Biochemical Studies in Embryos After Exposure of Pregnant Mice to Dietary Lead

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INTRODUCTION

Lead exposure of women can cause abortion and delay prenatal and postnatal growth (Weller, 1915). Earlier (Jacquet et al., 1975, 1976) we have observed that .125% to .5% of lead in the diet given after mating reduces the incidence of pregnancies, retards early divisions, diminishes embryonic weight and can cause death during the late stages of gestation. In order to elucidate the mechanism of embryonic retardation and death, we have now studied in embryos at the 16, 17 and 18th day of pregnancy several biochemical parameters thought to be related to the action of lead.

METHODS

Female mice of the C57Bl strain were mated as described earlier (Jacquet et al., 1975) and, when displaying a vaginal plug, were placed on a diet containing 0, .125, .25 or .5% of lead. The females were dissected at the 16, 17 or 18th day of gestation, the embryos were freed from their membranes, weighed and homogenized in 4 ml (embryos under 500 mg 2 ml) of ice cold water. Proteins were assayed in 50 µl of homogenate by the Biuret test (Colowick and Kaplan, 1957). Delta amino levulinic acid dehydratase was determined by the European standard procedure (Berlin, 1974) using 50 µl of homogenate. Porphyrins were estimated by a modification of the pro-

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cedure described by Schwartz (1966) (Sassa et al., 1973). Porphyrins are extracted into ethyl acetate acetic acid and re-extracted into HCl. Fluorescence was measured at 400 nm excitation and 600 nm emission using a Turner Modell 430 Spectrofluorometer.

The macromolecules were precipitated with PCA ethanol and washed 3 times with .2N perchloric acid. DNA was then extracted with hot trichloracetic acid and determined via the diphenyl amine reaction (Burton, 1968). Acetone was added to another .5 ml aliquot from the homogenate and hem was then extracted from the precipitate with acid acetone.

The extinction of the supernatant was determined at 412 nm using hemoglobine added to homogenate as a standard.

RESULTS AND DISCUSSION

Tables 1-3 present biochemical data on embryos from the 16, 17 and 18th day of pregnancy respectively. Normal development of the embryo is rapid at this time : the weight almost triplicated during these two days and the amounts of DNA, protein, hem (Fig. 1) and porphyrins parallel the weight increase, Aladehydratase increases, however, more gradually.

As described earlier (Jacquet et al., 1975) lead application causes a retardation in growth which becomes more prominent as pregnancy approaches its term. Increase in DNA - representing cell division - is reduced to the same extent as that of the weight, so that concentration of DNA in the embryo is not altered. The amount of proteins in the embryo, also is reduced under lead treatment, but it appears that concentration of proteins in the embryos increases slightly. No significant changes are seen with respect to hem content or hem synthesis related to body weight (Fig. 1), whereas content and concentration of porphyrins increase significantly in the lead-treated embryos. On the other hand, ALAdehydratase activity is reduced significantly to one third to one half of the controls under all regimens of lead treatment.

Several metabolic pathways in particular those leading to hem, and hem proteins, are affected by lead (Chisholm, 1971; Vallee and Ulmer, 1972; Goyer, 1971). ALAdehydratase appears to be the most sensitive indicator (Lauwerijs, 1972) for the action of lead and, in erythrocytes, is depressed already after small doses of lead. The synthesis of deltaaminolevulinic acid from glycine and the final assembly of hemoglobin are also susceptible to the toxic action of lead, and the simultaneous increase in porphyrins and decrease in hem level found in lead-intoxicated man suggest that inhibition of ALAdehydratase is only one aspect of lead action. Moreover, lead may have other

