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The Effect of Lead on Developing Rat Brain Lipids

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by

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THE EFFECT OF LEAD ON DEVELOPING RAT BRAIN LIPIDS

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The objective of this study was to determine the effect of lead on the brain lipids of developing rats.

Lead acetate was added to the drinking water of two pregnant rats. A control dam was maintained on the same diet without the lead. Following birth and weaning the lead concentration was reduced and an equal number of pups from each litter were sacrificed on days 19, 22, 26, 30, and 35 after birth. The brain lipids were isolated and fatty acid distribution analyzed by gas-liquid chromatography.

Variations in fatty acid distribution between the control and experimental pups were noted for palmitic and arachidonic acids. This difference was greatest in the younger pups but was diminished in the older pups.

These results are consistent with the idea that lead may cause abnormal brain growth as seen by a delay in brain maturation.

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Larry J. Stegink

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INTRODUCTION

The prevalence of lead throughout our environment has been well established. It has been mined and manufactured for over 2000 years and is now the most widely used nonferrous metal.[1]

The "natural" atmospheric lead level is estimated to be 0.0005 ug per m^3.[1,2] Today, in cities, this level is exceeded by a magnitude of one to ten thousand, much of it the result of the consumption of leaded gasoline which accounts for approximately 90% of all airborne lead.[3] Industrial facilities involved in lead recycling or processing have had lead levels recorded as high as 100 ug per m^3.[1]

The typical urban dweller inhales, on the average, 60 ug of lead per day, 35 to 40% of which is absorbed. Absorption of lead by the gastrointestinal tract however is just as high if not higher. Dietary intake averages 200 to 300 ug per day, with about 10% of that amount being absorbed.[1,4] In sum, an average of 30 to 40 ug of lead is absorbed per day by the adult human body. At low lead levels absorption equals excretion meaning no net lead retention[5] but at the levels stated above about 10 ug of lead is retained and incorporated into the human body per day, primarily the skeletal system which accounts for 90 to 95% of the total body burden of lead.[1,3] The increased environmental burden of lead and its incorporation into the human skeletal system is illustrated by the finding that skeletons of modern man contain lead at concentrations two orders of

magnitude greater than that in skeletons estimated to be 5,000 years old.[1,2]

It has long been known that children are more susceptible to lead poisoning than are adults and it is generally known that the earlier the exposure, the more severe the consequences. This is proposed to be due to increased absorption and retention of lead by the child and the increased vulnerability of developing organs to the toxic effects of lead.

A brief synopsis of the effect of lead on the developing organism shows first of all that the placenta is a poor barrier to lead transport. Studies[6-8] show that lead crosses the placental membrane rapidly and in significant amounts.

The transfer of lead during lactation has also been well established, as lead has been known to cross readily the mammary barrier.[5,9] This transfer is accentuated by an observed increase in the mothers blood lead level after giving birth, possibly due to a mobilization of minerals from the skeleton during lactation.[10,11]

Whole body retention of isotopic lead, injected intraperitoneally on the fifteenth day of lactation, was shown by Momcilovic[12] to be 20% lower in lactating rats as compared to non-lactating rats. The experimentals showed lower lead concentrations in urine, feces, and incisors but the litter showed a whole body concentration equal to 19.4% of the intraperitoneal injection, illustrating a high mobilization and transfer of lead to the pups. In a followup study,[13] lead was added to the

drinking water of the lactating mother. Pup whole body retention in this instance was 30% higher than the whole body retention of the mother. A recent study by Keller[14] indicated a transfer of 25% of the maternal dose to the pups.

Shigeta, et. al.,[15] while studying the absorption of lead during lactation, noted that lead concentration in the blood of young pups was 13 to 20% of that in the maternal milk while the adult blood lead level was only .03 to .05% of that in their drinking water indicating an absorption in the young of 400 times that in the adult. Mykkanen, et. al.,[16] in one of his studies, indicates that even though the lead concentration in maternal milk was 1/1000 of that in the dams drinking water, lead concentrations in the tissues of the suckling pups and the dams were similar.

Other studies also indicate increased absorption and retention in younger animals. Kostial[17] found that one-week-old suckling rats absorb lead from the intestinal tract more readily than adults. Absorption in young suckling rats, 13 to 20 days old, has been observed to be as high as 83 to 89%, which, at weaning, drops to adult levels, 15 to 16%.[18]

Kehoe's study,[19] quoted earlier, indicated that human adults absorb about 10% of ingested lead and probably retain only a few micrograms. In comparison, Alexander, et. al.,[20] studied the absorption and retention of lead in eight healthy children, ages three months to eight years. In these children an average of 50% of the ingested lead was absorbed with 18% eventually

retained. Another study by Ziegler[21] showed that with a dietary lead intake of greater than 5.0 ug per kg per day, net absorption averaged 41% and net retention, 32%.

