PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 175, 164–168 (1984)

Uptake and Transfer of Lithium in Pregnancy and Lactation in the Mouse[1] (41782)

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Abstract. The uptake of lithium in pregnant and lactating mice as well as its transfer to their respective fetuses (18-day postcoitum) and nurslings (11- to 15-day postnatal) were quantified. Lithium carbonate in concentrations of 1 or 2 mg/ml given *ad libitum* in drinking water produced plasma levels in adults ranging from 0.46 to 1.7 meq/liter. In pregnancy, plasma lithium of the adult was twice that of the fetal plasma. However, there was no statistical difference in brain lithium content between adults and fetuses at the 1- or 2-mg dosage. A significant decrease in bone lithium content was found in fetuses as compared to adults at the 2-mg level. During lactation the plasma lithium of nurslings was one-fourth to one-sixth that of the mothers' plasma. Lithium content in brain and in bone of adults was significantly lower than those of nurslings at both drug concentrations. No apparent effects on adults, fetuses, or nurslings were noted in the short term.

The impetus for this study stems from the therapeutic use of lithium salt in human manic-depressive illness (1, 2). The potential hazard of lithium as a teratogen has not gone unrecognized, yet the results of human and animal studies of mammals have been ambiguous (3). For example, nontoxic dosages equivalent to human therapeutic levels given to animals did not induce overt malformations (4, 5). However, lithium even at equivalent therapeutic dosages may be toxic to some fetuses (5). In addition, the potentiality of producing "behavioral defects" has been considered (6). Nevertheless, in spite of considerable effort neither the site nor the mechanism of action of lithium is known in the developing or maturing brain.

In view of the uncertainties mentioned and the need for ongoing experimentation, we estimated the amount of orally administered lithium in plasma, brain, and bone of the adult, the fetus, and the offspring of lactating mice.

Materials and Methods. *Administration of lithium during pregnancy or lactation.* Mice of strain 129/SvSl maintained in our laboratory were used throughout. Lithium carbonate was given *ad libitum* in the drinking water at concentrations of 0, 1, or 2 mg/ml. Pregnant animals were housed one or two per cage. Our commercial mouse food contained 0.064 μg/g of lithium. In the pregnancy series of experiments the lithium was started on Day 7 or 8 postcoitum (pc) and continued until Day 18 or 19 pc, 1 or 2 days prior to parturition. In the lactation series lithium administration was started on the first day of parturition and ended on postnatal Days 11 through 15 before offspring began eating food pellets available to the mother. Litter size was equalized to five pups per lactating mouse. In a few cases one or two excessive pups of similar age were added to litters less than five in number. At the end of the experiment the litter size averaged about four offspring.

Removal of plasma: adult. At the time of sacrifice the females were anesthetized with ether and blood was drawn from the orbital sinus with the aid of 32-mm-long heparinized microcapillary tubes ordinarily used for hematocrit determinations (Sherwood Medical Industries, St. Louis, Mo.). The blood passing through the capillary tube was collected in a disposable microtest tube of 400-μl capacity and centrifuged in a Beckman Spinco microcentrifuge for 5–10 min. The plasma was aspirated with a Pasteur pipet and a measured quantity was diluted to 1 ml with distilled water and readied for spectrophotometry. Under ideal circumstances, it was possible to obtain 400 μl of total blood and as much as 50–100 μl of plasma following centrifugation.

[1] Supported by the Minnesota Medical Foundation.

0037-9727/84 $1.50

Whenever possible, samples were measured in duplicate.

Removal of plasma: fetus. At sacrifice, viable fetuses were removed from the uterus and kept warm under a light bulb. The neck on one side was incised with a scalpel in the area of the jugular vein and blood was collected in a 75-mm heparinized microcapillary tube (Curtin Matheson, Houston, Tex.). The tube was then sealed (Seal Ease, Clay Adams, N.J.) and centrifuged for 5–10 min to separate plasma. The tube was scored, cut, and a measured quantity of plasma was diluted up to 1 ml with distilled water. In some cases pooled plasma from an entire litter was collected for lithium measurement.

Removal of plasma: lactating offspring. The plasma was collected with the same type of capillary tube as that used on fetuses. In the case of the pups, blood was obtained following decapitation. Enough blood was obtainable in most cases to determine plasma lithium in individual offspring.

