slices were added to tubes containing antagonists after incubation for 50 minutes and the other agents were added 10 minutes later. Incubations were terminated by placing the tubes in boiling water for 10 minutes. The samples were then centrifuged at low speed, and duplicate 50-μl portions of each supernatant were analyzed for cyclic AMP as described by B. L. Brown, J. D. M. Albano, R. P. Ekins, and A. M. Sgherzi [*Biochem. J.* **121**, 561 (1971)]. The protein content of the samples was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)]. The cyclic AMP content was proportional to the size of the portion tested, and known amounts of authentic cyclic AMP added as an internal standard were quantitatively recovered. Cyclic AMP content measured by this method was the same as that found by homogenizing the tissue in a mixture of ethanol (98 percent) and HCl (0.2*M*), centrifuging, evaporating the supernatant to dryness, and redissolving the residue for determination of cyclic AMP as described previously (*11*). In experiments where the cyclic AMP content of the slices and the incubation medium were determined separately, virtually all the cyclic AMP was found in the slices. The data are expressed as picomoles of cyclic AMP per milligram of protein. Each point represents the mean $\pm$ the standard error of the mean (S.E.M.) for six or more replicate samples.

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Lead Contamination around Secondary Smelters: Estimation of Dispersal and Accumulation by Humans

Abstract. *A high rate of lead fallout around two secondary lead smelters originated mainly from episodal large-particulate emissions from low-level fugitive sources rather than from stack fumes. The lead content of dustfall, and consequently of soil, vegetation, and outdoor dust, decreased exponentially with distance from the two smelters. Between 13 and 30 percent of the children living in the contaminated areas had absorbed excessive amounts of lead (more than 40 micrograms per 100 milliliters of blood and more than 100 micrograms per gram of hair) as compared with less than 1 percent in a control group. A relationship between blood and hair was established which indicated that the absorption was fairly constant for most children examined. It seemed that the ingestion of contaminated dirt and dusts rather than "paint pica" was the major route of lead intake. Metabolic changes were found in most of 21 children selected from those with excessive lead absorption; 10 to 15 percent of this group showed subtle neurological dysfunctions and minor psychomotor abnormalities.*

Attempts to establish a dose-response relationship between environmental lead concentrations and subtle changes in human health have met with only limited success, largely because of the multiplicity of sources and the consequent difficulties of quantifying individual exposure. We report here the health effects of lead contamination around two secondary lead smelters.

Although smelters A and B are located in different parts of Toronto, each smokestack (in this report all distances are measured from the respective smokestack) is about 100 m south of a residential area and 100 to 200 m north of an elevated expressway (10 to 20 m high carrying 50,000 to 150,000 cars per day). Lead emissions from the two smelters were estimated to be 15,000 and 30,000 kg/year, respectively. Procedures for the collection, preparation, and analysis of materials have been described extensively elsewhere (*1*).

A mosaic of lead concentrations in the soil was found in each urban-industrial complex, but extremely high values were recorded in localized areas around each of the smelters (Fig. 1a). Regression analysis of the concentrations indicated that an exponential decrease with distance, from values of 40,000 and 16,000 μg per gram of soil close to smelter A and smelter B, respectively, to an urban background of 100 to 500 μg per gram of soil, accounted for 60 to 80 percent of the variability in the data (*2*). The month-to-month variation in the lead content of dustfall was proportional to the mean, but regression analysis also indicated an exponential decrease with distance from each smelter (Fig. 1b). In this case the distance parameter accounted for only 40 to 60 percent of the data variability within the limit of influence. Because the lead fallout was very localized and consisted primarily of large particles, it appears that the emissions originated mainly from low-level, dust-producing operations rather than from stack fumes.

