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Effect of Prenatal Administration of Aluminum and Parathyroid Hormone on Fetal Development in the Rat[1] (40493)

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Aluminum (Al) is a light metal which, although a normal constituent of mammalian tissues, has no known essential metabolic function. In addition to its environmental ubiquity and presence in the food supply, Al is a major component of many preparations copiously consumed as antacids or phosphate binders to treat hyperphosphatemia and secondary hyperparathyroidism and to prevent metastatic calcifications (1, 2). A positive Al balance has been observed in normal subjects and uremic patients ingesting Al (3, 4). An elevated concentration of Al has been detected in certain areas of the brain in Alzheimer's disease and dialysis dementia suggesting an etiological role for Al in these encephalopathies (5, 6).

The neurotoxicity of Al, a trivalent cation, has been demonstrated in several animal species following systemic administration or application directly into the central nervous system (7–9). Neurofibrillary degeneration, similar although not identical to that seen in Alzheimer's disease, is the main pathqlogic lesion observed in brains of cats and rabbits treated with Al (7, 8). Behavioral and motor disturbances in cats following Al administration are positively correlated with the density of neurofibrillary degeneration and brain Al concentration (5, 8). Increased protein synthesis and decreased ribosomal RNA content are caused by Al which binds to nuclear chromatin (10, 11). Microtubular polymerization, a process involved in numerous cellular functions including mitosis, may also be decreased by Al (12).

Since there are critical periods of protein synthesis and cellular division during embryonic development, effects resulting from Al may be most pronounced following administration during organogenesis. The Al concentration in brain, bone and kidney is increased in Al fed rats by parathyroid hormone (PTH) (13) and serum PTH is elevated in women during pregnancy (14). Theoretically, elevated PTH may raise Al concentrations in maternal tissues and increase embryonic exposure to Al, especially if afflicted with hyperparathyroidism and consuming Al containing preparations. Therefore, it was of interest to determine: (a) The teratogenic potential of Al in rats following dietary exposure and (b) the effect of PTH on fetal Al concentration and severity of Al-induced fetal anomalies.

Materials and methods. Timed-pregnant Sprague-Dawley rats were obtained on day 2 of gestation (Spartan Farms, Haslett, MI). Animals were maintained in clear polypropylene cages at 22°C with a 12 hr light cycle (0700–1900 hr) and were allowed free access to food (Wayne Lab Blox) and water (distilled). On day 6 of gestation, rats were randomly assigned to the control group (ground Lab Blox) or one of the treatment groups. Treated groups received PTH (TCA-Inolex) and ground Lab Blox (containing 119 ppm Al, 12) supplemented with Al (aluminum chloride) and/or Ca (calcium chloride). Treatments were 500 or 1000 ppm Al and PTH (68 units/kg, s.c.) or vehicle (84.1% acid water, 15% gelatin, 0.1% phenol and 0.8% glycerine) on gestational days 6, 9, 12, 15 and 18 or 1000 ppm Al and 1.2% Ca (average Lab Blox) or 2.0% Ca (Ca supplemented Lab Blox).

On the 19th day of gestation, fetuses were removed by Caesarean section and the number and position of live, dead and resorbed fetuses was recorded. Fetuses were dried on absorbent paper, weighed, measured for

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crown-rump length with a vernier caliper, sexed and examined for external anomalies. Each litter was then divided into three subgroups for further examination and one subgroup for determination of whole carcass aluminum concentration. One sub-group was fixed in Bouin's solution for 2 weeks, after which the fetuses were sectioned by hand into 2–3 mm sections and examined for soft-tissue anomalies by the method of Wilson (15). A second sub-group was fixed in 95% ethanol, skinned and eviscerated. The fetal cartilage and ossified skeletons were subsequently stained with alcian blue 8GS and alizarin red S, respectively, by the method of Inouye (16). Stained skeletons were examined to assess skeletal anomalies. Brain, kidney and femur were removed from fetuses in a third subgroup, cut into small pieces and fixed in Karnovsky's fixative (500 milliosmoles) for 48 hr (17). Tissues were then washed in Zetterquists wash, pH 7.3, and postfixed in Zetterquists 1% osmium for 2 hr (18). Samples were rewashed in Zetterquists was, dehydrated and embedded in araldite. One micron sections were prepared for use in orientation of the tissue after which thin sections were cut, stained with lead citrate and uranyl acetate and examined under a Zeiss EM 952 electron microscope.

