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6. AUTHOR(S)
Reed HL, Quesada M, Hesslink RL Jr., D'Alesandro MM, Hays MT, Christopherson RJ, Turner BV, Young BA

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Medical Research Institute
Commanding Officer
8901 Wisconsin Avenue
Bethesda, Maryland 20889-5607

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National Naval Medical Center
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Changes in serum triiodothyronine kinetics and hepatic type I 5'-deiodinase activity of cold-exposed swine

H. LESTER REED, MARILEE QUESADA, ROBERT L. HESSLINK, JR., MICHELE M. D'ALESANandro, MARGuerite T. HAYS, ROBERT J. CHRISTOPHERSON, BRIAN V. TURNER, AND BRUCE A. YOUNG

Department of Medicine, Endocrine Service, Madigan Army Medical Center, Tacoma, Washington 98431–5000; Thermal Stress Adaptation Program, Naval Medical Research Institute, Bethesda, Maryland 20889–5055; Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814–4799; Veterans Affairs Medical Center, Palo Alto, California 94304; and Department of Animal Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

Reed, H. Lester, Marilee Quesada, Robert L. Hesslink, Jr., Michele M. D'Alesandro, Marguerite T. Hays, Robert J. Christopherson, Brian V. Turner, and Bruce A. Young. Changes in serum triiodothyronine kinetics and hepatic type I 5'-deiodinase activity of cold-exposed swine. Am. J. Physiol. 266 (Endocrinol. Metab. 29): E786–E795, 1994.—Swine exposed to cold air have elevated serum levels of total triiodothyronine (TT3) and free T3 (FT3). To characterize the mechanism by which these parameters increased, we measured in vivo kinetic parameters after a bolus intravenous injection of 125I-labeled T3 by use of both compartmental (MC) and noncompartmental (NC) methods and in vitro hepatic type I iodothyronine 5'-deiodinase (5'D-I) activity. Ten ad libitum-fed 5-mo-old boars were divided into two groups, living for 25 days in either control (22°C) or cold (4°C) conditions. Cold-exposed animals consumed 50% more calories than control animals but showed no difference in total body weight, percent body fat, or plasma volume. Thyroid gland weight was increased 86% (P < 0.004), as was serum total thyroxine (TT4) (48%), free T4 (FT4) (61%), TT3 (103%), and FT3 (107%), whereas serum thyrotropin (TSH) was not different in cold-exposed compared with control animals. The T3 plasma clearance rate was similar between groups when both MC and NC techniques were used. However, T3 plasma appearance rate (PAR) was elevated in cold-treated animals 110% over controls by MC (P < 0.001) and 83% by NC methods (P < 0.001). The animal total hormone pool of T3 was increased 76% (MC) and 53% (NC) compared with control (P < 0.01). The Michaelis constant of hepatic 5'D-I was not different between groups, but the maximum enzyme velocity increased (106%; P < 0.02). Therefore, cold exposure for 25 days is associated with increased energy intake, thyroid size, T3 PAR, and hepatic 5'D-I activity with little change in serum TSH.

considerable information exists regarding the role of triiodothyronine (T3) in brown adipose tissue (BAT) mediated thermogenesis (25). This specialized adipose tissue contributes significantly to circulating T3 in swine, which lack BAT as a possible source of the hormone, should provide information more applicable than rodent data as a model for human physiology and thermoregulation (7).

The outer ring 5' iodine of thyroxine (T4) is removed to form T3 in peripheral tissues by the enzyme 5'-deiodinase (5'D). Several forms of this enzyme exist, including type I (5'D-I), found primarily in liver and kidney, and type II (5'D-II), located in brain, pituitary, and BAT (14). Six- to eightfold increases in 5'D-II activity of BAT from cold-exposed rodents contribute significantly to increased circulating values of T3 in this species. By contrast, the 5'D-I activity that supplies circulating T3 under normal conditions in the rat is not consistently increased with cold exposure (20, 21, 25). We therefore hypothesized that if the T3 production rate is increased with cold in a species lacking BAT, an alternate hormone source should be present to offset the missing BAT contributions.

Swine are known to increase energy intake, serum concentrations of T4 and T3, and the disposal of T4 with constant cold exposure (12, 13, 16). We studied the mechanism of increase in serum T3 by comparing both the in vivo 125I-labeled T3 degradation and in vitro generation of T3 in animals exposed to either 4 or 22°C for 25 days. We hypothesized that an increased serum T3 concentration would be associated with either a decreased plasma clearance rate (PCR) and unchanged production rate (PR) or with unchanged PCR and increased PR. Because the T4 disposal rate (DR) is increased in this swine model, we favored an augmented T3 production to account for the change. Furthermore, if hepatic 5'D-I activity were contributing to the elevated serum T3 concentrations, the activity of this enzyme should either increase or be unchanged in the presence of increased serum T4.

METHODS

Animal characteristics and environmental exposure protocol. Protocols using 10 mature 5-mo-old boars (Sus scrofa) of the Large White breed (Pig Improvement, Calgary, AB, Canada) were approved for use by the Committee for the Use and Care of Animals at the Naval Medical Research Institute, Bethesda, MD, and the University of Alberta, Edmonton, AB, Canada. All studies were conducted in Edmonton from February to March, 1990.

For ~4 mo preceding the study, all animals were housed in climate-controlled conditions (20 to 25°C) and fed a highly digestible energy feed ad libitum (160 mg protein and 3.2 kcal
digestible energy per g dry matter (DM)). One day before temperature treatments, the animals were housed in metabolic crates within the climate chambers set to thermoneutral conditions (22°C) for familiarization. The floors of the metabolic crates were constructed of stainless steel rods 12.7 cm apart with reinforced clear Plexiglas inserts for walls and an open top. The floor of the crate was 65 cm from the floor of the climate chamber, and air freely circulated through the crate without significant lateral drafts.

