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Clinical manifestations of the presences of unnatural amounts of systemic lead depend upon the particular structures which are affected or damaged and these are determined by the intensity and duration of exposure. In some instances, the excretion levels of lead may reflect a person's on-going exposure, but the actual lead burden must also include an unmeasured quantity of lead stored in the central and peripheral nervous systems as well as in the skeleton, liver and kidney muscles. The persisting effects of the encephalopathy, such as, mental retardation-epilepsy and behavioral disorders, may be the result of damage occurring during the acute intoxication. It is important to recognize the possibility that such chronic cellular damage may be the result of the slow release of stored lead from certain tissues, as well as continued exogenous exposure and absorption. For, it is well known (Kehoe, 1961; Black, 1962) that it takes twice as long to excrete an excessive burden of lead from the body as it takes to acquire the burden. Byers (1959) felt that most children who have been damaged intellectually have been re-exposed to lead after treatment of the acute disease. Chisolm (1956) (1968) has repeatedly emphasized the importance of continued environmental exposure to lead as one factor which increases the incidence of severe permanent damage to the brain among survivors of an initial attack of acute lead encephalopathy. This "continued exposure" may be caused by the slow release of stored amounts of lead, which in themselves do not produce obvious symptoms, but nevertheless may be capable of poisoning certain intracellular enzyme systems and producing latent effects. An increased incidence of cerebral hemorrhage as a late effect in workers in lead-using industries has been ascribed to the prolonged heavy exposure to lead (Dingwall - Fordyce and Lane, 1963). Lane (1964) believed that many

persons carrying lead burdens insufficient to cause symptoms or to produce disability from work have shortened life expectancies because of the premature development of nephritis and in some instances cerebral hemorrhage. Harriet Hardy (1966) has also emphasized the potential danger in low level exposure to lead.

In this discussion I shall first review some of the clinical and pathological aspects of the effects of lead on the nervous system; secondly, relate some of the recent studies utilizing histochemistry and electronmicroscopy to offer better discription of some of the possible metabolic defects associated with lead poisoning; and thirdly, I shall present some of our own measurements of less obvious effects of lead on peripheral nerve function in children. In the latter part I hope to evaluate with you our findings as to their possible value as a delicate indicator of continued exposure to lead in otherwise asymptomatic individuals who have normal blood levels of lead.

A. Clinical Features

Nikander, the Greek physician of the second century before Christ described the neurotoxic effects of paralysis, and ocular disturbances of probable cases of poisoning in a poem: "His feeble limbs droop and all motion is still. His strength is now spent-". Paul of Aegina, in the 7th Century, recognized epilepsy as a result of what was probably lead poisoning. Francois Citois observed patients to have weakness of legs and feet, elbows and hands to follow the occurrence of convulsions in cases of "colick". Franchin the physician of Voltaire, in an essay entitled "De colica pictonum" published in 1757, showed that the colica pictonum in Amsterdam was caused by water passing over leaden roofs into cisterns where it was kept for drinking purposes. Ten years later, Sir George Baker

was able to prove that the Devonshire colic was due to lead-lined ceder presses. Indeed, when the farmers removed the lead linings from their cider presses the Devonshire Colic no longer occurred.

While the subtle symptoms and signs as contrasted with the obvious or "outspoken" are of greatest importance in making an early diagnosis. I shall restrict my remarks to the "outspoken" signs and symptoms as Minot (1938) used the word. Industrial lead workers have complained of lassitude, irritability, depression, constipation and abdominal discomfort. These symptoms are the same as those sometimes expressed by an emotionally depressed individual and could easily be overlooked.

Individual case reports have been many but several typical patient's histories may be of interest. One patient, was a lead melter, aged 34, who had worked at the same job for ten years without previous symptoms. He complained of a weak right hand, although examination revealed bilateral radial nerve palsies, Except for a mild anemia, no other signs or symptoms were present. A second example is that of a 53 year old business man whose only possible exposure to lead was a water pipe-line to his house. He complained of progressive weakness and atrophy of his shoulder and arm muscles. More recently, Simpson (1964) described an "acetylene burner" who was torch-cutting lead painted steel on ships who developed a left foot drop, hyperreflexia, spastic gait, and widespread muscle fasciculations. Electromyography confirmed these as myelopathic symptoms and signs which were attributed to the effects of chronic lead poisoning. Spastic paraplegia and hyperreflexia suggest upper aches and lower neuron damage. In such cases the differential diagnosis between lead myelopathy and spinal cord degenerations such as primary lateral sclerosis and amyotrophic lateral sclerosis may be quite difficult. Lead encephalopathy has been considered more common in