Processing of brain for lithium analysis. Immediately following sacrifice, the brains of adult, fetal, or nursing mice were placed on aluminum foil. The cerebral cortex was removed from the adult and the young mice while the entire fetal brains were pooled by litter. The brain tissues were dried in a 40°C oven for several days and the dried brains were weighed, homogenized with 0.5 ml 3 *N* HCl in small glass homogenizers and transferred into test tubes using a total of 2 ml of acid. The tubes were centrifuged at 3000 rpm for 20 min prior to spectrophotometric measurement.

Processing of adult bone. Lower extremities were disarticulated from the pelvis and placed in a test tube covered with distilled water and heated in a water bath at approximately 70°C overnight. The limbs were freed of muscle and connective tissue. The femora were dried, weighed and placed into tubes with 2 ml of 3 *N* HCl for several days to dissolve bone minerals. The tubes were centrifuged as before prior to spectrophotometry.

Removal of bones from fetus or suckling young mice. The entire litter of fetal mice or single young mice were skinned, placed in distilled water and heated overnight at 70°C in a water bath. After draining, 0.5 to 1.0% of papain in distilled water was added at about two times the volume of the sample. The vials were incubated at 37°C for 24 to 48 hr after which they were vigorously shaken resulting in soft tissue separating from denser bones. After repeated washings with distilled water an uncontaminated preparation of clean bones was obtained. If desirable, the sorting of bone free of soft tissue can be facilitated by using a 50% sucrose solution to take advantage of the disparate densities of tissues. After the last wash in water the bones were dried in an oven at 100°C, weighed, and 2 ml of 3 *N* HCl added and allowed to stand for several days along with intermittent shaking. The tubes were centrifuged and lithium was determined by spectrophotometry.

Determination by atomic absorption spectrophotometry. The sample solutions and standards were analyzed for lithium in a Jarrel–Ash spectrophotometer (Jarrell–Ash Co., Waltham, Mass.) and lithium content was calculated as μg/ml plasma and as μg/g dried brain and bone tissue.

Statistical evaluation. Statistical significance was determined by Student's t test; $P < 0.05$ was considered significant.

Results. *I. Pregnancy. Comparisons between mothers and fetuses. A. Control.* The results quantifying lithium in plasma, brain, and bone between mothers and fetuses of the control animals are presented in Fig. 1. Lithium was not detectable in plasma of the mothers or fetuses. Surprisingly, the brain and bone of mothers and fetuses contained small amounts of lithium, ranging from 0.56 to 1.19 μg/g of tissue.

The detection of small amounts of lithium in the tissues of control animals prompted sampling of water and food (Purina Mouse Chow). Lithium was not detectable in water but the mouse food contained 0.064 μg/g of lithium. (Purina Rat Chow contained 0.24 μ/g of lithium by comparison.)

B. Experimental: 1-mg/ml dosage. As seen in Fig. 1, chronic administration of 1 mg/ml of lithium carbonate in the drinking water produced a plasma lithium level of 3.18 μg/ml (0.46 meq/liter) in mothers which was about twice the fetal level (1.52 μg/ml).

Although adult brains contained higher lithium levels than fetal brains they were not significantly different. Lithium in brains of adults was higher than adult serum content

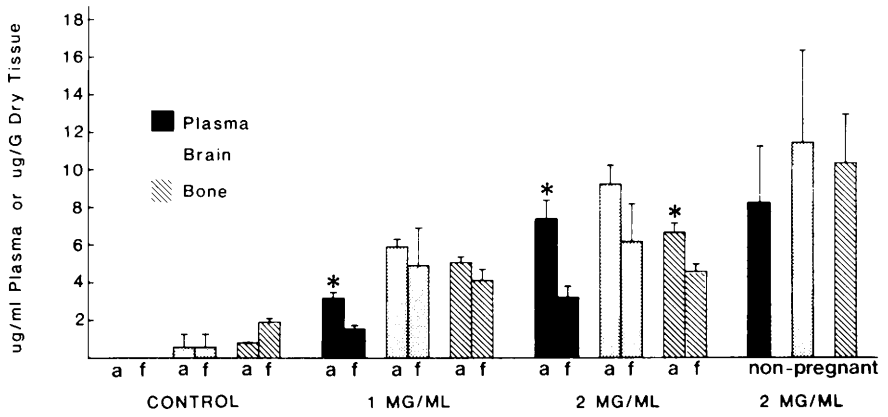

FIG. 1. Quantity of lithium contained within plasma, brain, and bone of pregnant or nonpregnant adults (a) and 18- or 19-day fetuses (f) after administration of 0, 1, or 2 mg/ml lithium carbonate in drinking water. The vertical bar represents standard error of the mean; the asterisk indicates statistical significance ($P < 0.05$) of adult (a) compared to fetus (f) (Student's t test).

and similarly fetal brains contained more lithium per gram dry weight than the fetal plasma value.