Tissue preparation for determination of whole fetal carcass aluminum concentration was similar to the method described by LeGendre and Alfrey (19). Whole pups were dried to a constant weight and 100 mg samples were ashed in a muffle furnace at 400° for 40 hr. The residue was then extracted in a saturated disodium ethylenedinitrilotetraacetic acid solution. Aluminum analyses were performed on a Varian Model 375 atomic absorption spectrophotometer equipped with a CRA90 graphite furnace assembly and a ASD-53 automatic sampler (Varian-Techtron, Chicago, IL). Five microliter aliquots of the EDTA extract were placed in the graphite rod which underwent a 60 sec dry cycle at 100°, a 40 sec ash cycle at 1000° and a 3 sec atomize cycle at 2550°. Our detection limit (defined as twice the background level) was 20 μg/L and the reproducibility of replicate samples was within 5–8%.

Data were analyzed statistically by analysis of variance, completely random design. Treatment differences were detected by the least significant difference (LSD) test (20).

Results. Concomitant treatment with PTH and 1000 ppm Al produced an increase in resorption rate but did not affect the number of live fetuses, fetal body weight or length (Table I). Other treatments with Al and/or PTH or Ca did not alter the embryo (or fetal) mortality rate, litter size, fetal body weight or length. Even control fetuses contained Al, however, the concentration of Al in whole fetal carcass was not modified by the dietary level of Al or by PTH or Ca (Table I). Although gross, soft-tissue or skeletal anomalies were detected in each treatment group,

TABLE I. RESORPTION RATE, FETAL SIZE AND WHOLE CARCASS ALUMINUM CONCENTRATION IN OFFSPRING OF PREGNANT RATS TREATED WITH ALUMINUM CHLORIDE (Al) AND PARATHYROID HORMONE (PTH).[a]

Treatment	No. of litters examined	No. of live fetuses	Percent resorbed or dead fetuses	Fetal body wt (g)	Fetal crown-rump length (cm)	Whole carcass aluminum (mg/kg body wt)
Control	8	14 ± 1	3.1 ± 1.9	3.96 ± 0.08	3.8 ± 0.1	0.68 ± 0.18
500 ppm Al	6	13 ± 1	6.2 ± 2.2	4.01 ± 0.06	3.8 ± 0.1	0.74 ± 0.03
1000 ppm Al	8	13 ± 1	6.6 ± 1.6	4.11 ± 0.08	3.9 ± 0.1	1.37 ± 0.57
Control + PTH	5	13 ± 1	4.8 ± 1.8	3.90 ± 0.13	3.8 ± 0.1	0.66 ± 0.16
500 ppm Al + PTH	5	13 ± 1	4.4 ± 3.0	3.91 ± 0.11	3.7 ± 0.1	1.25 ± 0.65
1000 ppm Al + PTH	7	12 ± 1	12.2 ± 2.5[b]	4.18 ± 0.05	3.9 ± 0.1	0.61 ± 0.13
% Ca	5	13 ± 1	4.0 ± 1.6	4.09 ± 0.10	3.8 ± 0.1	0.87 ± 0.24
% Ca + 1000 ppm Al	6	14 ± 1	5.3 ± 1.9	4.09 ± 0.13	3.9 ± 0.1	1.02 ± 0.06

[a] Rats were maintained on diets from day 6 of gestation until sacrifice at day 19 of gestation. Animals received subcutaneous injections of PTH (68 units/kg) on gestational days 6, 9, 12, 15 and 18. Values are the mean response/litter ± SEM.

[b] Significantly different from control value, $p < 0.05$.