children than adults. When it does occur in adults it may misdiagnosed as a brain tumor for example, there was a 28 year old woman who complained of intense headache, vomiting, diplopia, and periodic confusion for about one months duration. She had papilledema and ataxia of gait. This picture of increased intracranial pressure was attributed to a posterior fossa obstruction until the history of ingesting an ounce of abortifacient containing lead was obtained. We have seen other cases of encephalopathy ( ) in adults masquerading as focal brain disease often with increased intracranial pressure. Others have subtle changes in higher cortical function. Ophthalmological disturbances have been asociated in only 1 to 2 percent of cases of systemic lead poisoning. Sonkin (1969) reported finding lead particles, appearing upon ophthalmoscopic examination, as glistening; discrete, gray pigment concentrated in the tretina around the optic disk in 8 of 25 workers. He suggested that the ophthalmoscopic examination could predict those workers who would be found to excrete abnormal amounts of urinary lead. Grant (1962) stated that ocular symptoms of lead poisoning afe not usually among the early ones in adults, although it may be one of the first in children. In the majority of cases, the onset of ocular symptoms has been gradual. On the basis of clinical observations, it has been possible to recognize disturbances of the visual cortex and supra-geniculate pathways; optic nerve, both retrobulbar and bulbar; retina, intraocular muscles; the lens; and extraocular muscles. The optic nerves may be injured either by increased intracranial pressure or by direct toxic action of lead on the nerve. Pupils may be unreactive to light because of the damage to pathways rostral to the lateral geniculate body. Many cases of acute and transitory amaurosis have been found to have associated blurring of the borders of the optic nerve head, slight turbidity of the surrounding retina, and abnormal distension of the retinal veins. The onset

in some chronic cases has been accompanied by constriction in peripheral visual fields and in others there has been a central acotoma. Clinically, the optic neuropathy of chronic lead poisoning has been likened to "tobacco-alcohol amblyopia. Optic neuropathy caused by excessive lead intake has been induced in animals (Giannantoni, 1933) but the mechanism is still unclear. Ocular symptoms are fewer, it seems, with tetraethyl lead than with inorganic lead. Symptoms due to tetraethyl lead develop after long term exposure to low concentrations.(von Oettingen, 1958).

Peripheral neuropathy is considered a common neurologic manifestation of industrial hazards caused by several substances, such as lead, acrylamide, organophosphates, and thallium. The radial nerve palsy is quite common in lead poisoning. In fact, it seems there is a predilection for this nerve in the most active extremity, such as the right hand in a right handed lead worker. Some older authors felt that this was due to the proximity to the noxious material and that absorption through the skin was greater (Gusserow, 1861), while Reznikoff and Aub (1927) claimed that after an initial period of contraction, it was the "leaded" muscle, not nerves exposed to large concentrations of lead which became fatigued much more rapidly and completely. Fullerton (1968) studied lead poisoning on peripheral nerves in five men who were exposed to this substance during their work. They all had raised blood lead levels with mild anemia and one had had abdominal pain. They had no abnormal neurological symptoms or signs. While there was not a significant difference between these five men and twelve age-matched control subjects as to the conduction velocities in the peroneal nerves, a statistically significant difference in the proximal and distal amplitudes of the action potential extensor digitorum brevis muscle was noted. Fullerton suggested that the amplitude changes may be

a very sensitive way of detecting minimal peripheral nerve damage.

