular space in both white and grey matter, but this was more prominent in the former and contained fine granular material. The blood vessels in abnormal areas showed vacuolated cyto-

plasm, thickening of the basement membrane and, sometimes, absence of astrocytic foot processes. Colloidal thorium dioxide traversed vessel wall by passing between endothelial cells and through the basement membrane. The hyperpermeability was not correlated with the cytoplasmic vacuolation. Except for foot processes which contained vacuoles filled with amorphous material, the presence of astrocytic changes was specifically denied. Cells identified as microglia with phagocytosed material were numerous. No changes in neuronal bodies were described and tissue necrosis was not a prominent feature of the process even in the areas of hemorrhage.⁷ These results were subsequently confirmed and supplemented with chemical analyses for lead.¹³ The brains contained 1.2 g% and the blood level was 0.3 mg% of lead.

Lead encephalopathy has also been produced in the suckling mouse. Here, it is characterized by intervacular strands and minimal evidence of cerebral edema. On this basis, the similarity of the rodent and human disease was challenged.¹⁴

The administration of lead to adult monkeys and cats results in clinical symptoms in 2 weeks and death in 2 months.² This regimen did not produce changes in the brains comparable to those seen in children dying of lead encephalopathy. Much more promising are two recent studies in juvenile and adult baboons.^{15,16} Unfortunately, neither of these papers include histologic studies of the brains.

Accidental lead poisoning has also been demonstrated in zoo-dwelling primates.¹⁷ Although some of these animals developed convulsions and apparently blindness,¹⁸ the morphologic cerebral lesions described in these animals¹⁹ are not similar to those seen in controlled lead poisoning in juvenile rhesus monkeys.²⁰ Extraneous factors may have been operating in these animals.

The only chemical studies of the cerebral edema in lead encephalopathy of which we are aware reported higher than normal water content of the white matter²¹ in one child.⁹

Materials and Methods

Experimental

Pregnant Sprague-Dawley white rats were fed a normal laboratory diet supplemented with lettuce to avoid cannibalism of the newborn. About 1 week after delivery the mothers were placed on a synthetic diet containing 4% lead subacetate. The composition of this diet was the same as that previously published²² except that 0.32% magnesium sulfate was added to the basic diet. The vitamin fortification mixture was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. This diet was also made available to the weanlings. Control animals were maintained on the same diet without lead. The poisoned suckling animals failed

to develop normally and after 3 weeks many became paralyzed and died. The surviving animals were killed at 24 to 40 days of age along with paired control animals of the same age.

On the day before killing, the rats were injected intraperitoneally with about 10 μ Ci of radioactive ^{131}I -labeled human serum albumin (RISA[®]-Albumotope, Abbott Laboratories). The animals were anesthetized with ether and decapitated. Blood was collected from the trunk, and the serum separated by centrifugation. Protein was determined by methods previously described,²³ and the ^{131}I by routine counting. In some experiments, after counting, both brain homogenate and serum were mixed with 5 ml of 20% trichloroacetic acid (TCA). The mixture was centrifuged, the supernatant discarded and the process repeated. The tissue and serum were then recounted. The albumin content of brain was calculated by dividing the tissue counts by serum counts on the assumption that the specific activity of the cerebral albumin was the same as that of serum.

Serum sodium was determined by flame photometry and chloride by the Volhard method. The brain, including the cerebellum and brain stem, was homogenized and divided into two samples. One of these was counted and then wet-ashed in silver nitrate and nitric acid for chloride using the Volhard method. The other sample was dried to constant weight in a vacuum oven at 50 C after maceration with acetone. The dried sample was charred with 5 N sulfuric acid and dry-ashed at 500 C. The sodium and potassium were then determined by flame photometry. In 16 animals the role of hemorrhage was checked by analysis of tissue iron according to methods previously described.²⁴ For light microscopy the tissues were fixed by immersion in 10% buffered formalin and embedded in paraffin. The slides were stained by the hematoxylin and eosin and luxol fast blue-PAS-hematoxylin (LPH) technics. These studies were based on 18 lead-poisoned animals and 3 controls.

For electron microscopic studies, the animals were anesthetized with ether and killed by intracardiac perfusion using a modification of a technic previously described.²⁵ This involved perfusion under a pressure of 100 mm mercury of 100 ml of dilute fixative followed by full-strength fixative for about 20 minutes. The formulae of the fixatives are as follows: Dilute fixative, 1) 6.6 g paraformaldehyde in 500 ml distilled water, 2) 3.6 ml 50% glutaraldehyde, 3) 260 ml 0.2 N cacodylate buffer, pH 7.2 to 7.4, 4) 0.50 g calcium chloride, 5) 0.25 g aluminum chloride, 6) 400 g dextran (M.W. 60,000–90,000) Clinical Grade (National Biochemicals Corp, Cleveland, Ohio) and 7) *qs*, to 1 liter with distilled water; Full-strength fixative, as above, except that 10.0 g of paraformaldehyde are used.

Following perfusion, the brain was left *in situ* for about 2 hours. Samples were then taken, post-fixed in osmium, embedded in Epon and studied in an RCA electron microscope (Model EMU 3-G). For light microscopic studies of this material the sections were cut at 2 μ using a glass knife. The Epon was removed by exposing the sections to a saturated solution of sodium hydroxide in absolute ethyl alcohol for 1 hour, followed by four washings of absolute ethyl alcohol. The sections were placed in an aqueous phosphate buffer at pH 7.0, followed by thorough washing with distilled water. They were then stained by the PAS technic with a hematoxylin counterstain. Electron microscopic studies were performed on 11 poisoned rats with hind limb paralysis and 5 paired controls.

Clinical

Slides and paraffin blocks were obtained from autopsy material from 11 patients in which a clinical diagnosis of lead poisoning had been established. In all cases, six or more sections of brain and one to two sections of kidney were available. The brain sections were stained by the same technics used in the experimental material. For the kidney both the hematoxylin and eosin stain and the acid-fast stain were

used. In one instance in which perivascular globules were particularly numerous, the appropriate site was selected by light microscopy and the tissue was deparaffinized, rehydrated, fixed in osmium, embedded in Epon and studied by electron microscopy.

The cases of lead poisoning included 9 children ranging in age from 1 to 4 years. All showed the classic symptoms of lead encephalopathy. One patient was a 31-year-old male who had an industrial exposure to lead. He had chemical evidence of lead poisoning and symptoms of encephalopathy. This patient expired on the second hospital day. The second adult was a 50-year-old male with symptoms of peripheral neuropathy who died of renal failure. His kidneys, in addition to lead intranuclear inclusions, showed evidence of acute tubular necrosis.

Slides and blocks were also obtained from 6 children, all of whom had an episode of cerebral anoxia due to cardiac arrest some time before death. These cases ranged in age from 1½ to 7 years. Three patients had a clinical diagnosis of congenital heart disease; the others were diagnosed as having toxic impetigo, *Klebsiella* meningitis and bronchopneumonia. At least 10 sections were obtained from each brain and stained as above. A cerebral biopsy from an 8-year-old white child with an undiagnosed progressive neurologic disease was also studied. This child had an extensive medical work-up and no evidence of lead poisoning was demonstrated. The child was subsequently institutionalized and has been lost to follow-up.

Results

Histologic Changes in the Rat

The lead-poisoned infants showed gross and microscopic changes generally similar to those previously described.^{6,7} Animals with hind limb paralysis showed the most severe disease. The white matter of both the cerebellum and cerebrum contained a protein-rich fluid separating the myelinated fibers. This stained positively with the PAS technic and was associated with glial cells, the cytoplasm of which stained intensely (Figures 1 and 2). Similar focal collections of edema fluid were also observed in the grey matter of the caudate nucleus and septum (Figure 3) as well as in the Purkinje cell layer and in subpial tissues of the cerebellum. Vacuolation was also noted in the grey matter of the basal ganglia. This was seen both in association with pools of PAS-positive edema fluid (Figure 4) and as an independent change. Vessels with strands similar to those described in the mouse, but not previously described in the rat, were noted in both the cerebellum and basal ganglia (Figure 5). An additional change, not previously described in the experimental disease, consisted of perivascular PAS-positive globules (Figure 5). These correspond to Blackman's "droplets." Structures that were similar but smaller in size were noted in the controls (Figure 6). The latter appeared to be within the cytoplasm of perithelial cells. Massive collections of edema fluid and foci of tissue vacuolation occurred with the severe forms of the disease, but animals with no evidence of protein-rich edema fluid were infrequent. Animals with minimal disease showed

only the PAS globules and petechial hemorrhages. In rare cases only the latter were seen. The commonest sites of involvement were the cerebellum and basal ganglia. Lesions in the brain stem and hippocampus were rare. Except for some loosening of tissue structure adjacent to massive collections of edema fluid in the white matter of the cerebrum, the neocortex was not involved.

