notable. The general behavior of young adult mice of both strains and both sexes is benign. They show no fighting in either the large home cages or the small observation cages and are also easily picked up and handled.

In striking contrast the 32-week-old ICR male mice show remarkable changes in behavior. Many aggressive fights were observed in the home cage and in the small observation cages. All of these old males showed signs of bites on their tails and bodies. When picked up or handled, they immediately and vigorously attacked with biting, vocalizing and struggling making it necessary to wear heavy leather gloves when working with this group. As can be seen, these old ICR mice show increases in all 3 brain monoamines compared to young adults and particulary a 48% increase in brain DA level. This increased level is also reflected in an increase in DA turnover as well[2].

The old ICR females also show increases in brain DA (63%) but do not show behavioral changes and remain benign. The lack of aggressive behavior in the old ICR females despite comparable increases in brain amines again emphasizes the important permissive role of hormones in patterns of aggressive behavior.

The old C57BL6J male mice showed only small increases in brain monoamine levels when compared to young adults and showed no fighting or changes in behavior in either males or females.

Discussion. These and previous studies[1] show that the levels of brain monoamines in young adults of a given mouse strain are remarkably constant. However, the genetic program of amine changes with aging may show marked strain differences. The increase in aggressive behavior in old ICR males may be related to the marked increase in brain dopamine, compared to the small increases in NE or 5HT. We have previously reported[3] that giving l-DOPA to young adult ICR mice (thus raising brain dopamine) results in aggressive behavior, which is comparable to that observed in the old ICR mice in the present study. As mentioned, the young adult males of the BALB strain show aggressive fighting and have a high level of brain dopamine compared to their NE and 5HT levels[1]. As expected, these modes of aggressive behavior are blocked by small doses of haloperidol and other dopamine blocking agents.

It is possible that these studies may be pertinent to the problems of disruptive behavior seen in some elderly patients and may have a similar biochemical basis. There are clinical reports showing that the tranquilizers are useful in such patients. Basic and clinical studies in the biochemistry of aging and geriatric pharmacology are all too scarce and much needed.

Thymus gland involution induced by lithium chloride

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Summary. Chronic treatment with lithium chloride produced significant involution of the thymus gland with histological evidence of reduced cellularity due to loss of thymic lymphocytes and a significant reduction in the weight of the gland in normal and adrenalectomized mice. Lithium also increased corticosterone levels in normal mice without changes in adrenal weights. The involution of the thymus gland is most likely due to an effect of lithium on the gland, and it is not mediated by adrenocortical mechanisms or stress.

The effectiveness of lithium salts in the treatment of manic-depressive psychosis with mania is now well-documented[2]. In spite of this, the mechanism of action of lithium is not understood. Various studies have suggested an effect of this cation on central neurotransmitter function[3,4]; on endocrine glands, especially the thyroid gland[5], and on adrenal enzymes[6]. In the present study, we report an hitherto unrecognized effect of lithium chloride (LiCl) on the thymus gland in normal and adrenalectomized mice.

Materials and methods. Experiments were carried out in male Swiss (Canadian Breading Farms and Laboratories, Ltd. S.t Constant, Quebec) and CBA (McGill University, McIntyre Medical Science Building) mice weighing approximately 30 g. Mice were caged in groups of 8 under controlled temperature (20 °C) and light (12 h-on, 12 h-off). Standard mice chow (Master Laboratory Cubes, Maple Leaf Mills, Montreal) and water were given ad libitum. Lithium chloride (crystalline powder, Allied Chemical, Morriston, N. J.) was administered i.p. to Swiss and CBA mice in doses of 3 mEq/kg, twice a day for 4 days. In another group of Swiss mice, 4 separate doses of 1, 3, 6 and 9 mEq/kg were given i.p., twice a day for 4 days. Control mice were injected i.p. with normal saline. Another control group was not injected or stressed. Normal saline and LiCl solutions were injected in volumes of 0.2 ml twice daily. Controls and treated mice were sacrificed by cervical dislocation after 4 days. The thorax was opened by 2 incissions parallel to the sternum and the thymus gland was dissected carefully and removed after exposing the upper mediastinum and heart. The thymus glands were weighed individually on an analytical balance (Mettler, model H 31, sensitivity $\pm$ 0.05 mg). Immediately after weighing, the thymus glands were fixed in 10% formalin-saline and embedded in paraffin.

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Sections of 3 to 4 microns thickness were stained with hematoxylin and eosine, or with Giemsa stain, for light microscopic examination. Simultaneously processed thymus glands from saline-injected, and unstressed mice, served as histologic controls.

Adrenal gland weights and blood corticosterone levels were also measured in these experiments. The adrenal glands were carefully dissected and weighed with a model H 31 Mettler balance; the weight of 2 adrenals was expressed in mg. Blood corticosterone levels were measured in pooled blood from 4 mice using a radioimmunoassay described elsewhere[7]. Relationships between blood corticosterone levels, adrenal gland weight, and thymus gland weights were done in Swiss and CBA mice receiving 3 mEq/kg of LiCl twice a day for 4 days.