Of the neurologic manifestations of lead absorption in children, peripheral neuropathies have not been the most "outspoken". Watters and Barlow (1967) refer to lead neuropathy as "infrequent". Pearlstein (1966) considered the central nervous system a more likely target in the child. Seto and Freeman (1964) found only nine well documented cases of lead neuropathy in children in the English literature and thirty-one others referred to but in less detail. One of their patients was a three and one half year old boy who showed proximal weakness, moderate wasting of the hands, reduced reflexes at the ankles but increased at the knees. Routine blood lead level was 30 mcg/100 grams of blood, urinary coproporphyrin was strongly positive, and delta amino levulinic acid excretion was 192.5 uM/day (normal 20 uM/day). Urinary excretion of lead was within the normal range prior to calcium disodium edetate. (EDTA), administration, after which it increased 1000 fold. Muscle contraction thresholds of the intrinsic muscles of the hands, wrist flexors and extensor digitorum communis as well as the extensors of the feet were elevated. Electromyography revealed numerous fibrillations and positive potentials in the same muscles, consistent with denervation. A two year old girl described by Millichap (1952) whose blood level was 40 mcg/100 gr. and whose admission to hospital was prompted by diaphragmatic paralysis, later developed a foot drop with electrical evidence of denervation. Seto and Freeman emphasized that of the nine cases adequately studied, two had gone unrecognized for several years prior to exacerbation and diagnosis; two had clinical evidence of peripheral neuropathy, viz, sensory loss and weakness and when tested electrically, evidence of denervation was observed. All 9 cases had evidence of lead in bones, anemia, and basophilic stippling.

The point was also made, in referring to the four of five children reported by Byers and Lord (1943), that in those instances of peripheral neuropathy later evidence of mental deficiency became apparent. This may imply that the neuropathy and encephalopathy may actually occur together more often than has been recognized.

A ten year old boy came to my attention with the obvious combination of encephalopathy and neuropathy. He had been admitted to hospital several times over the previous five years because of abdominal cramps, usually attributed to his sickle cell anemia which has been earlier diagnosed. His hemoglobin averaged 5.6 grams for the year before the current admission. In addition, his blood pressure had been running at about 160/120. His lethargy and headache had been attributed to the hypertension. Although he began to walk with a high steppage gait, probably because of the weakness in his extensor muscles of his feet, he was not brought to the hospital until several months later. A history of pica was obtained and a blood level for lead was found to be 13 mcg/100 gms of blood, urine coproparphyrins were positive, and x-rays of the distal metaphysis of the femurs, tibia, fibula, radius, and ulna showed dense bands consistent with exposure to heavy metal. EDTA proved the diagnosis. Nerve conduction velocity studies showed evidence of severe neuropathy in the peroneal nerves, muscle action potentials could not be elicited. Conduction velocity in the ulnar and median nerves was slowed. In this chronic case the encephalopathy and neuropathy had been neglected for various social reasons.

In another instance I know of, the acute encephalopathy was quite dramatic as it was heralded by a generalized convulsion which developed into status epilepticus. After the spinal fluid protein was found to be increased (153 mgm%) and the cerebrospinal fluid pressure elevated, arteriograms

were done to "rule out brain tumor". Not until the fifth hospital day was the urine tested for coproporphyrins and found positive. The blood lead level of 80 mcg/100 gm discovered. She was chelated with EDTA. This child, an 18 month old girl, was in coma for fifteen days. When she awoke by the 20th day, she appeared to be blind. Nerve conduction velocity in the peroneal nerve was normal at that time (68 m/sec). However, three months later, at a time when her hair still showed increased amounts of lead in the proximal portions, although the blood lead had come down, her conduction velocity was reduced (44.8 M/sec.)

These comments and cases indicate the likelihood that peripheral neuropathy may be a manifestation of chronic exposure to lead, in conjunction with encephalopathy. In some instances one condition overshadows the other. Reznikoff and Aub (1927) were convinced that muscle weakness was the result of lead's effect on muscle cell membrane permeability and was not due to the nerve supply. Other studies cite a direct correlation between the low phosphocreatine content of a poisoned muscle and a diminished capacity for contraction. Since resynthesis of the hydrolyzed phosphocreatine is essential for muscle excitability, the leaded muscle is rendered inexcitable. It is of interest that the development of fatigue in lead poisoning is accompanied by increased urinary excretion of creatine (Cantarrow, 1944).