Ultrastructural Changes in the Rat

The perivascular globules observed in the control caudate nucleus (Figure 6) proved on electron microscopy to be slightly osmiophilic membrane-limited bodies clearly located within the cytoplasm of perithelial cells (Figure 7). By contrast, the perivascular globules seen in the lead-poisoned animals were much more osmiophilic, more variable in size, not membrane-limited and located in the cytoplasm of perivascular astrocytes (Figure 8). The edema fluid appeared as a coarse granular precipitate (Figure 9). The inclusions within the cytoplasm of the macrophages (Figure 4) were osmiophilic and not membrane-limited (Figure 10). Areas of tissue vacuolation seen by light microscopy (Figure 4) proved to be foci of swollen astrocytes (Figure 11).

The vascular strands (Figure 6) consisted of endothelial cytoplasm surrounded by a basement membrane and lacking a lumen (Figure 11). In the case illustrated we were able to follow the strand across an entire grid opening. Endothelial cells often showed an increase in cytoplasmic vacuoles. Definite alterations in the vascular basement membrane were not identified, nor did we encounter any vessels devoid of perivascular astrocytic processes.⁷

Clinical Material

All cases showed the characteristic acid-fast renal inclusions,¹ and all children showed both exudative cerebral edema and perivascular PAS-positive globules. Intravascular globules of similar structure were found in a few cases. Most children showed vascular strands (Figure 12). These structures could be easily misinterpreted as tangential cuts of normal vessels. That this is indeed a misinterpretation is indicated both by their relatively great number in lead encephalopathy and essential absence in control material. Necrobiotic neurons were observed in 50% of the cases. The adult whose symptoms were those of peripheral neuropathy showed no significant cerebral changes. The adult with the industrial exposure showed the perivascular globules (Figure 13), but the protein-rich edema fluid was confined to the subarachnoid space. By electron microscopy, the perivascular globules resembled those seen

in the experimental disease and, like these, were principally located within the cytoplasm of perivascular astrocytes (Figure 14). Because of the nature of this material, fixation is understandably poor.

Perivascular PAS-positive globules were frequently found in the anoxic cases but they were clearly different from the lesions described above. These lesions were smaller and more uniform than the lead lesions and were located within the vessel wall, most likely within perithelial cytoplasm. This type of lesion is illustrated in Figure 15, which is from the brain biopsy referred to above.

Chemical Changes in the Rat

Serum protein and electrolyte values are given in Table 1. There is no demonstrable alteration in the sodium or chloride of the poisoned animals, but the total protein is significantly lower in this group, as the result of a diminished globulin content.

The brains of the lead-poisoned animals showed a three-fold increase in the uptake of serum albumin (Table 2). This difference is statistically significant both by comparison of means and by paired comparison testing. There is no indication of any trend in this value for control groups of different ages nor is there any indication of a period of maximum disease for the group of poisoned animals. The presence or absence of hind limb paralysis is also indicated on this table. Animals with hind

Table 1—Serum Analyses

| | | Control | Poisoned |
|-------------------------|-------------|---------|----------|
| Sodium (mEq/liter) | \bar{x} | 140.3 | 141.5 |
| | S \bar{x} | 1.4 | 1.4 |
| | N | 12 | 19 |
| Chloride (mEq/liter) | \bar{x} | 104.8 | 105.8 |
| | S \bar{x} | 2.3 | 1.4 |
| | N | 14 | 18 |
| Total Protein (g%) | \bar{x} | 6.14 | 5.65* |
| | S \bar{x} | 0.21 | 0.14 |
| | N | 17 | 24 |
| Albumin (g%) | \bar{x} | 3.30 | 3.50 |
| | S \bar{x} | 0.17 | 0.11 |
| | N | 17 | 24 |

* Significantly different from control: $t = 2.04$, $P < 0.05$

Table 2—Serum Albumin Content of Brain (g dry weight/100 g dry weight)

| Age of animal (days) | Control | N | Poisoned | N | Difference | Paralysis |
|-------------------------|---------|--------|----------|---|------------|-----------|
| 24 | 0.21 | 2 | 1.10 | 2 | 0.89 | + |
| 26 | 0.18 | 1 | 0.56 | 2 | 0.38 | 0 |
| 27 | 0.25 | 1 | 0.92 | 2 | 0.67 | 0 |
| 30 | 0.29 | 3 | — | — | — | 0 |
| 31 | 0.28 | 1 | 0.64 | 2 | 0.36 | + |
| 32 | 0.29 | 3 | 1.19 | 3 | 0.90 | + |
| 33 | 0.16 | 1 | 0.34 | 1 | 0.18 | 0 |
| 33 | 0.18 | 2 | 0.67 | 2 | 0.49 | + |
| 40 | 0.47 | 1 | 0.99 | 3 | 0.52 | 0 |
| 40 | 0.28 | 1 | 0.84 | 2 | 0.54 | 0 |
| \bar{x} | 0.26* | | 0.84* | | 0.46 | |
| S \bar{x} | 0.02 | | 0.07 | | 0.09 | |
| t | | 7.25 | | | 5.11 | |
| P | | <0.001 | | | <0.001 | |

* Computed from total number of animals in this and subsequent tables.

limb paralysis show more albumin ($\bar{x} = 0.66$) than those without ($\bar{x} = 0.44$), but the difference is not statistically significant.

The values for cerebral water and electrolytes are given in Tables 3–6. As with the albumin uptake, there is no clear trend for these figures in either the control or poisoned groups in the time interval tested. The water content of the poisoned brains is significantly greater than the control by a factor of about 7%. The sodium content is also increased in about the same range. This difference, however, is statistically significant only on paired comparison testing. The chloride and potassium are increased by about 4%, but neither figure is statistically significant.

Table 3—Water Content of Brain (g/100 g dry weight)

| Age of animal (days) | Control | N | Poisoned | N | Difference |
|-------------------------|---------|-------|----------|---|------------|
| 24 | 384.2 | 2 | 419.8 | 2 | 35.6 |
| 26 | 366.0 | 1 | 384.6 | 2 | 18.6 |
| 27 | 406.8 | 1 | 456.3 | 2 | 49.5 |
| 30 | 376.7 | 3 | 381.5 | 2 | 4.8 |
| 31 | 362.5 | 1 | 254.2 | 2 | 89.7 |
| 33 | 357.2 | 1 | 366.5 | 2 | 9.3 |
| 33 | 359.8 | 2 | 370.3 | 2 | 10.5 |
| 40 | 356.8 | 1 | 366.5 | 2 | 9.7 |
| \bar{x} | 372.2 | | 399.7 | | 28.5 |
| S \bar{x} | 4.4 | | 9.2 | | 10.3 |
| t | | 2.43 | | | 2.77 |
| P | | <0.05 | | | <0.05 |

Table 4—Sodium Content of Brain (mEq/100 g dry weight)

| Age of animals (days) | Control | N | Poisoned | N | Difference |
|-----------------------|---------|------|----------|---|------------|
| 24 | 25.0 | 2 | 27.2 | 2 | 2.2 |
| 26 | 23.4 | 1 | 26.4 | 2 | 3.0 |
| 27 | 28.0 | 1 | 35.3 | 1 | 7.3 |
| 30 | 21.4 | 2 | 22.3 | 2 | 0.9 |
| 31 | 22.8 | 1 | 33.9 | 2 | 11.1 |
| 32 | 20.3 | 4 | 22.8 | 4 | 2.5 |
| 33 | 22.2 | 1 | 23.9 | 2 | 1.7 |
| 33 | 22.6 | 2 | 23.4 | 2 | 0.8 |
| 40 | 22.1 | 1 | 22.3 | 3 | 0.2 |
| \bar{x} | 22.5 | | 24.2 | | 3.3 |
| $S\bar{x}$ | 0.6 | | 1.0 | | 1.2 |
| t | | 1.30 | | | 2.75 |
| P | | NS | | | <0.05 |

The iron values for brain and whole blood are given in Table 7. The brain shows an increase in iron, reflecting the morphologically observed hemorrhage. The blood shows a diminished iron, reflecting the well-known anemia of lead poisoning. The hematocrit of the control was 32.6% ($S\bar{x} = 1.5$, $N = 7$) and the poisoned group was 24.8% ($S\bar{x} = 1.9$, $N = 10$).

There was no difference in the percent of counts not precipitated by TCA in the brain and serum of control and poisoned rats. In the serum, the mean was 3.7% ($S\bar{x} = 0.79$, $N = 10$) and in the brain, 3.8% ($S\bar{x} = 1.09$, $N = 8$).

