## LITERATURE CITED

1. M. M. Gapparov, Vestn. Akad. Med. Nauk SSSR, No. 3, 29 (1978).
2. M. M. Gapparov, "Mechanisms of energy provision for hydrochloric acid secretion in the stomach and dietary factors," Author's Abstract of Doctoral Dissertation, Moscow (1978).
3. A. G. Perevoshchikov, I. B. Bukhvalov, and É. A. Mikhailov, Byull. Éksp. Biol. Med., No. 7, 58 (1978).
4. A. A. Pokrovskii, M. M. Gapparov, and L. G. Levin, Fiziol. Zh. SSSR, No. 10, 1567 (1973).
5. A. A. Pokrovskii, M. M. Gapparov, L. G. Levin, et al., in: Mitochondria. Regulation of Oxidation and Coupling Processes [in Russian], Moscow (1974), p. 119.
6. A. A. Pokrovskii, M. M. Gapparov, and P. P. Doronin, Tsitologiya, No. 2, 175 (1975).
7. A. A. Pokrovskii, M. M. Gapparov, G. Yu. Mal'tsev, et al., in: Mitochondria. Electron Transport and Transformation of Energy [in Russian], Moscow (1976), p. 23.
8. J. S. Hanker, C. J. Kusyk, F. E. Bloom, et al., Histochemie, 33, 205 (1973).
9. S. Kerpel-Fronius and F. Hajos, Histochemie, 14, 343 (1968).
10. A. M. Seligman, M. J. Karnovsky, H. L. Wasserkrug, et al., J. Cell Biol., 38, 1 (1968).


# MORPHOFUNCTIONAL CHANGES DUE TO LITHIUM CHLORIDE IN THE RAT THYROID GLAND


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Lithium preparations are nowadays being used on an ever-increasing scale in the treatment of various diseases. Lithium was originally used in psychiatry in the treatment of manic states [4, 8], but its range of application is by no means confined to this. A number of recent investigations have demonstrated the new fact that lithium acts on the tissue complexes of the thyroid gland [3, 6], but the mechanism of this action is variously explained. Some workers [11, 12] consider that lithium prevents the accumulation of iodine and secretion of thyroid hormones, whereas others consider that lithium has a direct inhibitory action on liberation of hormones from the tissue of the gland and the elimination of thyroxine from the body [5, 10]. There is also evidence that lithium inhibits the breakdown of thyroglobulin and the stimulating effect of thyrotrophic hormone [9].

Because of the ambiguous and, in some cases, the contradictory data on the effect of lithium salts on the thyroid gland it was decided to study the dynamics of morphological and functional changes in the thyroid gland under the influence of various doses of lithium chloride. No such investigations could be found in the accessible literature.

## EXPERIMENTAL METHOD

Experiments were carried out on 96 male albino rats divided into four groups: one control and three experimental groups, with 24 animals in each. Lithium chloride was given per os to the experimental rats daily for 6 weeks in the following doses: 0.5 meq/kg to group 1, 1.0 meq/kg to group 2, 2.0 meq/kg to group 3. Intravital function testing of the thyroid gland was then carried out by the radioindication method. For this purpose a subcutaneous injection of $^{131}I$ was given to 12 animals of each group in a dose of 740 Bq/kg body weight, and the iodine-accumulating function of the gland was tested by means of the DSU-61 apparatus 2, 4, 6, 12, 24, 48, and 72 h after injection of the isotope. After radiometry of the thyroid glands the animals were decapitated and the protein-bound $^{131}I$ (PBI-131), thyroid hormones ($T_3$ and $T_4$), the coefficient of effective


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TABLE 1. Changes in Relative Proportions of Structural Components of Thyroid Tissue under the Influence of Lithium Chloride (M ± m)

| Structural component of thyroid tissue | Bulk density, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | control | group 1 | P | group 2 | P | group 3 | P |
| Colloid | 27,7±0,53 | 24,3±0,36 | <0,05 | 35,1±0,33 | <0,05 | 40,3±0,38 | <0,005 |
| Follicular epithelium | 29,4±0,46 | 25,8±0,35 | <0,05 | 31,0±0,25 | <0,05 | 33,7±0,33 | <0,005 |
| Connective tissue | 24,5±0,55 | 28,1±0,4 | <0,05 | 18,2±0,35 | <0,05 | 14,6±0,28 | <0,005 |
| Interfollicular epithelium | 18,4±0,51 | 21,8±0,43 | <0,05 | 16,7±0,4 | <0,05 | 11,4±0,31 | <0,005 |

