Thyroid Thermogenesis

RELATIONSHIPS BETWEEN Na^+-DEPENDENT RESPIRATION AND $Na^+ + K^+$-ADENOSINE TRIPHOSPHATASE ACTIVITY IN RAT SKELETAL MUSCLE

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ABSTRACT The effect of thyroid status on Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity was examined in rat skeletal muscle. $Q_{O_2}(t)$ (i.e. Na^+-transport-dependent respiration) was estimated with ouabain or Na^+-free media supplemented with K^+. In contrast to the effects of ouabain on ion composition, intracellular K^+ was maintained at about 125 meq/liter, and intracellular Na^+ was almost nil in the Na^+-free media. The estimates of $Q_{O_2}(t)$ were independent of the considerable differences in tissue ion concentrations. The increase in $Q_{O_2}(t)$ accounted for 47% of the increase in Q_{O_2} in the transition from the hypothyroid to the euthyroid state and 84% of the increase in the transition from the euthyroid to the hyperthyroid state. Surgical thyroidectomy lowered NaK-ATPase activity of the microsomal fraction (expressed per milligram protein) 32%; injections of triiodothyronine (T_3) increased this activity 75% in initially hypothyroid rats and 26% in initially euthyroid rats. Thyroidectomy was attended by significant falls in serum Ca and P_i concentrations. Administration of T_3 resulted in further declines in serum Ca and marked increases in serum P_i concentrations. Similar effects were seen in ^{131}I-treated rats, but the magnitude of the declines in serum Ca were less. The effects of T_3 on Q_{O_2}, $Q_{O_2}(t)$, and NaK-ATPase activity of skeletal muscle were indistinguishable in the ^{131}I-ablated and surgically thyroidectomized rats.

In thyroidectomized or euthyroid rats given repeated doses of T_3, $Q_{O_2}(t)$ and NaK-ATPase activity increased proportionately. In thyroidectomized rats injected with single doses of T_3, either 10, 50, or 250 μg/100 g body wt, $Q_{O_2}(t)$ increased linearly with NaK-ATPase activity. The kinetics of NaK-ATPase activity were assessed with an ATP-regenerating system. T_3 elicited a significant increase in V_{max} with no change in K_m for ATP.

INTRODUCTION

Evidence has been presented that increased energy expenditure for transmembrane active Na^+ transport mediates a significant fraction of the thermogenic response to thyroid hormone (1-4). The proposal that Na^+ transport is a significant metabolic pacemaker in thyroid thermogenesis is based in part on the inference that mitochondrial oxidation remains coupled to phosphorylation in various thyroid states (1, 2). Thus, thyroid-dependent increases in hydrolysis of ATP linked to Na^+ transport would increase the rate of formation of ADP and P_i and the ADP and P_i so generated would pace mitochondrial oxidative activity, if substrate and O_2 remain in adequate supply.

Two techniques were used in earlier studies to esti-

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mate the respiration linked to Na^+ transport [$Qo_2(t)$]:[1] addition of ouabain to or removal of Na^+ from the media (1, 2). In liver, about 90% of the triiodothyronine (T_3)-dependent increase in Qo_2 in thyroidectomized or euthyroid rats was attributable to the increase in $Qo_2(t)$ (2). In kidney, the increase in $Qo_2(t)$ accounted for 29% of the increase in Qo_2 in thyroidectomized rats and 46% of the increase in Qo_2 in euthyroid rats. These respiratory effects were accompanied by a 54% increase in NaK-ATPase activity in crude liver homogenates after administration of T_3 to hypothyroid rats and an 81% increase in the activity of this enzyme in homogenates from similarly treated euthyroid rats. Corresponding results were obtained in kidney homogenates; T_3 produced a 69% increase in renal NaK-ATPase activity in hypothyroid rats and a 21% increase in euthyroid rats. These results supported the inference that thyroid hormone enhanced energy utilization linked to Na^+ transport by activation of the Na^+ pump, either directly or indirectly.

Liver, kidney, gastrointestinal mucosa, heart, smooth muscle, and skeletal muscle are well-characterized thermogenic targets of thyroid hormone (5). In contrast, Qo_2 of brain, spleen, or testis is not dependent on thyroid status (5, 6). Since skeletal muscle is the most abundant tissue in mammals [e.g., skeletal muscle constitutes about 40% of the body weight in man (7)], the thermogenic response of skeletal muscle is one of the important determinants of the respiratory response of the whole animal to thyroid hormone. The available evidence, although limited, suggests that modulations in active Na^+ transport also contribute significantly to thyroid thermogenesis in this tissue (1, 3). In T_3-treated, thyroidectomized rats the increase in $Qo_2(t)$ accounted for 45% of the increase in Qo_2 of diaphragm, and in euthyroid rats it accounted for 90% of this increase (1). Administration of T_3 to thyroidectomized and euthyroid rats decreased intracellular Na^+ and increased intracellular K^+ concentrations of skeletal muscle without a discernible effect on serum Na^+, K^+, or Cl^- concentrations (3). Information on the effects of thyroid hormone on NaK-ATPase activity of skeletal muscle, however, has not yet appeared. In this report, we describe the effects of T_3 on respiration and NaK-ATPase activity in this tissue.

METHODS

Animal preparations. All of the experiments were on male, Sprague-Dawley rats maintained on Purina chow (Ralston Purina Co., St. Louis, Mo.) ad libitum or on a low-iodine diet (see below). In preliminary studies, we found that the Qo_2 varied inversely with the thickness of the diaphragm when obtained from rats that weighed more than 150 g. Thus, all of the rats were selected to weigh 120–140 g at the time of the experiment.

Hypothyroidism was produced either surgically or by administration of $Na^{131}I$. Surgical thyroidectomy was performed on rats weighing 75–100 g, and the animals were used 1–4 wk postoperatively. Radiation ablation of the thyroid gland was achieved by placing rats, weighing 25 g, on a Remington low-iodine diet for 10 days and then injecting 600 μCi of carrier-free $Na^{131}I$/100 g body wt, intraperitoneally. The low-iodine diet was discontinued 24 h after injection, and the rats were maintained on the standard Purina chow diet until use 4–5 wk later. Success in producing hypothyroidism 2 wk after surgery or 4–5 wk after administration of ^{131}I was judged by three criteria: (*a*) body weight increased less than 5 g/wk, (*b*) the concentration of thyroxine iodine in serum was less than 1 μg/100 ml, and (*c*) the resting heart rate, measured with the electrocardiogram, was less than 50% of that of the euthyroid controls. Serum thyroxine-iodine concentrations were estimated by column chromatography with the C:T_4 kit (Curtis Nuclear Corporation, Los Angeles) (8).[2]