Animals were randomly assigned to the control or cold condition. The temperature in the cold chamber was gradually lowered over 5 days to the predetermined value of 4°C, with a range of 1.8–7.3°C; the control chamber remained at 22°C, with a range of 20.4–22.8°C. The chambers had floor areas of 40 and 50 m², respectively, and had similar airflows (< 0.5 m/s) and relative humidity (45–60%). Cyclic lighting was maintained with automatic timers set to illuminate between 0900 and 2100 h. The animals received water by an automatic nipple fountain and feed through a front end trough ad libitum. The feed contained 3.2 kcal/g, 16% crude protein, and 92% DM composed of 51% barley, 30% wheat, and 13% soybean meal. The animals were weighed weekly in a rolling metabolic cage similar to their chamber crates, a procedure allowing no more than 10 min/wk outside of the treatment environment. The animal groups were matched for weight and growth rate by allowing energy intake to increase in the cold-exposed group to achieve this similarity. Background music, as is customary, was provided to aid in normalization of environments. The animals were housed in the temperature treatment chambers 25.2 ± 2.8 days before the kinetic study and an additional 4.2 ± 0.4 days until necropsy. There was no group difference in either the duration of temperature exposure or time to death.

One week before the kinetic studies (see below), two Silastic catheters were inserted into the external jugular vein. They were positioned, brought to the surface in the midback region, and used for sampling and injecting radiolabeled hormones. The catheters were maintained patent by flushing with a solution of heparin sodium (100 U/ml) at 12-h intervals. One catheter was positioned distal for injection, and the other one was more proximal. This arrangement minimized contamination of the sampled blood with the injected substances. The catheters were placed while the animals were given a general anesthesia with a 2% halothane-oxygen mixture. Recovery from anesthesia and surgery was verified by normalization of feeding habits and a stable rectal temperature compared with the preoperative value. Prophylaxis against catheter-related infection was carried out in all animals with ampicillin (500 mg iv) every 12 h from the postoperative period until necropsy.

Kinetic experiments. L-3,5,3'-125I-labeled triiodothyronine (125I)T3 was purchased from New England Nuclear (Wilmington, DE; sp act 2,200 Ci/mmol). The purity was confirmed by high-pressure liquid chromatography (HPLC), and it was shown that > 98% of 125I was in the form of 125I(T3) at the time of analysis. The radiopharmaceutical was diluted with sterile water and sterilized by filtration through a 0.2-µm filter (Millipore, Bedford, MA). Thyroidal 125I uptake was blocked by the oral ingestion of a volume of saturated potassium iodide (KI) solution containing 250 mg of KI. This solution was delivered on apple slices given twice a day starting 24 h before the kinetic protocol and continuing through the 1st day of sample collection (250 mg iodide per 5 drops; Roxane Laboratories, Columbus, OH) (16, 24). KI was administered to minimize differences between human and swine kinetic protocols (24). 125I(T3) was diluted to a volume of 5 ml with 1% autologous animal serum 5 min before injection; it was then administered to nonfasted animals as a bolus intravenous injection of ~36.4 pmol, and the catheter was flushed with an equal volume of the stock heparin solution. Catheter dead space volume was replaced with this heparin solution. Kinetic studies with the control and cold-exposed animals were carried out at the treatment temperatures. The animals were studied in a pattern that alternated tracer injections between control and cold-exposed animals to limit between-group methodology differences. The dose vial, syringe, catheter, and three-way stopcock arrangement contained < 5% residual radioactivity, and the individual dose was corrected for this residue. There was no group difference with respect to absolute administered dose. Six milliliters of venous blood from the proximal port of the catheter were obtained before and at 0.08, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 8.0, 24, and 48 h after injection. A total of 90 ml of whole blood (< 2% of the total blood volume) was collected. Blood samples were allowed to clot at room temperature for a minimum of 2 h. Serum was separated by centrifugation at room temperature (1,000 g, 10 min) and stored at −70°C.

Further animal procedures. Approximately 4.2 days after the 125I(T3) kinetic studies, nonfasted animals were weighed, and blood samples were obtained just before a lethal injection of 0.3 mg/kg T-61 (Hoechst, Regina, SK). Animals were killed in the exposure temperature and were then immediately transported to the necropsy room.

Three hours before death, a second dose of 125I(T3) was administered to measure the tissue uptake later at death. As with the kinetic studies, the animals received saturated KI solution orally for the 24 h before death. This second intravenous dose of ~36.4 pmol 125I(T3) (prepared, handled, and administered exactly like the kinetic dose) was delivered as part of a companion study (22). Two animals were killed without receiving the second dose. The residual radioactivity from the kinetic study dose was calculated to comprise < 3.0% of the radioactivity found in the liver 3 h after the second injection of 125I(T3).

Pericapsular fat and connective tissue were quickly removed from the thyroid gland before weighing. The wet weight of the liver and thyroid gland was then obtained. Tissue samples and homogenates were frozen at −70°C within 20 min of death. Liver homogenates were prepared as described under the methods of the 5'D-I assay.

Hormone extraction procedure. The 125I(T3) radioactivity in the serum samples and standards was measured as previously described (24). Briefly, an aliquot of serum was acidified to pH 3.0 with trichloroacetic acid (TCA), extracted with 4 volumes of 9:1 (vol/vol) ethyl acetate-butanol, and centrifuged at 800 g to promote phase separation. The 125I content of the organic phase was determined in a Gamma Trac 1193 counter (TM Analytic, Elk Grove, IL) with 75% counting efficiency. Aliquots of 125I(T3) stock, handled in a manner identical with the serum samples, were used as counting standards as well as controls for determination of the extraction coefficient. The aqueous phase routinely extracts > 99% of the free iodide by this technique (24). HPLC separation of the organic phase confirmed < 2% of the 125I to be free iodide or butanol nonextractable iodine. This method extracts 81.6 ± 1.5% of labeled T3 into the organic phase. Serum samples obtained 5 min after injection had an extraction efficiency of 81.7 ± 0.8%, which was identical to the standard, and confirmed by HPLC, to be > 98% 125I(T3).

