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Experimental Study of Biological Effects of Lead and Aluminum Following Oral Administration

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A wide spectrum of the biological effects of lead and aluminum ions is noted during short-term and long-term oral administration to laboratory animals. The general toxic and gonadotoxic effects of these metals during a short-term experiment appeared to be identical, and the correlation of these effects was preserved during chronic experiments. Lead (0.03 mg/l.) and aluminum (0.5 mg/l.) concentrations in water may be dangerous to the health of the population, and hygienic standards are recommended for inclusion in the standard for drinking water quality.

Among the chemical pollutants in drinking water which are especially toxic to humans are the highly cumulative and stable heavy metals such as lead (*1, 2*). Until recently, aluminum was considered to be a metal with low toxicity and, accordingly, widely used as a coagulant in drinking water treatment facilities. In the last few years, however, it was found that aluminum can negatively influence the functional state of the central nervous system, can upset the metabolism of phosphorus compounds, and can accumulate in the gonads (*3, 4*). Unfortunately, the water standards for both lead and aluminum were set without consideration of the wide range of their biological effects. In this connection we set the following goals for our research: (1) evaluation of the various types of biological effects of lead and aluminum and the study of the interrelation of their general toxic, gonadotoxic, and mutagenic effects; (2) determination of the range of doses from toxic to nontoxic in humans to ascertain the maximum permissible concentrations of these metals in water.

The research techniques used in these investigations were developed by Krasovskiĭ et al. (*5*). The metals were administered orally each day to laboratory animals. Lead acetate was administered in doses of 0.05, 0.005, and 0.0015 mg/kg (based on the ions of lead) to white rats; aluminum chloride in doses of 50, 17, and 6 mg/kg (based on ions of aluminum) to rats and guinea pigs, 27, 9, and 3 mg/kg to rabbits in short-term exposures, and 2.5, 0.25, and 0.0025 mg/kg to rats in chronic exposures. These experiments were of 20-30 days and 6-12 months' duration.

Results and Discussion

The toxic effects of lead were observed when administered to rats in doses of 0.05 mg/kg. The activity of aldolase increased and the level of sulfhydryl groups decreased 5-10 days after intoxication ($p < 0.01$). The effect of the 0.005 mg/kg dose was expressed as a trend in the increase of aldolase in the blood serum. By the 20th day of intoxication the activity of aldolase normalized, and the activity of β-galactosidase, β-glucosidase, and acid phosphatase and cholesterol content of the blood serum did not change.

In addition, substantial increases in the weight coefficients of liver and kidneys were observed with the 0.05 mg/kg doses of lead. A histological examination of the rat livers indicated a definite decrease in RNA and glycogen and in the activity of SDH, LDH, and NAD-diaphorase at all doses, and pyknosis of Kupffer cells at doses of 0.05 mg/kg. Fur-

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Table 1. Effect of lead on functional state of spermatozoa.

Test	Control	0.5 mg/kg	0.005 mg/kg	0.0015 mg/kg
Motility of spermatozoa expressed in grades	3.83 ± 0.17	3.17 ± 0.17[a]	3.5 ± 0.22	3.83 ± 0.17
Acid stability of spermatozoa (pH)	3.04 ± 0.097	3.3 ± 0.028[b]	3.18 ± 0.037	2.83 ± 0.048
Osmotic stability of spermatozoa, % NaCl	2.3 ± 0.045	1.93 ± 0.042[c]	1.97 ± 0.033	2.27 ± 0.042
Time of motility of spermatozoa, hr	38.0 ± 0.52	33.5 ± 0.56[c]	37.33 ± 0.33	38.5 ± 0.34

[a] Significant at $p < 0.02$.
[b] Significant at $p < 0.05$.
[c] Significant at $p < 0.01$.

thermore, small droplets of lipid inclusions were observed in the cytoplasm of epithelial cells located in the convoluted tubules of the cortical substance of liver.