O_2 consumption. Unanesthetized hypothyroid, euthyroid, or T_3-treated rats were decapitated instantly with a guillotine. The muscle segments of the diaphragms were rapidly freed from the rib attachments and the central connective tissue by sharp dissection. Quarter segments were transferred immediately into the flasks of a Warburg respirometer (American Instrument Co., Silver Spring, Md.). The standard Na^+-Ringer's solution contained: Na^+, 135; K^+, 5.0; Mg^{++}, 0.5; Ca^{++}, 1.0; Cl^-, 139; $H_2PO_4^-$, 5.0; glucose, 10 (all in mM), pH 7.40, and osmolarity, 290 mosM. Rates of respiration were measured at 15–30 min intervals at 37°C for 1 h (9). Sodium-independent respiration (Qo'_2) was measured in parallel incubations by addition of ouabain (10^{-3} M, final concentration) to K^+-free Ringer's solution (NaCl substituted for KCl) or in Na^+-free solutions prepared by isoosmolar substitution of sucrose or choline Cl for NaCl, with supplemental KCl. $Qo_2(t)$ was computed as the difference between Qo_2 and Qo'_2 of quarter diaphragms from the same rat (1, 10). At the end of the incubations, the quarter segments were removed from the flasks, blotted briefly with filter paper, and transferred to tared aluminum cups, and the dry weights were determined gravimetrically after being heated to 91°C for 24 h. Reproducibility of the measurements of Qo_2 in the four quarter segments of diaphragm taken from single rats (euthyroid) was assessed either in the absence or in the presence of ouabain (10^{-3} M). The mean of the paired differences between these quarter segments was 0.68 μl/mg dry wt/h (range = 0.42–0.95; $n = 4$). This variability is small compared to the differences in diaphragmatic Qo_2 obtained from different rats in diverse thyroid states (cf. Table I, II, VII, IX, and XI).

Tissue electrolyte concentrations. Quarter segments of diaphragm were incubated in standard Na^+-Ringers solution containing 0.2 μCi/ml of [*carboxyl*-^{14}C]inulin at 37°C in the Warburg respirometer. Preliminary studies established that steady-state penetration of [^{14}C]inulin into the tissue was achieved in 60–90 min of incubation and that Qo_2 was constant up to 90 min. Thus, the segments were incubated for 90 min with [^{14}C]inulin in either standard Na^+-Ringers solution, or in K^+-free Ringers solution supplemented with ouabain (10^{-3} M), or in Na^+-free Ringers

[1] *Abbreviations used in this paper:* Mg-ATPase, Mg^{++}-activated ATPase; NaK-ATPase, $Na^+ + K^+$-activated ATPase; Qo_2, total tissue oxygen consumption; Qo'_2, Na^+-transport-independent oxygen consumption; $Qo_2(t)$, Na^+-transport-dependent oxygen consumption; T_3, L-3,5,3′-triiodothyronine.

[2] These measurements were made by the Lazaroni Medical Laboratories, San Francisco.

solution containing choline Cl or sucrose with additional KCl. The Na^+-free media were isoosmotic with the standard Na^+-Ringers solution. The segments were then blotted briefly and transferred to tared aluminum cups (9 mm diameter, about 10 mg) that had been pretreated with 0.1 N HNO_3 as described previously (11) and weighed. The segments were dried at 91°C for 24 h, reweighed, and transferred to stoppered Pyrex tubes each containing 1 ml of 0.1 N HNO_3 and shaken for 48 h at room temperature (12). Aliquots of the acid extracts and of the bathing media were analyzed for [^{14}C]inulin content in a liquid scintillation spectrometer (Mark I, Nuclear Chicago, Searle Analytic Inc., Des Plaines, Ill.) (1, 2) and for Na^+ and K^+ content by atomic absorption spectrometry (Model 303, Perkin-Elmer Corp., Norwalk, Conn.) (13).

ATPase assays. Skeletal muscle microsomal fractions were prepared by the method of Rogus et al. (14) with some modifications. All of the steps in the procedure were carried out at 0–4°C. Approximately 1 g of muscle was trimmed from the gastrocnemius of rats whose diaphragms were used for measurement of Q_{O_2}. Visible fat and connective tissue were removed by sharp dissection, and the muscle was weighed and minced with scissors into 6 ml of ice-cold homogenization medium (mannitol, 250 mM; histidine, 30 mM; Tris-EDTA, 5 mM; and Tris deoxycholate, 1%; pH 6.8). The suspension was homogenized in a Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) at setting 5 (medium speed) for 10 s and then resheared for another 10 s. The mixture was transferred to an all-glass Elvehjem-Potter homogenizer and homogenized further with five to seven full strokes of the motor-driven pestle. The homogenate was centrifuged at 9,000 *g* for 20 min. The supernates were collected and stored at −20°C for 18–24 h. These supernates were then thawed and centrifuged at 100,000 *g* for 35 min in a Spinco Model-L Ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The pellets were resuspended in 9 ml of medium (containing mannitol, 250 mM; EDTA, 1 mM; Tris, 125 mM; pH 7.2), by brief homogenization (two or three strokes) in a Teflon-glass Elvehjem-Potter homogenizer. The suspensions were centrifuged at 20,000 *g* for 50 min, and the pellets were resuspended in 6–8 ml of Tris EDTA (1 mM, pH 7.4). The ATPase assays were completed immediately after preparation of these fractions.

The assay medium contained NaCl, 100; KCl, 10; $MgCl_2$, 5; EDTA, 0.1; Tris-ATP, 5; Tris, 50; (all in mM); pH 7.4. 30–80 μg of enzyme protein was added to 2.0 ml of the medium with or without 1 mM ouabain (final concentration). The mixtures were incubated at 37°C for 15 min. Preliminary studies indicated that NaK- and Mg-ATPase activities were linear with protein content up to 100 μg/2 ml and with time up to 15 min. To estimate the ATP-dependent K_m and V_{max} of the NaK-ATPase reaction, the incubation mixture contained a regenerating system consisting of 4 mM phosphoenolpyruvate, 50 μg pyruvate kinase, and variable concentrations of Mg^{++} and ATP (1:1) as described previously (15). The use of a fixed (1:1) ratio of Mg^{++}:ATP is in accord with previous studies (15). The reactions were terminated by addition of 2.0 ml of 10% ice-cold trichloracetic acid (TCA). The mixture was centrifuged at 10,000 *g* for 10 min at 0–4°C, and the orthophosphate content of the supernate was determined by the method of Fiske and Subbarow (16). Protein content of the TCA precipitate was determined by the method of Lowry et al. (17). NaK-ATPase activity was computed as the difference between total activity (without ouabain) and Mg-ATPase activity (with ouabain).