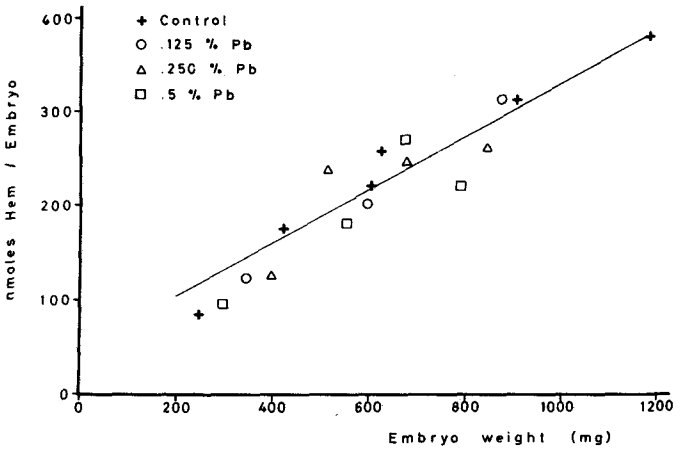

Fig 1. Plot of hem content on embryo weight for control and lead-treated embryos of different ages.

biochemical mechanisms of calcium. It can replace calcium, react with phosphates and histidyl residues, affect sulphydryl groups to mention only a few (Vallee and Ulmer, 1972).

Our data confirm the sensitivity of ALAdehydratase to intoxication by lead. The reduction found in the embryo is, however, much smaller than that seen in the blood of adult animals and this finding agrees with data on ALAdehydratase activity in different organs of the lead-intoxicated adult mouse. Thus, ALAdehydratase in liver, kidney or brain of several intoxicated mice was in the average reduced to only one third to one half of the controls. The little increase in porphyrins and the unaltered level of hem suggest indeed that total synthesis of hem is not greatly altered in these embryos. A specific effect of lead on DNA synthesis aside from the general retardation in growth appears unlikely from our data. This finding apparently contrasts with the impaired DNA synthesis seen in the brain of lead-intoxicated new born animals (Gerber, unpublished; Michaelson and Sauerhoff, 1974),the latter effect may also reflect a general retardation in development of the brain rather than a specific action on cell division. Further experiments dealing with synthesis of hem, the supply of substrates to the embryo, must decide whether the retardation in embryonic growth is due to a specific effect on the embryo or to the intoxication of the mother.

TABLE 1

Biochemical Parameters in Embryos from Control and Lead treated C57B1 Mice on the 16th day of gestation.

Parameter	Control [9]*	.125% Pb [12]	.25% Pb [12]	.5% Pb [6]
Weight (mg)**	389 ± 11 [375 ± 5]	375 ± 14 [366 ± 20]	406 ± 11 [385 ± 12]	312 ± 36 [320 ± 25]
DNA µg/Embryo	440 ± 38	503 ± 63	324 ± 24.5	330 ± 26
µg/g	1170 ± 151	1374 ± 132	969 ± 60	1094 ± 113
Proteins mg/Embryo	63.5 ± 5.6	65.8 ± 5.5	57.4 ± 3.6	51.7 ± 4.5
mg/g	163 ± 14	179 ± 15	149 ± 9	165 ± 14
Hem nmoles/Embryo	144 ± 5	120 ± 11	111 ± 9	88 ± 11
nmoles/g	370 ± 13	328 ± 30	331 ± 26	275± 34
Porphyrins ng/Embryo	59.5 ± 6.2	85.7 ± 3.9	78.2 ± 6.1	120 ± 27
ng/g	153 ± 15	228 ± 10	203 ± 15	375 ± 84
ALAdehydratase mU/Embryo	1.88 ± .09	1.44 ± .17	1.43 ± .06	1.35 ± .06
mU/g	5.02 ± .44	3.93 ± .21	3.71 ± .25	4.22 ± .20

*Values in parenthesis : number of embryos assayed/values single underlined difference significant at $p < .05$ level double underlined at $p < .01$ level.

**Means from embryos assayed. Values in parenthesis means from all embryos.

TABLE 2

Biochemical Parameters in Embryos from Control and Lead treated C57B1 Mice on the 17th day of gestation.