Modern biochemical studies have concentrated on the motor end plate region of muscle. Of note is the finding that lead precipitates at the esteratic site of acetylcholinesterase, (Koelle et al, 1968). A modification of the technique for binding of gold and lead complexes at the esteratic sites of motor end plates was accomplished by Kasa and Csillik (1966). A better electronmicroscopic delineation of the localization of cholinesterases

was achieved by using copper and lead together in the incubation. This slide (#1) shows the acetylcholinesterase activity of a motor-end-plate in the rat diaphragm, as outlined by the Cu-Pb thiocholine technique. The enzymatic activity of pre and post-synaptic membranes is conspicuous; slight activity within the synaptic cleft is present. A: terminal motor axon, filled with synaptic vesicles (SV) and (M) mitochondria. (JF): junctional folds of the post synaptic membrane, estending into the post synaptic sarcoplasm (Sp). Most of the acetylcholinesterase was present at the postsynaptic sites. Introducing the lead into this histochemical technique increased the electron density of the end product. Namba and Grob ( ) have attempted to delineate, further, the preferential binding of subneural apparatus of the motor end plate, by lead and other divalent metal ions. While the binding of divalent metal ions to the subneural apparatus included zinc, cadmium, copper and tin, it is pointed out that the appearance of lead-stained structures is identical with the subneural apparatus stained for cholinesterase activity. The binding sites are post-synaptic, as indicated by the observation that, following denervation, the axon terminal disappeared, but the cholinesterase activity did not. That the binding of lead to post-synaptic subneural apparatus is not completely identical with the characteristics of cholinesterase activity was shown by the fact that the latter was not affected by freezing the muscle then thawing it prior to staining, while binding of metal ions disappeared completely when the muscle specimen was treated in this way. On the other hand, binding activity of the subneural apparatus to lead and tin was not influenced by prior intramuscular injection of one millimolar solution of diisopropyl fluorophosphate or neostigmine. Cholinesterase activity was completely inhibited by these compounds. Also, cholinesterase activity was somewhat reduced in amount in muscle in which the subneural apparatus had been found

with lead. These studies of Namba and Grob are a part of attempts to identify some properties of the cholinergic receptor, but the differential characteristics of lead-binding at the motor end plate may shed light on some aspect of weakness found in lead poisoning. At the present time little is known about the influence of most metal ions on neuromuscular transmission. Metal ions do affect muscle contraction (Sandow and Isaacson, 1966). A change of external calcium ions has been found to cause a change in permeability of the post-synaptic membrane (Takeuchi, 1963), while lowering the calcium concentration may produce neuromuscular blockade by decreasing the pre-synaptic release of acetylcholine. (Elmquist and Eldman, 1965). Calcium is normally present at the motor end plate in bound form and is released as the free ion following nerve stimulation. Nakamura, Namba, and Grob (1967) were able to demonstrate, the release of calcium ions at the motor end plate, indicating a close relationship between the site of calcium release and the site of binding of divalent metal ions. This relationship between calcium and lead binding may have significance. Further work is needed. Such a relationship may explain the predilection for palsy in the more active muscles. The lead binding substance at post-synaptic membrane was postulated by Csillik (1963) to be an organized lipo-protein. Studzuiski and Love, 1964, demonstrated the binding of lead with nucleolar ribonucleoprotein of unfixed fresh HeLa cells.

Is it possible that Pb-Lipoprotein complexes are formed in a similar fashion suggested earlier by Sayer?

Neurones, and Encephalopathy:

Evidence for toxic effect on anterior horn cells and the peripheral nerve itself is convincing; some authors found changes in the anterior

roots and others also found changes in dorsal roots. Spinal roots have shown atrophy of anterior divisions. The anterior horn cells had eccentrically placed nuclei, Nissl bodies were dispersed showing chromatolysis (Haslett and Warrington, 1898). Edema and severe nerve cell degeneration seem to be prominent features of both acute and subacute intoxication, (Alkelaites, 1941, Marsden and Wilson). Emphasis has been placed upon mesodermal changes, some of which resemble atheroma with endothelial proliferation (Pelri, 1930). Blood vessels of the gray matter of the spinal cord and brain have been commonly involved.