Kinetic analysis. Kinetics of the serum 125I(T3) data were analyzed separately by multicompartmental (MC) and noncompartmental (NC) methods, and the analyses were done by different authors. Iterative least-squares curve fitting was used in both modeling methods.
MC analysis was done using the Simulation, Analysis, and Modeling methodology and applying a three-compartment mammary model that has been widely used for $T_3$ kinetics in other species. Before analysis, each pig's data were corrected for the measured plasma volume to obtain the percent dose in the plasma pool at each point in time. These values were used in the model as the content of compartment 1, the circulating $[125I]T_3$ compartment.

The model shown in Fig. 3 is not strictly identifiable in the form illustrated when all parameters are allowed to fit, yet the separate kinetics of the six- and fast-exchanging tissues are of interest. To handle this problem, a sequence of model fits was employed. First, the data for each pig were fitted to two "limiting" models, in which $k_{02}$ or $k_{03}$ was fixed at zero, so that all exit from the system was from compartment 2 or compartment 3, respectively. These fits provided outer bounds for the kinetic parameters for each pig. Results of this independent analysis of the two limiting model forms (shown in Table 3) showed that $k_{23}$ differed by only ~10% between the two extreme solutions. Hence, for the definitive solutions in which metabolism in both exchange compartments was examined, $k_{23}$ was fixed at its midpoint in the two limiting solutions. This left only five adjustable parameters, so that the model was now theoretically identifiable. The results of this final set of model solutions were used in the steady-state calculations and in the comparisons with the NC analyses.

The NC kinetic solution for comparison with an earlier human study (24) was based on a two-exponential fit of the serum $[125I]T_3$ decay data for each pig. The eigenvalues from these fits were then used to calculate the NC steady-state kinetic parameters (24).

The steady-state kinetic parameters from both the three-compartment and the NC solutions were then multiplied by the individual pig's serum $T_3$ levels to calculate the $T_3$ plasma disappearance rates (which equate to the minimum bound for production rate (PR) at steady-state) and the minimum bounds for $T_3$ content of the various compartments for the entire system (Q). $T_3$ produced and degraded in peripheral tissues without ever entering the blood will not appear in these steady-state calculations. Thus the PR and Q values calculated in this study represent lower bounds on $T_3$ production rate and tissue content. The upper bounds for PR and Q in the three-compartmental analyses presented here averaged 28 and 38% greater in the control and cold-exposed pigs, respectively, than the lower bounds of the three-compartmental solutions presented in RESAMTS. This underestimation is even more marked for the NC than for the MC solution method.

All steady-state parameters were normalized for body weight before statistical analysis.

Plasma volume and body composition measurements. Plasma volume and body composition were determined ~4 days before the kinetic studies at 0900–1100 h by injection of Evans blue dye and $^{3}H_2O$, respectively. Plasma samples were collected at 2, 6, and 24 h for calculation of these distribution spaces. The Evans blue dye concentration was determined spectrophotometrically, and the percent injected dose per liter was expressed for each animal. Extrapolation to time 0 provided estimates of plasma volume. Body composition was determined with live body weights and $^{3}H_2O$ distribution spaces (500 $\mu$Ci/animal; $sp$ act 70.27 $\mu$Ci/g; New England Nuclear). The $^{3}H_2O$ activity was determined in the same Packard Tri-Carb liquid scintillation analyzer, which has a counting efficiency of ~47%.

Body weights were determined 1 day before injection, and hematocrit (Hct) was measured by microcentrifugation the day of injection. Blood volume was calculated by (Evans blue distribution space) $\times$ [(1.0 – (0.96 × Hct/100))]$^{-1}$.

Miscellaneous assays. Immediately before injection of $[125I]T_3$ for the kinetic studies, blood samples were obtained for values of serum total thyroxine (TT$_4$), free $T_4$ (FT$_4$), total $T_3$ (TT$_3$), free $T_3$ (FT$_3$), thyrotropin (TSH), and Hct. Serum TT$_4$, FT$_4$, TT$_3$, and FT$_3$ determinations were carried out in quadruplicate with commercially available radioimmunoassay (RIA) kits that have been previously used with swine (Diagnostic Products, Los Angeles, CA) (12). Lower limits of detection for these assays were 4.0 nmol/l, 1.0 pmol/l, 0.11 nmol/l, and 0.77 pmol/l, respectively. The coefficient of variation (CV) of these assays, when repeated samples were analyzed on the same day, was ~3.8 and ~5.4% for samples measured on different days. Serum TSH was assayed using a commercially available kit (TSH-K-PR, CIS-US, Bedford, MA) with specificity for swine and sheep (12). This assay has an intra-assay CV of <5.0%. Hct values were measured by microcentrifugation and hemoglobin values by spectrophotometric analysis.

One gram of tissue from a few of the animals (liver, thyroid, skeletal biceps muscle) was homogenized, as described below, with tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4) and precipitated with TCA. Extraction was carried out with 4 volumes of ethyl acetate-butanol 9:1 (vol:vol). The organic solvent was dried with nitrogen, and the iodothyronines were separated using HPLC with 30-41 columns and a flow rate of 1.5-2 ml/min. Ultraviolet spectrophotometry (Waters, Milford, MA) was used for detection. Stock solutions of $T_3$, $T_4$, and reverse $T_3$ (r$T_3$, Sigma Chemical, St. Louis, MO) were used to identify migration peaks. Extraction efficiency was monitored using incubations of $[125I]T_3$ and was found to be consistent for the samples studied and similar to that for serum (~80%). The relative amounts of iodothyronine found in a single tissue had a pooled between-animal CV range of 12.7–36.5%.

Tissue homogenization. Liver tissue was dissected into 2-g sections and homogenized in 9 volumes of cold (4°C) Tris buffer (0.2 M, pH 7.4; Sigma Chemical) containing EDTA (2 mmol/l) and dithiothreitol (DTT; 2 mmol/l) (Buffer A) using a Bio-Homogenizer (Biospec Products, Bartlesville, OK). Aliquots of the homogenates were frozen at ~70°C within 20 min of death. Tissue samples of liver and thyroid were assayed by drying at 80°C for 24 h to determine percent DM. Kjeldahl digestion was used to estimate crude protein, and lipid content was analyzed after ether extraction.