TABLE 2. Changes in Diameter of Follicles and Height of Thyroid Epithelium under the Influence of Lithium Chloride (M ± m)

| Index | Control | Group 1 | P | Group 2 | P | Group 3 | P |
|---|---|---|---|---|---|---|---|
| Diameter of follicles, μm | 55,1±2,0 | 57,3±2,2 | >0,25 | 64,2±2,7 | <0,025 | 73,6±3,3 | <0,005 |
| Height of thyroid epithelium, μm | 5,4±0,12 | 5,1±0,10 | <0,10 | 4,8±0,09 | <0,005 | 4,4±0,08 | <0,005 |

thyroxine (CET), and the level of pituitary thyrotrophic hormone (TTH) were determined in blood collected separately from each rat by means of commercial radioimmunologic kits from the firms CEA-IRE-Sorin (France), Radiochemical Centre, Amersham (England), and Byk-Mallinckrodt (West Germany). Radioactivity of the biological specimens was measured in a NZ-138 well-type counter, connected to an energy-selective type NK-350 counter from the firm Gamma (Hungary). For histological analysis the thyroid glands were fixed in calcium-formol, neutral fixative, and Bouin's fluid. Sections 5-12 μm thick were cut from paraffin blocks and on a cryostat. Tissue for ordinary morphological examination was stained with hematoxylin and eosin and subjected to stereologic analysis [1]. An ocular micrometer was used for morphometry. Neutral and acid glycoproteins were demonstrated histochemically by the combined Ritter–Oleson method, acid and alkaline phosphatases by Gomori's method, DNA by Feulgen's method, and RNA by Brachet's method. Grimelius' impregnation method was used to demonstrate C cells. The C cells were counted in one conventional unit of area with a 10× ocular and 40× objective. The experimental results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

A study of the morphofunctional characteristics of the thyroid gland of the albino rats showed that lithium chloride differs in its action depending on the dose. In the thyroid tissues of animals of group 1 a morphological picture characteristic of the gland with certain features of hyperfunction was observed. These features were liquefaction of colloid, an increase in the diameter of the interfollicular capillaries, and a change in the ratio between structural components of the thyroid tissue toward an increase in the quantity of connective-tissue cells and interfollicular epithelium and a corresponding decrease in the bulk density of the colloid and follicular epithelium (Table 1). No significant changes in the histochemical parameters of the thyroid gland were observed in the animals of this group compared with the control. As Table 3 shows, the iodine-accumulating power of the organ was increased somewhat, but the rate of excretion of the isotope from the organ was reduced. The data in Table 4 indicate some decrease in the indices of the hormone-forming function of the thyroid (PBI-131, $T_3$, $T_4$, CET). However, in this group the changes in the various indices were not statistically significant except the blood TTH level of the animals.

When higher concentrations of lithium chloride were administered to the animals of groups 2 and 3 considerable changes were observed in all morphological and functional indices of the thyroid gland, corresponding to a hypofunctional state of the organ. These changes were most marked in the animals of group 3. Morphologically, the thyroid glands were represented by large and medium-sized follicles, usually polygonal in shape, and filled with homogeneous colloid with no signs of resorption. The thyroid epithelium was flattened (Table 2). The connective-tissue stroma of the gland was distinctly visible and contained single lymphoid cells. The results of stereologic analysis confirmed that follicular epithelium and colloid predominated in the sections through the gland. The content of neutral glycoproteins was reduced in the cytoplasm of the follicular cells compared with the control and they were localized mainly in the apical parts of the thyrocytes. A well-marked PAS-positive reaction was observed in the basement membranes of the follicles and in the colloid, which was uniformly and moderately stained. The intensity of staining of nuclei of the follicular epithelium for DNA and of staining of the cytoplasm for RNA was low. Acid phosphatase was detected mainly in the nuclei of the thyro-