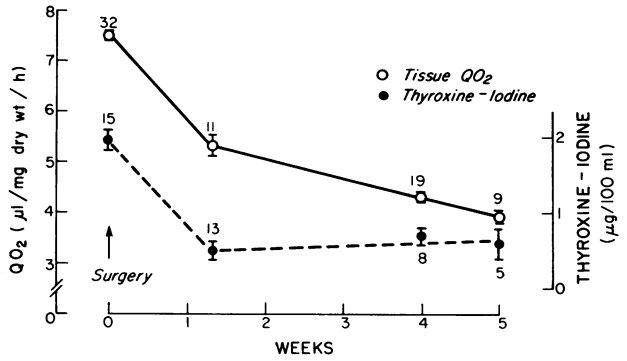

FIGURE 1 Q_{O_2} of rat skeletal muscle and serum thyroxine-iodine concentrations after surgical thyroidectomy. The vertical bar represents±1 SEM. The number of rats used for each point are indicated in the figure.

Serum calcium and inorganic phosphorus (P_i) concentrations. The rats were anesthetized with Inactin [5-ethyl-5-(1′-methyl propyl)-2-Na thiobarbiturate], 8 mg/100 g body wt, 48 h after the last injection of T_3 or diluent). Blood was collected in dry test tubes by percutaneous cardiac puncture and allowed to clot at room temperature. The supernatant serum was analyzed for total Ca by atomic absorption spectrometry and for P_i, total protein, and albumin in the AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (18, 19).[3]

Statistical calculations. The data are presented as means ±SEM and evaluated for significance by the unpaired Student *t* test. A P value < 0.05 was considered statistically significant.

Materials. All of the conventional reagents were analytical grade obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Na-L-3,5,3′-triiodothyronine, ouabain, and mannitol were obtained from Calbiochem, San Diego, Calif.; NaATP, Tris-ATP, choline Cl, histidine, phosphoenolpyruvic acid, and EDTA from Sigma Chemical Co., Inc., St. Louis, Mo.; Tris-base from Schwarz/Mann Div., Becton, Dickinson, & Co., Orangeburg, N. Y.; Inactin from Promonta, Hamburg; pyruvate kinase from Boehringer Mannheim Corp., New York; Remington low-iodine diet from General Biochemicals, Chagrin Falls, Ohio; and $Na^{131}I$ (25 Ci/mg) and [*carboxyl*-^{14}C]inulin (2 mCi/g) from New England Nuclear Corp., Boston, Mass.

RESULTS

Evaluation of hypothyroid status. Thyroidectomized rats were used from 1 to 4 wk after surgery. The differences in thyroid status over this time span were evaluated by measuring diaphragmatic Q_{O_2} and serum thyroxine-iodine concentrations. The results in Fig. 1 show that serum thyroxine-iodine concentrations were reduced from the normal value of 1.9 μg/100 ml to 0.5 μg/100 ml on the 9th postoperative day and remained at this low level thereafter. Diaphragmatic Q_{O_2} fell significantly by day 9 but continued to decline during the 1st to the

[3] These measurements were performed by the Central Laboratories of the University of California Medical Center at San Francisco.

TABLE I

Effects of T_3 on Respiration of Diaphragm from Thyroidectomized Rats

Rats	Medium	Na^+	K^+	Qo_2			Qo_2'			$Qo_2(t)$			$\Delta Qo_2(t)/\Delta Qo_2$
				$-T_3$	$+T_3$	Δ	$-T_3$	$+T_3$	Δ	$-T_3$	$+T_3$	Δ	
n		*meq/liter*					*µl/mg dry wt/h*						
26	Na^+-Ringers	135	5	4.3±0.2	7.6±0.1	3.3							
7	Na^+-Ringers $-K^+$ + ouabain	140	0				3.0±0.2	5.1±0.3	2.1	1.3±0.2	2.6±0.3	1.3	0.39
11	Choline-Ringers	0	20				3.0±0.1	4.7±0.2	1.7	1.2±0.1	3.0±0.2	1.8	0.55
4	Choline-Ringers	0	40				3.0±0.1	4.6±0.2	1.6	1.2±0.1	2.9±0.2	1.7	0.52
6	Sucrose-Ringers	0	40				3.0±0.1	4.7±0.2	1.7	1.2±0.1	2.6±0.3	1.4	0.42
		Mean		4.3	7.6	3.3	3.0	4.8	1.8	1.2	2.8	1.6	0.47

Qo_2 and Qo_2' were taken as the average of two successive 30-min readings, except in the case of Na^+-Ringers + ouabain, in which only the second 30-min reading was used because of variability in the manometer readings during the first 30 min. $Qo_2(t)$ is the difference between Qo_2 and Qo_2'. Rats (about 4 wk after thyroidectomy) were injected with 50 µg of T_3/100 g body wt or an equal volume of the diluent on alternate days for a total of three doses. Results are given as mean±SEM.

4th wk after surgery. The basis for the slower, late decline in Qo_2 in the face of constant serum thyroxine-iodine concentrations was not elucidated. Because of the changing pattern of the hypothyroid state after the operation, we elected to use the rats 1 wk after surgery for "acute" experiments (single injections of T_3) and 3–4 wk after surgery for "chronic" experiments (repeated injections of T_3). In the former case we wanted to evaluate the immediate response in the absence of late or secondary contributions to the hypothyroidism and in the latter to compare steady-state, untreated hypothyroid and steady-state, treated tissues.

Qo_2 and $Qo_2(t)$ estimated with ouabain or Na^+-free media. In the studies of Ismail-Beigi and Edelman (1) the contribution of changes in $Qo_2(t)$ to thyroid-dependent changes in Qo_2 of rat diaphragm were estimated by inhibiting Na^+ transport with ouabain in K^+-free media. These conditions promote intracellular gain of Na^+ and loss of K^+ (20, 21). The possibility that the estimate of Na^+-independent respiration (Qo_2') might have been spuriously low because of the effects of ouabain on intracellular Na^+ and K^+ concentrations has to be considered. If this were the case, thyroid-dependent differential sensitivity to ion dislocations might overestimate the change in $Qo_2(t)$ (i.e., $Qo_2 - Qo_2'$) elicited by the hormone. To maintain minimal intracellular Na^+ concentrations, Na^+-free media (choline Cl or sucrose) were used as alternatives to ouabain, and to limit the loss of K^+ these media were enriched with 20 or 40 mM K^+. The results are summarized in Tables I and II. Administration of T_3 to thyroidectomized rats doubled the $Qo_2(t)$, estimated with ouabain, which accounted for 39% of the increase in Qo_2 (Table I). This is in accord with the earlier results in which the comparable figure

