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EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON THE ELEMENTAL CONTENT OF RAT HEPATOCYTES

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SUMMARY: X-ray microanalysis has been used to determine elemental content in the cytoplasm of hepatocytes of hyperthyroid rats in comparison with euthyroid controls. No significant differences were found for any examined element (sodium, phosphorus, sulfur, chlorine, potassium, magnesium and calcium). In contrast to earlier reports from another laboratory, these data indicate that thyroid hormones do not substantially affect elemental content in rat hepatocytes.

INTRODUCTION. In a recent study (1) we found significantly lower Cl concentration (both in nucleus and in cytoplasm) and lower Na concentration (in nucleus only) in cardiocytes of Snell dwarf mice compared to phenotypically normal mice from the same strain. We concluded that lack of GH and PRL as well as deficiency of thyroid hormones in Snell dwarfs could be responsible for these elemental differences. Since all of these hormones have been reported previously to be involved in electrolyte distribution in animal tissues (2,3,4,5,6), we designed a series of experiments to investigate separately the effect of each of these hormones on intracellular elemental concentration. Thyroid hormones have been reported to produce similar changes in intracellular ionic concentrations in both skeletal and cardiac muscle as well as in liver slices (7,8). The present study employed the technique of electron probe X-ray microanalysis of freeze-dried, ultrathin tissue sections to examine the influence of experimental hyperthyroidism on the electrolyte content of the cytoplasm of rat hepatocytes.

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Abbreviations: ANOVA - analysis of variance, GH - growth hormone, PRL - prolactin, T_3 - triiodothyronine, T_4 - thyroxine.

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MATERIALS AND METHODS. Twelve Sprague-Dawley male rats weighing 200 ± 20 g each were used in the experiment. The animals were maintained in a room with controlled illumination (14 h light : 10 h dark, light on at 0600 h) and temperature (22 ± 2°C) and with constant access to standard laboratory rat chow and tap water. Six rats were made hyperthyroid by daily i.p. injection of 1.0 ml T_4 solution (L-Thyroxine, free acid, Sigma Chemical Co., 10 µg/ 100 g BW, dissolved in 0.9% NaCl containing 1% 0.1 N NaOH). The injections were continued for 12 days. Such a dose of T_4 has been shown previously to be sufficient for evoking the symptoms of thyrotoxicosis in rats (9). The remaining 6 rats served as controls and were injected with the corresponding volume of diluent. On the thirteenth day, the rats were sacrificed by decapitation. Small pieces of liver were quench-frozen in liquid propane. Thin (0.1 µm) cryosections were prepared at -100°C with an LKB cryoultramicrotome as previously reported (1). Freeze-dried sections between two layers of Formvar on a carbon slot grid were analyzed in the scanning transmission mode in a JEOL JSM-35 scanning electron microscope, using the same probe parameters, collection procedure, and data handling methods as previously reported (1). X-ray spectra were collected from the cytoplasm of 15 individual cells per animal. Data were statistically analyzed using the BMDP8V (10) computer program. A nested analysis of variance (ANOVA) was performed, using the following design: 2 treatment groups (controls and thyroxine-injected); 6 animals per group, nested within group; 15 cells per animal, nested within animal.

RESULTS AND DISCUSSION. The intracellular elemental concentrations and results of the statistical analyses are presented in Table 1. As shown, no statistically significant differences have been found between hyperthyroid and euthyroid animals for any element analyzed. However, it should be noted that Na and Cl concentrations were both somewhat lower in hepatocytes of T_4-injected rats. We have previously observed positive correlation between the concentrations of Na and Cl in various examined tissues (see 11 for review). Similar correlation was found in the present study. As a result of lower Na,

Table 1. Intracellular elemental concentration (mmole/kg dry weight) in cytoplasm of hepatocytes of control and hyperthyroid rats. Each value is the mean ± S.E.M. of 90 cells (6 animals, 15 cells per animal).

Element	Concentration		F_{group}	P_{group}	F_{animal}	P_{animal}
	Control	Hyperthyroid				
Na	280 ± 40	228 ± 35	1.43	0.26	10.54	<0.001
Cl	242 ± 30	208 ± 19	1.78	0.21	6.85	<0.001
K	392 ± 43	373 ± 27	0.18	0.68	14.32	<0.001
Mg	56 ± 18	53 ± 5	0.37	0.56	0.68	0.74
Ca	21 ± 9	20 ± 2	0.29	0.60	0.58	0.83
P	525 ± 81	556 ± 42	0.32	0.59	4.99	<0.001
S	151 ± 31	128 ± 15	0.71	0.42	11.74	<0.001

the intracellular Na/K ratio was about 14% lower in hyperthyroid animals when compared to saline-injected controls.

