Rats and rhesus monkeys were exposed for 22 hours per day to lead oxide at a concentration of 21.5 mg Pb cubic meter of air. The year-long exposure elicited a significant increase in the concentration of lead in the blood of both rats and monkeys. The blood-lead levels reached a maximum during the first few months of exposure and did not increase significantly after that time.

Elevated concentrations of lead were also detected in lung, liver, kidney and bone of both species. The concentration of lead decreased in the soft tissues of animals removed from the chamber after one year's exposure but remained elevated in bone.

The activity of the enzyme 3-aminolevulinic acid dehydrase (ALAD) in circulating erythrocytes was inhibited about 10% in rats exposed to the airborne lead. No reduction of the enzyme activity occurred in brain or liver tissue of either the rats or monkeys.

Two experiments were conducted to determine the effects of low level exposure to airborne lead particulates on humans. Male volunteers were exposed 23 hours per day for about 18 weeks to either 109 mg Pb cubic meter or 32 mg Pb cubic meter of air.

In both cases the blood lead levels increased correspondingly and appeared to plateau after about three months of exposure. Subsequent to that no further increases were observed. About two months after the men left the exposure chamber, their blood leads returned to near normal levels.

There was an increase in urinary lead excretion, but always within the limits usually considered normal.

* Present Address: International Center of Environmental Safety, Albany Medical College, P. O. Box 1027, Holloman AFD, New Mexico 88320
The activity of δaminolevulinic acid dehydrase was reduced by about 50% in the erythrocytes of men exposed to 10.9 μg Pb/cubic meter, but returned to normal after exposure was terminated. Practically no changes in the activity of the enzyme were noted in the men exposed to 3.2 μg Pb/cubic meter.

As a consequence of industrialization and in more recent years as a consequence of the use of lead alkyls in automobile fuels, airborne lead levels have tended to increase in the atmosphere over urban centers. Actual atmospheric lead levels in urban areas are highly variable and can range upward to more than 30 μg/cubic meter near highways during peak traffic hours. A number of cities in the United States have mean concentrations of lead in the atmosphere exceeding 2.0 μg/cubic meter and of the total lead emission in the United States, the major portion is accounted for from combustion of leaded gasoline (1). The Environmental Protection Agency has undertaken to control these emissions by means of limiting the amounts of allowable lead alkyls in gasolines for consumption by automobiles. In order that reasonable criteria for air quality may be set on a sound physiological basis, knowledge must be gained of the condition of the ambient air, the influence of increased concentrations of lead in the ambient air, the accumulation of lead by human beings, and the sensitivity to disease or impairment of any essential function as influenced by airborne lead (2).

The clinical consequences of intoxication by acute or chronic intake of large quantities of lead are well recognized, but there is less understanding of the biological events resulting from continuous exposure to particulate lead in atmospheric concentrations which are below those resulting in frank lead poisoning, but which are known to occur in urban centers. The most notable studies in this general field are those by Keene (3) who exposed small numbers of human volunteers both orally and by inhalation. The inhalation studies utilized intermittent rather than continuous exposure. It is not possible, therefore, to conclude with absolute certainty that his experimental exposures are an adequate model for studies of continuous exposure of the general population to lead in the ambient air. Our intent was to re-examine the effects of exposure to low levels of airborne lead particulates but to do so under nearly continuous exposure conditions.

Before initiating studies with human volunteers, we first conducted a thorough study of the effects of airborne particulate lead on rats and rhesus monkeys. The intent was not only to expose animals continuously to airborne lead, at least as nearly as could be managed in the laboratory, but also to subject the animals to tests which would serve as sensitive indicators of exposure and biological effect. The studies were meant to establish whether continuous exposure to airborne lead resulted in a continuous accumulation of lead by the animals or whether a stabilized response to lead would be established as evidenced by a stabilized level of blood-lead.
Two investigations with human volunteers were carried out at two different levels of exposure. The overall objective of these investigations was to delimit relationships between exposure under highly controlled conditions and biological responses measured by sensitive criteria. Major goals were to determine to what extent exposure would elicit an increase in body burden of lead as manifested by increased levels in blood and whether blood-lead would continue to increase throughout exposure or reach a stabilized concentration.