Unlike the extremely high soil and dustfall values, the monthly geometric means of the lead concentrations in suspended particles close to the smelters (1 to 5.3 μg per cubic meter of air) were only double those for urban sites (0.8 to 2.4 μg/m^3). However, the range of daily concentrations was much greater, producing a marked log-normal distribution (Fig. 1c). The episodal peaks were correlated with winds from the smelters, but extensive monitoring was necessary to separate emissions from the smelters and auto emissions on the expressway when winds were from the south. At two sites the same distance from the expressway as samplers north of smelter A and smelter B (that is, 300 and 200 m, respectively), the lead concentrations averaged 1.0 and 1.6 μg/m^3, in agreement with calculations from values reported by Daines *et al.* (*3*). The lead aerosol was predominantly submicron in size, having a mass median diameter (MMD) of 0.8 ± 0.2 μm. This was also the distribution at sites close to the smelters except for episodal days when, for example, concentrations at one site 100 m north of smelter A averaged 6.6 ± 1.0 μg/m^3 and the MMD increased to 4.6 ± 1.3 μm. The average concentrations of antimony and arsenic, also associated with smelter fumes, increased from 20 ng/m^3 to 440 and 180 ng/m^3, respectively, on episodal days, and the size distribution reflected similar changes between nonepisodal and episodal days. Bromine concentrations, associated with automobile emissions, were about 0.3 μg/m^3, irrespective of wind direction, with an MMD of 0.4 ± 0.2 μm. The average bromine/lead ratio at the control sites was 0.32 ± 0.04 as compared to 0.05 ± 0.02 close to smelter A on episodal days, which is a value similar to that found by Wesolowski *et al.* (*4*) for samples containing industrial lead emissions.

Between May and December 1973, the Toronto Board of Health collected capillary blood from 286 people close to smelter A, 1425 people close to smelter B, and 1231 people in a similar socioeconomic urban control group, and had these samples analyzed for lead content by anodic stripping voltammetry (*5*). The arithmetic mean for the control group was 19 μg per 100 ml of blood, and less than 1 percent of the children in this group (0 to 14 years) had lead contents in excess of 40 μg/100 ml. The means for children living withing 300 m of smelters A and B were 27 and 28 μg/100 ml, respectively. The log-normal distribution about these values was significantly higher than that of the control area on the basis of chi-square analysis ($p <$ .001), mainly because 28 percent of the children sampled during the summer months close to smelter A and 13 percent sampled during the winter months close to smelter B had blood lead contents in excess of 40 μg/100 ml. Although there was no significant trend for the adults, the blood lead concentrations of the children increased with proximity to both smelters (Fig. 1d). However, only 30 and 8 percent of the variation in blood lead concentrations within 300 m of smelters A and B, respectively, could be attributed to the distance variable. Similar increases in the blood lead content of people living in contaminated areas have been found by other workers (*6*), although such increases were only marginal in old lead-mining areas (*7*).

In order to determine the major source of increased lead absorption, a detailed survey of 16 families (out of a possible 30) living in houses within 150 m of smelter A and 8 urban control families living in houses of similar age and upkeep was carried out between April and November 1973. The analy-