Routine microscopic study of the pathologic changes in the brain in cases of lead poisoning show patchy nerve cell degeneration and slight vascular endothelial reaction. (Pentschew, 1958). Brun and Brunk (1967) studied the effects of lead experimentally in rats poisoned with lead. Animals killed one day after the cessation of administration of lead showed an increase in the quantity of lead in the brains of rats. An increase in alkaline phosphatase activity was found in the capillaries. Simultaneously, the acid phosphatase activity of the neurons appeared increased. Brun and Brunk (1967) explained these findings the pathology lead encephalopathy as being in part due to autolysis. Reduced alkaline phosphatase activity was thought to be a result of a toxic inhibition produced by the lead resulting in damage to the blood vessels, allowing transdate formation and perivascular cellular responses.

Small perivascular hemorrhages in all parts of the brain have been described. These are usually after acute poisoning. These were scattered in the walls of blood vessels as well. Granular perivascular material has been considered evidence of cerebral edema. Usually the brain is swollen with evidence of a pressure cone. In some instances, in the gross section

punctate hemorrhages are found. Microscopic sections show exudate about blood vessels. The vessels are dilated, narrowed capillaries, some with thrombi, necrotic cells, fat-laden cells. All lesions are patchy and irregular in distribution. Lesions in meningeal as well as cerebral vessels are prominent, but more abundant in the white matter of the centrum semivovale, internal capsule and in the corpus callosum. Large fibrous astrocytes are seen. Some laden with fat. The nerve cells show variable degrees of damage. The nuclei are more basophilic, nearly pyknotic, and the nucleoli are indistinct. Necrotic cells are scattered about and have irregular shapes. Most of the changes in nerve cells seem related to vascular changes and consequent presence of exudate in the tissues. They are similar to changes of ischemia and hypoxia.

The cerebellum is intensely affected in acute lead encephalopathy. Lesions are numerous in the molecular layer. Damaged capillaries, capillary thrombi, perivascular foci of necrosis, hemorrhages, and thin films of serous exudate about many capillaries are found. Purkinje cells are found to be vacuolated and even necrotic. Nerve cells of the dendate nucleus are lost. In white matter there are pools of perivascular serous exudate, perivascular foamy fat-laden cells, and pigmented cells. Basal ganglia and spinal cord showed similar changes. Obviously, lesions of a lesser degree must exist in individuals who survive lead encephalopathy.

Neurochemical studies of the brain in lead poisoning have been technically difficult but the fact that epileptic convulsions, psychotic states and excitement are common symptoms of lead poisoning, it is essential to consider certain aspects of brain metabolism in this condition. Tower ( ) cites lead as one heavy metal which interferes with brain oxidative metabolism. Because tetraethyl lead is a lipid soluble organic lead compound, it rapidly

penetrates the skin and because it is so volatile, it is rapidly absorbed by inhalation. Continued absorption of small amounts of tetraethyl lead may result in classical syndrome of chronic lead poisoning. Since the tetarethyl lead has high lipid solubility, large amounts gain access to the central nervous system. Cremer showed (1939) that the tetraethyl lead was converted in the liver to triethyl lead. Thus, the study of the effects of tetraethyl lead on the nervous system must be concerned with triethyl lead effects. Triethyl lead inhibits oxidation of glucose by brain cortex slices. This effect was observed whether in brain slices to which triethyl lead had been added in vitro, or in slices of the brain taken from poisoned animals (Cremer, 1962). Some studies of the effects in vitro of Pb on amino acids have been done. Varnadis and Quastel, (1961) showed that tetraethyl lead inhibits active transport of energy dependent amino acids into cortex at concentrations that show no effect on glucose metabolism of the slices. Tetraethyl lead inhibited the oxidation of glutamate by rat brain slices; it abolished, at low concentrations, potassium stimulated brain slice respiration in the presence of glucose, having no effect on unstimulated brain slice respiration. Varnadis and Quastel (1961) reported that when the brain is poisoned in the living animal by administration of tetraethyl lead, the only metabolic abnormality found is an inability of brain slices to transport actively glutamic acid and glycine. Since this disturbance coincides with the appearance of pathological symptoms it is reasonable to assume that some of the neurological symptoms of tetraethyl lead may be a consequence of the failure of the brain cell to transport amino acids, and possibly other ions, across the neuronal membrane.