Table 5—Chloride Content of Brain (mEq/100 g dry weight)

| Age of animal (days) | Control | N | Poisoned | N | Difference |
|----------------------|---------|-----|----------|---|------------|
| 24 | 17.4 | 2 | 21.9 | 2 | +4.5 |
| 26 | 15.5 | 1 | 14.2 | 2 | -1.3 |
| 27 | 19.6 | 1 | 21.4 | 2 | +1.8 |
| 30 | 18.0 | 3 | 18.1 | 1 | +0.1 |
| 31 | 14.0 | 1 | 23.3 | 2 | +9.3 |
| 32 | 14.9 | 3 | 14.2 | 6 | -0.7 |
| 33 | 14.4 | 1 | 16.8 | 2 | +2.4 |
| 33 | 14.1 | 2 | 16.4 | 2 | +2.3 |
| 40 | 16.1 | 1 | 14.4 | 3 | -1.7 |
| 40 | 15.5 | 1 | 15.2 | 2 | -0.3 |
| \bar{x} | 16.1 | | 16.9 | | +1.6 |
| $S\bar{x}$ | 0.5 | | 0.8 | | 1.0 |
| t | | 0.8 | | | 1.60 |
| P | | NS | | | NS |

Table 6—Potassium Content of Brain (mEq/100 g dry weight)

| Age of animals (days) | Control | N | Poisoned | N | Difference |
|--------------------------|---------|------|----------|---|------------|
| 24 | 52.6 | 2 | 49.8 | 2 | - 2.8 |
| 26 | 44.0 | 1 | 51.0 | 2 | + 7.0 |
| 27 | 45.4 | 1 | 47.6 | 2 | + 2.2 |
| 30 | 47.8 | 3 | 49.7 | 2 | + 1.9 |
| 31 | 44.5 | 1 | 55.2 | 2 | +10.7 |
| 32 | 50.7 | 4 | 53.8 | 4 | + 3.1 |
| 33 | 53.8 | 1 | 44.5 | 2 | - 9.3 |
| 33 | 49.9 | 2 | 49.9 | 2 | 0.0 |
| 40 | 53.3 | 1 | 56.4 | 3 | + 3.1 |
| 40 | 46.9 | 1 | 49.7 | 1 | + 2.8 |
| \bar{x} | 49.3 | | 51.3 | | + 1.9 |
| $S\bar{x}$ | 0.8 | | 1.1 | | 1.7 |
| t | | 1.43 | | | 1.11 |
| P | | NS | | | NS |

Discussion

It is to be emphasized that the present paper is not concerned with the broad aspects of the effect of lead on the human body²⁶ but with the overt encephalopathy which is essentially uniformly lethal in the experimental animal and, often so, in the human.²⁷ Recent clinical evidence suggests that children may incur neurologic and psychologic defects following exposure to lead without developing symptoms of overt encephalopathy.^{28,29} We are not aware of any studies demonstrating anatomic cerebral damage in such cases but structural lesions have been reported in rats exposed to lead but not developing overt neurologic symptoms or morphologic evidence of cerebral edema.^{30,31} Since the term *lead encephalopathy* is defined as "a brain disorder caused by lead poisoning," it should encompass such cases.

Table 7—Iron Content of Brain and Whole Blood

| | Brain (mg/k) | Blood (mg/ml) |
|------------|--------------|---------------|
| Control | | |
| \bar{x} | 15.4 | 0.35 |
| $S\bar{x}$ | 0.8 | 0.02 |
| N | 6 | 7 |
| Poisoned | | |
| \bar{x} | 20.7* | 0.24* |
| $S\bar{x}$ | 0.8 | 0.02 |
| N | 9 | 8 |

* Significantly different from control: brain, $t = 4.5$, $P < 0.001$; blood, $t = 3.9$, $P < 0.01$.

Cerebral edema was present in all of our patients, including the 1 adult who died with symptoms of acute lead encephalopathy. Gross cerebral swelling and/or microscopic edema was reported in all of the autopsy series cited in this paper with one exception.¹¹ In this exception, the staining methods used are not specified, but all of the illustrations are of the Nissl technic. This is an inappropriate method for studying cerebral edema. Furthermore, the author interpreted the diffuse astrocytic proliferation and capillary activation, which he observed, as a manifestation of dysfunction of the blood-brain barrier to which he applied the term *dysoria*.

Chemical Changes

The data presented here show that experimental lead encephalopathy is associated with an increase in cerebral water, sodium and serum albumin. In Table 8 the relationship between these variables is presented using a method of calculation previously described.²⁴ Briefly, this consists of calculating the amount of serum from a given group of poisoned animals needed to produce the measured change in the brains of the paired control group. The figure so obtained is defined as "serum equivalents." It should be noted that in making these calculations, individual figures rather than the means given in the tables are used. If the observed changes for two different substances are both due to the addition of serum alone, then the serum equivalents calculated from each substance should be the same. The data in Table 8 show that changes in sodium, water and albumin cannot be explained by this hypothesis but that there is a disproportionate increase in brain water. In comput-

Table 8—Serum Equivalents (g dry weight of poisoned animals based on 100 g dry weight of the control brains)

| Age of animal (days) | Water | Albumin | Difference from water | Sodium | Difference from water |
|-------------------------|-------|---------|--------------------------|--------|--------------------------|
| 24 | 4.88 | 2.48 | 2.40 | 1.47 | 3.41 |
| 26 | 2.43 | 0.96 | 1.47 | 2.02 | 0.41 |
| 27 | 7.14 | 1.63 | 5.51 | 5.12 | 1.08 |
| 30 | 0.62 | — | — | 0.59 | 0.03 |
| 33 | 1.19 | 0.44 | 0.75 | 1.13 | 0.06 |
| 33 | 1.35 | 1.16 | 0.19 | 0.52 | 0.83 |
| 40 | 1.24 | 1.18 | 0.06 | 0.13 | 1.11 |
| \bar{x} | 2.69 | 1.31 | 1.73 | 1.57 | 0.99 |
| $S\bar{x}$ | | | 0.83 | | 0.44 |
| t | | | 2.08 | | 2.25 |
| p* | | | <0.01 | | <0.01 |

* The hypothesis tested is that the observed differences are greater than zero.

ing the probabilities for this table, a one-tailed *t*-test was used, since all the differences are greater than zero.

Calculations of the degree of hemorrhage using the cerebral iron figures and the whole blood iron of the poisoned group, give a blood equivalent of 2.4 g for 100 g wet weight of brain. Using this mean and the values for rat blood in the poisoned group (water, 79% and sodium, 136 mEq/liter) changes due to hemorrhage may be computed for Tables 2, 3 and 4. The figures, using the same units given in the tables, are: albumin, 0.55; water, 372.3; sodium, 23.5. As can be seen, this degree of hemorrhage contributes essentially nothing to the water change. The serum equivalents, corrected for hemorrhage and using the units in Table 8, are: albumin, 0.67 and sodium, 0.45. Both are even less than the uncorrected figures. Thus, correction for hemorrhage does not alter the finding that the brain shows a disproportionate water increase.

The first point which should be considered in analyzing these results is whether the tissue counts accurately reflect tissue plasma albumin. There are two factors which may be important here, first the possibility that there has been catabolism of the cerebral albumin with loss of the tag, and second, whether the plasma albumin is in equilibrium with all cerebral albumin. Both factors could give an erroneously low figure for tissue albumin. It has been shown that in the cat after 1 hour of convulsive activity, only about 70% of the iodine attached to cerebral albumin remains protein bound.³² Lead encephalopathy in the infant rat is not a convulsive state, and our own findings using TCA precipitation would indicate that catabolism of the serum albumin is not a significant source of error. The problem of equilibrium is more difficult. Published studies on vital staining with trypan blue, with the dye being administered in the same manner as we administered the RISA, have shown intense staining of the same areas in which we observed the PAS-positive edema fluid.⁷ This staining indicates, under these conditions, the presence of serum albumin³³ and may be viewed as presumptive evidence that a state of equilibrium exists.

The figures in Table 8 also show a disproportionate water increase with respect to sodium. In this case a tagged substance was not used nor was there any demonstrable effect of the lead on the concentration of sodium in serum (Table 1), yet the results are the same as with albumin.