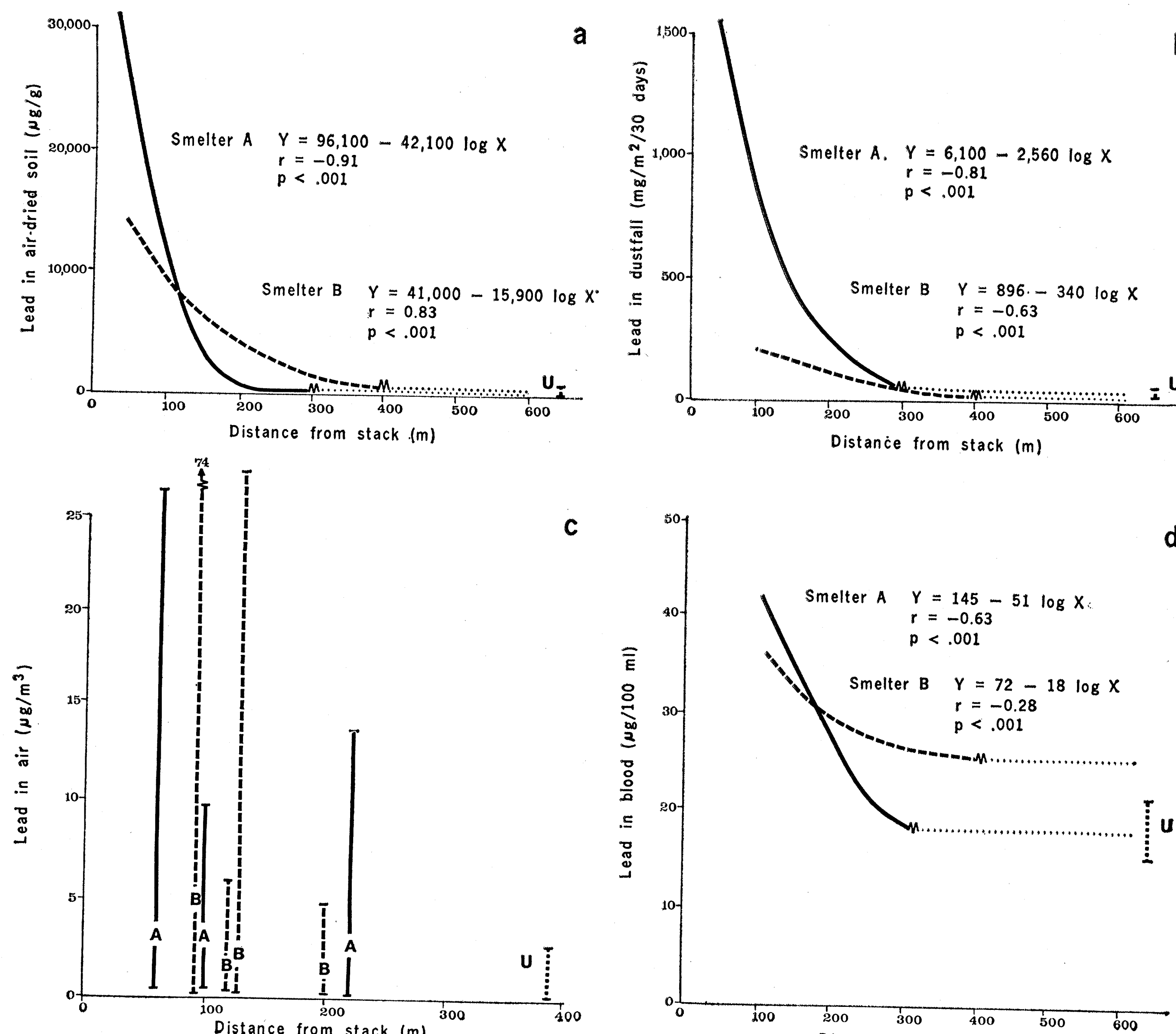

Fig. 1. Dispersal of lead contamination around two secondary smelters and accumulation by children. Solid lines and dashed lines in (a), (b), and (d) are fitted curves corresponding to the regression equations; dotted lines are an extrapolated fit; *U* indicates the corresponding values found in urban control areas; *A* and *B* in (c) are the geometric means ± range for 24-hour high-volume air samples close to smelter A and smelter B, respectively. In (a) the number of soil samples is 121 around smelter A and 146 around smelter B. In (b) the total number of monthly dustfall samples is 96 at 16 sites close to smelter A and 34 at 6 sites close to smelter B. In (c) the number of 24-hour air samples is 96, 57, and 94 at sites 60, 100, and 220 m from smelter A and 101, 64, 73, and 101 at sites 90, 120, 130, and 195 m from smelter B. In (d) the number of children is 84 close to smelter A (0 to 14 years) and 1041 close to smelter B (0 to 15 years).

sis of the lead content of capillary blood and entire lengths of head hair of the residents gave geometric means of 17 μg per 100 ml of blood and 13 μg per gram of hair in the control group as compared to 27 μg/100 ml and 41 μg/g for the smelter group (Table 1). The natural logarithm of the hair lead content was correlated with unaltered blood lead contents (correlation coefficient $r = 0.76$) for 68 of the 73 individuals sampled, which is in agreement with the results of Hammer *et al.* (*8*). Such a relationship might be expected if the rate of absorption is fairly constant. The observation that the lead content of hair segments did not vary appreciably with the distance of the segment from the scalp for children who fell into the correlated group strongly suggests that this is the case. The relationship does not hold true for children exposed to acute doses of lead (*9*), and it did not occur in three children of an occupationally exposed worker or two children with anecdotal evidence of soil pica.

The lead content of tap water was low in both study areas, the consumption of homegrown vegetables was not extensive, and, although the lead content of paint was extremely variable, there was no difference in the lead content of the house paint between smelter and control houses (Table 1). Suspended lead in air was higher close to the smelters than at a distance, but 70 percent of the increase was attributable to large nonrespirable particles; indoor values (during the winter months) were only 20 to 30 percent of those recorded outdoors. Ingestion of contaminated dirt and dusts may be the major route of intake by children (*10*), particularly in this case, as 20 to 60 percent of the lead in surface soil was extractable in 0.1*N* HCl compared with less than 10 percent extractable from paint samples.