TABLE II

Effects of T_3 on Respiration of Diaphragm from Euthyroid Rats

Rats	Medium	Na^+	K^+	Qo_2			Qo_2'			$Qo_2(t)$			$\Delta Qo_2(t)/\Delta Qo_2$
				$-T_3$	$+T_3$	Δ	$-T_3$	$+T_3$	Δ	$-T_3$	$+T_3$	Δ	
n		*meq/liter*					*µl/mg dry wt/h*						
32	Na^+-Ringers	135	5	7.5±0.1	9.7±0.1	2.2							
11	Na^+-Ringers $-K^+$ + ouabain	140	0				4.7±0.3	4.9±0.3	0.2	2.8±0.3	4.8±0.3	2.0	0.91
9	Choline-Ringers	0	20				4.1±0.1	4.6±0.2	0.5	3.4±0.3	5.1±0.3	1.7	0.77
7	Choline-Ringers	0	40				4.5±0.1	4.7±0.3	0.2	3.0±0.2	5.0±0.3	2.0	0.91
9	Sucrose-Ringers	0	40				4.4±0.2	4.9±0.2	0.5	3.1±0.2	4.8±0.2	1.7	0.77
Mean				7.5	9.7	2.2	4.4	4.8	0.4	3.1	4.9	1.8	0.84

See footnote to Table I for description of the calculations. Euthyroid rats were injected with T_3 (50 µg/100 g body wt) or the diluent on alternate days for three doses. Results are given as mean±SEM.

was 43% (1). The experiments with Na^+-free media (K^+ = 20 or 40 meq/liter) yielded estimates of Qo'_2, $Qo_2(t)$ and $\Delta Qo_2(t)/\Delta Qo_2$ that approximated those obtained with ouabain. In euthyroid rats, T_3 increased $Qo_2(t)$ estimated with ouabain by 71%, which accounted for 91% of the increase in Qo_2 (Table II). This result also agrees with the earlier findings (1). As in the hypothyroid rats, the estimates of Qo'_2, $Qo_2(t)$, and $\Delta Qo_2(t)/\Delta Qo_2$ obtained with the Na^+-free media were similar to those in which ouabain was used. Thus, whether the media, contained ouabain or Na^+-free, K^+-supplemented solutions, the estimates of energy expenditure coupled to Na^+ transport were similar. The composition of the media, however, altered intracellular ionic concentrations profoundly.

As shown in Table III, incubation of diaphragm from thyroidectomized rats (with or without T_3) in choline-Ringers reduced intracellular Na^+ concentration to less than 2 meq/liter and intracellular K^+ concentration to less than half normal in 20 mM K^+ or to two thirds of normal in 40 mM K^+. More complete results were collected on tissue from euthyroid rats with or without T_3 (Table IV). Incubation of diaphragm in ouabain (K^+-free media) reduced cell K^+ concentration to about 15 meq/liter and increased Na^+ concentration to about 155 meq/liter. In choline Ringers or sucrose-Ringers supplemented with 40 mM K^+, cell K^+ concentration was maintained at about 125 meq/liter, and Na^+ concentration was less than 5 meq/liter. The similarity of the estimates of Qo'_2 and $Qo_2(t)$ in these solutions (cf. Tables I-IV) implies that respiratory indices were independent of the ion composition of the diaphragm during the intervals required to make the measurements, i.e., 60–90 min.

NaK-ATPase and Mg-ATPase activities. The results summarized in Table V indicate that thyroidectomy or injections of T_3 had statistically insignificant effects on Mg-ATPase activities (expressed per milligram of microsomal protein). In contrast, surgical thyroidectomy lowered NaK-ATPase activity from the control level of 13.1±0.8 to 8.9±0.6 μmol Pi/mg/protein/h. Administration of T_3 over a 1-wk period increased NaK-ATPase activity 75% in thyroidectomized rats and 25% in euthyroid rats; both changes were statistically significant. Inasmuch as thyroid hormone has a protein-anabolic effect (*22*), the results were also analyzed on the basis of wet weight of tissue by measuring the protein yield in the microsomal fraction per gram of wet weight of muscle (Table VI). In the microsomal fraction, thyroidectomy tended to decrease the protein yield, and T_3 increased this yield. As a result, the enzyme changes expressed per gram of wet weight of muscle were more pronounced. Injections of T_3 increased Mg-ATPase 35% (NS) in thyroidectomized rats and 39% (significantly) in euthyroid rats. The changes in NaK-ATPase activities were also correspondingly greater: + 110% in thyroidectomized rats and + 54% in euthyroid rats.

Respiratory and enzymatic effects of extended treatment with T_3. In earlier studies on the respiratory and enzymatic effects of T_3 in the liver, some evidence was obtained that the fractional contribution of $Qo_2(t)$ to Qo_2

TABLE III

Na^+ and K^+ Content of Diaphragm from Thyroidectomized Rats ($\pm T_3$) Incubated in Various Solutions

				Intracellular concentrations			
				$-T_3$		$+T_3$	
Rats	Medium	Na^+	K^+	Na^+	K^+	Na^+	K^+
n		*meq/liter*		*meq/liter*			
7	Na^+-Ringers	135	5	54±4	143±6	53±5	156±5
7	Choline-Ringers	0	20	1.8±0.2	64±4	1.6±0.3	66±4
7	Choline-Ringers	0	40	2.0±0.3	104±3	1.7±0.2	98±6

The rats were injected with T_3 or diluent as described in the footnote to Table I. Quarter segments of diaphragm were incubated at 37°C for 90 min in the various media, which contained [^{14}C]inulin as described under Methods.

TABLE IV

Na^+ and K^+ Content of Diaphragm from Euthyroid Rats ($\pm T_3$) Incubated in Various Solutions

				Intracellular concentrations			
				$-T_3$		$+T_3$	
Rats	Medium	Na^+	K^+	Na^+	K^+	Na^+	K^+
n		*meq/liter*		*meq/liter*			
8	Na^+-Ringers	135	5	63±3	136±5	66±4	136±4
8	Na^+-Ringers $-K^+$ + ouabain	140	0	153±4	15±1	157±5	12±2
8	Choline-Ringers	0	20	2.9±0.5	65±3	2.3±0.4	65±4
8	Choline-Ringers	0	40	3.5±0.7	98±5	3.4±0.5	102±6
8	Sucrose-Ringers	0	40	4.7±0.5	123±5	4.5±0.4	128±2

The rats were injected with T_3 or diluent as described in the footnote of Table I. Quarter segments of diaphragm were incubated at 37°C for 90 min in the various media, which contained [^{14}C]inulin as described under Methods.

TABLE V

ATPase Activity of Membrane Fractions of Rat Skeletal Muscle

Thyroid status	Enzymes	$-T_3$	$+T_3$	Δ	*P*
		μmol P_i/mg protein/h			
Thyroidectomized	Mg-ATPase	9.7±0.6	10.7±0.8	+1.0	NS
	NaK-ATPase	8.9±0.6	15.6±1.0	+6.7	<0.001
Euthyroid	Mg-ATPase	7.9±0.8	9.0±0.8	+1.1	NS
	NaK-ATPase	13.1±0.8	16.4±1.1	+3.3	<0.05

Either diluent ($-T_3$) or T_3 (50 μg/100 g body wt) was injected into thyroidectomized (about 4 wk after surgery) or euthyroid rats on alternate days for a total of three doses. In each group, n = 22 rats, i.e. 11 injected with T_3 and 11 with the diluent. Results are means±SEM.