If the validity of these figures is accepted, we are then left with the problem of interpretation. The figures for protein and water could be explained on the assumption that the increase in albumin is confined to the expanded extracellular space while the water increase involves

both this and the observed intracellular swelling of astrocytes. In keeping with this is the presence of granular precipitate in the extracellular space and its absence in the swollen astrocytes (Figure 9). Extending this hypothesis to the sodium figures, however, would require the further assumption that the increase in this ion is also confined to the extracellular space. This seems unlikely, since for various reasons the astrocyte is thought to be a high sodium cell³⁴ and it is difficult to envision an abnormally swollen astrocyte excluding sodium. Furthermore, indirect measurements of intracellular ions in cold injury edema have shown an increase in sodium roughly proportionate to the water increase with respect to serum concentrations.³⁵

Another interpretation of these data could be that the accumulating fluid has a concentration of albumin and sodium which is less than that of plasma. This view has much to commend it. First, in the only situation of which we are aware in which cerebral edema fluid was directly analyzed, this was indeed found to be the case.²³ That this is not an artifact of the technic used for the isolation of the edema fluid is indicated by the fact that identical results have been obtained using whole edematous tissue.³⁶ Furthermore, if the concentration of chloride in the edema fluid is the same as that of serum, then, using the water figure as a standard (Table 8), the figure in Table 5 should have been 19.1 for the poisoned group. This is 13% greater than the measured figure and is well outside the error of the method used for measuring the chloride. We must therefore conclude that the concentration of chloride in the edema fluid is also less than that of serum and, indeed, approaches that of whole brain.

The resolution of the questions posed by these two interpretations of the electrolyte data could be obtained by direct measurement of electrolytes at specific sites in tissue sections. With modern electron probe technics, this is feasible. Unfortunately, the pyroantimonate precipitation technic for sodium³⁷ is not specific enough to provide an answer. Recent data obtained by electron probe analysis in normal brain have indicated that in normal astrocytes the antimonate precipitate also contains significant amounts of calcium and magnesium.³⁶

In a previous publication the evidence was reviewed that cerebral necrosis is associated with a disproportionate increase in tissue sodium.²⁴ The failure to demonstrate this in the present work suggests that experimental lead encephalopathy is not associated with a significant degree of tissue necrosis. This is in keeping with the morphologic studies of this condition as well as the failure to demonstrate decreased cerebral potassium in the lead-poisoned animals (Table 6).

The data presented in Tables 2-4 clearly demonstrate that experimental lead encephalopathy is associated with increases in tissue water and sodium as well as an increased albumin uptake. The data, however, fail to show any definite pattern of progression and regression which would allow the definition of a peak period of edema formation. These experiments were originally undertaken in an attempt to define such a peak period, which could then be used for the evaluation of antiedema therapy. The failure to do so will make this problem more difficult. There is also no clustering of neurologic signs at any definite time period.

Morphologic Changes

The morphologic observations of the experimental disease have generally confirmed those previously reported,^{6,7,13} and several new findings have been added. First, the PAS-positive character of the edema fluid has been defined, thus relating more fully the human and experimental disease. Second, the vascular strands observed in the mouse¹⁴ were found in both the rat and the human. In addition, the ultrastructure of these strands has been defined for the rat. Although such structures have been described as occurring normally in both human and animal brains,³⁸ they were at best extremely rare in our control material. These strands have been interpreted both as collapsed capillaries³⁹ and atretic blood vessels.¹⁴ Since there is a more than 300% increase in the number of blood vessels per unit volume of rat brain between the ages of 1 and 3 weeks,⁴⁰ arrested development would seem to be the best explanation for these structures in the rat. This is in keeping with the ultrastructure of those strands; a capillary lumen could not be identified as might be anticipated for a collapsed blood vessel. Comparable data are not available for the human. The vascular strands, however, constitute a second morphologic similarity to the human disease.

The most important feature with regard to similarity is, in our opinion, the perivascular globules. Ultrastructurally, these have been identified as non-membrane-limited cytoplasmic astrocytic inclusions. Similar structures have been observed by light microscopy around cerebral blood vessels damaged by mercury.⁴¹ By electron microscopy, however, these lesions have been described as being membrane-limited and located primarily within perithelial cytoplasm.⁴² The lesions in lead encephalopathy were originally interpreted as being inspissated plasma proteins¹ and, indeed, they might so be. The mercury lesions at least contain plasma proteins.⁴² A more intriguing question is whether or not these lesions are preferential sites of lead deposition. We were un-

able to demonstrate, by either light or electron microscopy, a sulfide precipitate in these structures (or elsewhere) using a histochemical technic which demonstrates mercury in tissue.⁴³ This problem might also be resolved by electron probe analysis.

The perivascular globules were found in all our human cases with clinically proven lead poisoning and symptoms of encephalopathy. They were absent in a series of children with cerebral anoxia. We have also been unable to find such lesions in the brains of children with a variety of diseases that were studied in our laboratory for the past 5 years. We propose that this lesion has sufficient specificity to justify a histologic diagnosis of lead encephalopathy when it is found in the brains of infants and children and, possibly, adults. In this regard it is important to differentiate between the perivascular PAS-positive globules of the lead lesion and the intravascular PAS-positive globules often found in the brains of infants and children (Figure 15). The latter are probably related to the membrane-limited inclusions of the perithelial cell (Figure 7). The failure to describe the perivascular globules in a recent study of lead encephalopathy¹¹ may be related to the staining methods used. The lesions do not stain with the Nissl technic, and with luxol-fast-blue they stain like red blood cells and would have been interpreted as perivascular hemorrhages.

PAS-positive macrophages, so characteristic of the experimental disease, were, in our human cases, limited to the perivascular space. The ultrastructure of the cytoplasmic macrophage inclusions in the rat is similar to the perivascular astrocytic lesions. Phagocytic glial cells with PAS-positive inclusions are also found in cryogenic edema, and such inclusions have been shown to contain plasma proteins.⁴⁵ It could thus be that both types of inclusions in lead encephalopathy represent intracellular uptake of extravasated blood proteins. The concentration of serum albumin in the edema fluid associated with cryogenic injury is 1.0 g%.⁴⁶ If our hypothesis about the edema fluid of lead encephalopathy is correct, its albumin concentration is 2.2 g%. If, however, a portion of the albumin is concentrated in intracellular inclusions, then the concentration of serum albumin in the edema fluid of these two forms of injury might be identical.

Even if the view is accepted that the cytoplasmic inclusions in macrophages and perivascular astrocytes are identical and contain serum proteins, the explanation of the lead lesion is not a nonspecific uptake of extravasated plasma protein. The phagocytic cells in cryogenic edema are random in distribution, while in lead encephalopathy the uptake is,

in general, selectively perivascular in terms of astrocytes and random only in terms of macrophages in the rat. Figure 9 is an exception rather than a rule. It may be that the presence of lead in the out-pouring fluid stimulates the perivascular astrocytes to take up blood proteins and perhaps with them lead, which is then sequestered.

The necrobiotic neurons, so frequent in the human, were absent in the rat. Blackman viewed these changes as being secondary to the anoxia associated with edematous process,¹ and there has been general agreement on this point.⁸⁻¹⁰ However, the symptoms of lead encephalopathy have been attributed to the direct effect of lead on the enzymatic processes of the neuron²⁷ and hence the necrobiosis could be a direct result of lead poisoning. The fact that chemical changes in the brain, including alterations in protein permeability, precede the clinical symptoms by many days argues against this and supports Blackman's original view.

It is to be emphasized that the production of the experimental disease in the infant rat requires that the exposure to significant amounts of lead be instituted at a critical time period in development. We have been unable to produce the disease by giving the lead after the age of 20 days or by using a 2% lead-containing diet. This would suggest that there is some factor in the developing rat brain which renders it susceptible to the toxic effect of lead. This may, however, be a species peculiarity of rodents. The adult human case reported here, as well as the reported results in the baboon,¹⁵ indicate that the adult primate is susceptible to the development of lead encephalopathy. It may be mentioned that the reported apparent negative results in the adult monkey are based on only 2 animals.² This question should, we feel, be left open at the present time.

The accumulated data support the following tentative view of the pathogenesis of the vascular changes and edema in lead encephalopathy. The primary site of injury is the blood vessel.⁴⁷ This results in the arrested development of growing blood vessels with the formation of intervascular strands and the outpouring of fluid through the endothelial junctions into the extracellular space of brain, which is thereby expanded. The outpouring fluid is similar to plasma in that it contains albumin and sodium chloride but differs from it in that the concentration of these substances is lower than in plasma. The cerebral edema causes tissue anoxia resulting in swelling of astrocytes and when prolonged necrosis of neurons and glia. The edema fluid contains lead which stimulates the uptake of plasma proteins by perivascular astrocytes resulting in the formation of perivascular PAS-positive globules.