ANIMAL INVESTIGATIONS

The rats and rhesus monkeys were exposed to airborne lead for a full year in a chamber designed similarly to that of Kehoe (3). The animals were exposed for 22 hours each day, seven days a week, the remaining two hours each day were reserved for servicing the chamber and caring for the animals. Details of the procedures used in biochemical studies are given elsewhere (4).

The mean level of airborne lead in the chamber during the year-long experiment was 21.5 \( \mu \text{g Pb/m}^3 \). The submicron size of the particles was determined by the use of a modified Andersen Cascade Impactor (5). The results of this study are shown in Table 1. As can be seen from the table, nearly 80% of the particles had a diameter of 0.18 \( \mu \text{m} \) (mass median equivalent diameter) or less and nearly 54% were smaller than 0.18 \( \mu \text{m} \).

The excretion of lead by the animals was studied by measuring the lead contained in the urine and feces. Levels of lead excreted in the feces of the animals are shown in Figure 1 for both species. With few

<table>
<thead>
<tr>
<th>Mass median equivalent diameter in ( \mu \text{m} )</th>
<th>Animal chamber</th>
<th>Human chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, 2.6, 1.5, 0.9, 0.4, 0.18, (&lt; 0.18)</td>
<td>7.3, 6.5, 7.3, 6.9, 17.6, 20.2, 34.9</td>
<td>0.6, 1.4, 1.1, 1.7, 9.4, 32.2, 53.6</td>
</tr>
</tbody>
</table>

* 50% cut-off size of collection plate
** Data courtesy of Ethyl Corporation
exceptions, the average lead content of the feces from exposed animals was greater than the controls. In most cases, however, due to the large individual variation in lead excretion, the averages were not significantly different from one another. Concentrations of lead found in the urine of the animals are shown in Figure 2. In most instances lead content averaged higher in the urine of exposed animals. As in the case of fecal excretion, the individual variations in rate of excretion of lead during any given collection period were too great to make any other generalizations.
Changes were observed in the lead content of blood of the rats and the monkeys during exposure to airborne particulate lead. In the rats (Figure 3) there was an increase through the third month of exposure followed by a stabilization of the blood-lead level at about 28 µg/100 ml which was maintained throughout the remainder of the exposure phase of the experiment. Upon cessation of exposure to airborne particulate lead, the blood-lead level decreased in the rats to about 5 µg/100 ml with a rate of change similar to the rate of initial increase. Five months after removal of the rats from the chamber the blood-lead was only slightly higher than the level in the control rats which averaged 3.7 µg/100 ml.

A similar, though somewhat slower, increase in blood-lead was observed in the monkeys (Figure 3). After four months of exposure the
Fig. 3. Blood lead of animals exposed to airborne lead

corporations of lead in the exposed monkeys averaged 16.0 µg/100 ml but only 3.7 µg/100 ml in the controls. No significant further increase in blood-lead was observed in the exposed group after that time. When some of the monkeys were removed from the chamber after 12 months of exposure, their blood-lead levels decreased to 5.7 µg/100 ml.

Increases in the lead content of tissues other than blood also occurred in the animals as a result of exposure to airborne lead. Measurements were made in the rats after six and twelve months of exposure and also in some rats after they had recovered for six months after the exposure phase of the study (Figure 4). None of the tissues of the control rats ever exceeded 0.50 µg of lead per gram but the levels increased nearly ten-fold in the exposed rats. The largest concentrations were found in bone where they rose to slightly more than 5 µg per gram after 12 months. There was less increase in lung tissue. There were no striking differences between the levels found at six and twelve months in any of the tissues.
Fig. 4. Lead in tissues of rats exposed to airborne lead

Decreases in the content of lead in soft tissues of the rats followed their removal from the exposure chamber. After six months recovery the highest level found in soft tissue was in the liver of exposed rats. The concentration was about three times that of controls, but averaged only 0.71 µg per gram. By contrast there was apparently no reduction in the amount of lead in bone (entire femur) after cessation of exposure to airborne lead.