$^{3}D$-I assay. Measurement of $^{3}D$-I kinetic parameters was by measurement of the percentage of $^{3}D$-I labeled $T_3$ to total $^{3}D$-labeled $T_4$ or reverse $T_3$ and free $T_3$. $^{3}D$-labeled $T_3$, T4, and reverse $T_3$ were used to identify migration peaks. Extraction efficiency was monitored using incubations of $^{[125]}T_3$ and was found to be consistent for the samples studied and similar to that for serum (~80%). The relative amounts of iodothyronine found in a single tissue had a pooled between-animal CV range of 12.7–36.5%.

Tissue homogenization. Liver tissue was dissected into 2-g sections and homogenized in 9 volumes of cold (4°C) Tris buffer (0.2 M, pH 7.4; Sigma Chemical) containing EDTA (2 mmol/l) and dithiothreitol (DTT; 2 mmol/l) (Buffer A) using a Bio-Homogenizer (Biospec Products, Bartlesville, OK). Aliquots of the homogenates were frozen at ~70°C within 20 min of death. Tissue samples of liver and thyroid were assayed by drying at 80°C for 24 h to determine percent DM. Kjeldahl digestion was used to estimate crude protein, and lipid content was analyzed after ether extraction.

$^{3}$I assay. Measurement of $^{3}$I-1 kinetic parameters was by measurement of the percentage of $^{3}$I-1 labeled $T_3$ to total $^{3}$I-1 labeled $T_4$ or reverse $T_3$ and free $T_3$. $^{3}$I-1 labeled $T_3$, T4, and reverse $T_3$ were used to identify migration peaks. Extraction efficiency was monitored using incubations of $^{[125]}T_3$ and was found to be consistent for the samples studied and similar to that for serum (~80%). The relative amounts of iodothyronine found in a single tissue had a pooled between-animal CV range of 12.7–36.5%.

Miscellaneous assays. Immediately before injection of $[125I]T_3$ for the kinetic studies, blood samples were obtained for values of serum total thyroxine (TT$_4$), free $T_4$ (FT$_4$), total $T_3$ (TT$_3$), free $T_3$ (FT$_3$), thyrotropin (TSH), and Hct. Serum TT$_4$, FT$_4$, TT$_3$, and FT$_3$ determinations were carried out in quadruplicate with commercially available radioimmunoassay (RIA) kits that have been previously used with swine (Diagnostic Products, Los Angeles, CA) (12). Lower limits of detection for these assays were 4.0 nmol/l, 1.0 pmol/l, 0.11 nmol/l, and 0.77 pmol/l, respectively. The coefficient of variation (CV) of these assays, when repeated samples were analyzed on the same day, was ~3.8 and ~5.4% for samples measured on different days. Serum TSH was assayed using a commercially available kit (TSH-K-PR, CIS-US, Bedford, MA) with specificity for swine and sheep (12). This assay has an intra-assay CV of <5.0%. Hct values were measured by microcentrifugation and hemoglobin values by spectrophotometric analysis.

One gram of tissue from a few of the animals (liver, thyroid, skeletal biceps muscle) was homogenized, as described below, with tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4) and precipitated with TCA. Extraction was carried out with 4 volumes of ethyl acetate-butanol 9:1 (vol:vol). The organic solvent was dried with nitrogen, and the iodothyronines were separated using HPLC with 30-41 columns and a flow rate of 1.5-2 ml/min. Ultraviolet spectrophotometry (Waters, Milford, MA) was used for detection. Stock solutions of $T_3$, $T_4$, and reverse $T_3$ (r$T_3$, Sigma Chemical, St. Louis, MO) were used to identify migration peaks. Extraction efficiency was monitored using incubations of $^{[125]}T_3$ and was found to be consistent for the samples studied and similar to that for serum (~80%). The relative amounts of iodothyronine found in a single tissue had a pooled between-animal CV range of 12.7–36.5%.

Tissue homogenization. Liver tissue was dissected into 2-g sections and homogenized in 9 volumes of cold (4°C) Tris buffer (0.2 M, pH 7.4; Sigma Chemical) containing EDTA (2 mmol/l) and dithiothreitol (DTT; 2 mmol/l) (Buffer A) using a Bio-Homogenizer (Biospec Products, Bartlesville, OK). Aliquots of the homogenates were frozen at ~70°C within 20 min of death. Tissue samples of liver and thyroid were assayed by drying at 80°C for 24 h to determine percent DM. Kjeldahl digestion was used to estimate crude protein, and lipid content was analyzed after ether extraction.
The reaction mixture consisted of Buffer A, $[^{125}]^T_4$ (100,000–150,000 cpm), thyroxine (0.5–20 μmol/l), and tissue homogenate (300–400 μg protein) in a total volume of 500 μl, with a pH of 7.0 (6.9–7.1).

Tubes were incubated at 37°C in a shaking water bath, and the reaction was terminated after 120 min by the addition of 500 μl serum and 1,000 μl 10% TCA. The mixture was centrifuged at 1,000 g for 10 min at 4°C, and the radioactive in the supernatant was determined in the same Gamma Trac 1193 counter (TM Analytic) with a 70% counting efficiency and confirmed by HPLC to be >95% free $^{125}I$. Repeated washes recovered >97% free $^{125}I$ from the reaction. The products in the TCA precipitate were extracted with a mixture of ethyl acetate-butanol (9:1) as previously described (24), and then they were separated and identified using HPLC. Nonradioactive $T_3$, $T_4$, and $rT_3$ were used as controls for identification of the peaks. The iodothyronine reaction products generated under these pH and incubation conditions were 29.3% $T_3$, 15.5% $rT_3$, and 5.6% diiodothyronines. Therefore inner ring deiodination (24), with its subsequent extremely rapid conversion of $rT_3$ to diiodothyronine, should not have accounted for more than ~8.0% of the generated free iodine.