The gonadotoxic effect of lead was observed in animals which received the maximum dose. The functional condition of spermatozoa changed (Table 1) and the acid phosphatase activity in the gonadal tissue increased being 25 ± 1.0 for controls, 34.4 ± 2.3 at 0.05 mg/kg, and 27.8 ± 1.4 at 0.005 mg/kg; $p < 0.05$. The weight coefficients of gonads were increased only in animals which were exposed to the dose of 0.005 mg/kg. Small disruptions in the permeability of vessels and dystrophic changes in the Leydig cells were observed in the gonads, and the activity of oxidizing enzymes increased.

In animals which were exposed to 0.0015 mg/kg of lead, no deviations in functional state was observed compared to the control group of animals.

Lead Acetate, Long-Term Exposure

In the 6-12 month exposures, special attention was paid to the study of the excretion of δ-aminolevulinic acid and coporphobilinogen in urine (6). The investigations showed that, beginning with the second month of intoxication, the excretion of δ-aminolevulinic acid and of perphobilinogen gradually increased in animals which received lead in doses of 0.05 and 0.005 mg/kg (Fig. 1).

In order to evaluate the effect of lead on the behavioral responses of animals, their motor activity was recorded by a method which tested conditioned reflexes. Animals exposed to lead at 0.05 mg/kg and 0.005 mg/kg had disruptions in their conditioned responses (Table 2), and the wavelike motor activity underwent a shift of phase, depending on the season of the year.

The investigation of functional and morphological conditions of spermatozoa and gonads indicated the gonadotoxic effect of lead in doses of 0.05 mg/kg

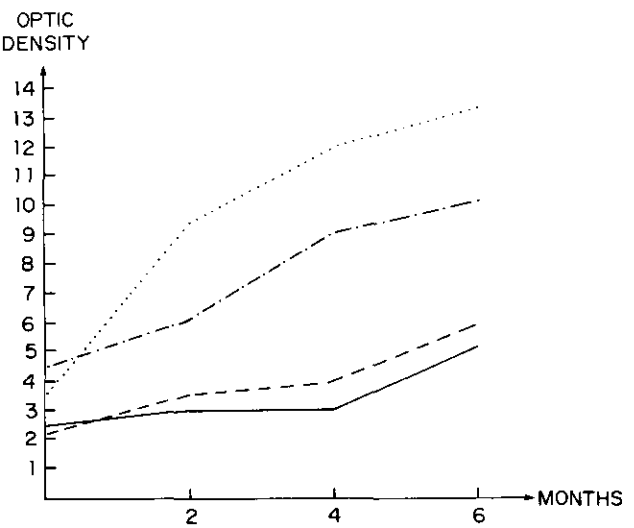

FIGURE 1. Effect of lead acetate on excretion of δ-aminolevulinic acid in urine in chronic intoxication in white rats: (——) controls; (– –) 0.0015 mg/kg; (– · –) 0.005 mg/kg; (· · ·) 0.05 mg/kg.

(Table 3). The histological study of gonads indicated a swelling of the follicular epithelial cells; the vascular network was full of blood. The RNA content and sulfhydryl group content of some tubules increased, but decreased in the remaining part of the parenchyma. The activity of AIDH, SGH, NAD, and NADPH-diaphorase was substantially depressed in the spermatogenic epithelium and less depressed in the interstices. The indices of activity of acid phosphatase and of β-glycosidase in gonadal tissues exposed to lead in doses of 0.05 mg/kg, 0.005 mg/kg, and 0.0015 mg/kg were, respectively, 58.5 ± 3.0, 49.3 ± 1.5, and 36.2 ± 2.0 (controls 37.4 ± 1.5) and 38.9 ± 1.9, 64.0 ± 5.4, and 9.04 ± 4.8 (controls 13.0 ± 4.5).

The histochemical study of the liver at doses of lead of 0.05 mg/kg and 0.005 mg/kg revealed a de-

Table 2. Effect of lead on conditioned reflex activity in case of chronic exposure.