Jugo[22] has listed several reasons proposed for this increased absorption, ranging from the theory that milk is directly or indirectly involved in increasing lead absorption to the proposal that immune globlins and colloids, absorbed by pinocytosis until three weeks after birth, could contribute to an increased absorption of lead.

Several theories have also been raised to explain increased lead retention. A study[23] showed that in two-week-old pups only 15% of lead acetate was excreted in the first six days after administration while the adult excreted as much as 55% over the same time period.

Jugo[23] has proposed that the lower excretion could be due to a higher stability of metal-body ligand linkages resulting in a lower amount of "free-metal" in the growing animal.

Others base their theories on the observation that younger rats accumulate lead in their kidneys at a much lower rate than do adults indicating an inability of the young kidneys to handle increased amounts of lead, due to a smaller number of nephrons or the inability of the pup to synthesize metallothioneins, a detoxifying protein.[24,25]

Once lead is absorbed into the body a difference in distribution is also noted between younger and older animals.

When lead is absorbed into the body the blood lead level

increases as the lead is distributed between blood cells and plasma in what is theorized to be a constant ratio[26] although some have proposed that blood plasma has a ceiling value for lead.[27]

The blood of younger animals, after lead administration, generally has higher blood lead levels than does the adult.[16,25] Also, Jugo, in an unpublished report, has shown that, after an intravenous injection of lead citrate, greater amounts of lead can be found in the plasma of young rats as compared to adults. This may be significant because Hilburn states[3] that the lead in the plasma may be the potentially active portion of lead in the human body.

Lead distribution in the body is frequently described with reference to three differing lead pools or compartments.[3,26]

1. A relatively slow, non-diffusible pool of lead in the skeletal system.

2. An accumulation of lead in the skin, muscle, and bone marrow which has an intermediate exchange rate with the blood.

3. Lead found in some soft tissues which is quite rapidly exchanged with blood lead.

Momcilovic used the above three categories to propose that, due to the relatively slow incorporation of lead into the skeletal system, high concentrations of lead in the blood may lead to a higher storage of lead in the soft tissues such as the brain. In a recent study of his[12] this was confirmed. At increased lead levels a lower percent of the lead was incorporated into the skeletal system and a higher percentage was

incorporated into the soft tissues relative to that incorporated at lower lead levels.

This, as well as the increased susceptibility of young animals to lead, has caused many to believe that the immature brain may absorb greater amounts of lead. This has been verified in many studies.[16,25,28-30] An underdeveloped blood-brain barrier is frequently cited as being partly responsible for this observation.[31,32] Lead itself has been implicated in one study[33] as being responsible for changing the blood-brain barrier permeability. Mice, when fed solutions containing 0.5% lead acetate, were determined to have increased blood-brain barrier permeability.

Mykkanen[16] has proposed that the increased permeability is due to the lower metabolic stability in the growing brain. The greater anabolism of the young brain may mean a greater influx of substances including lead if it is present.

Shigeta, et. al.,[15] found no difference in lead absorption between mature and immature brains and concludes that greater damage occurs because the young brain is developing at the time of exposure.

The fact that greater brain damage does occur in the growing organism is not questioned. Various degrees of brain damage are produced in suckling rats at concentrations tolerated by their mothers[34-36] and suckling pups of dams who received lead in their diets on days 1 to 10 demonstrated decreased learning ability when compared to pups whose mothers received lead on days 11 to 20.[37]

Generally, behavioral symptoms resulting from lead exposure are detectable at blood lead levels greater than 60 ug per 100 ml, the recommended blood lead level limit. Mild borderline retardation, hyperkinesis, varying manifestations of conduct disturbance, perceptual motor dysfunctions and learning disabilities are all indicative of severe lead poisoning.[38] With continuing exposure, paralysis, blindness, and death can result.[2]

Blood lead levels below 60 ug can lead to what is termed minimal brain dysfunction (MBD), characterized by any combination of the following: defective memory, irritability, mental dullness, inability to concentrate, fatigue, vague abdominal pains, appetite loss, listlessness, vague personality changes and others.[2,38-40] Because these symptoms can be produced by other disturbances such as a virus or stress, low level lead exposure is difficult to diagnose. Much work has recently been done in this area and Grandjean[1] has summarized many testing procedures correlating low level lead exposure with MBD symptoms such as personality changes, intellectual and sensory dysfunction, and impaired psychomotor and neuromuscular function.