All assays were performed in triplicate along with control tubes containing no homogenate. The nonspecific deiodination determined in these control tubes was minimal and subtracted as background. The enzyme assays were carried out within 14 days of each other with a single animal studied on a given day. Each animal's homogenate was selected randomly after alternation between control and cold treatment groups. An aliquot of stock homogenate was used to study the effect of freezer storage (~70°C). No detectable difference could be found in the activity of the stock homogenate over 1.5 mo of storage, which is in agreement with others who report that the enzyme stored in this manner is stable for up to 1 yr (30). Data were analyzed by the double reciprocal method of Lineweaver and Burk for estimates of the apparent Michaelis-Menten constant ($K_m$) and maximum enzyme velocity ($V_{max}$) during the period when the reaction rate was constant. This incubation period used ~10% of the substrate and represents the linear portion of the velocity curve, which is also in agreement with that of others (8, 20). A linear model, using least-squares regression, was fitted to the individual data. This model correlated well with the actual data, indicated by a P < 0.001 level of significance for the majority of fits. A single assay for one animal (B-3) was fitted to the individual data. This model correlated well with the control group, suggesting a slight increase in red cell mass. During exposure of the two groups to their respective temperatures, the skin temperature was not different between groups.

### Results

#### Physiological and anatomic measurements.

The cold-exposed animals consumed more dietary energy than the controls (P < 0.007), although the body weights and growth rates were not significantly different between groups (Table 1). The percent adipose, calculated from the body weight of the living pigs, was similar in both groups. The liver weight in the cold group tended to be greater than in controls but not quite significantly greater (P = 0.051). The absolute weight of the thyroid in cold-exposed animals was 86% greater than in control animals (P < 0.004). The amounts of protein, lipid, and water in thyroid and liver tissues were not different between control (14, 2.3, and 79% thyroid and 19, 1.1, and 74% liver) and cold-exposed animals (11, 1.8, and 83% thyroid and 18, 0.7, and 73% liver), respectively, when expressed as percentages of wet weight.

Blood volume and plasma volume were not significantly different between groups, although the total blood volume tended to be greater in the cold-exposed group (Table 2). Hct (P < 0.04) and hemoglobin (P < 0.05) were increased in the cold-exposed group compared with the control group, suggesting a slight increase in red cell mass. During exposure of the two groups to their respective temperatures, the skin temperature of the ear was reduced by 16.5 ± 2.3°C in the cold-exposed group (P < 0.0002), whereas the rectal temperature was not different between groups.

#### Thyroid hormone values.

Serum $TT_4$ [47.31 ± 2.18 vs. 31.96 ± 3.97 nmol/l (48%)], $FT_4$ [7.59 ± 0.81 vs. 4.72 ± 0.46 pmol/l (61%)], and $TT_3$ [1.43 ± 0.11 vs. 0.70 ± 0.04 pmol/l] were similar between control and cold-exposed animals.

#### Values are means ± SE of 5 boars in each treatment temperature

### Table 1. Total energy intake, organ and body weights, growth rates, and percent body fat for control and cold-exposed animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cold</th>
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<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>7.22 ± 3.2</td>
<td>81.0 ± 3.2</td>
</tr>
<tr>
<td>Liver weight, kg</td>
<td>2.6 ± 0.2</td>
<td>3.1 ± 0.1*</td>
</tr>
<tr>
<td>Thyroid weight, g</td>
<td>9.56 ± 0.32</td>
<td>17.77 ± 1.99†</td>
</tr>
<tr>
<td>Lipid content, %</td>
<td>21.7 ± 0.27</td>
<td>22.3 ± 0.30</td>
</tr>
<tr>
<td>Energy intake, g/day</td>
<td>2525 ± 277</td>
<td>3817 ± 216†</td>
</tr>
<tr>
<td>Growth rate, kg/day</td>
<td>1.17 ± 0.18</td>
<td>1.02 ± 0.10</td>
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Values are means ± SE of 5 boars in each treatment temperature after 25 days of exposure to either 22 or 4°C. Wet weights of liver and thyroid and %body lipid were determined by in vivo $^3$H$_2$O injection, mean energy intake/day at completion of study, and linear regression of growth rate over study period. Significantly different from control: *P < 0.05; †P < 0.007.
COLD AND $T_3$ KINETICS

A few animals ($n = 1–3$ for each assay). The molar ratio of $T_4$ to $T_3$ ($T_4/T_3$) in the tissues from control animals was, for liver, 15.0:1 (1); thyroid, 108.2:1 (1); and skeletal muscle, 0.059:1 (2). In cold-exposed animals the ratio was, for liver, 7.30:1 (1); thyroid, 37.9:1 (3); and skeletal muscle, 0.053:1 (1). The cold-exposed animals showed a reduction in $T_4/T_3$ by 51.3% for liver, 65.0% for thyroid, and 10.2% for skeletal muscle. When the cold treatment changes for liver and thyroid were pooled and compared with those of muscle, this difference was 48.0 ± 6.9% greater for liver and thyroid ($P < 0.02$).

**Serum kinetic parameters.** The mean serum values for the two groups of animals are presented in Fig. 2, together with a two-exponential fit to the mean data as used with the NC analysis of the data for individual pigs.

The compartmental parameters resulting from the initial "limiting" fits of the three-compartmental model (Fig. 3) to the serum data are presented in the first two segments of Table 3. These values can be considered outer bounds for these parameters. The third segment of Table 3 shows the compartmental parameters for the model solutions in which we assume a midpoint value for $k_{21}$, with separate solutions of the exit parameters from both peripheral compartments. Although there appears to be a trend toward slower exchange rates for both peripheral compartments in the cold-exposed animals, this is not statistically significant. However, the increased rate of exit from the cold-exposed animals' slow-exchange compartments (noted in the third solution set) is significant ($P < 0.05$).