The content of lithium in bone was similar to that of brain and the relationship of adult and fetal bone was also not significantly different. Lithium levels in dried adult and fetal bone were relatively higher than their respective plasma content.

C. Experimental: 2-mg/ml dosage. Doubling the dosage resulted in an approximate two-fold increase in plasma lithium content in both adults and fetuses. As before the plasma values of adults were about twice those of fetal plasma. Brain and bone levels of lithium were elevated in adults and fetuses, but only the values in adult brain rose proportionately (Fig. 1). Brain lithium content of adults was not significantly higher than that of fetal brain, but adult bone content of lithium was significantly higher than that of fetal bone. Brains of adults and fetuses, and fetal bone, but not adult bone lithium content, were higher than their respective plasma levels. As was the case with the lower dosage, lithium content was somewhat higher in brain than that in bone in both pregnant females and fetuses. On comparing pregnant with nonpregnant mice given 2 mg/ml lithium carbonate (Fig. 1) one finds that nonpregnant mice contained more lithium on the average in plasma, brain, and bone. However, none of these differences was statistically significant.

II. Lactation. Comparisons between mothers and offspring. A. Control. As seen in Fig. 2, mothers and their suckling offspring receiving no lithium in drinking water (but some in food) showed a slight amount of lithium in plasma, 0.24 μg/ml, which was barely detectable in the expanded range of the spectrophotometer. The quantities in brain and bone of mothers and offspring were 0.68 and 1.63 μg/g/dry weight, respectively.

B. Experimental: 1-mg/ml dosage. Lactating mice on a dosage of 1 mg/ml yielded lithium levels in their plasma averaging 5.16 μg/ml (or 0.8 mEq/l) which was approximately 6 times the level found in their suckling offspring (0.83 μg/ml).

The amount of lithium in brains of adults was 18.35 μg/ml compared to 4.3 μg/ml in offspring, a ratio of 4.3 to 1, while the ratio between maternal and offspring lithium in bone was about 3 to 1, (10.5 versus 3.4). Lithium levels in brain exceeded that in bone both in adult or offspring.

C. Experimental: 2-mg/ml dosage. Following an increase in dosage from 1 to 2 mg/ml there was an increased uptake of lithium in all tissues. Adult plasma rose 2.4 times while offspring plasma increased 3.4 times. The mean levels in adult brains increased but the variation increased as well. Brains of offspring showed significant increases. Bone of adults and offspring showed the least increase in lithium content.

In this higher-dosage group the plasma levels of lithium were 12.3 μg/ml (or 1.8 meq/

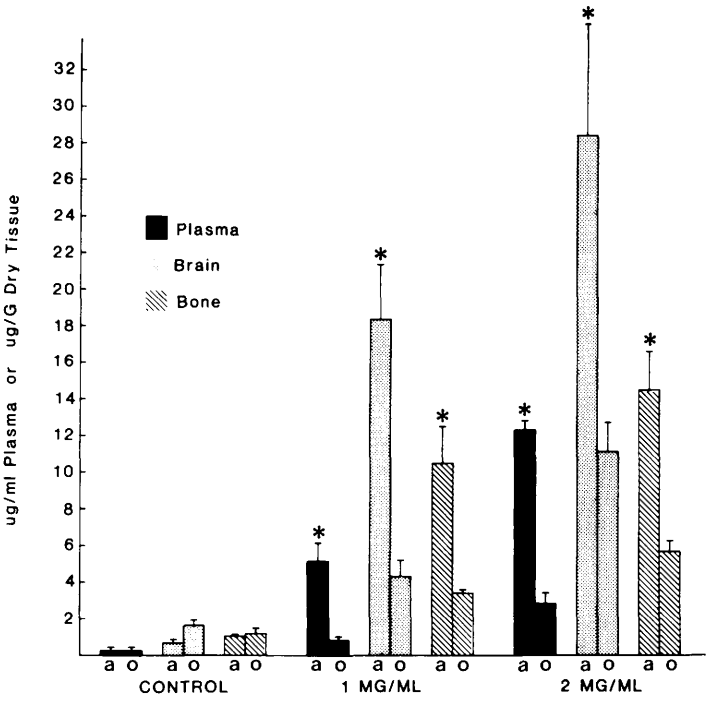

FIG. 2. Quantity of lithium contained within plasma, brain, and bone of lactating adults (a) and nursing offspring (0) after administration of 0, 1, or 2 mg/ml lithium carbonate in drinking water. The vertical bar represents standard error of the mean; the asterisk indicates statistical significance ($P < 0.05$) of adult (a) compared to offspring (o) (Student's t test).