These symptoms indicate an obvious interference of lead with neurological functioning. However, no definitive process has been determined by which this occurs. To date lead has generally been implicated for reducing or slowing brain growth and development and interfering with the concentration and activity of several neurochemicals.

A delay in the increase of cytochrome concentration in the

brains of lead-treated rats,[41] delays in the development of metabolic compartmentation,[42] reduced synaptic densities in the cerebral cortex and less mature synaptic profiles[43] all indicate a slowing of brain development in lead-treated animals. Postnatal lead exposure has also been shown to affect the development of Purkinje cells in the rat.[44] According to this study, animals with lead encephalopathy had a lower number of Purkinje cells, granule cells, and a decreased cell density. There was also a 34.8% reduction in dendritic length due to a decrease in total segment number and length of distal segments. Abnormal branching of dendrites was also observed. Krigman and Hogan[45] observed an alteration of growth and maturation of neurons, including a reduction in the number of synapses per neuron. In one of the very few papers directly relating behavioral modifications with delays in brain development, Croften et. al.[46] noted that lead-induced delays in exploration and locomotor activity appear to be associated with delays in synaptogenesis and biochemical development of the cerebral cortex.

Studies on the effects of lead on the neurochemical systems are numerous and varied, as neurochemical transmitters in lead-treated animals have been observed to increase, decrease, or to show no change depending on the region of the brain studied, experimental methods and so on.

In general, lead seems to inhibit central cholinergic functioning. Some sources[47-49] have reported a decrease in acetylcholine release in vitro including decreased acetylcholine

release in the sympathetic ganglion[46] and at the neuromuscular junction.[50,51]

Acetylcholine and choline levels are usually observed to be the same or, in a few cases, elevated[52] and in vitro acetylcholine turnover rates are significantly reduced with lead exposure.[53] This agrees with the findings that acetylcholinesterase activity is usually found to be unchanged or reduced in response to lead treatment whereas cholinesterase activity is either increased or unchanged.[54,55]

Studies of the effect of lead on the aminergic systems tend to show an increase in dopamine release and a decreased uptake of both dopamine and norepinephrine[56,57] leading some to believe that lead interrupts this system postsynaptically as compared to presynaptically in the cholinergic system. Dopamine levels are either decreased or unchanged in response to lead while norepinephrine levels have been reported to be either elevated or, in a few instances, decreased or unchanged.[52,58-61]

Both seem to undergo a high turnover as shown by high levels of homovanillic acid and vanillylmandelic acid, the two primary metabolities of norepinephrine and dopamine, respectively, in the brain and urine of lead-treated animals.[61-63] Monoamine oxidase, the enzyme involved in the breakdown of the transmitters, was reported to show a 20% increase in activity.[63]

Hyperactivity is assumed to be caused by a combination of decreased cholinergic activity and an increase in the activity

of the aminergic system. This is supported by the experimental findings that hyperactivity is suppressed by cholinergic agonists and aminergic agonists and the anticholinergic agent, antropine.[61,64] The findings then that lead decreases cholinergic activity reaffirms the observation that lead causes hyperactivity. The corresponding increase in aminergic activity however is not seen, as dopamine and epinephrine do not show an increased uptake. However, some have suggested that there could be an increased sensitivity of dopamine receptors leading to an increased activity.[65] This has yet to be shown.

Because of the effect of lead on neurotransmitter levels it is hardly surprising that studies show the interference of lead with nerve transmission. Lead causes reduced peripheral and motor nerve conduction velocities,[66-68] reduced size of endplate potentials,[51] decrease in forces of contraction and significant increases between indirect nerve stimulation and the initiation of contractile response.[50,69]

The precise mechanism by which lead interferes with neurological functioning is not known. Studies aimed at determining the distribution of lead within the brain show high concentrations of lead in the hypothalmus and striatum,[70] both believed to play a key role in behavior and regulation of motor activity, and the hippocampus.[71] Within the cell it appears that lead has a particular affinity for cell mitochondria, as some studies report a decrease in phosphorylative and respiratory enzymatic activity with lead treatment.[72-74] Goyer and Krall[75] observed impaired

mitochondrial oxidative and phosphorylative metabolism as well as ultrastructural evidence of defective mitochondrial membranes as a result of lead treatment.