TABLE II. PERCENT INCIDENCE OF ANOMALIES AMONG OFFSPRING OF PREGNANT RATS TREATED WITH ALUMINUM CHLORIDE (Al) AND PARATHYROID HORMONE (PTH).[a]

Treatment	Control	500 ppm Al	1000 ppm Al	Control + PTH	500 ppm Al + PTH	1000 ppm Al + PTH	2% Ca	2% Ca 1000 ppm Al
Anomalies								
Runted fetuses	0	2.5 ± 1.8	0.9 ± 0.9	1.5 ± 1.5	0	0	1.5 ± 1.5	0
Imperforate anus	0	1.2 ± 1.2	0	0	0	1.2 ± 1.2	0	0
Microcaudia	0	1.2 ± 1.2	0	0	0	1.2 ± 1.2	0	0
Gonad agenesis	0	0	1.8 ± 1.8	0.8 ± 0.8	0	0	3.0 ± 3.0	0
Ischium or pubic absent or small	0	0	0	0	6.2 ± 4.1	0	0	0
Tibia or fibula absent or small	0	0	0	0	0	0	3.0 ± 3.0	0
Ribs:								
Supernumerary	1.8 ± 1.8	0	0	0	3.1 ± 3.1	0	0	2.7 ± 2.7
Fused	0	0	0	0	3.1 ± 3.1	0	0	0
Absent	0	0	0	0	0	1.2 ± 1.2	0	0
Vertebrae:								
Fused	0	2.5 ± 2.5	0	0	3.1 ± 3.1	2.3 ± 2.3	0	0
Absent or not ossified	1.8 ± 1.8	2.5 ± 2.5	0	0	0	2.3 ± 2.3	0	0
Digital bones: Front or hind limbs absent or not ossified	10.0 ± 6.6	9.5 ± 4.7	9.3 ± 4.0	10.2 ± 5.3	13.0 ± 6.2	14.6 ± 6.5	7.5 ± 4.2	7.0 ± 4.1
Sternebrae:								
Absent or not ossified	25.8 ± 8.6	31.5 ± 9.8	17.5 ± 12.1	36.6 ± 11.7	31.0 ± 10.5	37.9 ± 14.8	13.1 ± 7.3	27.1 ± 8.9

[a] Rats were maintained on diets from day 6 of gestation until sacrifice at day 19 of gestation. Animals received subcutaneous injections of PTH (68 units/kg) on gestational days 6, 9, 12, 15 and 18. Values are the mean response/litter SEM.

there were no statistically significant increases in the incidence of any abnormality (Table II). Examination of cells and their ultrastructures from numerous areas of brain, kidney or femur did not reveal changes which could be attributed to any experimental treatment.

Discussion. Resorption rate was increased following 1000 ppm Al and PTH suggesting that this metal and hormone may be embryotoxic when administered throughout organogenesis and late fetal development (day 6–19). Neither PTH nor 1000 ppm Al alone had any effect on mortality and the apparent no-effect dose level for embryotoxicity after combined treatment is 500 ppm Al and PTH. This effect was not correlated with an increased whole fetal carcass Al concentration. However, the concentration of Al was not determined in stillborn or partially resorbed fetuses or individual fetal tissues such as brain, bone and kidney which have elevated Al concentrations in adult rats following combined treatment with Al and PTH (13). Nevertheless, histopathological changes were not detected in tissues examined and the incidence of gross, soft-tissue or skeletal anomalies was not affected by any treatments employed in this investigation.

Transplacental movement of Al resulted in an Al concentration in control fetuses similar to that previously reported for whole carcass of adult rats maintained on Lab Blox containing 119 ppm Al (13). Since the concentration of Al in whole fetal carcass was not affected by the dietary level of Al or by PTH or Ca and presumably did not reflect maternal Al burdens, the rat placenta may, at least partially, preclude transport of Al and/or PTH. Thus, the placenta may assume a protective role by restricting Al accumulation in the unborn rat. There have been few studies regarding Al passage across the placenta; however, there is evidence that maternal PTH does not cross the placenta in nonhuman primates (21, 22).

In summary, combined treatment with 1000 ppm Al and PTH throughout organogenesis and late fetal development resulted in an increased resorption rate. Administration of Al and/or PTH or Ca did not increase the incidence of teratogenic anomalies or whole fetal carcass Al concentrations. These results suggest that in rats 1) dietary exposure to Al at levels as high as 1000 ppm throughout organogenesis may not result in teratogenic or histopathological alterations and 2) although combined maternal treatment with

1000 ppm Al and PTH throughout organogenesis may increase embryolethality, it may not enhance the potential for Al-induced toxic effects in surviving fetuses.