liter) in the mothers and 2.82 μg/ml in their offspring for a ratio of 4.4 to 1. Interestingly, the ratios of mother versus offspring as regards brain or bone content were the same, 2.6 to 1. As before, brain levels were higher than bone levels in corresponding adult or offspring.

Discussion. That lithium would pass from pregnant female to fetus or from lactating female to suckling offspring was certainly not unexpected since it is a remarkably small molecule (atomic wt 6.9). Many studies in animals and in humans have verified its uptake into numerous adult tissues (cf. (7)). However, the transfer of lithium during pregnancy or nursing has not been studied extensively in animals (8) or in humans (9, 10).

Any comparisons between pregnant and/or lactating mice or humans treated with lithium are weakened by lack of adequate knowledge of the two physiologic states in either species. In particular fluid and food intake as well as drug clearance are likely to be different. For example, clearance of lithium has been reported to be higher during pregnancy than during lactation (9).

In our experiments we relied on the plasma level as a reflection of intake. Changes in lithium content in plasma as well as in brain and bone with increased dosage served as criteria for most conclusions.

Of the many relationships which emerged, the most consistent was that the lithium in plasma of pregnant mice was about twice that of fetuses. In the dog (8) by comparison, lithium was not detectable in the fetus until much higher than therapeutic levels were attained in adult serum during a 95-min infusion. Plasma levels in lactating mice were four to six times that of nursing pups. Similar results were obtained after both dosages. These data may be compared with those from humans in which the serum lithium levels of lactating mothers were approximately equal to mothers' milk and from two to three times the serum level of the nursing offspring (10).

The response of pregnant females and of lactating females after the 1- or 2-mg/ml dosage was similar. A twofold increase in drug concentration resulted in a proportionate elevation in plasma levels (Figs. 1 and 2). The

absolute quantity of plasma lithium was highest in lactating females (1.8 meq/liter) accountable perhaps by intake or by clearance differences as alluded to above. This level of lithium may be compared with rat mean plasma level of sodium, 151 meq/liter and potassium, 5.9 meq/liter (11). Interestingly, mean plasma values of lithium in nonpregnant females fell in between those of the other two groups.

The brain content of lithium of pregnant females was higher but not significantly more than brain of fetuses at either dosage. During lactation the mothers' brain lithium content increased 54% with increased dosage while the nurslings' brain levels were elevated 175%. At either drug concentration the mothers' brain contained significantly more lithium than her pups' brain. It should be recalled that the plasma levels of mother was four to six times that of her pups. Comparison of lithium content in brain of nurslings with fetal brain would suggest the rapidly growing postnatal brain has a higher capacity for drug uptake than the developing brain on unit-weight basis. The reasons for these differences remain to be determined.

The lithium content of adult bone was not significantly different from fetal bone. On increased dosage, fetal bone lithium content increased only 12% while adult bone lithium content was elevated 29%. The bones of lactating females contained significantly more lithium than bones of nursing offspring at both concentrations tested. Increasing the concentration resulted in a 68% elevation in nursling bone and a 38% increase in nursing mothers' bone. The slight increase of 12% in fetal bone lithium content after increased dosage as compared to the significant 68% elevation in bone lithium content of nursing offspring was not unexpected. Fetal bones being highly cartilaginous with less mineral content are likely to take-up less lithium than the highly mineralized rapidly growing bone of the postnatal pups. The exchange of lithium with other ions and its storage in bone for extended periods has been well established (12, 13).

It is believed that lithium ingestion may be a potential hazard to the pregnant and nursing adult (14). The findings reported here confirm that lithium is transferred from the mother to the fetus as well as to the nursling through the placenta and through the milk, respectively. The quantity of the element taken up by brain or bone is dose dependent. On cursory examination the high lithium levels in brain and/or bone did not appear to produce untoward effects to the adult or their offspring in the short term, however, long-term effects remain to be determined.

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Received June 8, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted October 5, 1983.