Goldstein et. al.[76,77] presents some interesting findings related to the interaction of lead with cellular organelles. Brain capillaries, isolated and incubated with 10^{-4} M lead nitrate, showed a large increase in calcium transport as endothelial cell uptake of calcium tripled.[77] Goldstein proposes that this may be due, in part, to the inhibition of active calcium efflux by the cell which is abolished by 10^{-5} M lead nitrate. Kim et. al.[78] has also reported a decrease in calcium efflux in brain slices pretreated with lead carbonate. Also, endothelial cells contain a large number of mitochondria and electromicroscopy revealed that the lead in the endothelial cells was located almost exclusively within the mitochondria near the inner surface of the cristae. The mitochondria are considered to be the "buffering system" in the cell, closely regulating ionic concentrations within the cytoplasm. At concentrations of 5×10^{-6} M lead nitrate, lead inhibited calcium uptake by the mitochondria by 50%.[76]

Taking the results from these two studies by Goldstein it appears that lead increases calcium concentration in the cytosol by decreasing calcium efflux from the cell and calcium uptake by the mitochondria, presumably by interfering specifically with the calcium transport system. Potassium transport is not altered.[77]

This increasing intracellular concentration of calcium can influence several key membrane enzymes such as cation ATPases,

adenyl cyclase, and protein kinases. It is worthwhile noting that certain ATPases have been found to be sensitive to low lead concentrations.[79,80] Adenyl cyclase as well is inhibited in the presence of lead.[81,82]

This competition of lead with calcium is not surprising. Lead is believed to compete with calcium for absorption from the gastrointestinal tract,[83,84] and also displaces calcium for incorporation into the bone.[84,85] The proposed presynaptic effect of lead on the cholinergic system is presumably due to the competition between lead and calcium at the synapse.[46,50,86,87]

Could it be possible that lead also alters lipid and fatty acid distribution in the brain? Previous studies have shown that the presence of certain lipids as well as the fluidity of the membrane, determined in part by the fatty acid distribution within the cell membrane, can alter the activity of certain membrane-bound proteins and enzymes such as adenylate cyclase,[88] acetylcholinesterase,[89] mitochondrial-bound succinate oxidase,[90] various ATPases,[91,92] monoamine oxidase,[93] and many others, some of which have been shown to have altered activities in the presence of lead, as mentioned earlier. Other studies have shown a possible direct involvement of lipid fluidity with nerve conduction,[94] synaptosomal neurotransmitter binding,[95] and ionic transport and binding[96-98] which is so important in nerve conduction.

It is also possible that delays in brain development, as mentioned earlier, can also be reflected in the fatty acid distribution in lead-treated rats as compared to controls. Fatty acid

distribution is known to change with age.[99-101]

Yet, despite this intimate involvement of lipids with membrane integrity and membrane integrity with the operation of the neurological system, very little work has been done in this area.[102,103] In this study we will look at the effect of pre- and postnatal lead exposure on the fatty acid distribution in the developing rat brain.

MATERIALS AND METHODS

Three pregnant rats were purchased from Charles River (Wilmington, Massachusetts). The dams, 14 days pregnant, were weighed and housed in separate compartments. Two of the dams, designated Pb-A and Pb-B, received water containing lead acetate at a concentration of 2.07 mg per ml (0.01 M) Pb^{2+}, an amount which has previously been observed to be transferred to suckling pups[13,15] and to affect rat behavior and physiology.[41,43,46,70] The control dam received plain water and all three were given Purina Rat Chow ab libitum.

On the 23rd day of pregnancy the Pb-A and control dams gave birth to 10 and 14 pups, respectively. The following day, Pb-B gave birth to 15 pups. The number of pups in each litter was reduced to nine. Nineteen days after the birth of the Pb-A and control pups, the smallest pup from each of the three litters was sacrificed. On day 21 the remaining pups were weaned and their mothers sacrificed. The lead concentration in the pups drinking water was decreased to 0.41 mg per ml (0.002 M) Pb^{2+}. Then on days 22, 26, 30, and 35, the two smallest pups from each litter were sacrificed.

Throughout the procedure the amount of food and water consumed by each group was measured and recorded. The mothers were weighed and the pups were weighed as a group throughout the study.

After being sacrificed, the brain from each rat was removed, placed in a vial, and frozen by placing the vial in dry ice and

ethanol. The vial was purged with nitrogen and placed in the freezer.