To date, 21 of the children with excessive lead absorption have been admitted to the hospital for closer observation. Metabolic changes, as indicated by increased excretion of δ-aminolevulinic acid (ALA) and coproporphyrins (*11*), were observed in most of these cases. Increased density in bone metaphyses was observed in four children. The criteria used as indicating the need for chelation therapy (*12*) were urinary excretion of ALA in excess of 2 mg per square meter of body surface over a 24-hour period and urinary lead excretion in excess of 1 mg per gram of ethylenediaminetetraacetic acid (EDTA) over a 24-hour period, after mobilization with 25 mg of the calcium salt of EDTA per kilogram of body weight. The difficulty of determining a level of "significant toxicity" is shown by the fact that 14 cases exceeded the former criterion whereas five cases exceeded the latter. Two individuals exhibited neurological abnormalities of a nonspecific nature, and a reduction in peripheral nerve conduction velocity was recorded in three cases (*13*). Seven children were hyperactive, but it is still unclear whether lead is an etiological factor in, or the consequence of, hyperactivity (*14*). These findings are consistent with other studies (*15*), but the functional consequences of asymptomatic but elevated lead absorption remain equivocal (*16*).

We conclude that poor personal hygiene and play habits of children living in areas where the lead content of house dust, outdoor dust, and soil is considerably elevated can result in excessive lead absorption. The increases recorded do not appear sufficient to cause clear-cut symptoms of lead poisoning but may cause metabolic and subtle neurological changes.

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Table 1. Lead accumulation by people living close to a secondary smelter compared to an urban control group, and some potential sources of lead uptake. Abbreviations: A.M., arithmetic mean; G.M., geometric mean; D.W., dry weight; and F.W., fresh weight.

Matrix units	Smelter area			Urban control area		
	A.M.	G.M.	Range	A.M.	G.M.	Range
Blood (μg/100 ml)	31	27	12–61	19	17	8–40*†
Hair (μg/g)	52	41	14–166	17	13	3–39*†
Tap water (μg/liter)	6.8	5.9	2–14	1.40	1.3	1–2.4*
Paint (% by weight)	1.23	0.72	0.13–4.64	1.30	0.89	0.11–2.62‡
Dustfall (mg/m² per 30 days)	553	158	35–2334	25	23	17–36*
Suspended particles (μg/m³)	3.84	3.01	1–10	0.93	0.82	0.30–2.54§
Outdoor dust (μg/g, D.W.)	5828	2416	578–39,500	1002	924	500–1561‖
House dust (μg/g, D.W.)	2055	1550	470–8400	845	713	351–2010‖
Garden soil (μg/g, D.W.)	2626	1715	355–8750	110	99	57–240*
Vegetables, washed						
Lettuce leaf (μg/g, F.W.)	8.6	2.9	0.7–40	0.9	1.0	0.8–1.1‖
Radish tuber (μg/g, F.W.)	4.6	1.7	0.6–28	0.3	0.3	0.2–0.4‖
Tomato fruit (μg/g, F.W.)	0.33	0.32	0.25–0.40	0.24	0.23	0.10–0.30‡

* Frequency distribution significantly different at the 0.1 percent level by Student's *t*-test after log-transformation. † Frequency distribution significantly different by chi-square analysis at the 0.1 percent level. ‡ Frequency distribution not significantly different. § Frequency distribution significantly different at 0.1 percent by paired comparison analysis of 24-hour air samples. ‖ Frequency distribution significantly different at the 5 percent level by Student's *t*-test after log-transformation.

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Viability of Stored Seed: Extension by Cathodic Protection

Abstract. *Placing seeds on a negatively charged conductor extended their viability during artificial aging. Such cathodic protection may reduce free radical attack by providing a source of electrons. The results support the hypothesis of free radical damage to cellular components and are consistent with such damage being important in deteriorative senescence changes.*

It is well known that free radicals initiate peroxidative degradation of unsaturated tissue lipids, and it has been suggested that this could give rise to damage to cellular membranes (*1*). Lipid peroxidation in monomolecular and bimolecular films leads initially to an increase in membrane permeability and then to a decrease in membrane stability (*2*). Damage to biological membranes associated with lipid peroxidation has been shown in mitochondria (*3*), microsomes (*4*), and lysosomes (*5*). Lipofuscin granules accumulate with chronological age in some animal tissues and these granules appear to contain protein and peroxidized lipid (*6*), indicating that lipid peroxidation occurs throughout the life span of an organism. It has been suggested that this free radical peroxidation of unsaturated lipids is a basic deteriorative mechanism in cellular aging (*7*). There is as yet little direct evidence for the accumulation of membrane damage during senescence. However, extremely swollen mitochondria, with disorientated and fragmented cristae, indicative of membrane damage, have been observed in the flight muscle of old (25 to 30 days) male houseflies (*8*).

