

Similarly, in the case of the MTT assay, lithium at 0.4 mM, 0.8 mM, 1.0 mM, 1.6 mM and 2 mM concentrations and in the absence of TSH stimulation significantly increased the absorbance at 570 nm when compared with the controls without lithium (Fig. 2). However, under stimulation by TSH in the 1 mU/ml concentration, lithium at 0.4 mM, 0.8 mM, 1 mM, and 1.6 mM concentrations did not change the absorbance at 570 nm when compared with the controls without lithium. Surprisingly, lithium at the 2 mM concentration significantly diminished the absorbance at 570 nm when compared with the controls without lithium (Fig. 3).

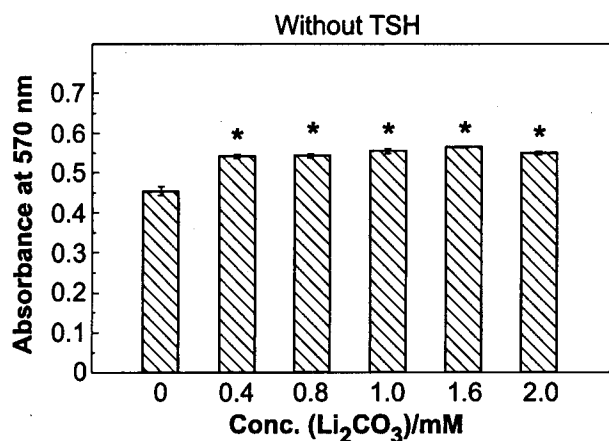


Fig. 2. Influence of lithium on proliferation of FRTL-5 cells (MTT assay). Bars represent average values of quadruplicate determinations  $\pm$  SD. Asterisks represent statistically significant ( $P < 0.05$ ) results when compared with controls without lithium.

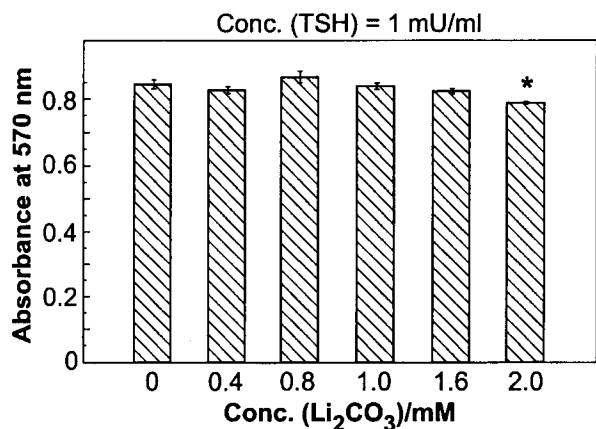


Fig. 3. Influence of lithium + TSH on proliferation of FRTL-5 cells (MTT assay). Bars represent average values of quadruplicate determinations  $\pm$  SD. Asterisks represent statistically significant ( $P < 0.05$ ) results when compared with controls without lithium.

In the absence of TSH, lithium at 1.4 mM, 1.7 mM and 2 mM concentrations significantly increased <sup>51</sup>Cr release from the FRTL-5 cells in the cytotoxicity assay (Fig. 4). These results are confirmed by the data shown in Table 1. The higher was the lithium concentration, the lower was the percentage of viable cells, as assessed with the semiquantitative trypan blue exclusion method (second column). Additionally, a higher percentage of specific lysis was observed at higher lithium concentrations (third column). Although lithium with 1 mU/ml of TSH did not influence <sup>51</sup>Cr release from FRTL-5 cells (Fig. 5), again, a higher percentage of specific lysis was observed at higher lithium concentrations (Table 1, fourth column).

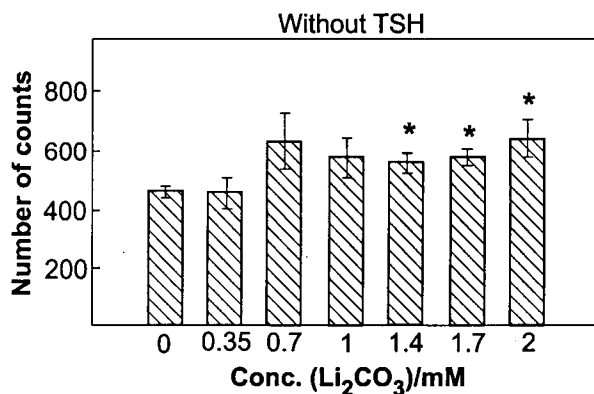


Fig. 4. Influence of lithium on <sup>51</sup>Cr release from FRTL-5 cells (cytotoxicity assay). Bars represent average values of quadruplicate determinations  $\pm$  SD. Asterisks represent statistically significant ( $P < 0.05$ ) results when compared with controls without lithium.