TABLE VI

Microsomal Protein Yield and Total ATPase Activity of Rat Skeletal Muscle

Rats	Thyroid status	Analyses	$-T_3$	$+T_3$	Δ	*P*
n						
14 (7/group)	Thyroidectomized	Protein	1.69±0.12	2.03±0.09	+0.34	NS
		Mg-ATPase	16.0±1.2	21.6±2.2	+5.6	NS
		NaK-ATPase	16.2±1.5	34.1±2.0	+17.9	<0.001
20 (10/group)	Euthyroid	Protein	1.91±0.08	2.38±0.11	+0.47	<0.005
		Mg-ATPase	15.2±1.7	21.2±2.3	+6.0	<0.01
		NaK-ATPase	25.5±1.7	39.3±3.0	+13.8	<0.001

The rats were injected as described in the footnote of Table V. Protein content was assayed as milligrams per gram wet weight of muscle, and enzyme activity was calculated as micromoles Pi per gram wet weight per hour. Results are means±SEM.

increased with continued administration of the hormone (4). Accordingly, we studied the respiratory and enzymatic effects of extended treatment with T_3. Surgically thyroidectomized rats were given 50 μg of T_3/100 g body wt every other day for a total of seven doses over a two-wk interval. Extended treatment with T_3 more than doubled the Q_{O_2} and tripled $Q_{O_2}(t)$ (Table VII). The "anabolic" effect of the hormone was exhibited by the marked increase in the protein yield in the microsomal fraction and the almost twofold increase in NaK-ATPase activity. In this group, the mean $\Delta Q_{O_2}(t)/\Delta Q_{O_2}$ ratio was 0.58, suggesting that the relative contribution of Na^+-dependent respiration to total respiration was somewhat but not strikingly greater than in the group given three doses of T_3 (cf. Tables I and VII).

Q_{O_2}, $Q_{O_2}(t)$, and ATPase activities in ^{131}I-treated rats. The most pronounced effects of T_3 on $Q_{O_2}(t)$ and NaK-ATPase activity were observed in the surgically thyroidectomized rats. We considered the possible contribution of effects on parathyroid status to these responses because surgical thyroidectomy often results in damage to the parathyroid glands. Rats that developed signs of tetany (i.e., hyperirritability) during the first 6 days after the operation were always eliminated from the study. To obtain further information on parathyroid status, serum was analyzed for total Ca and inorganic phosphorus (P_i) content in various thyroid states. The results in Table VIII indicate that mean serum Ca and P_i concentrations were lower after surgical thyroidectomy (i.e., 9.2±0.2 vs. 10.0±0.2 for Ca, and 6.4±0.2 vs. 8.7±0.4 for P_i) and that administration of T_3 elicited a further significant fall in serum Ca concentration only in the thyroidectomized rats. T_3 also produced significant increases in serum P_i concentrations in both thyroidectomized and euthyroid rats, but the effect was greater in the thyroidectomized group. The possibility that changes in serum Ca and P_i concentrations contributed to the observed effects of T_3 on $Q_{O_2}(t)$ and NaK-ATPase activity was explored in rats subjected to thyroid ablation with ^{131}I. Radiation ablation of the thyroid gland resulted in about the same changes in serum Ca and P_i concentrations as surgical thyroidectomy (Table VIII). Administration of T_3 to the ^{131}I-treated group, however, had an insignificant effect on serum Ca concentration but raised serum P_i concentration almost as much as in the surgically thyroidectomized group.

The lesser effect of ^{131}I-ablation on serum Ca concentrations prompted us to evaluate the respiratory and enzymatic effects of T_3 in this group. The effectiveness of ^{131}I-ablation of the thyroid is indicated by the low Q_{O_2} (4.1±0.1 μl/mg dry wt/h), as compared to that in euthyroid rats (7.5±0.1) (cf. Tables I and IX). Injections of T_3 resulted in equivalent changes in Q_{O_2}, $Q_{O'_2}$, and $Q_{O_2}(t)$ of diaphragm in the ^{131}I-treated and surgically thyroidectomized rats (cf. Tables I and IX). In the former group, $\Delta Q_{O_2}(t)/\Delta Q_{O_2}$ was 0.46 and in the

TABLE VII

Respiratory and Enzymatic Effects of Extended Treatment with T_3 in Skeletal Muscle of Thyroidectomized Rats

Rats	Analyses	$-T_3$	$+T_3$	Δ	*P*
n					
20 (10/group)	Q_{O_2}	4.4±0.1	9.7±0.2	5.3	<0.001
	$Q_{O'_2}$	3.4±0.1	5.6±0.2	2.2	<0.001
	$Q_{O_2}(t)$	1.0±0.1	4.1±0.3	3.1	<0.001
18 (9/group)	Protein yield	1.66±0.08	2.63±0.16	+0.97	<0.001
	Mg-ATPase	21.3±2.0	25.7±2.8	+4.4	NS
	NaK-ATPase	15.0±0.8	42.1±3.5	+27.1	<0.001

Surgically thyroidectomized rats were injected either with the diluent or T_3 (50 μg/100 g body wt) on alternate days for a total of seven doses (i.e., over a 2-wk interval). Q_{O_2}' was determined in diaphragms by incubation in K^+-free Ringers solution containing 10^{-3} M ouabain. The respiratory indices are given in microliters per milligram dry weight per hour. See the footnote to Table I for a description of the procedure. The enzyme assays are expressed as micromoles of P_i per milligram of wet weight of skeletal muscle per hour. Results are means±SEM.

TABLE VIII

Serum Ca and P_i Concentrations in Various Thyroid States

Rats	Thyroid status	Analyses	$-T_3$	$+T_3$	Δ	P
n			*mg/100 ml*			
42	Surgically	Ca	9.2±0.2*	7.4±0.4	−1.8	<0.001
(21/group)	thyroidectomized	P_i	6.4±0.2	12.9±0.7	+6.5	<0.001
22	Euthyroid	Ca	10.0±0.2	9.9±0.2	−0.1	NS
(11/group)		P_i	8.7±0.4	9.9±0.4	+1.3	<0.05
22	^{131}I-treated	Ca	9.4±0.3	8.8±0.3	−0.6	NS
(11/group)	hypothyroid	P_i	6.5±0.3	12.2±0.8	+5.7	<0.001

Rats were injected either with the diluent or T_3 (50 μg/100 g body wt) on alternate days for a total of three doses. Results are means±SEM.