References

1. Blackman SS Jr: The lesions of lead encephalitis in children. *Bull Johns Hopkins Hospital* 61:1-61, 1937
2. Ferraro A, Hernandez R: Lead poisoning: histopathological study of nervous system of cats and monkeys in acute and subacute stages. *Psychiatr Q* 6:121-145, 329-350, 1932
3. Hass GM, Brown DVL, Eisenstein R, Hemmens A: Relations between lead poisoning in rabbit and man. *Am J Pathol* 45:691-737, 1964
4. Oyasu R, Battifora HA, Clasen RA, McDonald JH, Hass GM: Induction of cerebral gliomas in rats with dietary lead subacetate and 2-acetylaminofluorene. *Cancer Res* 30:1248-1261, 1970
5. Blackman.¹ p 34
6. Pentschew A, Garro F: Lead encephalomyelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases: with special reference to the permeability disorders of the nervous system capillaries. *Acta Neuropathol (Berl)* 6:266-278, 1966
7. Lampert P, Garro F, Pentschew A: Lead encephalopathy in suckling rats, Symposium on Brain Edema, Vienna, 1965. Edited by I Klatzo, F. Seitelberger. New York, Springer-Verlag, 1967, pp 207-222
8. Akelaitis AJ: Lead encephalopathy in children and adults: a clinicopathological study. *J Nerv Ment Dis* 93:313-332, 1941
9. Smith JF, McLaurin RL, Nichols JB, Asbury A: Studies in cerebral edema and cerebral swelling. I. The changes in lead encephalopathy in children compared with those in alky tin poisoning in animals. *Brain* 83:411-424, 1960
10. Popoff N, Weinberg S, Feigin I: Pathologic observations in lead encephalopathy: with special reference to the vascular changes. *Neurology* 13:101-112, 1963
11. Pentschew A: Morphology and morphogenesis of lead encephalopathy. *Acta Neuropathol (Berl)* 5:133-160, 1965
12. Raimondi AJ, Backman F, Evans JP: Fine structure of cerebral damage: toxic and mechanical, Impact Injury and Crash Protection. Edited by ES Gurdjian, AB Lange, CD Patrick, EF Thomas. Springfield, Ill, Charles C Thomas, Publishers, 1970, pp 160-179
13. Thomas JA, Dallenbach FD, Thomas M: Considerations on the development of experimental lead encephalopathy. *Virchows Arch [Pathol Anat]* 352:61-74, 1971
14. Rosenblum WI, Johnson MG: Neuropathologic changes produced in suckling mice by adding lead to the maternal diet. *Arch Pathol* 85:640-648, 1968
15. Hopkins A: Experimental lead poisoning in the baboon. *Br J Ind Med* 27:130-140, 1970
16. Cohen N, Kneip TJ, Goldstein DH, Muchmore EAS: The juvenile baboon as a model for studies of lead poisoning in children. *J Med Primatol* 1:142-155, 1972
17. Zook BC, Sauer RM, Bush M, Gray CW: Lead poisoning in zoo-dwelling primates. *Am J Phys Anthropol* 38:415-423, 1973
18. Zook BC, Sauer RM, Garner FM: Acute amaurotic epilepsy caused by lead poisoning in nonhuman primates. *J Am Vet Med Assoc* 161:683-686, 1972
19. Sauer RM, Zook BC, Garner FM: Demyelinating encephalomyelopathy associated with lead poisoning in nonhuman primates. *Science* 169:1091-1093, 1970

20. Clasen RA, Pandolfi S, Coogan PS, Laing I, Becker RA, Hartmann JF: Experimental lead encephalopathy in the rhesus monkey. *J Neuropathol Exp Neurol* 32:176, 1973
21. Katzman R, Pappius HM: *Brain Electrolytes and Fluid Metabolism*. Baltimore, The Williams and Wilkins Company, 1973, p 4
22. Battifora H, Eisenstein R, Laing GH, McCreary P: The kidney in experimental magnesium deprivation: a morphologic and biochemical study. *Am J Pathol* 48:421-437, 1966
23. Clasen RA, Sky-Peck HH, Pandolfi S, Laing I, Hass GM: The chemistry of isolated edema fluid in experimental cerebral injury,⁷ pp 536-553
24. Clasen RA, Pandolfi S, Russell J, Stuart D, Hass GM: Hypothermia and hypotension in experimental cerebral injury. *Arch Neurol* 19:472-486, 1968
25. Coogan PS, Morris F: An improved histologic technique for studying primate retina. USAF, SAM-TR-69-53, 1969
26. Goyer RA, Rhyne BC: Pathological effects of lead. *Int Rev Exp Pathol* 12:1-77, 1973
27. Coffin R, Phillips JL, Staples WI, Spector S: Treatment of lead encephalopathy in children. *J Pediatr* 69:198-206, 1966
28. Pueschel SM, Kopito L, Schwachman H: Children with an increased lead burden; a screening and follow-up study. *JAMA* 222:462-466, 1972
29. de la Burd e B, Choate MS Jr: Does asymptomatic lead exposure in children have latent sequelae? *J Pediatr* 81:1088-1091, 1972
30. Krigman MR, Druse MJ, Wilson MH, Hogan L: Myelination in lead-intoxicated suckling rats. *Am J Pathol* 62:1a, 1971 (Abstr)
31. Krigman MR, Reitz RC, Wilson MH, Newell LR, Hogan EL: Morphometric and sphingolipid composition of myelin in lead-intoxicated and undernourished suckling rats. *Am J Pathol* 66:1a, 1972 (Abstr)
32. Eisenberg HM, Barlow CF, Lorenzo AV: Effect of dexamethasone on altered brain vascular permeability. *Arch Neurol* 23:18-22, 1970
33. Clasen RA, Pandolfi S, Hass GM: Vital staining, serum albumin and the blood-brain barrier. *J Neuropathol Exp Neurol* 29:266-284, 1970
34. Katzman R: Electrolyte distribution in mammalian central nervous system. *Neurology (Minneapolis)* 11:27-36, 1961
35. Reulen HJ, Sami M, Fenske A, Hev O, Hase U: *Energy metabolism, fluids and electrolyte distribution in cold injury edema, Head Injuries*. Baltimore, The Williams and Wilkins Company, 1971, pp 232-239
36. Clasen RA: (Personal observations)
37. Hartmann JF: High sodium content of cortical astrocytes. *Arch Neurol* 15:633-642, 1966
38. Cammermeyer J: A comparative study of intervascular connective tissue strands in the central nervous system. *J Comp Neurol* 114:189-208, 1960
39. Tang TT, Cyrus AE, McCreddie SR: Some hitherto undescribed morphologic changes in lead encephalopathy. *Lab Invest* 18:336, 1968
40. Caley DW, Maxwell DS: Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. *J Comp Neurol* 138:31-47, 1970
41. Broman T, Grontoft O, Steinwall O: Comparative intravital and postmortem studies on chemically induced blood-brain barrier damage tested with trypan blue. *Acta Neurol Scand* 41:527-538, 1965

42. Olsson Y, Hossmann KA: Fine structural localization of exudated protein tracers in the brain. *Acta Neuropathol (Berl)* 16:103-116, 1970
43. Steinwall O, Klatzo I: Selective vulnerability of the blood-brain barrier in chemically induced lesions. *J Neuropathol Exp Neurol* 25:542-559, 1966
44. Chang LW, Hartmann HA: Electron microscopic histochemical study on the localization and distribution of mercury in the nervous system after mercury intoxication. *Exp Neurol* 35:122-137, 1972
45. Klatzo I, Miquel J, Otenasek R: The application of fluorescein labeled serum proteins to the study of vascular permeability in the brain. *Acta Neuropathol* 2:144-160, 1962
46. Hindo WA, Clasen RA, Rayudu GVS, Pandolfi S: Technetium in cryogenic cerebral injury and edema. *Arch Neurol* 27:526-534, 1972
47. Thomas JA, Dallenbach FD, Thomas M: Distribution of radioactive lead (^{210}Pb) in the cerebellum of developing rats. *J Pathol* 109:45-50, 1973

[Illustrations follow]

Fig 1—Control rat, neocortex at left, head of caudate at right (Paraffin section, PAS-luxol fast blue-hematoxylin, $\times 130$).

