intestinal mucosa, greatly increasing the utilization of glucose and production of lactate in a similar *in vitro* preparation(8). It would seem unlikely however, that stimulation of glycolysis would inhibit bile salt uptake.

Summary. Studies on the effect of Tween 80 on bile salt absorption *in vitro* do not support the hypothesis that this surface active detergent enhances intestinal absorption of bile salts. At high concentrations of Tween 80 inhibition of absorption occurred.

1. Jones, C. M., Culver, P. J., Drummey, B. S., Ryan, A. E., *Ann. Int. Med.,* 1948, v29, 1.

2. Du Bois, J. J., Holt, P. R., Kuron, G. W., Hashim, S. A., Van Itallie, T. B., PROC. SOC. EXP. BIOL. AND MED., 1964, v117, 226.

3. Norman, A., *Arkiv. Kemi,* 1955, v8, 331.

4. Holt, P. R., *Am. J. Physiol.,* 1964, v207, 1.

5. Wilson, T. H., Wiseman, G., *J. Physiol.,* 1954, v123, 116.

6. Culver, P. J., Wilcox, C. S., Jones, C. M., Rose, R. S., *J. Pharmacol. Exp. Therap.,* 1951, v103, 337.

7. Nissim, J. A., *Nature* (London), 1960, v187, 305.

8. Holt, P. R., Haessler, H. A., Isselbacher, K. J., *J. Clin. Invest.,* 1963, v42, 777.

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Effect of Toxic Doses of L-Thyroxine on Tissue Water, Electrolytes and Plasma Protein in Rats. (29544)

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The metabolic stimulating effect of thyroid hormones has long been recognized. The functional capacity and biochemical composition of the heart, liver and skeletal muscle were found to be altered in hyperthyroidism(1-3). In thyrotoxicosis, changes in the heart are characterized by an increase in rate and strength of contraction, an elevated cardiac output and development of myocardial hypertrophy. Among other pathological conditions in human subjects or animals, liver glycogen depletion and muscular weakness are most commonly seen(4). The purpose of this work was to study and reevaluate the toxicity of L-thyroxine as concerned with electrolyte and water distribution patterns in various organs as well as the changes in plasma protein fractions of rats.

Methods and materials. The 25 male Sprague-Dawley rats used in this study weighed from 250-350 g. All animals were kept in separate cages and maintained on Purina chow *ad libitum.* Thirteen rats received a daily subcutaneous injection of 0.5 ml of 0.02 N NaOH containing 0.5 mg of L-thyroxine, whereas 12 rats were injected with the same alkaline solution without thyroxine. Administration of thyroxine was continued for 24 days when one of the experimental rats died. At 25 days, the rectal temperature of all surviving animals was measured with a thermistor probe and was followed by injection of an anesthetic dose of Na pentobarbital intraperitoneally (40 mg/kg in 1.5% solution). Blood samples were taken from the dorsal aorta. As soon as an adequate amount of blood was withdrawn into a heparinized syringe, the ventricles of the heart, random muscle samples of both hind limbs and the whole liver were immediately taken for biochemical analyses.

Hematocrit was determined by a microcapillary technique. Plasma was separated from the red blood cells by centrifugation at $4500 \times g$ for 10 min. Glucose concentration was measured immediately in plasma and packed red blood cells according to Nelson-Somogyi's method(5,6). Total plasma protein was determined(7) and the individual protein fractions were separated by paper electrophoresis(8). Plasma and tissue Na^+ and K^+ were determined in a Perkin-Elmer flame

photometer with lithium (10.1 mEq Li/liter) as an internal standard(9). Chloride was measured by the method of Cotlove *et al* (10). The plasma Ca^{++} and Mg^{++} were extracted with an equal volume of 10% TCA and the extract was titrated with EDTA(11).

Immediately after the surface blood of tissue samples was blotted and the visible connective tissue removed, approximately 0.5 g of muscle and liver were placed into 80% methanol. Tissue glycogen was analyzed by the method of Kemp *et al*(12). Water content in the tissues was determined by drying 0.5-1.0 g samples to a constant weight in an oven at 110°C. Plasma and red blood cell water contents were measured by the same procedure. Tissue total lipids were extracted with 2:1 (v/v) chloroform and methanol (13) and the lipid extract was further purified according to Folch's procedures(14). Weight of total lipids was measured gravimetrically after complete evaporation of the purified extract.

Techniques for a single extraction and determination of liver and muscle electrolytes have been described(15). The intracellular concentration of skeletal muscle electrolytes and water was calculated according to Benson *et al*(16). Because of the small size of the rat ventricular muscle, all determinations were made using the same sample. Water and lipid content of the heart were determined first and the dried specimens were ashed in a furnace at 600°C for 6 hours. The Na^+ and K^+ of the cardiac muscle-ash was dissolved in 10 ml of lithium (10.1 mEq Li/liter) for flame photometric determinations.