Table 4 presents the steady-state kinetic parameters calculated from the third (combined) set of these model parameters for comparison with the results of the NC model steady-state parameters derived from the eigen
values of the two-exponential fits. The steady-state T₃ content and clearance values, calculated from the steady-state kinetic parameters and the observed serum T₃ concentrations, are also presented. The kinetic steady-state parameters — the plasma-equivalent volumes of distribution (V), plasma clearance rates (PCR), as well as the fractional clearance rates (FCR) and mean residence times (MRT) — show little, if any, change with cold exposure. There is a marginally significant (P < 0.05) decrease in V in the slow-exchange tissues, which cold exposure. There is a marginally significant (P < 0.05) decrease in V in the slow-exchange tissues, which is balanced by an increase in FCR (P < 0.05) from these same tissues. The product of V and FRC is the PCR, which remains unchanged with cold exposure. The FCR and MRT data are given later in the text.

On the other hand, as might be expected, when V and PCR are multiplied by the serum T₃ concentrations to calculate the Qs and PRs, the increased circulating T₃ levels in the cold-exposed animals are reflected in marked increases in Q and PR.

Table 3. Parameter values resulting from solution of three-compartment model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>V, l/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment 1 (plasma)</td>
<td>0.079±0.005</td>
<td>0.073±0.008</td>
</tr>
<tr>
<td>Compartment 2 (fast)</td>
<td>0.16±0.02</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Compartment 3 (slow)</td>
<td>0.93±0.09</td>
<td>0.69±0.02*</td>
</tr>
<tr>
<td>Total compartmental</td>
<td>1.17±0.11</td>
<td>0.91±0.02*</td>
</tr>
<tr>
<td>Noncompartmental</td>
<td>0.95±0.07</td>
<td>0.72±0.04*</td>
</tr>
<tr>
<td>Q, nmol/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment 1 (plasma)</td>
<td>0.055±0.001</td>
<td>0.113±0.010†</td>
</tr>
<tr>
<td>Compartment 2 (fast)</td>
<td>0.11±0.02</td>
<td>0.22±0.03†</td>
</tr>
<tr>
<td>Compartment 3 (slow)</td>
<td>0.65±0.07</td>
<td>1.10±0.10†</td>
</tr>
<tr>
<td>Total compartmental</td>
<td>0.81±0.07</td>
<td>1.43±0.47</td>
</tr>
<tr>
<td>Noncompartmental</td>
<td>0.66±0.05</td>
<td>1.01±0.06†</td>
</tr>
<tr>
<td>PCR, l·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment 2 (fast)</td>
<td>0.79±0.09</td>
<td>0.68±0.06</td>
</tr>
<tr>
<td>Compartment 2 (slow)</td>
<td>0.84±0.09</td>
<td>0.86±0.10</td>
</tr>
<tr>
<td>Compartmental whole body</td>
<td>1.63±0.17</td>
<td>1.55±0.12</td>
</tr>
<tr>
<td>Noncompartmental</td>
<td>1.56±0.15</td>
<td>1.43±0.10</td>
</tr>
<tr>
<td>PAR, nmol·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment 2 (fast)</td>
<td>0.56±0.07</td>
<td>1.07±0.11†</td>
</tr>
<tr>
<td>Compartment 2 (slow)</td>
<td>0.69±0.05</td>
<td>1.22±0.13†</td>
</tr>
<tr>
<td>Compartmental whole body</td>
<td>1.14±0.11</td>
<td>2.40±0.16†</td>
</tr>
<tr>
<td>Noncompartmental</td>
<td>1.10±0.11</td>
<td>2.01±0.11†</td>
</tr>
</tbody>
</table>

When the total body results of the three-compartment and NC solutions are compared, the Vs and PCRs and their T₃ counterparts, the Qs and PRs, are greater with the three-compartment models, as expected. Also, as noted above, even the MC solution gives only lower bounds for V, Q, PCR, and PR, the upper bounds being 26 and 38% higher in the control and cold-exposed pigs, respectively.

FCR measured in percent per day, which we would expect to be equivalent in the two methods, is smaller in the MC solutions (cold vs. control): FCRfast 504±52 vs. 512±28; FCRslow 124±14 vs. 91±5 (P < 0.05); FCRwhole body 171±12 vs. 140±6 (P < 0.05); FCRNC 198±8 vs. 166±10 (P < 0.05). There is a corresponding lengthening of MRT with the MC over the NC solutions measured in hours: MRTfast 5.0±0.6 vs. 4.7±0.2; MRTslow 20.3±1.8 vs. 26.8±1.3 (P < 0.05); MRTwhole body 14.4±0.9 vs. 17.3±0.8 (P < 0.05); MRTNC 12.2±0.5 vs. 14.7±1.0 (P < 0.05). These differences are probably due to the choice of a two-exponential fit of the data for the NC solutions, which tends to underemphasize the late flattening of the decay slope.

The calculated experimental changes were in the same direction and of magnitude comparable to the different model solution methods. We believe, therefore, that these discrepancies are not important in interpretation of the experimental results and, unless specified, we will refer to the NC solutions.

Table 4. In vivo serum T₃ kinetic parameters of control and cold-exposed animals calculated by both multicompartamental and noncompartmental methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cold Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>V, l/kg</td>
<td></td>
<td></td>
</tr>
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<td>Compartment 1 (plasma)</td>
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<tr>
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<td></td>
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</tr>
<tr>
<td>PCR, l·kg⁻¹·day⁻¹</td>
<td></td>
<td></td>
</tr>
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<td>Compartment 2 (fast)</td>
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<td></td>
<td></td>
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<td>Compartment 2 (fast)</td>
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</tr>
</tbody>
</table>