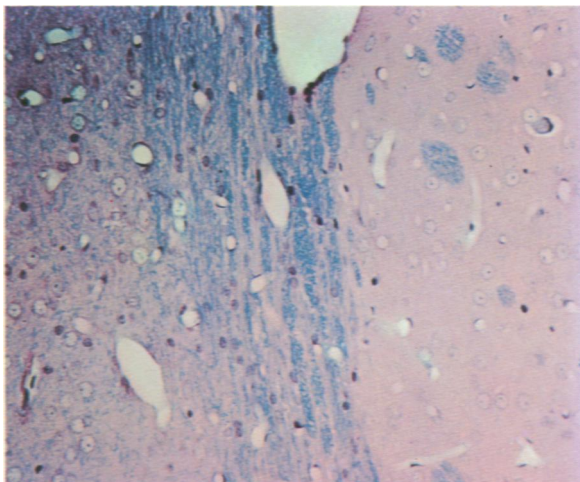


Fig 2—Lead-poisoned infant rat. Cortex and caudate nucleus as in Figure 1. The fibers of the white matter are separated by a PAS-positive fluid. This area also contains numerous glial cells with PAS-positive cytoplasm ($\times 130$).

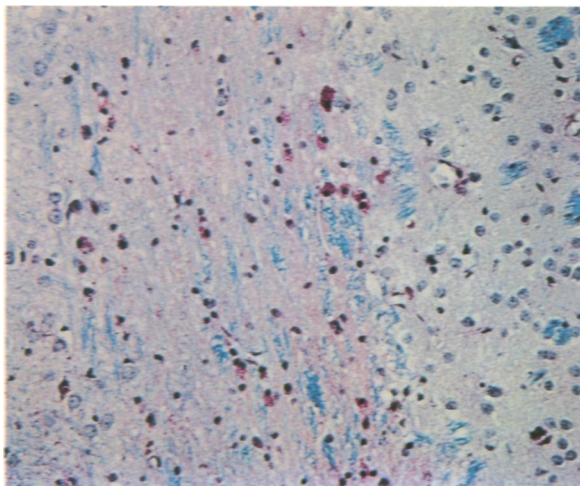
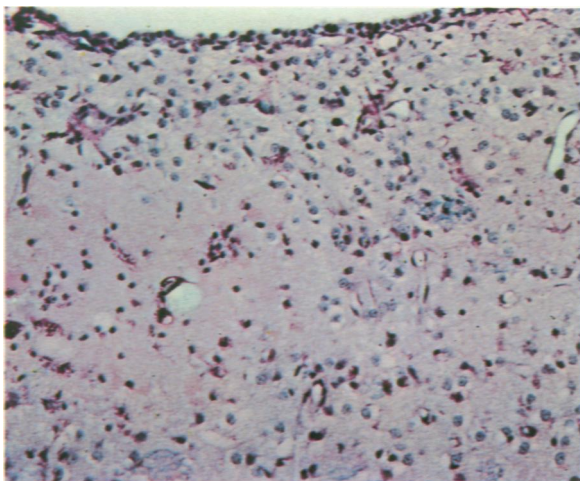
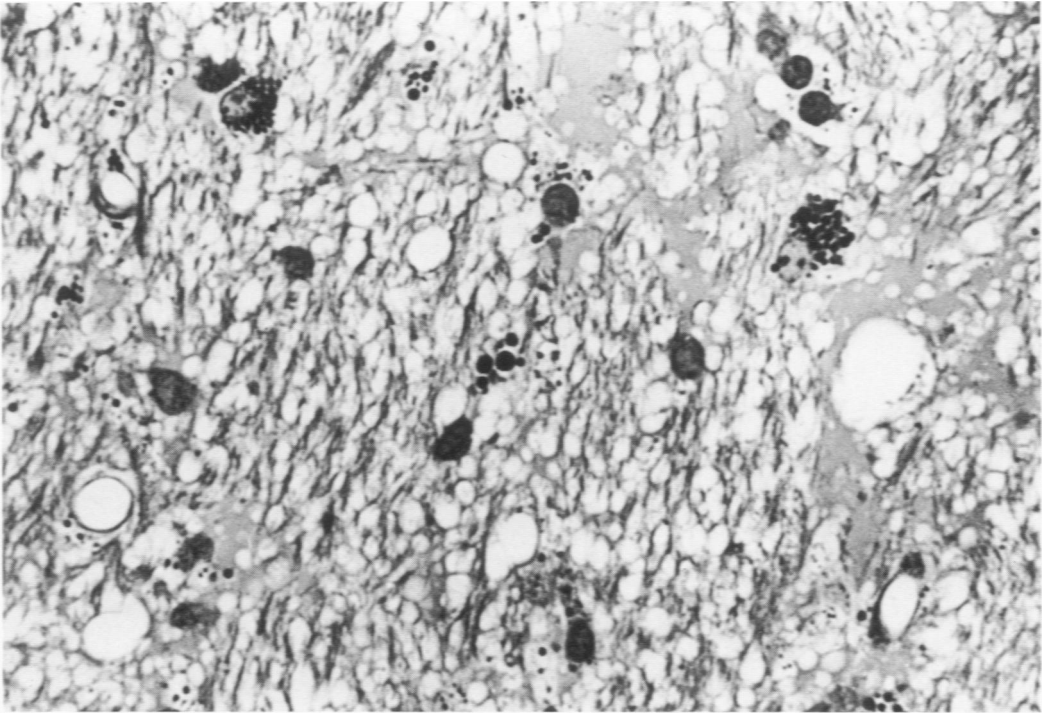


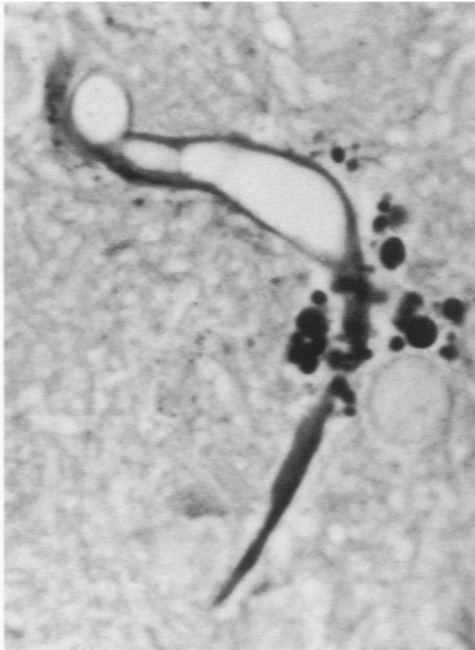
Fig 3—As above. Head of caudate nucleus. At the left, a collection of PAS-positive fluid is seen. The tissue generally has a vacuolated appearance ($\times 130$).