All values were analyzed statistically. The "Null" hypothesis was rejected at 5% level and a P value of 0.05 or less was considered significant.

Results. Rectal temperature. Rectal temperature of rats was elevated from 37.5 to 38.5°C ($P<0.01$) upon injection of a toxic dose of L-thyroxine for 25 days (Table I).

Plasma and red blood cells. Despite the fact that plasma Na^+ and K^+ concentrations remain unchanged, the concentration of plasma Cl^- and water content increased ($P<0.05$, <0.001) and plasma levels of Ca^{++}, Mg^{++}, glucose and total protein decreased ($P<0.001$, <0.05, <0.01, <0.001) (Tables I and II). Alpha 1 and gamma globulins were diminished significantly ($P<0.001$, <0.02), but there was no significant change in concentrations of albumin, alpha 2 and β-globulins nor A/G ratios (Table II).

Hematocrit was elevated in the thyroxine-injected animals as compared to the normal animals ($P<0.05$)(Table I). In the former group, glucose concentration in red blood

TABLE I. Effect of l-Thyroxine on Rectal Temperature, Electrolyte, Water and Glucose Concentrations in Plasma and Erythrocytes.†

Group	Rectal temperature °C	Plasma							Erythrocyte		
		Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	H_2O	Glucose	H_2O	Glucose	Hematocrit
		mEq/liter					g%	mg%	g%	mg%	
Control (12 rats)	37.5 ±.5	136.6 ±2.2	5.4 ±.4	5.2 ±.2	7.1 ±.3	105.9 ±2.3	92.9 ±.1	143.8 ±14.9	56.8 ±2.8	34.1 ±7.5	40.6 ±.99
Thyroxine (12 rats)	38.5* ±1.1	138.6 ±3.6	5.8 ±.8	4.3* ±.2	6.8* ±.3	108.2* ±2.1	93.9* ±.2	122.1* ±12.0	62.8* ±3.6	26.6* ±6.6	43.8* ±2.4

* Statistically significant.
† All values are means ± standard deviation.

TABLE II. Changes of Total and Individual Plasma Proteins in Thyroxine-Injected Rate (g%).†

Group	Total protein	Albumin	Globulins α_1	α_2	β	γ	A/G ratio
Control (12 rats)	6.7 ±.43	3.3 ±.41	.92 ±.28	.51 ±.12	1.2 ±.12	.91 ±.26	.96 ±.26
Thyroxine (12 rats)	5.8* ±.35	3.0 ±.29	.35 ±.06	.57 ±.16	1.2 ±.15	.68* ±.16	1.09 ±.15

* Statistically significant.

† All values are means ± standard deviation.

cells was reduced ($P<0.05$) whereas water content was increased ($P<0.001$) (Table I). It should be noted that the glucose content in the red blood cells is much lower than in the plasma for both groups of animals studied.

Cardiac and skeletal muscles. Response of the rat heart to injected thyroxine is characterized by an increased weight and a reduced water content ($P<0.001$, <0.05) (Table III). Concentrations of cardiac Na^+, K^+ and total lipids were not significantly altered. On the other hand, skeletal muscles showed a considerable change in water and electrolyte distribution pattern (Table IV). Intracellular Na^+ and Ca^{++} concentrations were elevated ($P<0.02$, <0.001) and a diminished intracellular K^+ and a water content were observed ($P<0.001$, <0.001). No change was found for glycogen, total lipids or Mg^{++} concentrations in skeletal muscle of the thyroxine-treated animals.

Liver. Besides a drastically reduced liver glycogen in the hyperthyroid rats ($P<0.001$), there was also a significant reduction of total liver K^+ and Ca^{++} concentrations ($P<0.01$, <0.01) (Table V). The reduction of liver K^+ and Ca^{++} levels was associated with elevation of liver Na^+, Mg^{++} and water contents ($P<0.001$, <0.05, <0.001). Liver total lipids and Cl^- concentrations were found unchanged in the thyroxine-injected rats.

Discussion. Although thyroxine is known as a stimulant which increases cellular metabolism, little work has been done concerning the action of thyroxine in altering the distribution patterns of electrolyte and water in animals(17). The heart is recognized to be one of the most susceptible organs in hyperthyroidism. In this study it was demonstrated that Na^+ and K^+ concentrations in the hypertrophic heart of the thyroxine-injected rat were not altered even though cardiac dehydration and tachycardia were observed.