* Significantly different from euthyroid control ($P < 0.05$).

latter group, 0.42. Moreover, T_3 had the same effects on Mg-ATPase and NaK-ATPase activities in these groups (cf. Table V and IX). In neither group was there a significant effect on Mg-ATPase activity. In the ^{131}I-treated rats, T_3 augmented microsomal NaK-ATPase activity 80% and in the surgically thyroidectomized rats, 75%. In view of the relatively small change in serum Ca elicited by T_3 in the ^{131}I-treated rats, it seems unlikely that changes in serum Ca concentration play an important role in the effects on $Q_{O_2}(t)$ or NaK-ATPase activity. Further studies are needed, however, to evaluate the possible role of changes in serum P_i in these processes. In any case the method used to produce hypothyroidism did not affect the T_3-dependent changes in Q_{O_2}, $Q_{O_2}(t)$, and NaK-ATPase activity.

Quantitative relationships between $Q_{O_2}(t)$ and NaK-ATPase activity. The inference that activation of the Na^+ pump accounts for the observed increases in $Q_{O_2}(t)$ was explored further by an analysis of the quantitative relationships between the respiratory and enzymatic effects of T_3.

The experiments described above provided sustained (three doses of T_3 every other day) differences in thyroid status (4). The results shown in Table X were abstracted from Tables I, II, VI, and VII. On a percentage basis, the increase in $Q_{O_2}(t)$ was greater than in NaK-ATPase activity. The ratio of the absolute increase in $Q_{O_2}(t)$ to that in NaK-ATPase activity (i.e., $\Delta Q_{O_2}(t)/\Delta$NaK-ATPase), however, was about the same in all three groups, consistent with the conclusion that the changes in $Q_{O_2}(t)$ were dependent on the changes in Na^+ pump activity. To explore this issue further, a dose-response protocol was used. 1 wk after thyroidectomy, rats were given 10, 50, or 250 μg of T_3/100 g body wt, or the same volume of the diluent, in a single injection. Skeletal muscle (diaphragm for Q_{O_2} and $Q_{O_2}(t)$ and gastrocnemius for NaK-ATPase activity) was sampled at 48 h, the time of the maximal increase in Q_{O_2} in the liver (4). The results are summarized in Table XI and Fig. 2. The mean change in absolute $Q_{O_2}(t)$ was about half that in Q_{O_2} at all three dose levels. The similarity in the dose-response curves of NaK-ATPase activity and Q_{O_2} and

TABLE IX

Respiratory and Enzymatic Effects of T_3 in Skeletal Muscle of ^{131}I-Treated Rats

Rats	Analyses	$-T_3$	$+T_3$	Δ	P
n					
14	Q_{O_2}, *μl/mg dry wt/h*	4.1±0.1	7.2±0.2	+3.1	<0.001
(7/group)					
	Q_{O_2}', *μl/mg dry wt/h*	2.9±0.1	4.6±0.2	+1.7	<0.001
	$Q_{O_2}(t)$, *μl/mg dry wt/h*	1.2±0.04	2.6±0.3	+1.4	<0.001
20	Mg-ATPase, *μmol P_i/mg protein/h*	13.8±1.1	14.2±0.6	+0.4	NS
(10/group)	NaK-ATPase, *μmol P_i/mg protein/h*	6.5±0.8	11.3±0.5	+5.2	<0.001

Hypothyroid rats (^{131}I-treated) were injected either with the diluent or T_3 (50 μg/100 g body wt) on alternate days for a total of three doses. Q_{O_2} was determined in sucrose (Na^+-free) Ringers supplemented with 40 mM K^+. See footnote to Table I for a description of the procedure. Results are means±SEM.

TABLE X

Quantitative Relationships between the Changes in $Q_{O_2}(t)$ and NaK-ATPase Activity of Skeletal Muscle

Thyroid status	ΔQ_{O_2}	$\Delta Q_{O_2}(t)$	ΔNaK-ATPase	$\Delta Q_{O_2}(t)$/ΔNaK-ATPase
Hypothyroid (+T_3 × 3)	3.3	1.6	17.9	0.09
Hypothyroid (+T_3 × 7)	5.3	3.1	27.1	0.11
Euthyroid (+T_3 × 3)	2.2	1.8	13.8	0.13

These data were taken from Tables I, II, VI, and VII.

$Q_{O_2}(t)$ is obvious. As shown in Fig. 3, the increases in $Q_{O_2}(t)$ varied linearly with those in NaK-ATPase activity and the regression line intersects the origin. The proportionate changes in $Q_{O_2}(t)$ and NaK-ATPase activity support the concept that augmentation of Na^+ pump activity mediates the changes in $Q_{O_2}(t)$.

Effects of thyroid hormone on K_m and V_{max} of NaK-ATPase. Thyroidal stimulation of the NaK-ATPase enzyme system could be a consequence of activation of a fixed number of enzyme sites or of an increase in the total enzyme population. Activation of a fixed number of enzymes might be revealed by changes in enzyme kinetics. Accordingly, hypothyroid rats (1 wk after surgical thyroidectomy) were given a single dose of T_3 (250 μg/100 g body wt); 48 h later the gastrocnemius was removed, and the K_m for ATP and V_{max} were determined in an ATP-regenerating system. The results in Fig. 4 indicate that the data fit Michaelis-Menten kinetics (23, 24), irrespective of thyroid status. The computed values for K_m(ATP) and V_{max} are given in Table XII. T_3 had no effect on the K_m and increased V_{max} by 39% ($P < 0.005$).

DISCUSSION

In studies on the rat diaphragm, Ismail-Beigi and Edelman (1) estimated that the increase in $Q_{O_2}(t)$ accounted for 45% of the increase in Q_{O_2} produced by T_3 in hypothyroid rats and for 90% of the increase in euthyroid

TABLE XI

Dose-Response Relationships after a Single Injection of T_3

T_3, μg/100 g body wt	0 (n = 11)	10 (n = 9)	50 (n = 9)	250 (n = 8)
Analyses				
Q_{O_2}, μl/mg dry wt/h	5.3±0.2	6.6±0.2	7.9±0.4	8.3±0.3
$Q_{O_2}(t)$, μl/mg dry wt/h	2.4±0.2	3.0±0.2	3.7±0.3	4.1±0.2
NaK-ATPase, μmol P_i/mg protein/h	7.8±0.5	9.0±0.4	10.2±0.8	11.0±1.0

Rats were injected with a single dose of T_3 1 wk after surgical thyroidectomy and assayed 48 h later. Respiratory analyses were done on diaphragm and enzyme activity on gastrocnemius. Results are means±SEM.