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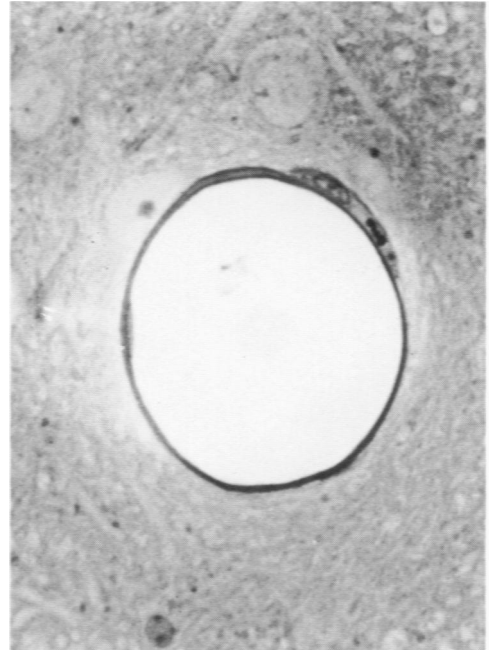
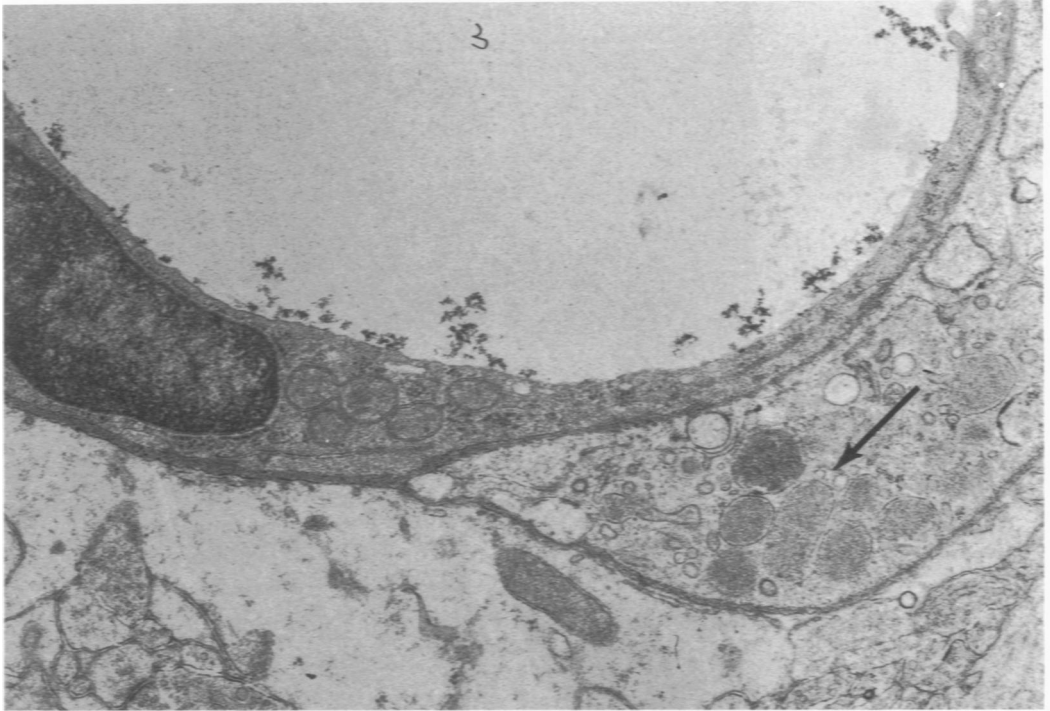
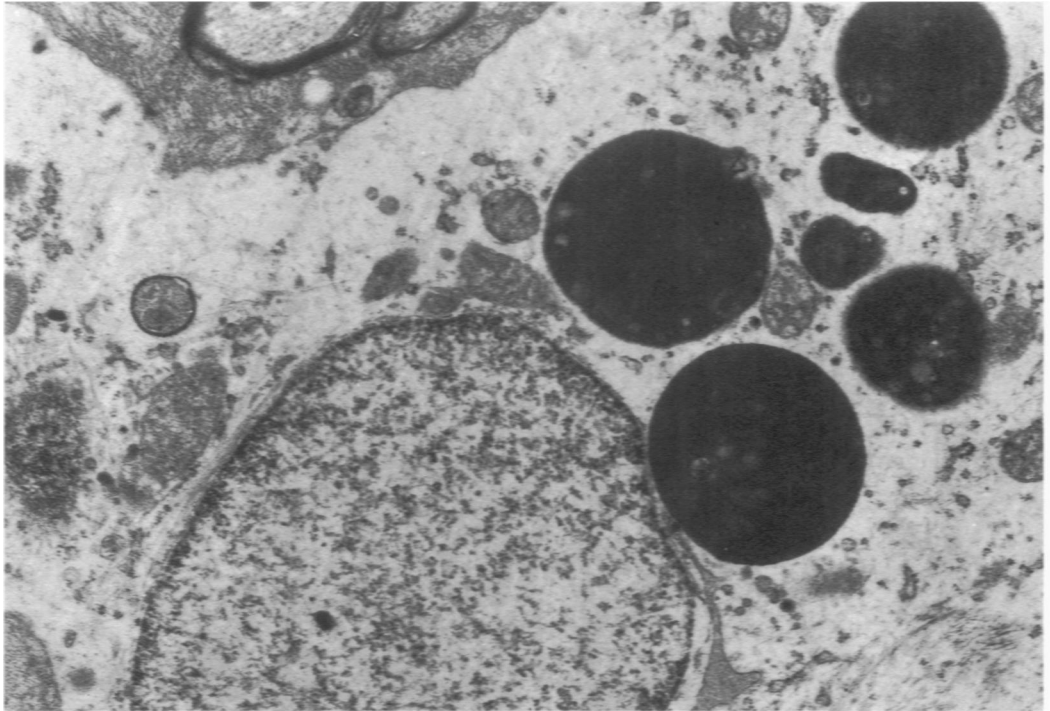


Fig 4—Lead-poisoned infant rat. Head of caudate. Pools of edema fluid are prominent, and the tissue generally has a vacuolated appearance. There are numerous glial cells with PAS-positive granules (Epon section, PAS, $\times 330$). **Fig 5**—The vessel shows both perivascular PAS-positive globules and a vascular strand (Epon section, PAS $\times 660$). **Fig 6**—Control. Note the PAS-positive granules within the cytoplasm of the perithelial cell (Epon section, PAS, $\times 660$).



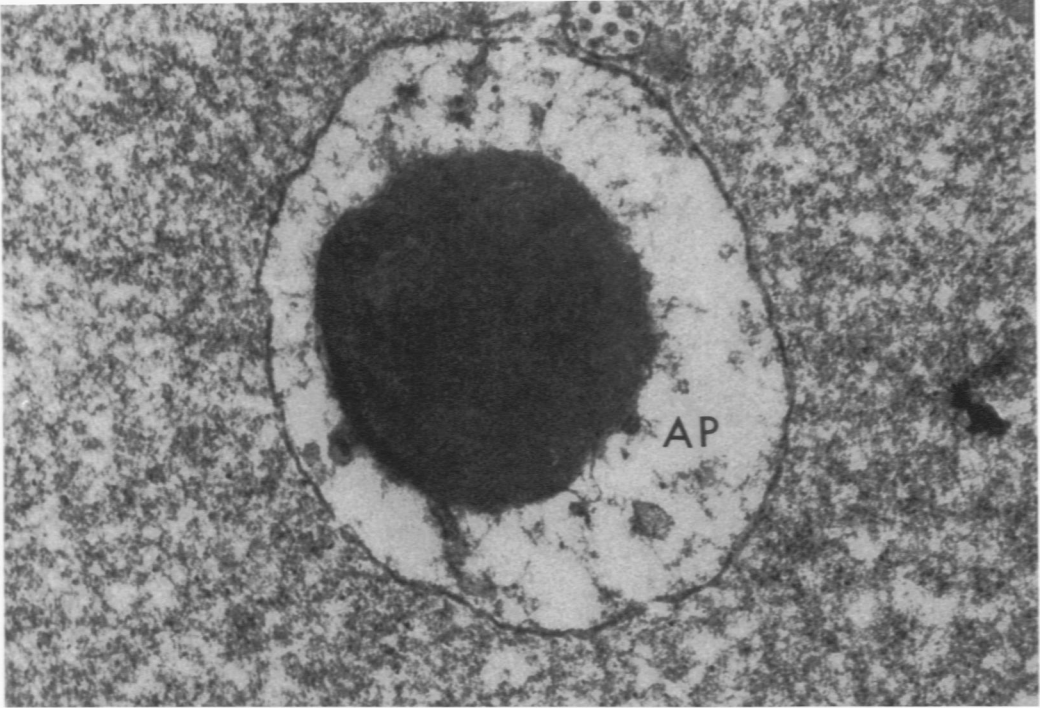
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Fig 7—Control rat. Head of caudate nucleus. The perithelial cytoplasm contains slightly osmiophilic membrane-limited inclusions (*arrow*) ($\times 22,670$). **Fig 8**—Lead-poisoned rat. Head of caudate nucleus. The perivascular astrocyte contains glial fibers and markedly osmiophilic non-membrane-limited inclusions ($\times 16,440$).