Millikan and Haines(4) pointed out that the effect of thyroxine appears to be primarily upon intrinsic muscle metabolism rather than upon the nervous system. Zierler

TABLE III. Effect of l-Thyroxine on Changes in the Rat Heart.†

Group	Na^+ (mEq/kg fat-free wet tissue)	K^+ (mEq/kg fat-free wet tissue)	H_2O g%	Total lipids g%	Wt of ventricle g
Control (12 rats)	33.5 ±1.3	71.3 ±4.6	77.1 ±.3	1.4 ±.2	.85 ±.08
Thyroxine (12 rats)	32.0 ±3.2	72.0 ±3.6	76.1* ±.4	1.6 ±.2	1.02* ±.09

* Statistically significant.

† All values are means ± standard deviation.

TABLE IV. Effect of l-Thyroxine on Electrolyte, Glycogen and Total Lipid Concentrations in the Rat Skeletal Muscle.†

Group	$(Na)_T$	$(K)_T$	$(Ca)_T$	$(Mg)_T$	$(Cl)_T$	$(H_2O)_T$	Glycogen, mg%
Control (12 rats)	23.7 ±2.1	155.0 ±9.8	5.6 ±.55	33.0 ±2.1	13.8 ±1.7	762.0 ±5.6	351.8 ±49.4
Thyroxine (12 rats)	30.1* ±1.8	132.0* ±11.1	10.0* ±1.8	31.3 ±2.7	16.1* ±1.8	749.0* ±4.2	320.9 ±113.0
	$[Na]_I$	$[K]_I$	$[Ca]_I$	$[Mg]_I$		$(H_2O)_I$	Total lipids, g%
Control	17.6 ±4.5	223.1 ±11.3	7.9 ±.96	47.7 ±2.7		692.1 ±18.3	1.7 ±.34
Thyroxine	22.8* ±4.3	201.3* ±14.6	13.6* ±3.8	46.9 ±4.9		660.8* ±8.6	1.7 ±.30

* Statistically significant. † All values are means ± standard deviation.
$(\ )_T$ = Total electrolyte mEq/kg fat-free wet tissue.
$[\ ]_I$ = Intracellular electrolyte mEq/kg intracellular water.
$(H_2O)_T$ = Total water g/kg fat-free wet tissue.
$(H_2O)_I$ = Intracellular water g/kg fat-free wet tissue.

TABLE V. Effect of l-Thyroxine on Rat Liver Electrolyte, Water, Glycogen and Total Lipid Concentrations.†

Group	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	H_2O	Glycogen	Total lipids
	mEq/kg fat-free wet tissue					g%	g%	g%
Control (12 rats)	30.6 ±3.8	117.5 ±5.3	2.3 ±.25	19.2 ±2.3	29.2 ±1.8	69.4 ±.32	4.8 ±2.1	4.8 ±.45
Thyroxine (12 rats)	37.3* ±2.3	107.9* ±9.9	2.0* ±.25	21.3* ±2.0	28.8 ±2.0	71.5* ±1.6	.30* ±.18	4.8 ±.53

* Statistically significant. † All values are means ± standard deviation.

(18) and Thorn and Eder(19) suggested that muscular disease in hyperthyroidism may be caused by an excess synthesis of creatine. An inadequate utilization or storage of creatine in the involved muscle produces hypercreatinemia and creatinuria. However, the severity of the thyrotoxic myopathy did not prove to have any correlation with the amount of creatine excreted in urine (20). Similar findings were also demonstrated in human hyperthyroid patients by Satoyoshi *et al*(21). In the present study, the alteration of water and certain electrolyte concentrations in the skeletal muscle of the thyroxine-injected rat may partially explain how the muscular disease is developed. Thus, it may be postulated that during hyperthyroidism the energy supply mechanism is impaired as indicated by an unchanged glycogen content and an accumulation of Na^+ and Ca^{++} within the muscle cells. It is also possible that the cellular operation of "sodium pump" and "calcium pump" become less effective in thyrotoxicosis.

If excess of thyroxine is present in the circulation, liver damage may result. Clinically, liver cirrhosis with fatty infiltration is observed in severe thyrotoxicosis(22). In our study, we failed to confirm such findings in rats through evidence that liver total lipids, plasma albumin and A/G ratios in the thyroxine-injected rats were not significantly altered. Nevertheless, the experimental animals showed a decreased concentration of total plasma protein, alpha 1 and gamma globulins. This may indicate some damage to the liver and reticulo-endothelial cells(23). Drill and Gunn(24) were unable to show any significant histological changes in the hyperthyroid rat liver except a depletion of liver glycogen. Drastic reduction of liver glycogen was also demonstrated in this study. This may be due to an hepatic defensive mechanism(25) or a reduction of lactic dehydrogenase activity(26). The increased liver water content in the thyroxine-injected rats may imply that the liver was congested with blood.