TABLE 3. Indices of $^{131}I$ Uptake by Thyroid Gland of Albino Rats during Lithium Therapy (M ± m)

| Time after injection of isotope, h | Accumulation of $^{131}I$ in thyroid gland, % of standard | | | | | | |
|---|---|---|---|---|---|---|---|
| | control (n=12) | group 1 (n=12) | P | group 2 (n=12) | P | group 3 (n = 12) | P |
| 2 | 27,7±1,7 | 30,9±1,6 | <0,25 | 18,9±0,9 | <0,025 | 13,5±1,0 | <0,005 |
| 4 | 32,9±2,1 | 35,7±2,3 | >0,50 | 21,6±1,1 | <0,005 | 15,1±1,3 | <0,005 |
| 6 | 38,8±1,9 | 42,6±2,9 | >0,25 | 24,7±2,4 | <0,005 | 19,5±1,8 | <0,005 |
| 12 | 44,2±2,5 | 48,9±3,3 | >0,25 | 30,6±3,0 | <0,005 | 22,4±1,6 | <0,005 |
| 24 | 58,8±4,0 | 63,5±5,1 | >0,50 | 40,0±3,4 | <0,005 | 30,7±2,3 | <0,005 |
| 48 | 46,4±3,5 | 50,3±4,3 | >0,25 | 32,1±3,0 | <0,01 | 25,4±3,7 | <0,005 |
| 72 | 40,2±3,4 | 41,7±4,1 | >0,50 | 23,6±2,3 | <0,005 | 20,1±2,5 | <0,005 |

TABLE 4. Indices of Hormone-Forming Function of Thyroid under the Influence of Lithium Chloride (M ± m)

| Index | Control (n=12) | Group 1 (n=12) | P | Group 2 (n=12) | P | Group 3 (n=12) | P |
|---|---|---|---|---|---|---|---|
| PBI - 131 | 0,27±0,03 | 0,25±0,08 | >0,25 | 0,21±0,02 | <0,1 | 0,17±0,01 | <0,05 |
| Tri-iodothyronine, conven. un. | 0,73±0,07 | 0,75±0,11 | >0,5 | 0,63±0,04 | <0,05 | 0,54±0,02 | <0,05 |
| Thyroxine, mg % | 3,25±0,54 | 2,75±0,37 | >0,5 | 2,45±0,12 | <0,05 | 2,0±0,09 | <0,005 |
| CET, conven. units | 1,06±0,07 | 1,04±0,03 | >0,5 | 0,88±0,08 | <0,05 | 0,66±0,02 | <0,005 |
| TTH, ng/ml | 0,56±0,05 | 0,25±0,10 | <0,005 | 0,19±0,03 | <0,005 | 0,11±0,01 | <0,005 |

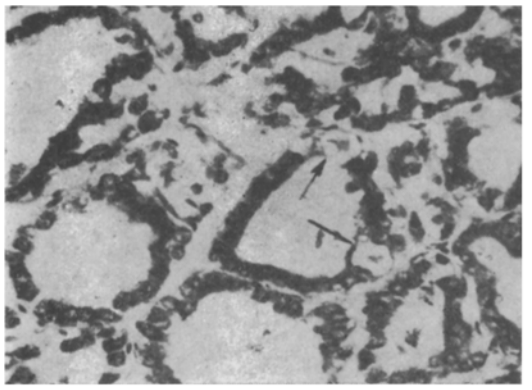

Fig. 1

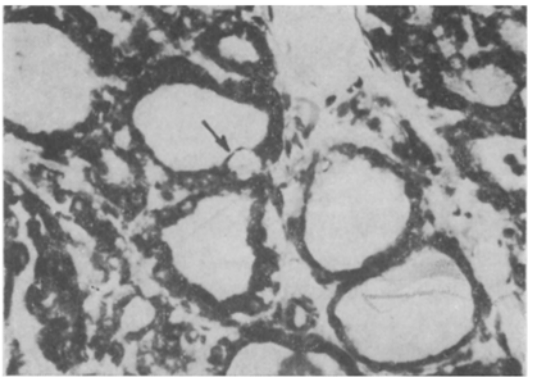

Fig. 2

Fig. 1. Thyroid gland of intact albino rats. Localization of C cells in follicle wall (arrow). Here and in Fig. 2, magnification: ocular 10, objective 20. Impregnation by Grimelius' method.