Additional experiments were performed in adrenalectomized male Swiss mice (Charles River, University of Missouri-Columbia, Division of Animal Services) weighing 30 g. Mice were caged in groups of 3 to 4 under the above environmental conditions and given food and maintained with 0.9% saline ad libitum. LiCl was injected i.p. in 2 separate groups of adrenalectomized mice in doses of 6 and 9 mEq/kg day. 2 adrenalectomized control groups were used. One control adrenalectomized group was not injected or stressed; and another was injected with normal saline twice daily for 4 days. Saline and LiCl solutions were injected in volumes of 0.2 ml twice daily. The adrenalectomized mice were sacrificed by cervical dislocation after 4 days. The thymus was resected and weighed as described above and frozen for future histological and biochemical analyses. Completeness of adrenalectomy was verified by abdominal exploration. Blood lithium levels in mEq/l were determined with a flame photometer in whole blood by diluting 100 µl of whole blood in 2 ml of distilled water. Means and SD of measurements were obtained for controls and treated mice. The statistical significance of the data was determined by 2-tailed Student's t-tests for paired observations between controls and treated animals[8].

Results. Thymus glands observed in situ were small in mice treated for 4 days with LiCl. Histologic examination revealed reduced cellularity due to loss of small lymphocytes from both the cortex and medulla compared with control tissue. No histological difference was evident between thymus glands removed from saline-injected and unstressed controls. The weights of the thymus glands were significantly decreased by 47.1% in Swiss and by 60% in CBA mice given LiCl, 3 mEq/kg, i.p., twice a day for 4 days, as shown in table 1. No significant difference in thymus weight was evident between glands removed from saline-injected controls and unstressed mice.

Table 2 shows the weights of the thymus glands in 4 groups of Swiss mice given 1, 3, 6 and 9 mEq/kg of LiCl, i.p., twice a day for 4 days. The decrease in thymus weight was directly proportional to the dose used. A small, but significant, reduction in the weight of the thymus gland (−38.5%) was observed at a dose of 1 mEq/kg whereas doses of 3, 6 and 9 mEq/kg produced highly significant reductions in the weight of the gland from −41.7% to −87.1%. Doses of 9 mEq/kg of LiCl produced marked reductions in gland weight and almost complete involution of the thymus gland, and evidence of toxicity such as, neuromuscular irritability, polydipsia, ruffled fur and diarrhea. Mortality in this group approached 50%. The lower doses caused no mortality and were not associated with behavioral or physiologic evidence of toxicity.

Adrenal gland weights in Swiss and CBA mice were not changed by chronic lithium treatments. The average weight of the adrenal glands in unstressed normal controls (n = 7) was 9.2 ± 0.7 mg, in saline-injected con-

Table 1. Weight of thymus gland in mice treated with LiCl for 4 days

A. Swiss mice	Thymus weight (mg ± SD)	B. CBA mice	Thymus weight (mg ± SD)
Controls	58.6 ± 14.7 (10)	Controls	30.5 ± 14.0 (8)
LiCl	31.0 ± 7.1 (10)**	LiCl	12.2 ± 4.5 (8)**
Percent decrease	−47.1%**	Percent decrease	−60.0%**

**$p < 0.01$ (see text for statistical analysis). Number of mice in parenthesis. LiCl was given i.p. in a dose of 3 mEq/kg, twice a day, for 4 days. SD, standard deviation.

Table 2. Effect of different doses of LiCl on the weight of the thymus gland

Conditions	Weight of the thymus gland (mg ± SD)	Percent change
Controls (saline)	40.3 ± 13.2 (9)	–
LiCl (1 mEq/kg)	24.8 ± 5.4 (4)*	−38.5*
LiCl (3 mEq/kg)	23.5 ± 5.7 (7)**	−41.7**
LiCl (6 mEq/kg)	10.8 ± 3.9 (4)**	−73.2**
LiCl (9 mEq/kg)	5.2 ± 1.0 (3)**	−87.1**

*$p < 0.05$ (see text for statistical analysis). **$p < 0.01$. Number of Swiss mice in parenthesis.

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Table 3. Weight of thymus gland in adrenalectomized mice treated with LiCl for 4 days

Conditions	Treatment (mEq/kg day)	Thymus weight (mg ± SD)	Percent change
Control adrenalectomized (unstressed)	None	62.3 ± 8.2 (6)	–
Control adrenalectomized	Saline	65.5 ± 17.9 (4)	–
Treated adrenalectomized	6	40.3 ± 10.9 (9)***	−35.0%***
Treated adrenalectomized	9	43.6 ± 12.4 (7)**	−30.0%**

**$p < 0.01$; ***$p < 0.001$. Percent change in thymus weight is in comparison with weights in unstressed mice. Number of mice in parenthesis. LiCl was given i.p. twice a day for 4 days.