Isolation of Lipid Fractions

Glass distilled solvents were used (Burdick and Jackson Laboratories, Muskegon, Michigan) except as noted. Chloroform was reagent grade and was distilled before use. Solvent mixtures are expressed as v/v, and quantities used are per 1 g of tissue.

The brain was homogenized in 19 ml of 2:1 chloroform:methanol (contained 0.005% butylated hydroxytoluene (BHT) as antioxidant).[104] The suspension was filtered through a sintered glass funnel. The residue was washed with 3.5 ml of 2:1 chloroform:methanol, followed by another washing with an equal volume of 1:2 chloroform:methanol. Both washings were mixed with the original filtrate and the chloroform-methanol ratio was adjusted to 2:1 by the addition of chloroform.

The filtrate was washed with 6.0 ml of 0.1 M KCl. The upper layer was removed and the lower layer was rewashed with the same volume of 3:48:47 chloroform:methanol:0.1 M KCl. After removing the upper layer from the second washing, the lower layer was brought to dryness on a rotary evaporator. Benzene:ethanol 1:1 was added to aid in removing the water and to prevent excessive foaming and splashing. The crude lipid was dried in a desicator for two days under a nitrogen atmosphere at reduced pressure.

The next step, a transmethylation procedure, was done with three variations.[105]

1. For the mothers, 19, 22, and 26 day pups, 15 ml of 2:1 chloroform:methanol (0.005%) was added to the crude lipid. One third of the resulting solution was pipetted into a round bottom flask containing 5 ml of methyl pentadecanoate (400 ug per ml) and the mixture brought to dryness on the rotary evaporator. The methyl pentadecanoate served as an internal standard for gas-liquid chromatography (GLC).

The transmethylating reagent was prepared by mixing 2 volumes of chloroform with 1 volume of 0.21 M sodium methoxide in methanol. For every 30 mg of crude lipid, 10 ml of this reagent was added and the solution stirred for one hour at room temperature. After being stirred, about one-fifth volume acetic acid was added, and two layers formed. 0.1 M NaOH or 0.36 M acetic acid was added to the solution if necessary until the pH of the upper layer was about 6.7. The top layer was then discarded. The lower layer, containing the esterified fatty acids, was washed with 3:48:47 chloroform:methanol:water. A volume equal to that of the 0.36 M acetic acid was used. The upper layer was discarded and the lower layer was brought to dryness on the rotary evaporator. Again, 1:1 benzene:ethanol was added to remove water and prevent foaming and splashing.

2. The brains from the 30 day pups were treated in essentially the same way with the exception that no internal standard for GLC was added and the entire crude lipid fraction, rather than 1/3, was used. Therefore, after removing the crude lipid from the desicator, the transmethylating reagent was immediately

added.

3. The crude lipid from the 35 day brains was applied to a thin-layer chromatography (TLC) plate in an effort to separate the various lipid components. This procedure, however, did not give consistent results.

Isolation of the methyl ester derivatives was carried out by Florisil column chromatography.[106] Florisil, 60-100 mesh (Fisher Scientific Co., Fair Lawn, N.J.) was used. The Florisil was activated by heating it for 1 hour at 600°C. After cooling, deionized water was added (7% by weight) and the Florisil allowed to equilibrate for at least 24 hours before use.

The column was prepared using a ratio of 40:1, Florisil to lipid based on the crude lipid weight. A column diameter was chosen to give a column height of 10-11 cm. After applying the lower layer lipid to the column three elutions were done. Hexane:benzene 9:1, 25 ml per gram of Florisil, was added to elute the methyl esters. This was followed by hexane:ether 8:2, 30 ml per gram of Florisil, which eluted cholesterol. The third fraction, containing cerebrosides and sulfatides, was eluted with chloroform:methanol 3:1, 30 ml per gram of Florisil. The purity of each of the three fractions was affirmed by TLC.

GLC of the Methyl Esters

An F and M, model 402, dual column, flame ionization detector, high efficiency chromatograph was used for the methyl ester analysis.

The glass columns were U-shaped, 6 ft X 3 mm. Methyl esters from the 30 day rat brains were chromatographed on columns packed with 10% diethylene glycol succinate on Chromosorb W - acid washed (Applied Science Laboratories, State College, PA). The remaining methyl ester chromatography was done on columns packed with 2% Silar-5CP on Gas-Chrom Q, 100-120 mesh (Applied Science Laboratories). These two columns give nearly identical separations. The methyl esters were chromatographed between 160°C and 225°C with a temperature gradient of 4°C per minute. A standard containing a wide range of fatty acid methyl esters, each at a known concentration, was chromatographed before each run (Standard NHI-F, Applied Science Laboratories). The analyzed composition of the standard corresponded to the stated values with less than a 5% relative error.