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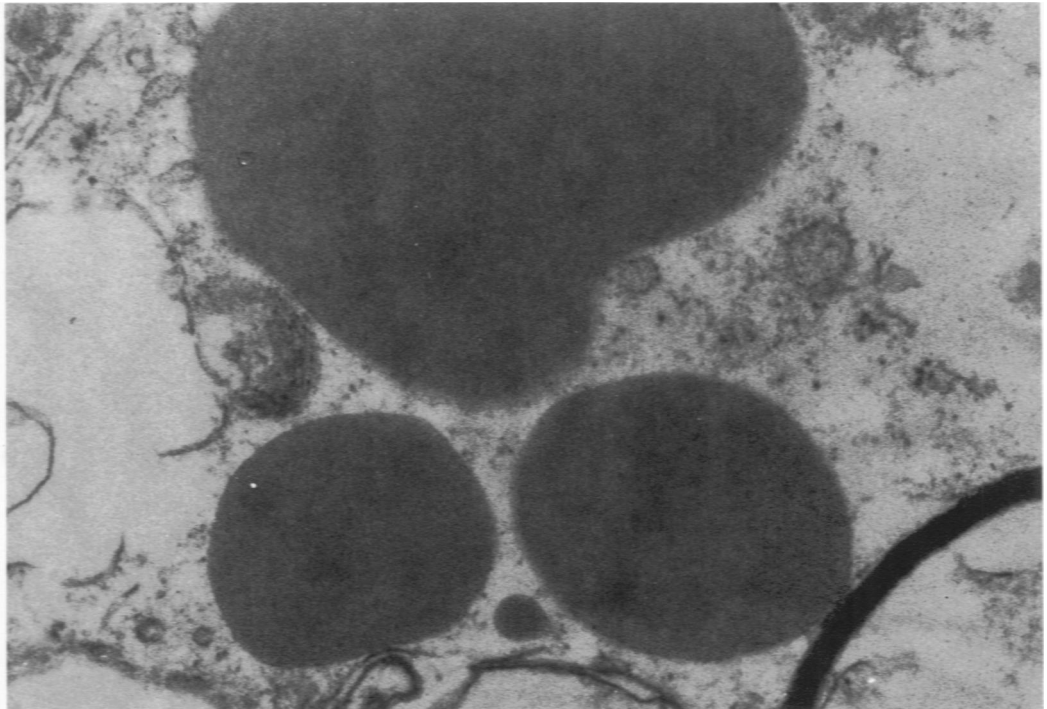
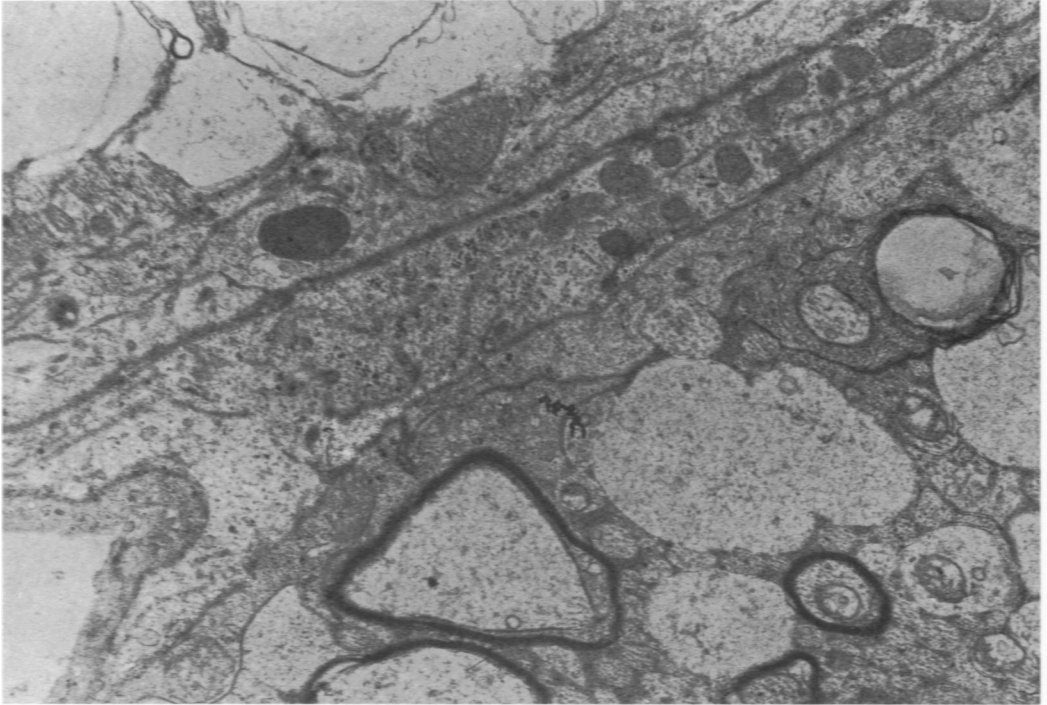
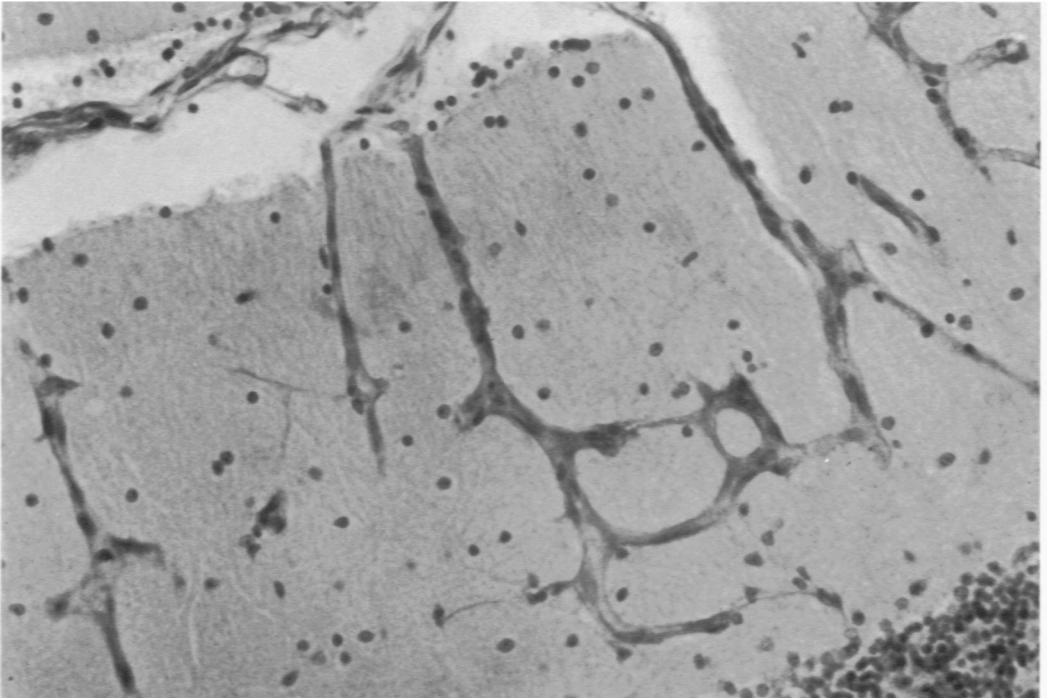


Fig 9—As above. Astrocytic process (AP) containing an inclusion and located in a pool of edema fluid ($\times 48,160$). **Fig 10**—As above. Macrophage containing non-membrane-limited osmiophilic inclusions ($\times 54,260$).

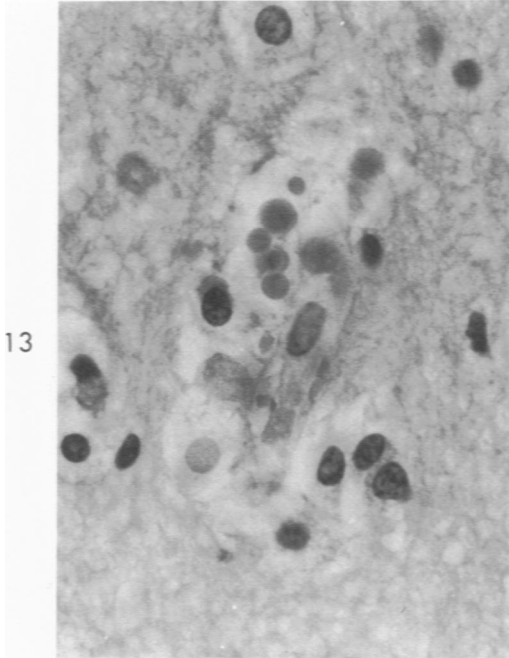


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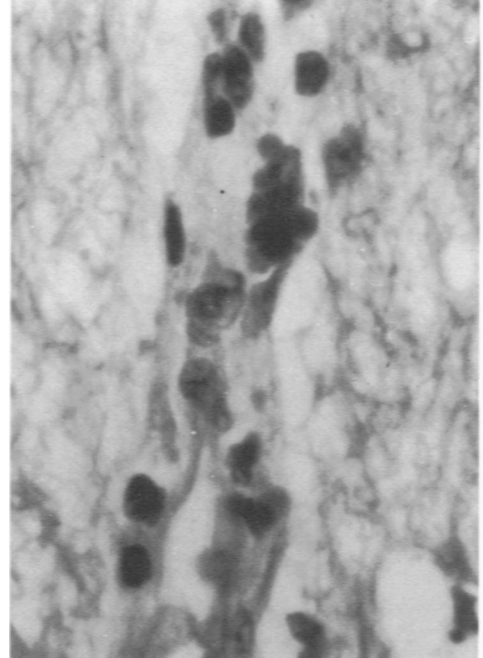


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Fig 11—As above. A vascular strand runs diagonally across the figure. Swollen astrocytic processes are present at the upper left ($\times 16,440$). **Fig 12**—Lead-poisoned child. Celloidin section of cerebellum (LPH stain, $\times 190$).



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Fig 13—Lead-poisoned adult. Paraffin section of brain (H&E, $\times 390$). **Fig 14**—Child with lead poisoning. The perivascular astrocytic cytoplasm contains a non-membrane-limited osmiophilic inclusion (*arrow*). It is situated adjacent to a perithelial cell ($\times 21,410$)
Fig 15—Biopsy from child with no clinical evidence of lead poisoning. Paraffin section of subcortical white matter. The perivascular globules are contained within the vascular basement membrane (LPH, $\times 650$).