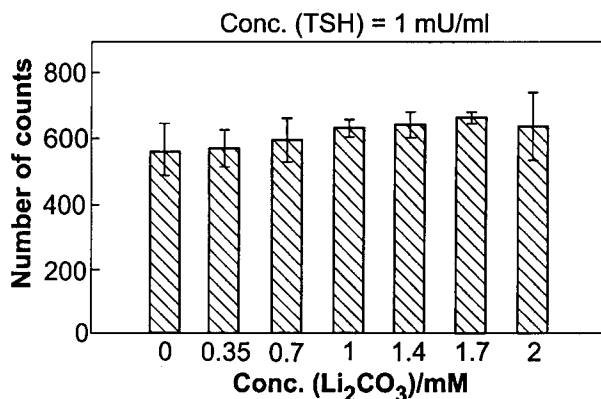


Fig. 5. Influence of lithium + TSH on <sup>51</sup>Cr release from FRTL-5 cells (cytotoxicity assay). Bars represent average values of quadruplicate determinations  $\pm$  SD.

Table 1. Viability (second column) and specific lysis without TSH (third column) and with TSH (fourth column) of FRTL-5 cells with respect to concentration of lithium as  $\text{Li}_2\text{CO}_3$  (first column).

Conc. ( $\text{Li}_2\text{CO}_3$ )/mM	% of viable cells	% of specific lysis – without TSH	% of specific lysis – with TSH
0	99.0	0	0
0.35	90.2	0.5	0.7
0.7	86.4	17.7	3.4
1	85.1	11.9	7.5
1.4	87.2	10.2	8.5
1.7	86.3	11.7	10.8
2	84.1	15.5	8.1

## Discussion

In the present study, we have confirmed the stimulative effect of selected lithium concentrations on proliferation of FRTL-5 cells, and, surprisingly, we have noticed an inhibitory effect of higher lithium concentrations on FRTL-5 cells proliferation under the stimulation by TSH. Additionally, a cytotoxic effect of higher lithium concentrations has been observed.

The results obtained by the  $^3\text{H}$ -thymidine incorporation assay and by the MTT assay in the absence of TSH stimulation confirm the *in vivo* observations that lithium stimulates proliferation of thyroid cells and, therefore, leads to enlargement of the thyroid gland (Perrild et al., 1984; Boccheta et al., 1991). These results are compatible with those obtained with porcine thyroid follicles (Tsuchiya et al., 1990), where the 0.5 mM concentration of lithium without TSH was found stimulative. Additionally, our results agree with a study of Urabe et al. (1991), where lithium without TSH stimulated proliferation of FRTL-5 cells. Tasevski et al. (2000) observed the same effect at concentrations equal or higher than 1 mM. Lithium has been shown to have an effect on thyroid cells through a number of cell-signalling pathways (Manji et al., 1995), but probably not through the stimulation of adenylate cyclase and the synthesis of cAMP (Wolff et al., 1970; Mori et al., 1989; Tsuchiya et al., 1990; Van Sande et al., 1990). The lithium ion could have influence on the thyroid cells partly through the protein kinase C system (Urabe et al., 1991) and, as recently shown by Tasevski et al. (2000), through *de novo* cholesterol synthesis and G-protein prenylation.

We have observed an inhibitory effect of a higher lithium concentration (2 mM) on FRTL-5 cells proliferation under stimulation by TSH. The results are partly in contradiction with the findings of Urabe et al. (1991), who found a significant stimulation of  $^3\text{H}$ -thymidine incorporation in FRTL-5 cells in the presence of 37  $\mu\text{U}/\text{ml}$  of TSH and at the 10 mM concentration of lithium. Our results are in agreement with the observation in porcine thyroid cells (Urabe et al., 1991), where thyroid cell growth in the presence of both 1 mU/ml of TSH and lithium was found to be suppressed when

compared with lithium alone. Presumably, the reduction of proliferation could be partly due to the inhibitory effect of higher lithium concentrations on cAMP synthesis, stimulated by TSH (Mori et al., 1989; Tsuchiya et al., 1990), and consequently, on cell proliferation, and partly due to the toxic effect of higher lithium concentrations (Kontozoglou and Mambo, 1983). Another possibility in the MTT assay is a changed mitochondrial activity rather than the decreased number of FRTL-5 cells. In the absence of TSH, the proliferative effect of lithium probably prevails over its cytotoxic effect. Only under the stimulation by TSH, which in this case controls the cell proliferation, the cytotoxic effect of lithium might become evident.

Indeed, the assumption of a cytotoxic effect of lithium has been confirmed by our results with the cytotoxicity assay without TSH, by the percentage of specific lysis and by the results obtained with the trypan blue exclusion method. Our results are in agreement with literature data, where several cases of lithium-associated thyroiditis have been reported. McLaren and Toft (1981) observed atypical changes in thyroid cell morphology, pleomorphism of the cells, disrupted architecture of the gland from a hyperthyroid patient treated with lithium and antithyroid drugs. Mizukami et al. (1995) published data on histopathological alterations of the thyroid gland with extensive follicular cell disruption. Lithium might directly damage thyroid follicular cells.

In conclusion, lithium was found to stimulate proliferation of FRTL-5 cells in conditions without TSH stimulation. Surprisingly, under the stimulation by TSH, lithium diminished proliferation of FRTL-5 cells. A cytotoxic effect of higher lithium concentrations in the absence of TSH was observed.

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