Values stated for the relative concentrations of fatty acids are averages of values obtained from 2 or 3 chromatograms in which the values for all the major methyl esters; palmitic(16:0), stearic (18:0), oleic(18:1), arachidonic(20:4), and docosahexaenoic(22:6); had deviations of less than 5%. Area calculations were based on peak height and peak width at one-half peak height.

Preliminary unpublished work on the effect of lead on developing rat brain lipids is included in this thesis. The procedures used were the same as those described above. This work was done by Tom Stiger and Victoria Hagus.

In the preliminary study, two pregnant rats were used. One was a control and the other was given water in which the lead

concentration, as lead acetate, was 1.42 mg per ml (6.86 mM) Pb^{2+}. The pups, two from each litter, were sacrificed on days 15, 18, 22, and 25.

RESULTS AND DISCUSSION

The food and water consumed by each group of animals is summarized in tables 1 and 2. After weaning the dams were killed and values are for pups only. The lower amount of water and food consumed by the experimentals, relative to the controls, is reflected in the decreased weight of the pups born to the lead-treated dams. Brain weight was also consistently reduced in the experimental animals. However, when expressed as a percentage of the body weight, brain weight was greater for the lead-treated rats with this difference diminishing with age. Rats on lead diets, in general, have been observed to eat less and show less weight gain than those on diets without lead,[53,58,60] leading

Table 1 - Food Consumed by Dams and Pups
(grams per gram body weight per day)

	Control	Pb-A	Pb-B
Before Birth	0.068	0.076	0.066
Birth to Weaning	0.17	0.15	0.15
Day 22	0.11	0.084	0.060
Days 23-26	0.11	0.12	0.12
Days 27-30	0.12	0.14	0.13
Days 31-35	0.10	0.11	0.11

Table 2 - Water Consumed by Dams and Pups
(ml per gram body weight per day)

	Control	Pb-A	Pb-B
Before Birth	0.16	0.16	0.16
Birth to Weaning	0.34	0.33	0.30
Day 22	0.21	0.21	0.16
Days 23-26	0.24	0.28	0.24
Days 27-30	0.23	0.28	0.23
Days 31-35	0.26	0.33	0.24

To weaning, Pb^{2+} concentration - 2.07 mg per ml, after weaning - 0.41 mg per ml.

many to question whether the effects of lead on the body are the direct result of lead, an indirect consequence resulting from lead-invoked malnutrition, or a combination of both. The question can be raised again here.

The crude lipid fraction, when expressed as a percentage of brain weight, deviated little between control and lead-treated samples.

The cholesterol and cerebroside fractions, isolated along with the methyl esters by column chromatography of the methanolized crude lipid, were weighed and expressed as a weight percentage of crude lipid and of whole brain. In both cases, the cholesterol values of lead-treated pups tended to be greater than those of the controls (table 3). Day 22 showed considerable variation.

Table 3 - Cholesterol Levels in Young Rat Brains as Weight Percent of Brain and (Crude Lipid)

	Control	Pb-A	Pb-B
19 Day	0.91% (16.4%)	1.02% (18.6%)	1.11% (17.6%)
22 Day			
1.	1.37% (21.5%)	1.76% (34.0%)	1.07% (14.9%)
2.	1.50% (33.3%)	1.74% (22.9%)	1.69% (26.5%)
26 Day			
1.	1.05% (16.2%)	1.37% (19.0%)	1.31% (17.5%)
2.	1.19% (18.0%)	1.21% (18.0%)	1.29% (19.0%)
30 Day			
1.	1.05% (17.6%)	1.36% (20.3%)	1.36% (19.2%)
2.	1.01% (14.5%)	1.22% (23.5%)	1.32% (19.3%)

Cerebroside values, on the other hand, showed no significant deviations amongst the pups.

Table 4 lists the relative fatty acid concentrations found from ester bound fatty acids. Nine peaks were measured on each chromatogram, 5 of which were major; palmitic (16:0), stearic (18:0), oleic (18:1), arachidonic (20:4), and docosahexaenoic (22:6). Of these, the relative fatty acid concentrations of palmitic and arachidonic acids in the experimentals show consistent deviations from the control concentrations.