trols (n = 9) the adrenal glands weight was 9.2 ± 0.8 mg, and in mice treated with 3 mEq/kg of LiCl (n = 7) the weight was 9.6 ± 1.0 mg. These results show that a reduction in thymus weight is not accompanied by changes in the weight of the adrenal glands. On the other hand, the average levels of corticosterone from pooled plasma of 4 mice were elevated from 12.7 ± 5 ng/ml, in saline-treated mice, to 50.2 ± 8 ng/ml in mice treated with the 3 mEq/kg dose for 4 days. The increases in the levels of corticosterone were not associated with changes in adrenal weights and inversely related to the weights of the thymus glands in both Swiss and CBA mice. Corticosterone levels were directly related to increments in lithium dosage. In mice injected with saline the average level of plasma corticosterone was 14.3 ± 1.2 ng/ml whereas the average levels of corticosterone in mice treated with 1 mEq/kg and 3 mEq/kg of LiCl were 49.6 ± 2.0 ng/ml and 60.2 ± 4.0 ng/ml respectively.

As shown in table 3, the weight and size of the thymus glands were significantly decreased ($p < 0.001$) in adrenalectomized mice treated with LiCl doses of 6 to 9 mEq/kg day for 4 days. The reduction in thymus weights in adrenalectomized mice treated with LiCl was 35% when compared with unstressed adrenalectomized mice and 37% less than adrenalectomized mice treated with daily injections of saline. No significant difference in the weight or size of the thymus gland was observed between control unhandled adrenalectomized and saline-injected adrenalectomized mice. No corticosterone was detected in adrenalectomized mice confirming completeness of adrenalectomy in this group of animals. Abdominal exploration revealed no adrenal tissue.

Blood lithium levels were proportional to the dose of lithium injected i.p. Mice treated with i.p. doses of 1 mEq/kg, twice a day for 4 days showed a blood lithium level of 0.1 mEq/l; for the 3 mEq/kg dose the mean blood level was 0.6 mEq/l; for the 6 mEq/kg dose, 0.84 mEq/l; and for the 9 mEq/kg dose, 1.70 mEq/l. In adrenalectomized mice the mean blood lithium level, for mice treated with the 6 mEq/kg day dose, ranged from 0.3 mEq/l to 0.7 mEq/l; in adrenalectomized mice treated with a dose of LiCl of 9 mEq/kg day the blood lithium levels ranged between 0.7 mEq/l and 1.2 mEq/l.

Discussion. Our results show that LiCl produces an obvious reduction in size and a significant reduction in the weight of the thymus gland after repeated i.p. administration for 4 days in normal and adrenalectomized mice. This change was associated with apparent loss of small lymphocytes on light microscopic examination of the thymus sections. This involution of the thymus is not strain specific.

Chronic stress is known to cause involution of the thymus gland[9]. Our results are unlikely to be due to the injection or handling of the animals since no involution of the thymus gland occurred in saline injected mice when compared with unstressed mice. This study also shows that lithium effects on the thymus gland are not due to stress or to an increase in plasma corticosterone levels, but probably these effects are likely due to an effect of lithium on the thymus gland. Our findings indicate that the reduction in the weight of the thymus gland after LiCl treatments in adrenalectomized mice are slightly less than in mice with intact adrenals suggesting that the involution of the thymus may be slightly potentiated by increments in plasma corticosterone. Furthermore, an inverse relationship between the incremental doses of lithium and the involution of the gland was observed indicating that the concentration of the circulating cation and not stress is responsible for the involution.

Previously we have shown that lithium induces adrenal tyrosine hydroxylase and phenyl-N-methyl transferase enzymes in the rat[6]. Others have shown that the increase in the production of aldosterone after lithium treatments is due to an effect of the cation on the zona glomerulosa of the adrenal gland[10]. The fact that no increase in adrenal gland weight was observed after chronic lithium treatments suggests that the significant increases in plasma corticosterone are not due to gross adrenocortical hyperplasia. Lithium is also known to alter thyroid gland function producing a variety of interesting changes in the endocrine function of this gland[5]. Hypothetically the above effects of lithium on the adrenals and the thyroid gland are mediated by an effect of the cation on these glandular tissues; therefore, it is likely that the effects of lithium on the thymus gland are due to an effect of the cation on the thymus tissue.

The exact mechanism responsible for the involution of the thymus after lithium will need further investigation. The fact that histological examination of the tissues revealed reduced cellularity due to loss of thymic lymphocytes suggests an interference with thymic lymphocytic function. Studies now in progress have shown a persistent lymphopenia in mice treated chronically with lithium. Furthermore, rubidium and cesium, 2 other members of the group 1, alkali metal series, do not produce involution of the thymus gland when administered at the same mEq/kg dose range effective in these experiments (unpublished observations). Corticosterone may contribute to the involution of the thymus but this factor was ruled out in the experiments with adrenalectomized mice and it is unlikely that it plays a major role in the involution of the gland after lithium.

The thymus gland is known to play an important role in immunological mechanisms mediated by T-lymphocytes[11], and disordered immune mechanisms are thought to be responsible for various medical diseases[12]. The significant involution of the thymus gland after chronic treatment with lithium shows that this cation exerts profound pharmacological effects on this gland.

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