The importance of glutamic acid is emphasized by the fact that it is the sole substrate capable of sustaining cerebral metabolism in the

absence of glucose (Geiger, A., 1958). Glutamic acid is of central importance in the transfer and metabolism of ammonia. During seizure states ammonia levels rise as a result of extra glucose oxidative metabolism secondary to high level of neuronal activity. It is conceivable that defects in glutamate-transamiantion reactions may be partly responsible for initiation of seizure activity in certain situations. Although, seizures encountered in acute lead encephalopathy may be due to neuronal exicitation related to local edema or vascular reasons, interference with flutamic acid metabolism by lead may result in reduction of (GABA) gamma aminobutyric acid production. This might be done by affecting the enzymes and cofactors such as

1. Glutamic acid decarboxylase (GAD) which decarboxylates glutamate to GABA.
2. Glutamic acid dehydrogenase (GHD) which reduces alpha-ketoglutarate to glutamic acid.
3. Pyridoxal phosphate metabolized from pyridoxine (B_6P), which functions as the coenzyme for transamination reactions used for glutamic acid decarboxylase in the synthesis of GABA.

Gamma aminobutyric acid has been considered an inhibitary transmitter or depressant moderator, and interference with GABA production might produce seizures by increasing neuronal excitability (Curtis, 1960, 65). Cremer (1964) reported that normal brain slices incubated in vitro with ($-^{14}C$) glucose showed that GABA attained the highest specific activity whereas both glutamine and aspartic acid had specific activities about half that of glutamic acid. Triethyl lead had no inhibitory action on glutamic acid decarboxylase (GAD) activity. Brains of rats, given triethyl lead which showed excitable behavior progressing to permanent rremors, contained increased

amounts of free glutamine after electrical stimulation, but not after metrazol or picrotoxin seizures. Further clinical evaluation of this might include study of the use of pyridoxine or glutamine in children with seizures due to acute lead poisoning.

Peripheral Nerves

Gombault (1880) and de Villaverde ( ) demonstrated segmental parenchymatous degeneration of myelin sheaths and axis cylinders affecting the distal parts of the motor fibers and motor end plates. Recent careful electron microscopic stidues of experimental lead neuropathy by Lampert and Schochet (1968) showed that while Schwann cells and myelin sheaths were primarily damaged, axons also showed degenerative and reactive changes. Degenerating axons at a distance from an area of trauma are characterized by granular disintegration and clumping of axoplasmic organelles, while reactive axons show abundant mitochrondria, vesicular elements and membranous dense bodies. Remyelination takes place as Schwann cells wrap themselves around the denuded but other wise normal axons. The formation of new myelin lamellae by the apposition and fusion of the enfolded plasma membranes does not differ from normal myelination or from remyelination as described inhuman hypertrophic neuropathies. Before some of the sheaths became fully restored, their supporting Schwann cells again degenerate. Each degenerated Schwann cell leaves behind a basement lamina that serves as a scaffolding along which other Schwann cells grow. Repeated degeneration and regeneration or proliferation of Schwann cells leads to the formation of "onion bulbs" around axons.

(Slide 3 Lampert)

(#1) A transverse section of a sciatic nerve of a rat on a lead diet for four months showed no pathological changes, stained with paraphenylene-diamine.

(#2) At six months of lead diet, myelinated axons are separated from each other and there are wide distensions of the sheaths (arrow).

(#3) After seven months there is disintegration of myelin sheaths and macrophages filled with myelin debris are seen.

(#4) A sciatic nerve from a rat after 8 months of lead diet shows numerous remyelinated axons, some of which are surrounded by concentric layers of Schwann cell processes (arrow).

The next slide (4) shows a portion of a disintegrating myelin sheath from a rat on a lead diet for seven months. It shows splitting of minor and major dense lines and transformation of myelin lamellae into membranous blebs (x60,000). Slide #5 (A17). An axon from a rat on a lead diet for seven months is enclosed by a Schwann cell forming a new myelin lamellae. Other cytoplasmic processes are in the space between the Schwann cell and the remnants of the old neurolemmal tube or basement lamina (x10,000)

(#18) In the lower electronmicrograph showing an axon enclosed by a proliferated Schwann cell. Processes from other Schwann cells grow along the inner surface of the basement lamina or old neurolemmal tube (arrow).

Slide (#23) The onion-bulb formation around an axon showing beginning remyelination from a rat on a lead diet for 8 months. Macrophages (MP) are seen between the concentric layers of Schwann cell processes (x6000).