In the 19, 22, and 26 day lead-exposed pups, the relative

Table 4 - Fatty Acid Composition of Young Rat Brains

	A-19	B-19	C-19	A-22-1	A-22-2	B-22-1	B-22-2	C-22-1	C-22-2
16:0	25.5	26.2	26.6	23.9	22.9	24.5	23.7	24.3	24.3
16:1	1.6	1.8	1.6	1.3	1.4	1.4	1.2	1.6	1.4
18:0	20.7	20.4	20.7	22.3	21.1	21.3	22.7	21.2	21.7
18:1	16.7	16.0	16.0	18.0	19.2	18.7	18.5	18.9	18.6
18:2	1.2	1.1	1.2	1.2	1.3	1.2	1.3	1.3	1.3
20:1	0.8	0.7	0.6	0.8	1.1	0.9	0.9	1.0	1.0
20:4	12.8	13.0	12.2	12.3	12.2	11.7	11.8	11.4	11.2
22:5	3.9	4.3	4.2	3.9	4.0	4.0	4.0	4.0	3.9
22:6	16.8	16.6	16.7	16.3	16.9	16.4	15.9	16.5	16.7
Total fatty acid mass (mg)	9.40	9.67	8.86	10.5	11.5	11.4	10.8	11.3	11.0
Percent unsaturation	53.8	53.5	52.5	53.8	56.1	54.3	53.6	54.7	54.1

See next page for explanation of symbols

Table 4 - Continued

	A-26-1	A-26-2	B-26-1	B-26-2	C-26-1	C-26-2	A-30-1	A-30-2	B-30-1	B-30-2	C-30-1	C-30-2
16:0	23.1	22.7	22.0	22.7	24.5	23.2	22.3	22.2	21.6	21.5	21.4	22.1
16:1	1.3	1.3	1.4	1.3	1.1	1.2	1.3	1.0	1.2	1.2	1.1	1.1
18:0	21.2	21.4	21.3	21.2	21.7	21.2	21.1	21.5	21.3	20.9	21.5	21.4
18:1	19.7	20.0	19.7	20.0	19.5	20.2	20.7	20.4	21.4	21.1	21.2	21.1
18:2	1.1	1.3	1.2	1.2	1.2	1.2	1.0	1.1	1.2	1.2	1.2	1.2
20:1	1.3	1.2	1.2	1.3	1.3	1.5	2.0	1.7	1.6	1.8	1.7	1.8
20:4	11.4	11.3	11.9	11.2	10.7	10.8	11.4	11.4	11.3	11.5	11.0	11.0
22:5	3.9	4.0	4.2	4.1	3.7	3.8	4.0	4.0	4.0	4.0	3.8	3.8
22:6	17.0	16.8	17.1	17.1	16.4	16.9	16.2	16.7	16.4	16.8	17.0	16.5
Total fatty acid mass (mg)	12.4	11.5	11.3	12.1	11.7	13.3	-	-	-	-	-	-
Percent unsaturation	55.7	55.9	56.7	56.2	53.9	55.6	56.6	56.3	57.1	56.6	57.0	56.5

"A" represents the Pb-A pups, "B" the Pb-B pups, and "C" the control pups; the second identifying symbol represents the day after birth the animal was sacrificed, either day 19, 22, 26, or 30; the final number merely distinguishes between the two pups from each litter that were killed on that day.

concentration of palmitic acid was lowered, with the exception of B-22-2 which was slightly elevated, in relation to the 22 day control average. In general the deviation was greater for the younger animals. Arachidonic levels, on the other hand, were elevated in all Pb-A and Pb-B pups. As with palmitic acid, this difference was observed to be greater in the youngest group.

An analysis for variance was run on the data obtained for these two fatty acid methyl esters. This computer analysis compared the data of the experimentals with that of the controls and reported an overall value called a P value. This P value represents the probability that the results obtained were merely coincidental. The P value for the palmitic acid methyl ester data was less than 0.10 meaning that there is less than a 10% chance that the difference between the control and experimental data was coincidental. In other words, the data was significantly different at the 90% confidence level. The data for the arachidonic acid methyl esters was significantly different at the 99% confidence level as the P value was less than 0.01, the lowest value possible.

The average 22:5 values also appeared to be slightly elevated in the 26 and 30 day experimentals, with the greatest difference appearing at 26 days.