Steady-state parameters derived from 3rd set of 3 compartment model solutions presented in Table 3 and observed steady-state serum triiodothyronine (T₃) content, presented in comparison with results of an independent noncompartmental analysis. Plasma-equivalent volume of distribution (V), T₃ content (Q), plasma-equivalent clearance rate (PCR), and T₃ plasma absorption rate (PAR) values represent minimum bounds (see text) for those values. All results are presented as means ± SE of 5 animals. Significantly different from control: *P < 0.05; †P < 0.01; ‡P < 0.001.
5'D-I activity. The Lineweaver-Burk plots support a major effect on $V_{\text{max}}$ shown by the change in the $y$-intercept and slope and a minor effect on the $K_m$ represented by the $x$-intercept (Fig. 4) that are upheld in the analysis of individual animal parameters. In the cold-exposed group, $V_{\text{max}}$ of 5'D-I was increased to $2.50 \pm 0.34 \text{ pmol I} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$, compared with the mean control group value of $1.21 \pm 0.25 \text{ pmol I} \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$; $P < 0.02$. There was no significant difference in $K_m$ between cold ($2.22 \pm 0.33 \text{ pmol/l}$) and control ($1.88 \pm 0.33 \text{ pmol/l}$) groups. The ratio $V_{\text{max}}/K_m$ (30) was different between groups and increased ~70% in the cold-exposed swine ($P < 0.01$) compared with control [1.15 ± 0.06 vs. 0.68 ± 0.12 pmol I \cdot \text{min}^{-1} \cdot \text{mg protein}^{-1} \cdot (\text{pmol/l})^{-1}].

Correlations. A significant positive correlation with a linear regression ($n = 10$) was determined between PAR and thyroid size ($r = 0.818, P < 0.002$; Fig. 5), between PAR and serum $\text{TT}_4$ ($r = 0.761, P < 0.005$), and between PAR and hepatic 5'D-I activity ($r = 0.564, P < 0.044$). Serum $\text{TT}_4$ was not significantly correlated with 5'D-I activity.

DISCUSSION

Twenty-five days of continuous cold exposure doubles serum $\text{TT}_3$, $\text{FT}_3$, thyroid weight, $\text{T}_3$ PR, and hepatic 5'D-I $V_{\text{max}}$ with smaller increases in $\text{TT}_4$ and $\text{FT}_4$, whereas serum TSH remains unchanged. Energy intake increased ~50% in the cold-exposed animals to maintain growth rates similar to those of controls. These findings establish that the physiological response of cold-exposed adult male swine involves an increased $\text{T}_3$ production, with hepatic 5'D-I probably having a significant role in this adaptation.

This swine model (6, 11, 16) mimics some human responses. Men exposed to cold (~4–11°C) increase oxygen utilization. If they are subjected to these temperatures for 12–14 days while partially clothed, thyroidal iodine clearance and serum $[125\text{I}]\text{T}_4$ removal increase (15). Furthermore, as recently reported with this type of cold exposure, human $\text{T}_3$ production and clearance increase independent of TSH, even though humans have little contribution from BAT (1, 23). Our major objective was to study the influence of cold on $\text{T}_3$ kinetics with animals in energy balance. Cold-exposed swine maintain growth rates by increasing their energy intake (12, 13, 18); thus feed consumption cannot be isolated from the other effects of cold in animals fed ad libitum as done in our study. This study, therefore, cannot separate the causal relationship between the two variables of cold exposure and increased energy intake on $\text{T}_3$ generation. The increased energy intake was necessary to maintain nutritional balance as defined by the similar amounts of body fat and rates of growth of the two groups. Calorie restriction, on the other hand, decreases hepatic $\text{T}_3$ production (27) and in vivo $\text{T}_4$ degradation (9) in swine. Consequently, equalizing the energy intake between treatments to that of the control group would result in possibly confounding conditions. Further studies that limit energy intake during cold exposure will need to be carried out to expand our understanding of this relationship.

The increased serum concentrations of $\text{TT}_3$, $\text{FT}_3$, and $\text{TT}_4$ with cold exposure are similar to those reported in
younger swine living for 3 wk in temperatures between 10 and 12°C (12, 13). Macardi has shown in thyroidectomized swine that increased serum thyroid hormone values are critical for sustaining the increased energy intake found during cold exposure (18). Taken together, these earlier observations and our findings support the hypothesis that increases in thyroid hormone production are adaptive physiological responses to cold exposure associated with increased energy intake and a normal growth rate.

Contrary to Herpin et al. (12), we do not find a significant difference in the TSH values between the groups. Animal maturity, circadian fluctuation, or energy balance at the time of death may account for this discrepancy between studies in serum TSH concentrations. Alternatively, homeostatic feedback should result in decreased serum TSH when circulating free T4 is markedly elevated, suggesting the possibility of decreased pituitary sensitivity in both studies. Conversely, thyroidal enlargement may be mediated by small changes in TSH, antibodies to the TSH receptor, or the sensitivity of the gland to growth factors. Unfortunately, we have no histological assessment of these glands to help clarify the mechanism of enlargement. The sensitivity of this porcine TSH assay may not be sufficient to detect small differences between groups, and thus subtle changes may be missed.

The elimination rate of [125I]T4 doubles if energy intake is allowed to increase in 8- to 12-wk-old swine exposed to cold (5°C) for 4 days (6). However, when energy intake is restricted to that of the control period, the T4 removal rate with cold is also similar to that of the control period (6, 16). Because increased energy intake is required by swine during exposure to cold, then these data reported by both Evans and Ingram (6) and Ingram and Kaciuba-Uscilko (16) are in general agreement with human studies by Suda et al. (28) and with swine studies by Griggio and Ingram (9). These findings may be explained by a degree of environmental undernutrition interacting with cold exposure to result in a relatively decreased T4 plasma clearance rate, a mechanism possibly mediated by decreased 5'D-I activity. Gastrointestinal loss of conjugated T3 and T4 through bile secretion is usually small but may increase in parallel with energy intake and therefore represent another possible mechanism to explain these changes.

The serum [125I]T3 kinetic parameters in our study are difficult to compare with other published porcine data (5, 9). These earlier studies used less mature animals and terminated the experiment 3 h after injection, reporting only a single rate constant. This type of analysis is limited by a single distribution rate constant model for T3 kinetics. The more complex kinetics of T3 with slow or late plasma disappearance, is not accounted for in earlier reports (Fig. 2) (5, 9). The values for control swine that we provide using NC techniques are similar to canine and feline values calculated over a period to include both rate constants by use of NC techniques (10).