The decreased plasma glucose concentration resulting from injection of L-thyroxine in rats is probably due to an increased utilization of tissue glucose(27). The diminished erythrocyte glucose concentration might be due to an expansion of cellular water, a reduction of plasma glucose or a direct increased utilization of glucose within the red blood cells. Since plasma water content is elevated in the thyroxine-injected rats, the increased hematocrit is not due to a dehydration of the blood. Meineke and Crafts(28) demonstrated an increased oxygen consumption and total erythrocyte volume in thyroxine-treated hypophysectomized rats. Furthermore, Chang(29) observed an increased blood volume in the patients who suffered from hyperthyroidism. It is possible that thyroxine acts directly on the bone marrow and stimulates the process of hemopoiesis.

Summary. 1. Rats injected with L-thyroxine revealed an increased hematocrit and plasma water content. Concentrations of plasma Ca^{++}, Mg^{++}, glucose, total protein and erythrocyte glucose were reduced. No change was found in plasma levels of Na^{+} and K^{+}. 2. By fractionation of total plasma protein in the thyroxine-injected rats, reductions in alpha 1 and gamma globulins were observed. Concentrations of albumin, alpha 2 and β-globulins as well as A/G ratios were unaltered. 3. Weights of the ventricles of the heart were increased. Concentrations of cardiac Na^{+}, K^{+} and total lipids were not changed. 4. Despite a dehydration of the muscle cells, a reduced concentration of intracellular K^{+} and an elevation of Na^{+}, Cl^{-} and Ca^{++} in the hyperthyroid state occurred. Muscle Mg^{++}, glycogen and total lipid levels were not modified. 5. The liver showed increased concentrations of water, Na^{+} and Mg^{+} which corresponded with a reduction of liver glycogen, K^{+} and Ca^{++} contents. The liver total lipids remained unchanged.

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1. Andrus, E. C., *Am. Heart J.,* 1932, v8, 66.
2. McIver, M. A., *Surgery,* 1942, v12, 654.
3. Morgan, H. J., Williams, R. H., *Southern Med. J.,* 1940, v33, 261.
4. Millikan, C. H., Haines, S. F., *A.M.A. Arch. Int. Med.,* 1953, v92, 5.
5. Nelson, N., *J. Biol. Chem.,* 1944, v153, 375.
6. Somogyi, M., *ibid.,* 1952, v195, 19.
7. Kingsley, G. R., *J. Lab. Clin. Med.,* 1942, v27, 840.
8. Dunn, W. L., Pearce, R. H., *Canad. M. A. J.,* 1961, v84, 272.
9. Overman, R. R., Davis, A. K., *J. Biol. Chem.,* 1947, v168, 641.
10. Cotlove, E., Trantham, H. V., Bowman, R. L., *J. Lab. Clin. Med.,* 1958, v51, 461.
11. Friedman, H. S., Rubin, M. A., *Clin. Chem.,* 1955, v1, 125.
12. Kemp, A., Adrienne, J. M., Van Heijninger, K., *Biochem. J.,* 1954, v56, 646.
13. Folch, J., Ascoli, I., Lees, M., Meath, J. A., Le Baron, F. N., *J. Biol. Chem.,* 1951, v191, 833.
14. Folch, J., Lees, M., Sloane Stanley, G. H., *ibid.,* 1957, v226, 497.
15. Liu, C. T., Ph.D. Thesis, Univ. of Tennessee, Memphis.
16. Benson, E. S., Freier, E. F., Hallaway, B. E., Johnson, M. J., *Am. J. Physiol.,* 1956, v187, 483.
17. Timiras, P. S., Woodbury, D. M., Agarwal, S. L., *J. Pharm. Exp. Therap.,* 1956, v115, 154.
18. Zierler, K. L., *Bull. Johns Hopkins Hosp.,* 1951, v89, 263.
19. Thorn, G. W., Eder, H. A., *Am. J. Med.,* 1946, v1, 583.
20. Hed, R., Kirstein, L., Lundmark, C., *Neurol. Neurosurg. Psychiat.,* 1958, v21, 270.
21. Satoyoshi, E., Murakami, K., Kowa, H., Kinoshita, M., Noguchi, K., Hoshina, S., Nishiyama, Y., Ito, K., *Neurology,* 1963, v13, 645.
22. Shaffer, I. M., *Arch. Path.,* 1940, v29, 20.
23. Grant, G. H., Wallace, W. D., *Lancet,* 1954, v2, 671.
24. Drill, V. A., Gunn, F. D., *Endocrinology,* 1944, v35, 477.
25. Lichtman, S. S., *Ann. Int. Med.,* 1941, v14, 1199.
26. Vestling, C. S., Knoepfelmacher, A. A., *J. Biol. Chem.,* 1950, v183, 63.
27. Mirsky, I. A., Broh-Kahn, R. H., *Am. J. Physiol.,* 1936, v117, 6.
28. Meineke, H. A., Crafts, R. C., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 121.
29. Chang, H. C., *J. Clin. Invest.,* 1931, v10, 475.

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