A similar response to airborne lead was observed in tissues of exposed monkeys (Figure 5). The levels of lead in control monkeys were similar (except for bone) to those of control rats but the degree of increase due
to exposure was less in the monkeys. In general, the concentrations increased less than ten-fold in monkey tissues. This lesser increase in monkey tissues was similar to that observed in blood-lead levels of the animals. In all the monkey tissues studied there was a trend toward higher levels of lead after twelve months exposure than after six months exposure although the differences were small.

Additional information concerning the effects of exposure to airborne particulate lead in relation to the synthesis of heme was obtained in studies of δ-aminolevulinic acid dehydratase (ALAD) activity in blood and other tissues. After the animals had been in the exposure chamber

Fig. 5. Lead in tissues of monkeys exposed to airborne lead
for several months, examinations were made of ALAD activity in the blood of the animals using the Bonsignore (6) method. It soon became apparent that normal levels of the enzyme in the rhesus monkeys were too low to permit meaningful results to be obtained and no further attempts were made to assess the activity of the enzyme in the blood of the monkeys. Higher activities were present in the blood of rats thereby yielding more useful results. Figure 6 demonstrates that after twelve months exposure of the rats to airborne lead, the level of ALAD activity in the erythrocytes of these animals was only 3.8 units compared to 12.6 units in the unexposed controls. The effect was reversible, however, and within one month from the time the animals were removed from the chamber, the blood ALAD had returned to the same level as that of the control animals.

![Graph](image)

*Fig. 6. ALAD in rat erythrocytes*

The results of ALAD estimations of liver and brain tissue obtained during the twelve month autopsy are shown in Table 2. Despite the depression of ALAD activity seen in the blood of exposed rats, there was no reduction of the enzyme activity in either liver or brain tissue from either species.

No evidence of lead toxicity was found through extensive hematological or serum chemistry studies.
Table 2
Activity of δ-aminolevulinic acid dehydratase in tissues of animals exposed to airborne particulate lead at 21.5 μg/m³ for one year

<table>
<thead>
<tr>
<th></th>
<th>Liver, units/g</th>
<th>Brain, units/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exposed</td>
</tr>
<tr>
<td>Rate* Monkeys</td>
<td>144 ± 22</td>
<td>199 ± 19</td>
</tr>
<tr>
<td></td>
<td>258</td>
<td>262</td>
</tr>
<tr>
<td></td>
<td>10.1 ± 0.7</td>
<td>9.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation
** Values are means only. There were insufficient numbers for calculation of standard deviation

INVESTIGATIONS WITH HUMAN VOLUNTEERS

The exposure of human volunteers was conducted in cooperation with the New York State Department of Corrections at the Clinton Correctional Facility, Dannemora, New York. A ward in the prison hospital was converted into an appropriate environmentally controlled exposure chamber shown diagrammatically in Figure 7. The men participating in the study were selected from a group of inmates of the correctional facility who had volunteered for the study. The men were fully informed about the nature of the study and were apprised of any possible dangers inherent in the experiment. Each man was also informed of his freedom to discontinue his participation in the experiment at any time.

Fig. 7. Cut-away view of human exposure chamber showing the air conditioning and distribution system. The indicated components of the chamber are: 1. air supply duct — 2. air return duct — 3. air sample filter and baffle — 4. air supply register — 5. air return register — 6. lead oxide injection point — 7. lead oxide generator — 8. passageway to toilet and showers — 9. air sample ports — 10. air conditioning system.
Two studies were conducted with this chamber: one at 10.9 μg Pb/m³ and one at 3.2 μg Pb/m³. Particle size data for these studies are shown in Table 1. In both cases the men were exposed for about 23 hours daily and, in both cases, the duration of the exposure phase of the studies was about 18 weeks. Details of the air sampling procedures and other analytical and biochemical techniques used in the studies have been reported elsewhere (7).