Test	Control	0.05 mg/kg	0.005 mg/kg	0.0015 mg/kg
Manifestation of reflex (number of combinations)	6.33 ± 0.94	17.66 ± 0.75[a]	10.16 ± 1.19[b]	6.83 ± 1.16
Consolidation of reflex (number of combinations)	3.66 ± 2.21	35.16 ± 1.53[a]	31.83 ± 1.85[b]	24.16 ± 2.89
Latent period	1.67 ± 0.23	2.66 ± 0.04[b]	2.03 ± 0.15	1.69 ± 0.19
Conditioned reflex	36.99 ± 6.31	2.66 ± 0.04[b]	21.65 ± 4.53	39.16 ± 5.51
Unconditioned reflex	24.47 ± 6.17	5.52 ± 3.25[b]	46.34 ± 4.17[c]	25.47 ± 5.26
Intersignal time	38.65 ± 3.28	73.36 ± 6.9[b]	44.5 ± 6.20	35.41 ± 5.0
Decrease of conditioned reflex	23.66 ± 2.66	41.5 ± 1.83[a]	20.5 ± 1.54	26.16 ± 2.8
Restoration of conditioned reflex	3.83 ± 0.86	10.66 ± 0.55[a]	7.0 ± 0.72[c]	3.33 ± 0.41

[a] Significant at $p < 0.01$.
[b] Significant at $p < 0.05$.
[c] Significant at $p < 0.02$.

Table 3. State of structural-functional elements of gonads in case of chronic lead intoxication.

Tests	Control	0.05 mg/kg	0.005 mg/kg	0.0015 mg/kg
Spermatogenesis index	3.75 ± 0.008	3.61 ± 0.052[a]	3.72 ± 0.0013	3.74 ± 0.015
Number of tubules with cast-off epithelium	3.88 ± 0.9	5.5 ± 2.9	5.88 ± 1.8	4.0 ± 1.48
Average number of spermatogonia	25.51 ± 0.81	21.64 ± 0.6[b]	20.84 ± 1.35[b]	25.0 ± 0.9
Number of tubules with 12th meiosis stage	2.16 ± 0.26	2.5 ± 0.5	2.33 ± 0.2	2.33 ± 0.5

[a] Significant at $p < 0.05$.
[b] Significant at $p < 0.01$.

crease in glycogen content, RNA, sulfhydryl groups and activity of oxidizing enzymes in the central regions of lobules and an increase of NAD and NADPH-diaphorase at the periphery of the lobules. In the kidneys the highest tested dose of 0.05 mg/kg only insignificantly increased chromosome aberrations. The 0.0015 mg/kg dose of lead (which corresponds to 0.03 mg lead/l. water) did not cause any changes. It can be considered that this does not have an effect on the organism.

Aluminum Chloride, Short-Term Exposure

In the investigation of aluminum, a toxic biological effect was indicated by the substantial decrease in the activity of the alkaline phosphatase in blood serum and by the change in adenine nucleotides in blood. This was the effect of the largest dose (Table 4). The minimum effective dose of aluminum that affected these indices is set at the level of 17 mg/kg for rats and guinea pigs; for rabbits it is 9 mg/kg (based only on changes in the activity of alkaline phosphatase in blood serum).

In these investigations no changes were recorded in the activity of aldolase in blood or in blood content of erythrocytes and of sulfhydryl groups. All the tests of animals were taken 3 hr after administration of the compound. These results show that three types of laboratory animals have a similar sensitivity to the general toxic effect of aluminum. From the parameters of acute toxicity, rats, guinea pigs, and rabbits have the same sensitivity to the effect of aluminum, because their LD_{50} levels are about the same (380, 400, and 400 mg/kg, respectively). At the end of the short-term experiment, the spermatozoa of all three types of animals clearly showed moderate dystrophy of protein in the epithelium of the proximal tubules as well as an increase in desquamation of epithelium, pyknosis, and rexis of some cells in the endothelium of the glomeruli, and hyperplasia of the cellular nuclei in the epithelium of the medullar rays.

Anatelophase analysis of the cells of the bone marrow of white rats revealed a small increase in the percentage of chromosomal aberrations among animals receiving the largest dose compared to the controls: controls, 2.6 ± 0.14; 0.05 mg/kg, 3.6 ± 0.22; 0.005 mg/kg, 3.0 ± 0.37; 0.0015 mg/kg, 2.5 ± 0.14; $p < 0.05$.