Work done earlier with somewhat less lead gave similar results (table 5). In that study the palmitic acid concentration was lowered in the 15 and 18 day experimental rats with no apparent deviation in the 22 and 25 day rats. Stearic acid (18:0) was also lowered in the youngest group. Arachadonic acid concentrations

Table 6 - Fatty Acid Composition of Young Rat Brains

	E-15-1	E-15-2	C-15-1	C-15-2	E-18-1	E-18-2	C-18-1	C-18-2
16:0	27.8	27.6	28.6	28.5	24.4	24.8	25.0	25.8
16:1	2.2	2.3	2.9	2.5	1.5	2.6	1.4	2.0
18:0	19.1	19.2	19.6	20.2	19.8	19.9	20.4	19.8
18:1	15.3	15.2	15.0	15.8	17.5	17.7	16.8	17.8
18:2	1.2	1.2	1.2	1.2	1.2	1.3	1.2	1.5
20:1	0.4	0.6	0.6	0.5	0.7	0.9	1.0	1.0
20:4	12.8	13.0	12.5	12.0	13.0	12.5	12.2	12.1
22:5	4.8	4.3	4.5	4.5	4.9	4.4	4.8	4.3
22:6	16.2	16.6	15.1	14.7	16.3	16.1	16.4	15.6
Percent Unsaturation	53.1	53.2	51.8	51.3	55.8	55.3	54.6	54.4

See next page for explanation of symbols

Table 5 - Continued

	E-22-1	E-22-2	C-22-1	C-22-2	E-25-1	E-25-2	C-25-1	C-25-2
16:0	23.0	22.8	22.4	24.2	21.6	21.5	21.7	21.5
16:1	1.2	1.5	1.7	1.5	0.9	1.0	1.0	1.1
18:0	20.7	21.8	20.4	20.9	20.8	20.4	20.7	21.0
18:1	18.2	19.6	19.2	18.8	20.6	19.8	20.7	21.0
18:2	1.2	1.6	1.5	1.5	1.1	1.2	1.1	1.2
20:1	1.2	1.4	1.3	1.4	1.5	1.5	1.8	1.6
20:4	12.2	11.6	11.6	11.4	11.3	11.3	10.5	10.9
22:5	4.6	4.6	4.5	4.6	4.7	4.6	4.5	4.6
22:6	16.7	14.8	16.7	15.6	16.6	17.6	17.2	16.8
Percent unsaturation	56.3	55.4	57.2	54.9	57.6	58.1	57.6	57.5

"E" represents the experimental pups and "C" represents the control pups; the final two numbers are the same as described earlier

were elevated in all of the experimental rats with the exception of one 22 day pup which had a relative arachadonic acid concentration the same as that of one of the control pups. The deviation showed little change over the age range studied. Docosapentaenoic acid (22:5) showed no deviation from the controls in this study. On the other hand, docosahexaenoic acid (22:6) concentration in the lead-treated rats showed a large increase over the controls in the 15 day pups.

The 15 and 18 day pups showed an overall higher percentage of unsaturated fatty acids. This is also seen in the 19 and 26 day brains of the present study.

In general, the results are consistent with the idea that lead causes abnormal brain growth[41-46] as both palmitic acid and arachidonic acid concentrations in the younger pups deviate from those of the controls. The fact that this difference diminishes with age, indicates that lead may only delay brain maturation rather than completely inhibit it. In connection with this, it may be significant that the most rapid myelination occurs at the time that the greatest changes in fatty acid distribution are noted. Any changes in the fatty acids incorporated into myelin during this period could quite conceivably interfere with normal brain development.

It has also been shown that changes in overall fatty acid concentration, especially those changes which may affect the unsaturated/saturated fatty acid ratio has a definite effect on the kinetics of membrane-bound enzymes.[90,92,107,108] The theory that

lipids and membrane fluidity may be directly involved in nerve transmission is further substantiated by studies showing that pesticides may interfere with nerve transmission by altering lipid fluidity[89] as do certain anesthetics.[109] Haines[110] has carried through with this idea and proposed a mechanism by which lipids, particularly unsaturated fatty acids, may be involved in ionic transport.

As to whether the change in fatty acid composition seen in this study is sufficient to cause changes in membrane fluidity and therefore, changes in the kinetics of membrane-bound enzymes, is difficult to say.

However, the changes in lipid composition do seem to verify that lead absorption may lead to delayed brain maturation. Even the seemingly temporary abnormal fatty acid distribution of the phospholipids may in turn affect other more permenant aspects of brain development.

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