Exposure of the men to 10.9 μg Pb/m³ resulted in a small but definite increase in the rate of excretion of lead in urine as shown in Figure 8. The urinary concentration of lead was much higher in the men after three months exposure to airborne lead than it was before the exposure began and was also higher than it was in the control men. After the men left the chamber the levels of lead in their urine decreased to levels as low as the pre-exposure concentrations.

Excretion of lead by men in the second exposure, at 3.2 μg Pb/m³, is shown in Figure 9. In several cases, very slight increases in the rate of excretion were noted in the exposed men, although this was not observed in any of the controls. In all cases, the rates of excretion were well within the normal limits.

![Graph](image)

Fig. 8. Urinary excretion of lead by men exposed to 10.9 μg/m³ airborne lead
Measurement of the fecal content of lead several times during exposure of men to 10.9 μg Pb/m³ did not reveal any consistent differences between exposed and control groups. Feces were not examined in the men exposed to 3.2 μg Pb/m³.

Blood-lead levels increased in the exposed men as shown in Figure 10. After about 12 weeks of exposure there was no further increase in the mean concentration of lead in the blood of the eight members of the original group remaining in the chamber at the time. At this point the level apparently stabilized in these men and showed no further increase during the latter part of the experiment. The stabilized blood-lead level in the men was about twice the level present in their blood before the exposure began and averaged about 37 μg/100 ml of whole blood. After the men left the exposure chamber, their blood-lead levels began to decrease at about the same rate that they had increased previously and by about five months following the termination of the exposure, most of the men's blood-lead had returned to levels similar to those before exposure.

Levels of lead in the peripheral blood of the volunteers during the second study are shown in Figure 11. Blood-lead levels in all of the exposed men increased during the study with the exception of two volunteers. In both of these cases, exposure to 3.2 μg Pb/m³ did not cause an increase in the level of lead in the circulating blood. However, the level of lead in the blood of these men during the baseline period was...
Fig. 10. Blood lead of men exposed to 10.9 µg/cu m airborne lead

Fig. 11. Blood lead of men exposed to 3.2 µg/cu m airborne lead
about as high as the average blood-lead in the rest of the exposed group following exposure to the airborne lead. The average blood-lead increased during the first twelve weeks of exposure and then leveled off during the rest of the exposure period at about 27 μg/100 ml of blood. Although it was not possible to conduct complete recovery studies in all of the volunteers, it appeared that the blood levels returned to near-normal levels after about two months from the time exposure was terminated.

Assessment of the effects of exposure of the human volunteers to airborne particulate lead on the synthesis of heme was made by measurements of ALAD in erythrocytes. The results of ALAD determinations during the exposure at 10.9 μg Pb/m³ are shown in Figure 12 and indicate that the response of ALAD was generally inverse to that of blood-lead. Significant reduction of ALAD occurred in the exposed men, and this reduction was maintained throughout the exposure period. Among all the men in the study at 10.9 μg Pb/m³ who were exposed for two months or longer, the reduction of ALAD amounted to about 50% of the baseline level. As in the case of blood-lead levels, this altered level of enzyme activity apparently became stabilized at the reduced level, and continuation of exposure did not cause further reduction. Recovery of ALAD activity began almost immediately after cessation of exposure.

![Figure 12](image_url)
and, in fact, the recovery of ALAD may have preceded the recovery of blood-lead levels to pre-exposure values. This effect was difficult to assess due to the inherent variability in the ALAD assay procedure.