The results of the assay of the dominant lethals during the 12th month of intoxication indicated that lead has a negative influence on the reproductive function of animals (Table 5). The exposure of animals to lead at 0.005 mg/kg during the full period of pregnancy influenced the development of embryos (Table 6).

Table 4. Effect of aluminum chloride on blood content of ATP, ADP, AMP of experimental animals in case of short-term intoxications.

Animals	Aluminum dose, mg/kg of weight	ATP	ADP	AMP
Guinea pig	Control	9 ± 0.9	1.096 ± 0.09	0.671 ± 0.01
	50	4.5 ± 1.0[a]	2.88 ± 0.12[a]	0.901 ± 0.03[a]
	17	5.04 ± 0.6[a]	2.0 ± 0.09[a]	0.86 ± 0.09[a]
	6	8.8 ± 0.4	1.056 ± 0.11	0.655 ± 0.04
Rat	Control	8.65 ± 0.8	2.33 ± 0.07	0.33 ± 0.04
	50	3.25 ± 1.1[a]	7.7 ± 0.97[a]	0.5 ± 0.01[a]
	17	4.5 ± 0.1[a]	1.5 ± 0.06[a]	0.44 ± 0.08
	6	6.3 ± 0.65	2.74 ± 0.03	0.37 ± 0.04
Rabbit	Control	7.65 ± 0.8	0.525 ± 0.03	0.14 ± 0.02
	27	4.95 ± 0.3[a]	0.735 ± 0.04[a]	0.35 ± 0.05[a]
	9	6.4 ± 0.75	0.535 ± 0.04	0.45 ± 0.03
	3	6.75 ± 0.6	0.33 ± 0.1	0.14 ± 0.02

[a] Significant at $p < 0.05$.

Table 5. Assay of dominant lethals in chronic exposure to lead of white rats.

Test	Control	1.0 mg/l.	0.1 mg/l.	0.03 mg/l.
General embryonic mortality, %	12.41	51.08	32.14	13.43
Preimplantation mortality	0.12	0.35	0.30	0.13
Postimplantation mortality	0	0.24	0.03	0.0
Total number of progeny	120	68	95	116
Mean litter size ± S.E.	12.0± 0.45	6.8± 1.16	9.5± 1.75	11.6± 0.46
p (statistical significance)	—	<0.01	<0.01	—
External abnormalities in development	None	None	None	None

Table 6. Indices of embryotoxic effect of lead.

Test	Control	0.005 mg/kg	0.0015 mg/kg
Number of yellow bodies	11.76 ± 0.04	11.0 ± 0.67	10.9 ± 1.07
Number of live embryos	9.76 ± 0.6	8.75 ± 0.54	9.7 ± 1.0
Number of resorptions	0.58 ± 0.19	0.69 ± 0.4	0.62 ± 0.34
General mortality of embryos	16.8 ± 1.76	20.6 ± 0.4[a]	14.18 ± 5.7
Preimplantation mortality	0.15 ± 0.04	0.13 ± 0.09	0.04 ± 0.02
Postimplantation mortality	0.04 ± 0.017	0.08 ± 0.03	0.06 ± 0.039
Average weight of embryo, g	2.35 ± 0.21	2.18 ± 0.15	2.29 ± 0.06
Amount of lead in placenta, mg/g	5.33 ± 0.85	9.47 ± 2.4	5.4 ± 1.42
Amount of lead in embryo, mg/g	2.74 ± 0.62	5.6 ± 1.1[a]	2.7 ± 0.6

[a] Significant at $p < 0.05$.

The progeny of these animals, when raised to full sexual maturity without additional exposure to lead, suffered a diminished development of conditioned reflexes with a tendency toward increase of the latent stage. It is interesting to note that the progeny had increased coefficients of weight of the same organs (liver, kidneys, gonads) like the animals subjected to chronic intoxication by lead. When compared to those of the controls, the gonads did not show changes.