The major increase in T3 Q in the face of little or no change in V is supported by the in vitro assay of these tissues after administration of a bolus [125I]T3 dose 3 h before death, as previously reported (22). The serum-to-tissue ratio of the tracer was similar for most tissues, resulting in increases in Qtissue. An exception was subcutaneous hip fat, which showed an increased uptake even though the extraction coefficient was constant, suggesting a subtle tissue-dependent distribution effect not detectable with our NC analysis of the kinetic data. The three-compartmental analysis using fast, slow, and plasma compartments suggests that the increase in the tissue FCR of T3 noted in the NC solution is due to the slowly exchanging tissues such as fat. The increase of in vivo plasma T3 appearance rate that we describe may be associated with either increasing thyroidal T3 production or with an augmented peripheral production of T3 by 5'D-I. Factors such as immunoglobulins (19) or sympathetic nervous system stimulation that may shift thyroid gland or peripheral 5'D-I hormonogenesis to favor T3 production have not been eliminated by this study.

The relative tissue content of T4 and T3 appears to change with cold exposure in two of the three tissues studied (liver and thyroid). The tissue uptake of T4 is not likely decreased by cold air treatment (6), and T3 uptake is not increased from these tissues using our compartmental analysis or in vitro techniques (22). The apparent decline (~50%) in T4/T3 implies either increased tissue production of T3, increased degradation of T4, or both. The two tissues that decrease this ratio are liver and thyroid, both of which have very effective 5'D-I activity. Swine skeletal muscle, on the other hand, has only ~3% the activity per gram of protein as swine liver (26) and shows little change during the same period of cold exposure. These preliminary results suggest to us that the thyroid with its 5'D-I activity may have a significant role in contributing to the increased in vivo T3 production rate found in the present study.

We are not able to distinguish the sequence for increasing T4 (6) and T3 production in swine fed ad libitum. Two possibilities seem likely: 1) the increased serum T4 production stimulates an increase in 5'D-I activity and production of T3, or 2) increased 5'D-I activity stimulated by a circulating substance increases T3 production in tissues with known 5'D-I activity such as liver, kidney, and thyroid, whereas the thyroid alone, responding as if to TSH, also produces T4.

The activity of 5'D-I is not PTU sensitive, whereas type II thyroidyronine 5'-deiodinase activity is relatively unaffected by PTU (8). PTU inhibition of the enzyme that we assayed characterizes it predominately as type I, which is commonly found to be decreased in fasted swine (27). Type I enzyme activity in rodents is increased with hyperthyroidism, decreased with hypothyroidism, and variable with cold exposure (20, 21). A cold stimulus and fixed T4 replacement in hypothyroid animals increases 5'D-I even though serum T4 values may be very low, suggesting that the regulation with cold exposure is not dependent on increased circulating T4 concentrations (20). In contrast, however, Pazos-Moura et al. (21) found no change in hepatic 5'D-I activity compared with
large changes in BAT 5'-D-II activity of iodine-deficient rats replaced with iodine and exposed to cold.

Our values for $V_{\text{max}}/K_m$ [0.68 ± 0.12 pmol I·min$^{-1}$·mg protein$^{-1}$·(μmol/l)$^{-1}$ in the control group] are in agreement with values determined in swine up to age 42 days (26), where thyroxine was also used as a substrate for this enzyme. Differences in $V_{\text{max}}$ between studies may depend on the age of the animals (26) and the state of nutrition at death because only moderate energy restriction results in an approximately threefold decrease in the net $T_3$ produced by the liver homogenates (27). These earlier studies also differ from our own in that the reaction product $T_3$ was measured by RIA (3, 26, 27), possibly contributing to the substantial within-group variability of adult animals (26).

Hepatic and renal contributions to daily $T_3$ in vivo production have been reported to be between 50 and 80% (14). Because the liver and kidneys together are thought to account for the majority of the fast-exchanging tissues assessed in the three-compartment model, this model form gives additional information about the distribution of $T_3$ production and disposal. PR in our control animals was approximately the same in the fast and the slowly exchanging compartments, but the fraction attributable to the slowly exchanging tissues increased slightly (from 51 to 55%) in the cold-exposed animals.

Calculations from absolute in vitro enzyme activity involving many estimated variables predict only 16% of total daily $T_3$ production (30). This inability to predict accurately the in vivo $T_3$ production may be attributed to in vitro studies that do not use natural cofactors such as reduced glutathione. Another reason for these discrepancies might be that the concentration of the substrate near the enzyme is increased with an active pump that may depend on the age of the animals (26) and the state and the target tissue relevance of these findings.

In conclusion, we report that, after 25 days of cold exposure, $T_3$ production was increased along with thyroidal size and the deiodination of thyroxine by hepatic 5'-D-I. During cold exposure, increased serum $T_3$, concentrations and thyroid weights were not associated with changes in serum TSH. Further study is required to completely understand the time course of trophic events and the target tissue relevance of these findings.

We thank Everett Dixon, Fran Hoeksema, David McKinnay, Jack Francis, Paul Gregory, Sam Castro, and Geraldine Licauco for their technical assistance. Munnaza Malik for HPLC determinations, Drs. Inver Chopra and Louis Homer for discussion of the 5'-D-I assay, Dr. Beth Walker for mathematical modeling of energy requirements, and Patricia Mullinix and Christine Reed for manuscript preparation.

Experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Publication (NIH) 86-23 (1985).

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Present addresses: R. L. Hesslink, 10850 Sabre Hill Dr. #236, San Diego, CA 92128; M. M. D'Alessandro, Department of Chemistry, US Naval Academy, Annapolis, MD 21402-5026; B. A. Young, Dept. of Animal Production, Gatton College, University of Queensland, Lawes, QLD 4343, Australia.

Address for reprint requests: H. L. Reed, HSHL-ME, Endocrine-Metabolic Service 7D, Department of Medicine, Walter Reed Army Medical Center, Washington, DC 20307-5001.

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