Fig. 2. Degranulation of cytoplasm of C cells in thyroid gland of animals of group 3 (arrow).

cytes in the form of pale to dark brown granules. Single, small, pale brown granules of lead sulfide were frequently found in the cytoplasm of the epithelial cells. Alkaline phosphatase was detected in the follicular epithelium as diffuse pale gray staining, evidence of low activity of the enzyme. Higher alkaline phosphatase activity was observed in the capillary epithelium and also in the fibrocytes. The low activity of the enzyme reactions reflects the low level of oxidation–reduction processes in the thyrocytes. The morphological changes in the thyroid gland described above were confirmed also by function tests. The absolute quantity of $^{131}I$ taken up by the thyroid gland in the inorganic phase of iodine metabolism (during the first day) was significantly lower than in the control group and also in groups 1 and 2, and the rate of elimination of the isotope from the tissue also was retarded. This fact indicates delay of hormone formation and depression of the concentration function of the thyroid gland under the influence of lithium salts both in the inorganic phase and in the organic transport phase of the iodine cycle. This conclusion is also supported by reliable data showing a decrease in the PBI level in the plasma and in the thyroxine and tri-iodothyronine levels. The blood TTH concentration was reduced by more than 80% compared with the control, suggesting not only that lithium has a direct influence on thyroid tissue, but also that the pituitary is involved.

The results of the study of the reaction of the parafollicular cells, or C cells, of the thyroid gland to administration of lithium salts are interesting from the point of view of this investigation. The C cells were found to be unevenly distributed in the gland, as other workers also have found [2, 7]. Practically none are found at the periphery of the thyroid gland, most of them are in the central part, where on average they numbered 5-7 for every 50 cells of the follicular epithelium. C cells are distributed singly in the follicle walls between ordinary thyroid cells and are separated from the lumen of the follicles by a narrow strip of the apical part of the thyrocytes (Fig. 1). Parafollicular cells are found more often in the wall between follicles, or they form small groups of two or three cells between follicles. These cells are usually round or slightly oval in shape and larger than the thyrocytes, with well-defined borders. The nuclei of these cells are large and poor in chromatin. Fine granules are unevenly distributed in the cytoplasm of individual cells. Degranulation of the cytoplasm was observed in the tissue of the experimental animals more frequently than in the control (Fig. 2). Significant changes in size of the C cells were not seen under the influence of lithium, but their number per conventional unit of area of section through the thyroid gland was 29.2% less in the rats of group 3 than in the control. Consequently, lithium affects not only true thyroid tissue but also the population of C cells, although the mechanism of this action requires further study.

Analysis of the results of this experiment thus demonstrates an inhibitory action of lithium chloride both on the phase of hormone formation in the thyrocytes and on the phase of liberation of thyroid hormones into the blood stream, and this effect is directly dependent on the dose of lithium. The significant fall in the plasma TTH level in the animals of all experimental groups suggests that lithium not only has a direct inhibitory action on thyroid tissue, but also an indirect influence on the thyroid gland through the adenohypophysis.

## LITERATURE CITED


1. G. G. Avtandilov, N. N. Yabluchanskii, and V. G. Gubenko, Byull. Éksp. Biol. Med., No. 1, 93 (1977).
2. A. I. Briskin, V. A. Odinokova, V. F. Kondarenko, et al., Byull. Éksp. Biol. Med., No. 2, 111 (1971).
3. V. A. Glumova, N. M. Petrov, and V. N. Markov, Byull. Éksp. Biol. Med., No. 6, 604 (1979).
4. J. Candy, Br. Med. J., 3, 349 (1972).
5. H. E. Carlson, R. Temple, and J. Robbins, J. Clin. Endocrinol., 36, 1251 (1973).
6. T. Eulry, J. Orgiazzi, and R. Mornex, Nouv. Presse Méd., 6, 2955 (1977).
7. M. Label, Endocrinology, 67, 315 (1976).
8. J. H. Lazarus and E. H. Bennie, Acta Endocrinol. (Copenhagen), 70, 266 (1972).
9. A. Radvilla, R. Roost, H. Burgi, et al., Acta Endocrinol. (Copenhagen), 81, 496 (1976).
10. A. Rifkin, F. Quitkin, A. G. Blumberg, et al., J. Psychiatr. Res., 10, 115 (1974).
11. G. Sedvall, B. Jonson, V. Petterson, et al., Life Sci., 7, 1257 (1968).
12. B. Shopsin, Dis. Nerv. Syst., 30, 237 (1970).