The smallest dose of lead which resulted in general toxic, gonadotoxic, and embryotoxic effect was 0.005 mg/kg. Lead can be considered a weak mutagen because a gonadotoxic effect is evident only under the influence of the largest tested doses (27-50 mg/kg). However, one of the investigated indices, the motility time of spermatozoa, decreased somewhat in comparison with controls in guinea pigs and white rats exposed to an aluminum dose of 17 mg/kg. The general toxic and gonadotoxic effects of aluminum became evident at the same doses.

Aluminum-Potassium, Long-Term Exposure

A change in the activity of phosphatase in the blood serum of rats receiving an aluminum dose of

2.5 mg/kg was observed in the six-month, chronic exposure experiment. Other effects of the same dose were a slow-down in development and reinforcement of conditioned reflexes and a recorded high value of index of the latent stage of the conditioned reflexes. The study of motor activity of rats, recorded on an Animex instrument, showed that changes occurred only in small movements, i.e., those which are not related to complex behavioral responses of animals (controls, 33.9 ± 3.8; experimental animals, 20.7 ± 2.0; $p < 0.05$). The animals exposed to aluminum at 0.25 mg/kg showed a change in the alkaline phosphatase activity in the blood serum only during the first month of intoxication, but later it normalized to the level of the controls. At the end of the chronic exposure experiment, no changes were observed in the β-lipoproteins, erythrocytes, or the transaminase activity in blood.

The gonadotoxic effect of aluminum was weak. Changes in the number of spermatozoa and in their motility were observed only in animals which received the highest dose of aluminum (control, 208 ± 10; experimental group, 154 ± 17; $p < 0.05$). Histochemical analysis of the testes revealed that a thickened basal membrane with a layer of Sertoli cells remained in empty tubules; there were some pathological forms of spermatogenic epithelium in the tubular lumen. There was a substantial proliferation of interstitial cells (Leydig cells). It is typical for the parenchyma of the gland to have a substantial reduction of RNA content and to have a suppression of activity of oxidizing and hydrolytic enzymes. However, in some tubules there was an increase of RNA and an increased activity of the oxidizing enzymes. Histochemical changes of the opposite type were observed on the side which is close to interstitial tissue: the oxidizing activity of the oxidizing enzymes in Leydig cells was increased as was the level of ATPase in basal membranes of seminiferous tubules. The biochemical method used for assaying the content of nucleic acids in tissues of gonads (*7, 8*) did not indicate that there were significant changes of DNA in the test group compared with the control animals. At the same time, a tendency for decreased RNA was observed for a dose of 2.5 mg/kg. When smaller doses were administered, no changes were recorded in oxidizing phosphorylation in mitochondria and in the content of adenine nucleotides in the fibers of the testes of rats. The percentage of chromosomal aberrations in cells of bone marrow of all animals remained generally on the same level (controls, 4.01 ± 0.051; 0.025 mg/kg, 4.08 ± 0.086; 0.25 mg/kg, 4.86 ± 0.085; 2.5 mg/kg, 5.31 ± 0.082). The results of chronic exposure indicate that aluminum in relatively large doses (2.5 mg/kg) has general toxic and gonadotoxic effects. An aluminum dose of 0.25 mg/kg can be considered nearest to the threshold levels; the dose of 0.025 mg/kg (which corresponds to the concentration in water of 0.5 mg/l. of aluminum) does not affect the organism.

Conclusions

Lead and aluminum cause a wide spectrum of biological effects. The picture of the general toxic and gonadotoxic effect of these metals in the short-term experiment was the same as was obtained in chronic exposure experiments, and the interrelations of these effects remained the same. The observed consistent regularity could be useful for predicting the harmful effect of these metals on the human organism.

In order to assay the gonadotoxic effect in an experiment with animals, an array of physiological, biochemical, and morphological methods for assessing gonadal functions should be used in conjunction with the method of dominant lethals.

A concentration in water of 0.03 mg/l. of lead and 0.5 mg/l. of aluminum could be considered safe for the health of the general public, and these could be recommended for inclusion into the public health standards for drinking water.

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