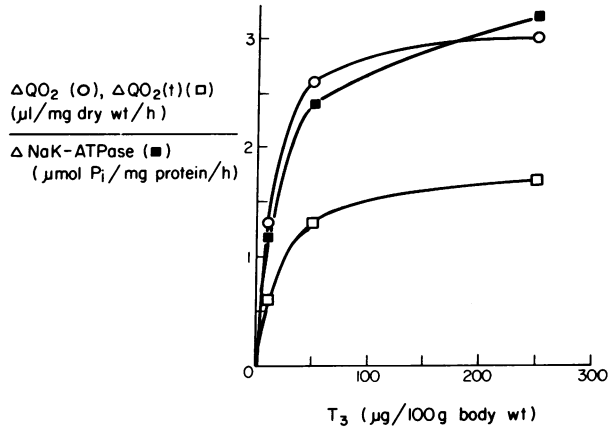

FIGURE 2 The dependence of the change in Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity of skeletal muscle on the dose of T_3. Pairs of rats were injected with various doses of T_3 1 wk after thyroidectomy, and skeletal muscle was sampled 48 h later. These values were computed from the data given in Table XI.

rats (1). These estimates were obtained by inhibiting Na^+ transport with ouabain in K^+-free media. That ouabain did not have a direct, toxic effect on mitochondrial function was indicated by the finding of no effect of this inhibitor on respiration in Na^+-free (choline-Ringers) media (1). That Na^+ transport-dependent respiration can be measured as the difference between Q_{O_2} and Q_{O_2}'s rests, in part, on the assumption that ouabain-dependent intracellular Na^+ and K^+ concentration do not, in themselves, alter oxidative metabolism (20). Van Rossum (25) suggested that two-thirds of the fall in

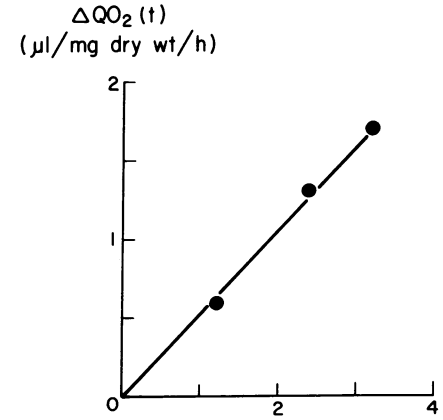

FIGURE 3 The relationship between the change in $Q_{O_2}(t)$ and the change in NaK-ATPase activity of skeletal muscle in response to various doses of T_3. These results were computed from the data given in Table XI.

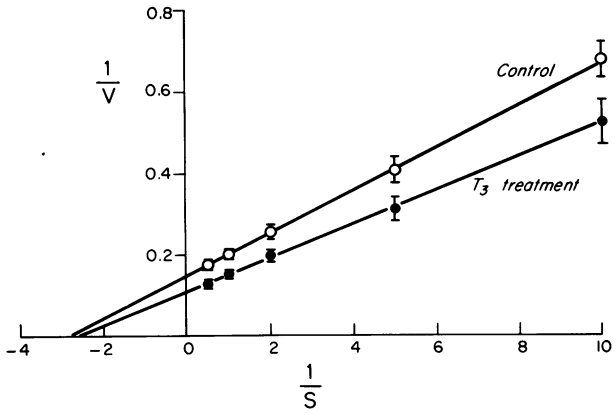

FIGURE 4 Effect of T_3 on skeletal muscle NaK-ATPase kinetics as a function of ATP concentration. Pairs of thyroidectomized rats were injected with the diluent or T_3 (250 μg/100 g body wt) and skeletal muscle was assayed for NaK-ATPase activity 48 h later. The height of the vertical bar represents±1 SEM. V denotes NaK-ATPase activity in micromoles P_i per milligram protein per hour of the microsomal fraction and S the millimolar ATP concentration of the assay medium. $n = 10$ for each point.

Q_{O_2} on addition of ouabain to conventional media may result from changes in intracellular ion composition. In his studies, however, no distinction was made between K^+ antagonism of the inhibitory action of ouabain on the Na^+ pump and the effects of changes in composition, per se (26). In experiments from our laboratory, when the K^+ concentrations of the media were increased to 20 mM after addition of ouabain in rat liver slices, there was no effect on $Q_{O'_2}$, despite a more than twofold increase in tissue K^+ content (27). To explore further the possibility that inhibition of the Na^+ pump with ouabain might give spurious estimates of $Q_{O_2}(t)$, Na^+-free K^+-supplemented media were used (Tables I-IV). These results indicate that in the interval in which Q_{O_2} was measured, distortions in intracellular Na^+ and K^+ concentrations had no effect on the estimates of $Q_{O_2}(t)$.

TABLE XII
The Effects of T_3 on Kinetics of NaK-ATPase Activity of Skeletal Muscle

	K_m(ATP)	V_{max}
	mM	*μmol P_i/mg protein/h*
Hypothyroid	0.35±0.02	6.6±0.3
Hypothyroid + T_3	0.40±0.05	9.2±0.7
Δ	+0.05	+2.6
P	NS	<0.005

Hypothyroid rats were given a single injection of T_3 (250 μg/100 g body wt) or the diluent (control group) 1 wk after surgical thyroidectomy. Gastrocnemius was assayed 48 h after injection in an ATP-regenerating system. K_m(ATP) and V_{max} were computed from the data shown in Fig. 3 (24). Results are means±SEM. [n = 20 (10/group)].

In the present studies, the respiratory measurements were made on diaphragm and the enzyme (NaK-ATPase, Mg-ATPase) measurements on gastrocnemius. Diaphragm was chosen for study of Q_{O_2} and $Q_{O_2}(t)$, as minimal damage to the muscle fibers is produced in isolation and dissection, because of the protection provided by the serosal linings. The muscular portion of the diaphragm from one rat, however, is insufficient for accurate measurement of both the respiratory indices and enzymes; average wet weight is less than 250 mg/diaphragm. To circumvent the problems that would have attended measuring Q_{O_2} and NaK-ATPase in separate animals, two sources of muscle, i.e., diaphragm and gastrocnemius, were obtained from the same animal. In preliminary studies, we found no significant differences in NaK-ATPase or Mg-ATPase activities in diaphragm and gastrocnemius of the same rat.

Thyroidal augmentation of NaK-ATPase activity is somewhat selective. The increase in NaK-ATPase activity exceeded the increase in Mg-ATPase activity and in the protein yield of the microsmal fraction (cf. Tables V and VI). In previous studies, the activity of hepatic 5′-nucleotidase activity was not significantly altered by administration of T_3 to hypothyroid or euthyroid rats (2). Similarly, thyroid hormone had no effect on cardiac adenyl cyclase activity or on cyclic AMP content (28-30). Jones et al. (31) found no difference in adenyl cyclase activity, basal or epinephrine-stimulated, in liver from hypothyroid, euthyroid, or hyperthyroid rats. It appears, therefore, that the dependence of NaK-ATPase activity on thyroid status is not simply an expression of a general or nonselective increase in membrane-bound enzymes.