Attempts have been made to prolong the life span of organisms, and promising results have been obtained by supplying membrane-stabilizing drugs to *Drosophila* (*9*) and by including various antioxidants in the diet of mice (*7, 10*). However, these results are far from unequivocal: not all treatments are effective (*7, 9*), different results are obtained with the same antioxidant on different strains of mice (*7*), and in some experiments lipid peroxidation appeared to be reduced without any effect on life span (*11*).

Molnár (*12*) attempted to reduce free radical attack, not by supplying antioxidants in the diet, but by maintaining mice in a cage with an applied negative potential in a manner analogous to the technique of cathodic protection of metals against corrosion. The average and maximum life spans of mice exposed to a negative charge were, respectively, 25 and 32 percent greater than those of mice exposed to a positive charge.

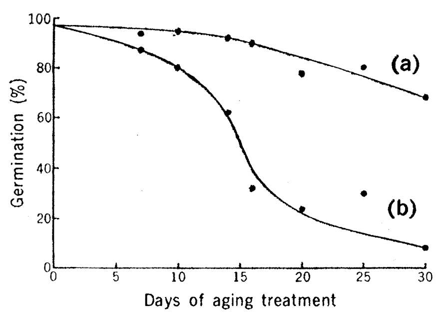

Fig. 1. Viability of stored seeds of *Zea mays* as a function of the duration of the aging treatment. Seeds with a moisture content of 13.6 percent were placed, embryo side down, on aluminum foil in a small enclosed chamber (to maintain the moisture content) and kept at 40°C to accelerate their aging (reduce their storage life). The entire contents of a single chamber were taken for each sample. The seeds were soaked in tap water overnight and then placed on moist paper toweling. Germination was recorded 84 hours after the start of soaking. (a) Seeds provided with cathodic protection by applying a negative potential of 300 volts to the aluminum foil; (b) control seeds.

Air-dry seeds can be rapidly aged artificially by maintaining them at a relatively high moisture content (13 to 15 percent) and an elevated temperature (40°C) (*13*). Air-dry seeds probably do not have sufficient moisture to permit aqueous-phase enzyme reactions to occur, and repair mechanisms are unlikely to be operative in such a system. Thus, damage induced by free radicals would accumulate in dry seeds, whereas in hydrated tissue such damage could be repaired (*14*). Seeds of *Zea mays* (Indian corn) that have been artificially aged show aberrations of mitochondrial membranes as an early sign of aging, before the viability of the seeds declines (*13*).

Seeds of *Zea mays* were subjected to artificial aging treatment by maintaining them in a small enclosed chamber at 40°C. The seeds were provided with cathodic protection by placing them, embryo side down, on aluminum foil within the chamber and applying a negative potential of 300 volts to the foil. Control seeds were maintained under similar conditions without the applied charge. The effect of storage time on the viability of the seeds is shown in Fig. 1.

Considerable decreases in viability loss were achieved with cathodic protection. In addition, the conductivity of the water in which the seeds were soaked before germination was consistently greater for the control seeds than for the seeds which had been exposed to the negative charge. This indicates that more material was leached from the control seeds during soaking, which suggests that greater membrane damage had occurred in them than in the seeds provided with cathodic protection. Chromosome aberrations are known to accumulate in nondividing cells of seeds under conditions of accelerated aging (*15*). Free radical peroxidation may affect macromolecules other than lipids; thus, our observation that after 10 days of the aging treatment the percentage of chromosome aberrations was 12.6 in the control seeds and 4.3 in the protected seeds may be of significance. The reductions in mortality rate for dry seeds, which are relatively nonhydrated systems, were far greater than any achieved for animals, which are hydrated systems.

Cathodic protection should reduce free radical attack on biological macromolecules by providing a source of electrons to react with the free radicals (*12*). Thus, these results provide strong evidence for free radical damage to