It is of interest to compare our results, which indicate that thyroid hormones do not substantially affect intracellular Na, Cl, and K distribution in rat hepatocytes, with results which have been reported by others. It has been hypothesized by one laboratory (7,8,12) that the increased energy consumption associated with thyroid calorigenesis is related to direct stimulation of the Na-K-pump, which leads to a decrease of intracellular Na and an increase of K. These investigators have reported (7) decreases in Na (25%) and Cl (25%), an increase in K (29%), and a resultant 43% decrease in the Na/K ratio in liver tissue of euthyroid rats to which T_3 was administered. They found changes similar in direction but of less significance for diaphragm muscle (7). In a later study (8), they reported no significant changes in electrolytes in diaphragm and cardiac muscle. Direct comparison of our results with those cited is not straightforward for several reasons. [1] They used atomic absorption spectrophotometry to measure Na and K and potentiometric titrimetry to measure Cl in whole tissue slices. [2] Their concentration calculations are based on corrections for extracellular space as measured by inulin-^{14}C, a technique which is of questionable accuracy and reliability (13). [3] Their tissue slices were incubated in modified Ringer's solution with inulin at 37°C for 30 min prior to sampling for analysis. It has been demonstrated by X-ray microanalysis (14,15) that even the slightest rinsing or processing of tissue leads to redistribution and loss of intracellular ions. When expressed in the same concentration units (mEq/kg dry weight) that we used and then compared, all Na, Cl and K values reported in the previous study (7) are low compared to those obtained in our study, indicating the possibility of leaching in the former case. [4] Their thyroid hormone dose (T_3, 50 μg/100 g BW, three times, on alternate days) was higher than that used in the present study.

It has been admitted (8) that the intracellular ionic concentrations in the previous series of studies were inexact since they were calculated from several measured values, including total tissue water, whole tissue electrolytes, serum

electrolytes, and plasma and tissue inulin-^{14}C assays. The method of electron probe X-ray microanalysis of intracellular elemental content overcomes many of these disadvantages but still has some problem with precision of the measurements, which is probably due mostly to the difficulty of reproducibility of such a complex preparative procedure for the tissue. The nested design ANOVA which we used made it possible to sort out variability in concentration measurements due to group effect (treatment), animals within groups, and cells within animals. As is seen in Table 1, the between-animal variability was highly significant ($P < 0.001$) for all elements except Ca and Mg (each of which was present near its minimum detectable level and hence was detected with high variability relative to its concentration level).

In the present study, the tendency of Na and Cl to decrease in hepatocytes of T_4-treated rats is consistent with the above-mentioned theory of calorigenesis. However, K concentration did not increase, as would be predicted by the theory. It should be pointed out that the theory that thyroid calorigenesis is mediated by direct stimulation of the Na-K-pump has been criticized (4,16,17,18) and hence its prediction of intracellular ion concentration alterations may not be valid.

In addition to direct stimulation of the Na-K-pump, one should also consider the possibility that passive movement of ions through the membrane is of importance in explaining the effect of thyroid hormones on intracellular ionic content. The intimate relationship of active transport and passive permeability is well known (19). It is also known that thyroid hormones can facilitate passive movement of ions across the cell membrane (4). One can hypothesize that these passive movements, with resultant elevation of intracellular Na and reduction of K, are the stimulus for activation of the Na-K-pump and subsequent decrease in Na and increase in K concentration. Since passive membrane permeability for K is much higher than that for Na (20), it is possible that thyroid hormone-induced efflux of K (initial loss of K) could be relatively greater than the simultaneous influx of Na (initial gain of Na) and that the Na-K-pump might not be capable of completely restoring the balance

(21). Such an interpretation is plausible in the present study, where we have found slightly lower Na and Cl but no increase in K in liver of T_4-treated rats. However, in a time course analysis of cation concentrations and Na-K-ATPase activity in rat heart after a single injection of T_3, no evidence was found that the T_3-dependent increase in Na-K-ATPase was a secondary, adaptive response to a prior effect of thyroid hormone on intracellular ion concentrations (5).

With regard to the lack of clearcut results regarding the effect of thyroid hormones on ionic concentrations in various tissues, it has been pointed out (8) that ion concentration may not be the critical parameter and that perhaps thyroid hormone-induced changes in ion activities at the pump site could be of greater magnitude than those revealed by measurements of changes in concentration of Na and K.

With respect to our previous study (1) regarding hormonal influence on intracellular ionic content, the results of the present study suggest that lack of GH and PRL rather than hypothyroidism was responsible for lower Na and Cl in cardiocytes of dwarf mice. However, it is unquestionable that in the case of severe hypothyroidism, as occurs in Snell hypopituitary mice, the deficiency of thyroid hormones results in salt wasting and in dilutional hyponatremia and hypochloremia. Possible mechanisms accounting for the hyponatremia in hypothyroidism include renal tubular dysfunction (22) or adrenocortical insufficiency (23). PRL seems to exert that most potent hormonal influence on electrolyte distribution in animal tissues (see 24 for review), and its absence in Snell dwarfs appears to account for the changes in intracellular elemental content.

Our present results lead us to hypothesize that if an excess of thyroid hormones affects electrolyte distribution at all, the action is exerted directly on cells rather than on general regulatory mechanisms. Some other reports (25, 26, 27) have shown changes in concentrations of ions other than Na and Cl (mostly in the extracellular fluid) due to an excess of thyroid hormones. In the present study, we were not able to demonstrate changes of intracellular concentration for K, P, S or Ca in hepatocytes of thyroxine-injected rats. In

conclusion, the results obtained in the present study cast doubt on the substantial role which thyroid hormones have been purported to play in electrolyte distribution in animal tissues. Further studies are required to explain in detail the role of thyroid hormones in this respect.

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