Lampert and Schochet offer as an explanation for the process of demyelination and remyelination in lead neuropathy two possibilities:

1. that a substance having porphobilinogen as a precursor is somehow essential for the maintenance of myelin. Such occurs in porphyria, in which a block occurs in the liver resulting in excretion of excess porphobilinogen and

delta amino levulinic acid, and at the same time causes demyelination. 2nd. is based on the possibility that lead causes vasodilation and produced altered vascular permeability. Evidence of intraendoneural edema was seen, and such accumulation of exudation of protein rich material within the endoneural compartment could produce damage to the Schwann cell by pressure or indirectly by ischemia.

Slide 10

The most conspicuous capsular cell changes were seen in those animals showing Wallerian degeneration in the psoterior nerve roots and in sciatic and tibial nerves. Schlaepfer suggested that the dense bodies contained a particulate material which has electron density characteristics of heavy metal compatible with lead.

Slide 11

Evidence of segmental demyelination and remyelination was noted.

(a) seen here is a (8) whole-mount teased preparation of tibial nerve showing linear arrangement of osmiophilic droplets in a fiber undergoing Wallerian degeneration. (arrow).

(b) (9) shows segmental loss

(c) (10) unequal thicknesses indicate remyelination

Slide 12

Axoplasm undergoing granular degeneration

Slide 13

Summarizes histochemical studies. In section 11 and 12 is seen the acid phosphatase activity in the nodal axoplasm with unilateral spread into the adjacent axoplasm; (13) accumulation of acid phosphatase activity along 3 successive nodes, and (17) acid phosphatase activity following the arrays of lipid debris after Wallerian degeneration.

Schlaepfer (1969) produced neuropathy by feeding rats 1% lead acetate solution for periods of 3-18 months. He observed widespread segmented demyelination and remyelination in peripheral nerves and the spinal nerve roots. A selective involvement of the capsular cells in spinal ganglia was characterized by a proliferation of these cells and an accumulation of dense bodies within their cytoplasm. These dense bodies contained a particulate material which has the electron density characteristics of a heavy metallic substance, compatible with lead. An increase in neurofilaments and a relative paucity of endoplasmic reticulum was seen in some associated sensory ganglion cells. More striking neuronal alteration, however, occurred in the distal peripheral nerves and in the posterior nerve roots, in the form of Wallerian degeneration. The accumulation of acid phosphatase activity in the nodal axoplasm of some peripheral nerve fibers appeared as an early form of axonal injury and attested to a special reactivity of the nodal region. Schlaepfer concluded that the coexistance of segmental degeneration and Wallerian degeneration in peripheral nerves of lead neuropathy may result from a common metabolic lesion of the supporting Schwann and capsular cells.

Slide 9 -- Phase contrast examination of dorsal root ganglia from the experimental animals revealed an increase of capsular cells which occurred in a circumferential manner around individual ganglion cells and focally in the intervening tissue (fig 1-2). The arrow in figure 1 showed accumulation of dense bodies within cytoplasm of proliferating capsular cells. Figure 2 shows a ganglion cell surrounded by a seam of capsular cells containing dense ganules (arrow).

Pamela Fullerton (1966) had reproduced Gombault's experiment and (guinea Pig) demonstrated teased nerve fibers undergoing segmental degeneration. (Slide 14) Different stages of demyelination were usually found in the same animal and in different parts of the same nerve. In the poisoned animals (Slide 15) there was a marked reduction in large diameter fibers but no decrease in the number of small diameter fibers. She felt that the prolonged latencies (Slide 16) were not due to unmasking of more slowly conducting fibers but rather due to degeneration of nerve fibers. Fullerton measured motor nerve conduction velicity in these animals and showed that it was below normal (Slide 17). As mentioned earlier (1969) Fullerton measured conduction latencies in 5 lead workers and found no significant slowing of conduction velocity in the peroneal nerve. However, by using the amplitude of the potential following stimulation at th- knee expressed as a percentage of that following stimulation at the ankle, a difference emerged between the lead workers and 12 control subjects. The difference between the two means is significant at less than 1.01 level (Student "t" test). The explanation given is that conduction is reduced in only a few fibers. This would not affect maximal velocity but the potential would become more dispersed the greater the conduction distance and thus affect the ratio. Fullerton suggested this method as a sensitive way to measure peripheral nerve damge. She asked what level of lead poisoning might be needed for such small changes in mm? This question was raised years ago by Minot ( ) when he reviewed studies of the physiological effects of small amounts of lead. He emphasized the need to reassess the margin of safety between the lead exposure of the average individual and those amounts generally recognized as dangerous.