An attempt was made to determine whether changes in serum Ca and P_i concentrations resulting from "subclinical" damage to parathyroid glands contributed to the respiratory and enzymatic consequences of surgical thyroidectomy or the response to T_3 in the thyroidectomized rats. Surgical thyroidectomy was attended by falls in serum Ca^{++} and P_i concentrations even in rats that gave no outward signs of tetany (Table VIII). Administration of T_3 accentuated the fall in total serum Ca concentration but raised serum P_i concentration above the normal level. In ^{131}I-ablated rats, the effects on serum Ca were similar in direction but lesser in magnitude. This is in agreement with Gorbman's findings (32) of damage to the parathyroid glands after treatment with ^{131}I. It is probable, however, that free (ionized) Ca^{++} concentrations were maintained near normal as serum albumin concentrations fell significantly in both surgi-

cally and ^{131}I-thyroidectomized rats given T_3.[4] The fall in serum protein concentration has also been documented in hypothyroid patients given thyroid hormone (33). In view of the small changes in serum total Ca and the probability of a lesser change in free Ca^{++} concentration, it seems unlikely that the T_3-dependent increases in $Q_{O_2}(t)$ and NaK-ATPase activity of skeletal muscle are mediated by changes in serum Ca^{++} concentration (cf. Tables VII and IX). This conclusion is supported by the results of Katz and Lindheimer (34); no difference was seen in the T_3-dependent increment in renal NaK-ATPase activity and tubular reabsorption of Na^+ in surgically thyroparathyroidectomized (and therefore completely deprived of parathyroid hormone) and in ^{131}I-treated rats. The role of changes in serum P_i concentrations in these processes, however, remains undecided. In all of the groups studied (surgically thyroidectomized, ^{131}I-thyroidectomized, and euthyroid rats), T_3 raised serum P_i concentrations significantly. There was no correlation, however, between the absolute concentration of P_i in serum and either the respiratory or enzymatic status of skeletal muscle (cf. Tables I-VI, VIII, and IX). For example, Q_{O_2} was higher in hyperthyroid rats than in hypothyroid rats but serum P_i concentration, on the average, was lower.

The results in Tables I, II, VI, and VII show significant effects of thyroid hormone on $Q_{O_2}(t)$ and NaK-ATPase activity. The latter is an index of the maximum capacity of the enzyme system identified as the enzymatic equivalent of the Na^+ pump (26). If the T_3-dependent increase in $Q_{O_2}(t)$ is a consequence of an increase in total Na^+ pump activity, proportionate effects of thyroid status on $Q_{O_2}(t)$ and NaK-ATPase activity may be evident. As shown in Table X, the absolute increase in $Q_{O_2}(t)$ elicited by T_3 was proportionate to the absolute increase in NaK-ATPase activity; the ratio varied only from 0.09 to 0.13, despite the considerable differences in the magnitude of the effects on Q_{O_2} among these groups (i.e., ΔQ_{O_2} was 2.2 μl/mg dry wt/h in the euthyroid and 5.3 μl/mg dry wt/h in the hypothyroid rats given seven doses of T_3). To explore the quantitative relationship between $\Delta Q_{O_2}(t)$ and ΔNaK-ATPase further, we used rats 1 wk after thyroidectomy and assessed the response 48 h after a single injection of T_3. A single-dose method was used to limit the analysis to the immediate response. The tissue was sampled 48 h after injection, the time of the maximum increase in Q_{O_2} and NaK-ATPase activity in rat liver (4). The results in Figs. 2 and 3 exhibit proportional increases in Q_{O_2}, $Q_{O_2}(t)$ and NaK-ATPase activity, and the regression line of $\Delta Q_{O_2}(t)$ on ΔNaK-ATPase activity intersects the origin. These findings are in accord with the inference that enhanced Na^+ transport activity contributes significantly to the respiratory effects of thyroid hormone.

Augmentation of NaK-ATPase activity by thyroid hormone could result from activation of a fixed number of enzyme sites or from an increase in the total number of sites. The latter effect would yield an increase in V_{max} of the enzyme and the former, an increase in K_m or in K_m and V_{max}. To obtain preliminary information on these questions, the kinetics were measured with ATP as the variable. In these studies, we also used a single-injection format, 1 wk after surgical thyroidectomy. As expected, the increase in V_{max} was significant (+ 40%) but no change in K_m was detected (Table XII). The K_m (ATP) values given in Table XII are in accord with those obtained in other tissues, e.g., 0.24 mM for calf heart (35), 0.46 mM for embryonic chick heart (36). Moreover, hyperbolic kinetics were evident in both sets of membrane preparations (Fig. 4). Further studies, however, are needed to evaluate the possible effects of thyroid hormone on the equivalent K_m's for Na^+ and K^+, and on the number of Na^+ pump sites evaluated by independent methods (e.g., binding of [^{3}H]ouabain). For example, recent findings from this laboratory indicate that T_3 increases the number of Na^+ pump sites in both kidney and intestinal mucosa as estimated by specific binding of [^{3}H]ouabain and by incorporation of ^{32}P into a Na^+-dependent phosphorylated intermediate from [γ-^{32}P] ATP.[5]

In the present study, we focused on energy expenditure in Na^+ transport. The results in Tables I and II imply that in hypothyroid muscle, about 50% of the increase in Q_{O_2} in response to T_3 involves Na^+-independent pathways. A variety of processes are candidates for mediating roles in Na^+-independent thermogenesis. Skelton et al. (37) proposed that thyroid hormone increases energy utilization in the cardiac contraction-relaxation cycle. Another candidate is active Ca^{++} transport in sarcoplasmic reticulum. Suko (38) found that Ca^{++}-ATPase and Ca^{++} uptake of cardiac sacroplasmic reticulum were reduced in hypothyroid rabbits and that treatment with thyroxine significantly increased both activities. In cats, however, administration of thyroxine had no effect on Ca^{++}-ATPase activity of skeletal muscle (39). The possible contributions of alterations in regulatory enzymes and mitochondrial function to thyroid thermogenesis have been considered by a number of investigators; thyroid-dependent changes in the activity ratios of hexokinase/citrate synthase and glycerolphosphate dehydrogenase/triosephosphate dehydrogenase have been posited to participate in the thermogenic response (40). Similarly, the finding of enhanced transport of reducing

[4] Liberman, U. A., Y. Asano, and I. S. Edelman. Unpublished observations.

[5] Lo, C. S., U. A. Liberman, and I. S. Edelman. Unpublished observations.

equivalents from glycerol to O_2 on treatment with thyroid hormone has been identified as an indication of direct modulation of energy metabolism (41). At the mitochondrial level, two effects of thyroid hormone have been described: (*a*) some degree of uncoupling or loosening of the coupling ratio between oxidation and phosphorylation (42, 43), and (*b*) increased carrier-mediated ADP transport into mitochondria (44). With the exception of the effects on coupling ratios (i.e., ADP to O), the evidence of thyroidal enhancement of glycolytic and mitochondrial enzymes and transport activity are all consistent with hormonally dependent increases in the capacity to do metabolic work but the pacemaker would be the rate of evolution of ADP from ATP.

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