Summary. Oral aluminum (Al) exposure is a consequence of the element's presence in food, cosmetics, drinking water and its use in preparations administered therapeutically in large quantities as antacids or phosphate binders. Al is neurotoxic in animals and elevated concentrations of Al have been detected in brains of patients with certain encephalopathies. Protein synthesis, ribosomal RNA content and microtubular polymerization may be altered by Al. Since there are critical periods of protein synthesis and cellular division during embryonic development it was of interest to determine the teratogenic potential of Al. Since parathyroid hormone (PTH) increases tissue Al concentrations the effects of combined treatment of Al and PTH were also evaluated. Rats were maintained on diet supplemented with Al and/or calcium and treated with PTH or vehicle from day 6 of pregnancy through day 19, when fetuses were removed by Caesarean section. Combined or separate treatment with PTH and Al did not alter the number of live fetuses, fetal size, incidence of gross, soft-tissue or skeletal anomalies. Al concentration in whole fetal carcass was not affected. Combined treatment was accompanied by a significant increase in resorption rate. Histopathological alterations were not detected in animals from any treatment group. These results suggest that although combined maternal treatment with PTH and 1000 ppm Al may increase embryolethality, it may not enhance the potential for Al-induced toxic effects in surviving rat fetuses.

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1. Rubini, M. E., Coburn, J. W., and Massry, S. G., Arch. Intern. Med. **124,** 663 (1969).
2. Verberckmoes, R., Bouillon, R., and Kremplen, B., Ann. Intern. Med. **82,** 528 (1975).
3. Kaehny, W. D., Hegg, A. P., and Alfrey, A. C., N. Engl. J. Med. **296,** 1389 (1977).
4. Clarkson, E. M., Luck, V. A., and Hynson, W. V., Clin. Sci. **43,** 519 (1972).
5. Crapper, D. R., Krishnan, S. S., and Dalton, A. J., Science **180,** 511 (1973).
6. Alfrey, A. C., Gendre, G. R., and Kaehny, W. D., N. Eng. J. Med. **294,** 184 (1976).
7. Klatzo, I., Wisniewski, H., and Streicher, E., J. Neuropathol. Exp. Neurol. **23,** 187 (1965).
8. Crapper, D. R., and Dalton, A. J., Physiol. Behavior **10,** 935 (1973).
9. DeBoni, U., Otvos, A., Scott, J. W., and Crapper, D. R., Acta Neuropathol. **35,** 285 (1976).
10. Miller, C. A., and Levine, E. M., J. Neurochem. **22,** 751 (1974).
11. DeBoni, U., Scott, J. W., and Crapper, D. R., Histochemistry **40,** 31 (1974).
12. Bonhaus, D. W., McCormack, K. M., Mayor, G. H., Mattson, J. C., and Hook, J. B., Proceedings of the American Society of Nephrology, in press (1978).
13. Mayor, G. H., Makdani, D. D., Keiser, J. A., and Ku, P. K., Science **197,** 1187 (1977).
14. Cushard, W. G., Jr., Creditor, M. A., Cantebury, J. M., and Reiss, E., J. Clin. Endocrinol. Metabol. **34,** 767 (1972).
15. Wilson, J. G., *in* "Teratology: Principles and Techniques" (J. G. Wilson and J. Warkany, eds.), p. 251. University of Chicago Press, Chicago (1965).
16. Inouye, M., Cong. Anom. **16,** 171 (1976).
17. Karnovsky, M. J., J. Cell Biol. **27,** 137A (1965).
18. Pease, D. C., "Histological Techniques for Electron Microscopy," p. 38, Academic Press, New York (1964).
19. LeGendre, G. R., and Alfrey, A. C., Clin. Chem. **22,** 53 (1976).
20. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics". McGraw-Hill, New York (1960).
21. Lerman, S., Fleichman, A. R., Oakes, G. K., Epstein, M. F., Chez, R. A., and Mintz, D. H., Clin. Res. **22,** 474 (1974).
22. Northrop, G., Misenhimer, H. R., and Becker, F. O., Amer. J. Obstet. Gynecol. **129,** 449 (1977).

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