Original Research - Preliminary Results

What percentage of the patients with acute lead encephalopathy may have evidence of peripheral neuropathy as well? Could these cases represent chronic exposure with an acute precipitation of encephalopathy, which may have been prevented if the minimal signs of neuropathy could have been detected? Are the children who show neuropathy later, after an acute encephalopathy, reflecting the continuing exposure to endogenous lead being slowly released from stores in bone and soft tissues? Or simply, are these changes in peripheral nerve in some patients and not in others the result of a dose - response relationship?

We have tried to answer some of these questions by measuring nerve conduction characteristics in a group of children known to have been exposed to lead. Seven children had had frank evidence of encephalopathy, with seizures, stupor and behavior change. Eleven were the siblings of patients with encephalopathy or were picked up as suspect in a clinic screening by the hair test of Kapito, Byers, and Schwachmann. None had obvious evidence in their hospital records of peripheral neurpathy, although several had ataxia of gait or unsteady stance. Most of these children were studied for hair lead concentration as well. In those patients in whom the blood lead was not significantly elevated but the hair lead levels were convincing, EDTA provocative tests proved the diagnosis by liberating lead into the urine from stored tissue sites. Conduction studies were done in three children within two weeks of a diagnosis and after chelation had been instituted. It takes 21 days after denervation to get slowed conduction. In these cases the studies were repeated three months later. Two children were studied 6 months

and two one year after initial diagnosis; five were done after 2 years and five were studied three years after initial diagnosis. The children were all awake and drugs were not used for sedation.

Stimulating electrodes were placed at the peroneal nerve as it coursed around the lateral head of the fibula. Surface recording electrodes were placed over the extensor digitorum brevis muscle. Distal stimulation was done by placing the stimulating electrode over the anterior tibial nerve, the branch of the peroneal which supplies the extensor digitorum brevis. Recordings were made on an oscilliscope and photographed for later measurements. Threshold stimulations at a constant duration (0.1 milliseconds) single shocks, delivered at a frequency of one per second and a voltage of fifty to one hundred and fifty volts were done. A ground lead was placed on the leg being studied.

Results were analyzed in the following manner. (Slide 18) Threshold curves were obtained. No consistent correlation was found in the minimal voltage required to obtain either a muscle response or an evoked response measured over the anterior tibial nerve when stimulating the peroneal nerve. Threshold for the patients range from 50 to 100 and the supramaximal response was obtained above 150. There seemed to be no correlation between the threshold or supramaximal stimulus and the conduction velocity or amplitude of response obtained. Supramaximal stimulation produced the type of muscle response recorded and shown on this slide 19. The time between the stimulus artifact and the initial upward deflection is the latency. The latency of the proximal response and the distal response are subtracted and the difference is divided into the distance between the two stimulating sites. In this way the conduction velocity is calculated. The amplitude of the response was measured from the baseline to the peak of the deflection.

Because of the paucity in available controls at the various ages necessary for study those values reported by Thomas and Lampert, have been used in tis study. (Slide 20). Our own controls fall into the same ranges. (Slide 21)

When the conduction velocities were plotted against the control values of Gamstorp according to the age groups 1 to 3; 4-6; 6-10; it is clear that the conduction velocities from our group of children with chronic exposure to lead after chelation still showed abnormalities in the ability of the peroneal nerve to conduct normally. These preliminary data are significant to the .05 level for the younger group and to the .01 level for the middle group. We do not have enough data to be sure about the older group.

Slide 22,23, 24

These values, grouped according to age so that the developmental and maturation factors affecting myelination of the peripheral nerve are taken into consideration, was studied in relationship to the blood level of lead at the time of the conduction studies as well as the hair lead at the time of the conduction studies as well as the hair lead concentrations for a given length (centimeter) of hair as measured by Kapito's laboratory. (A) conduction of velocities are generally lower than normal; (B) the blood lead levels are generally normal since these children have all been treated,

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