Parameter	Control [9]*	.125% Pb [10]	.25% Pb [12]	.5% Pb [6]
Weight (mg)**	617 ± 16 [645 ± 21]	605 ± 15 [597 ± 24]	587 ± 23 [607 ± 32]	331 ± 15 [366 ± 23]
DNA µg/Embryo	657 ± 45	566 ± 44	627 ± 55	470 ± 19
µg/g	1.018 ± 70	948 ± 77	1030 ± 90	1280 ± 130
Proteins mg/Embryo	101 ± 11	95 ± 10	87 ± 12	61 ± 8
mg/g	156 ± 12	159 ± 17	143 ± 19	183 ± 21
Hem nmoles/Embryo	256 ± 20	200 ± 17	219 ± 15	124 ± 20
nmoles/g	398 ± 15	335 ± 28	360 ± 25	338 ± 54
Porphyrins ng/Embryo	117 ± 11	194 ± 34	284 ± 26	340 ± 51
ng/g	194 ± 18	325 ± 57	407 ± 43	960 ± 114
ALAdehydratase mU/Embryo	3.31 ± .30	1.55 ± .32	.943 ± .05	.918 ± .057
mU/g	5.36 ± .49	2.60 ± .54	1.55 ± .09	2.76 ± .18

* Values in parenthesis : number of embryos assayed/values single underlined difference significant at $p < .05$ level double underlined at $p < .01$ level.

** Means from embryos assayed. Values in parenthesis means from all embryos.

TABLE 3

Biochemical Parameters in Embryos from Control and Lead treated C57Bl Mice on the 18th day of gestation.

Parameter	Control [28]*	.125% Pb [28]	.25% Pb [12]	.5% Pb [12]
Weight (mg)**	978 ± 21 [977 ± 19]	922 ± 15 [910 ± 8]	850 ± 17 [866 ± 15]	793 ± 30 [810 ± 6]
DNA µg/Embryo	985 ± 84	971 ± 112	1014 ± 100	762 ± 87
µg/g	1007 ± 90	1067 ± 120	1181 ± 120	940 ± 110
Proteins mg/Embryo	144 ± 6	153 ± 4	151 ± 7	138 ± 8
mg/g	147 ± 6	165 ± 4	177 ± 8	174 ± 10
Hem nmoles/Embryo	245 ± 8	319 ± 15	258 ± 10	219 ± 20
nmoles/g	250 ± 8	345 ± 16	303 ± 12	276 ± 26
Porphyrins ng/Embryo	155 ± 5	150 ± 5	187 ± 15	233 ± 32
ng/g	159 ± 5	163 ± 5	220 ± 17	294 ± 40
ALAdehydratase mU/Embryo	3.03 ± .18	1.61 ± .28	2.15 ± .21	1.61 ± .21
mU/g	3.09 ± .19	1.74 ± .19	2.52 ± .25	2.06 ± .26

* Values in parenthesis : number of embryos assayed/values single underlined difference significant at $p \leq .05$ level double underlined at $p \leq .01$ level.

**Means from embryos assayed. Values in parenthesis means from all embryos.

REFERENCES

BERLIN, A. : Z. Klin. Chem. Klin. Biochem. 12, 389 (1974)

BURTON, K. : Colowick Kaplan ed. Methods in Enzymology, Vol.XII p.163, Acad. Press New York (1968).

CHISHOLM, J.J.: Sci. Amer. 224, 15 (1971)

GOYER, R.A. : Am. J. Pathol. 64, 167 (1971)

JACQUET, P., A. LEONARD, G.B. GERBER : Experientia 31, 1312 (1975)

JACQUET, P., A. LEONARD, G.B. GERBER : Toxicology 6, 129 (1976)

LAUWERIJS, R. : Louvain, Med. 91, 19 (1972)

LAYNE, E. : Colowick Kaplan ed. Methods in Enzymology, Vol.III p.450, Acad. Press New York (1957).

MICHAELSON, I.A., M.W. SAUERHOFF : Env. Health Perspect. 7, 201 (1974).

SASSA, S., J.L. GRANICK, S. GRANICK, A. KAPPAS, R.D. LEVERE: Biochem. Med. 8, 135 (1973)

SCHWARTZ, S., M.H. BERG, I. BOSSENMAIER, H. DIMSMORE : Glick ed. Methods Biochem. Anal. Vol. VIII 221 (1966)

VALLEE, B.L., D.D. ULMER : Ann. Rev. Biochem. 41, 91 (1972)

WELLER, E.V. : J. Med. Res. 33, 271 (1915)