Studies of ALAD made periodically in the men exposed to 3.2 µg Pb/m³ are shown in Figure 13. In attempting to generalize from these results, it is difficult to conclude that exposure of the men to 3.2 µg of lead/cubic meter of air caused an overall lowering of the ALAD. In some of the men there appeared to be a slight decrease in the level of ALAD, while in other exposed men there were no reductions whatsoever. Those reductions that were observed were in the order of the normal variation that was observed in some of the control men.

![Graph showing ALAD levels](image)

Fig. 13. ALAD in erythrocytes of men exposed to 3.2 µg/m³ airborne lead

DISCUSSION

In the studies reported herein, the effects of exposure to low levels of airborne lead were evaluated using the most sensitive criteria available to compare these effects in two species of laboratory animals. A multifaceted approach to the investigations was pursued and chemical and
morphologic responses from exposure to airborne particulate lead were examined. One of the most noteworthy findings in the investigation was the apparent stabilization of blood-lead in both species following the initial period of increase. This stabilization may be interpreted as evidence for an approach to an equilibrium condition within the animals with respect to the metabolism of lead.

That such an equilibrium condition existed was also supported by the data obtained from analysis of lung, liver, kidney and bones. Both the rats and monkeys accumulated lead in all of these tissues. There was strong evidence, at least in the rat studies, that a stabilized level of lead in the tissues was achieved beyond which there was no additional accumulation due to continued exposure to airborne lead. If there was additional accumulation, it was only in the bone tissue where it probably represented relatively inert storage.

None of the usual manifestations of frank lead poisoning were observed in rats or monkeys exposed to 21.5 μg of lead/cubic meter of air. This was true throughout the year-long exposure of the animals. Although there was evidence of inhibition of one of the enzymes involved in heme synthesis, there was no evidence that heme synthesis per se was affected.

In the two investigations of human volunteers exposed almost continuously to elevated levels of airborne particulate lead, there was demonstrated a resulting increment in the concentration of blood-lead, even when the exposure level was a low as 3.2 μg Pb/m³. The concentration of blood-lead in the men increased to a maximum level in a dose related fashion. This observation of a rise to a constant or plateau level was similar to that noted in the laboratory animal studies but different from results of human investigations described by Koehne (3). Koehne studied total input and excretion of lead in volunteers ingesting known quantities of lead and he employed balance techniques not feasible in the present investigations. Koehne also studied absorption and excretion of lead in volunteers exposed intermittently to airborne lead sesquioxide particulates. He has concluded from his various studies that abnormal exposure to lead results in a continuing increase in total body burden of the metal. While it was not possible in our studies of men undergoing continuous exposure to airborne lead to examine total body burden, the blood-lead data strongly suggest that an equilibrium was established during the investigations. To conclude that total body burden was no longer increasing is tempting but an alternate explanation of the data might be that at the low levels of exposure studied, deposition of lead in such tissues as bone might have been too slow to have a measurable effect during the time limits of the investigations.

Irrespective of the quantity of lead in air or the source of excessive exposure to lead, the level of blood-lead has been related inversely to the activity of ALAD in erythrocytes by Hernberg and coworkers (8). Results obtained here tend to confirm the relationship, at least at the higher level of exposure where the ALAD activity of the exposed group was very
close to the predicted activities calculated from the measured blood-lead levels both before and after exposure. During the study conducted at the lower exposure level, however, the predicted ALAD was lower than that measured in both the exposed and control groups. This discrepancy is likely due to the inherent individual variability of measured ALAD levels.

ACKNOWLEDGMENT

This work was supported by Contract C-PA 70-85 from the Environmental Protection Agency and by grants from the International Lead Zinc Research Organization and the American Petroleum Institute.

References


Sažetak

BIOLOŠKI I KLINICKI UCINCI KONTINUIRANE EKSPOZICIJE ATMOSFERSKOM OLOVU

Povišene koncentracije olova u atmosferi u posljednje su vrijeme sve veći rad u zdravstvu i u industriji. Većina gradova sadrži olovo u zraku premašujuću 2 ug/ml šetna se da je olovo u benzincu najvažniji izvor ovog unokačenja. Biološki i klinički učinci olova u akutnom otrovanju rasanjeno su dobro proučeni, ali manjka ju podaci o mogućim stenim djelovanjima ovog metalna pri svakodnevnjoj ekspoziciji malim dozama.

Da bi se istražili mogući učinci olova na ljudima, trebalo je najprije provesti odgovarajuće istraživanja na pokusnim životinjama. Jakost i rezus majmunski ekspanoni su atmosferskom olovu (21,5 ug/ml) 22 sata dnevno kroz ciljev godinu i proučava koncentracija olova u krv, mokrim čestama i kostima te aktivnost dehidrataze delta-aminolevulinske kiseline. Upravljenje je da je ugradnja ekspozicije atmosferskom olovu izazvala znatno povećanje koncentracije olova u krv pokusnih životinji koje je doseglo vrhunac nakon nekoliko mjeseća ekspozicije i nivo joj dalje nije...
povećavao. Povećanje koncentracije olova utvrđeno je i u plućima, bubregu, te kostima i u šljaktnici u majmunica. Nakon odstranjivanja pokusnih životinja iz atmosfere s olovom, koncentracija olova opada negdje u mekim tijelima, ali je u kostima ostala povećana.

Aktivnost dehidrogenaza delta-aminolevulinске kiseline u eritrocitima šakora bila je smanjena za otprilike 70%, ali aktivnost ovog enzima u mozgu i jeliti nije se bilo mijenjala niti u šakora niti u majmunica. U neeksponiranih majmunica aktivnost ovog enzima bila je tako niska da rezultati dobiveni na eksponiranim životinjama ne oduzimaju nikakve značajke.

Nakon ovih pokusa zapoceta su istraživanja na dobromoljacima. U toku 18 tjedana ekspozicije 23 sati dnevno, ispitanici su uživali zrak u kojemu se nalazilo 3,2 ulazno 10,9 μg/m³ olova. U oba slučaja povećala se koncentracija olova u krvi koja je dosegla plata otprilike 3 mjesece od početka ekspozicije i nije bilo daljnjih povećanja koncentracije olova u krvi. Pri koncentraciji olova od 109 μg/m³, ispitanici su izlučivali veću količinu olova močnom, ali količina olova u stoliči nije bila povećana. Nakon napuštanja kontrole, izlučivanje olova močnom se normaliziralo.

Aktivnost dehidrogenaza delta-aminolevulinске kiseline bila je smanjena za otprilike 50% u ispitanika eksponiranih većoj koncentraciji olova, ali negdje nakon završetka ekspozicije aktivnost se normalizirala.

Rezultati ovih istraživanja pokazali su da nije bilo mjernih promjena u ekserciciji prekursora hemi niti je bilo ikakvih promjena u serumu ili krvnom sliči ispitanika eksponiranih manjoj ili većoj koncentraciji olova u zraku tijekom 18 tjedana.

**DISCUSSION FOLLOWING THE PAPER**

**BERLIN:** 1. In view of some of the fluctuations observed in the various biological parameters during exposure and following, it could be suggested either that the baseline levels prior to exposure?

2. In the case of exposure to 32 μg/m³ the control and exposed groups were not matched as to their blood levels indicated that they might have had different exposure prior to the experiment which would be reduced during exposure due to controlled conditions. How can then the elevation in blood levels be assessed?

**GRIFFIN:** 1. With regard to the first question, the blood-lead levels in the volunteers were monitored in each experiment over a two-week period prior to exposure.

2. To answer the second question, it must be recalled that while it is true that each individual in the study had a different lifetime history of exposure to lead, their confinement in the correctional facility with its relatively controlled environment for a period prior to the study probably tended to stabilize this exposure to lead before exposure started. In any case, we carefully examined the changes in each individual's blood-lead throughout the study as well as assessing group averages.