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Deiodination of *l*-thyroxine in vitro by peripheral leukocytes from rhesus monkeys with bacterial sepsis

FREDERICK R. DERUBERTIS* *Pittsburgh, Pa.*

The deiodination of l-thyroxine (T_4) in vitro by peripheral leukocytes isolated from healthy rhesus monkeys was compared to that of leukocytes from monkeys with acute **Salmonella typhimurium** sepsis, an infection associated with accelerated metabolism of T_4 in vivo. Deiodination of T_4 by leukocytes from septic monkey donors was significantly enhanced, with inorganic iodide identified chromatographically as the predominant product of T_4 degradation. Induction of phagocytosis in vitro potentiated the T_4 deiodinating activity of leukocytes from both control and infected monkeys. However, the proportion of added T_4 degraded by leukocytes from septic donors following stimulation of phagocytosis in vitro was nearly twice that of cells from controls. Although mixed populations of isolated leukocytes (predominantly neutrophils and lymphocytes) were studied, the metabolism of T_4 in vitro was almost exclusively an action of the neutrophil. By contrast with the enhanced T_4 deiodinating activity of neutrophils from septic hosts, the rate of ^{14}C-1-glucose oxidation in vitro by these cells was not detectably different from that of neutrophils from control monkeys, when assessed basally or after induction of phagocytosis. The data suggest that deiodination of T_4 by host neutrophils might contribute to the acceleration of T_4 metabolism observed in vivo during some acute infections. The quantitative importance of neutrophil metabolism of T_4 in vivo, the mechanisms mediating enhanced hormonal degradation by these cells, and the extent to which iodide released from T_4 is utilized in the myeloperoxidase-H_2O_2-halide antimicrobial system as part of a host-defense system against invasive bacteria remain uncertain.

Accelerated host metabolism of *l*-thyroxine (T_4) has been observed during acute bacterial pneumonia in man[1] and during bacterial sepsis in the rhesus monkey.[2-4] These illnesses are characterized by significant increases in the number of circulating leukocytes. Since leukocytes stimulated to phagocytize in vitro accumulate and deiodinate T_4 more rapidly than resting cells,[5,6] it seemed possible that the enhanced metabolism of T_4 accompanying some acute bacterial infections might, at least in part, be attributable to increased deiodi-

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In conducting the research described in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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Table I. Deiodination of ^{131}I-labeled-*l*-thyroxine (T_4) in vitro by peripheral leukocytes from rhesus monkeys

Group	Hours after inoculation			
	Basal	8	24	48
	(% added ^{131}I-T_4/10^7 neutrophils per 2 hours)			
Control (6)	11.3	12.4	10.5	9.6
	± 1.2	± 1.4	± 1.3	± 1.0
SALM (6)	9.4	12.9	11.0	11.7
	± 1.1	± 1.5	± 1.3	± 1.4
Infected (6)	10.8	16.5*	18.3*	17.4*
	± 1.0	± 1.7	± 2.1	± 1.9

Values shown are means ± S.E. Basal rates represent the average of 2 sequential preinoculation determinations made at 24 hours and immediately prior to inoculation. Monkeys were inoculated intravenously with either saline (control) or a saline suspension of 2 × 10^9 heat-killed (SALM) or viable (infected) *S. typhimurium* at hour 0. Reaction mixtures were adjusted to contain a total of 10^7 neutrophils.

*Indicates $p < 0.05$, comparing the postinoculation value to both its own basal value and to the value of control at the corresponding hour of study.

Table II. Effects of induction of phagocytosis in vitro on the deiodination of ^{131}I-labeled-*l*-thyroxine (T_4) by peripheral leukocytes from rhesus monkeys*

Reaction mixture	Control (4)		Infected (5)	
	^{131}I-T_4 deiodination			
	OM + I-	OM	OM + I-	OM
Leukocytes	9.9	0.9	16.8†	1.6
	± 1.3	±0.2	± 1.9	±0.4
Leukocytes + *S. typhimurium*	28.7*	5.2*	45.6*†	8.6*†
	± 2.8	±0.6	± 5.1	±1.1
Leukocytes + *E. coli*	25.1*	4.3*	41.3*†	7.9*†
	± 2.4	±0.7	± 4.6	±1.2

Leukocytes were isolated from 9 monkeys 24 hours after intravenous inoculation with saline (control) or a saline suspension of 2 × 10^9 viable *S. typhimurium* (infected). All monkeys were studied concomitantly as a single group. Reaction mixtures were adjusted to contain a total of 10^7 neutrophils with or without heat-killed bacteria (20 bacteria per neutrophil). Values shown are means ± S.E., expressed as per cent of added ^{131}I-T_4/10^7 neutrophils per 2 hours. OM represents product appearing as chromatographically immobile origin material; I-, that as iodide.

*Indicates $p < 0.05$ compared to corresponding value for leukocytes alone.

†Indicates $p < 0.05$ comparing corresponding conditions of control and infected.

nation of T_4 by activated leukocytes of the infected host. There is evidence to suggest that in vitro deiodination of T_4 by leukocytes from infected monkeys may be increased, although this possibility was not examined in detail.[2] Accordingly, in the present study, the degradation of T_4 by peripheral leukocytes from monkeys with *Salmonella typhimurium* sepsis was assessed in vitro. This infection has been shown to markedly potentiate hepatic metabolism of T_4 in vivo and is associated with a neutrophilic leukocytosis.[4]

Methods

Healthy male rhesus monkeys (*Macaca mulatta*), weighing between 2.7 and 4.3 kilograms, were secured in primate chairs, fed a standard diet, and allowed water ad libitum. After a 7-day period of adaptation, monkeys were inoculated intravenously with 1 ml. of saline

Table III. Deiodination of ^{131}I-labeled-*l*-thyroxine (T_4) in vitro by peripheral lymphocytes from rhesus monkeys

Monkey	Reaction mixture	Exp. 1	Exp. 2
		(% added ^{131}I-T_4/10^7 cells per 2 hours)	
Control	A. Mixed Leukocytes	11.8 (39%)	9.5 (31%)
	B. Lymphocytes	0.5 (4%)	0.4 (3%)
	C. Lymphocytes + *S. typhimurium*	0.9 (4%)	0.7 (3%)
Infected	A. Mixed leukocytes	17.0 (86%)	14.2 (81%)
	B. Lymphocytes	0.8 (5%)	1.1 (7%)
	C. Lymphocytes + *S. typhimurium*	1.1 (5%)	1.5 (7%)
	D. Mixed leukocytes + lymphocytes	16.2 (30%)	14.9 (32%)

Mixed peripheral leukocytes and purified lymphocytes were isolated from monkeys 24 hours after inoculation with saline (control) or with 2×10^9 viable *S. typhimurium* (infected). Experiments 1 and 2 were conducted on separate days, employing cells from a different control and infected monkey pair. Reaction mixture A (mixed leukocytes) was adjusted to contain a total of 10^7 neutrophils and results are expressed per 10^7 neutrophils, uncorrected for any contribution of lymphocytes. B and C contained 10^7 purified lymphocytes, and results are expressed per 10^7 lymphocytes. The cell population of D consisted of a total of 10^7 neutrophils, added as mixed leukocytes, plus approximately 1.8×10^7 purified lymphocytes from the same monkey, added to reduce the proportion of neutrophils in the final reaction mixture. Results in D were expressed per 10^7 neutrophils. The final proportion of neutrophils in each reaction mixture is shown in parentheses. The ratio of heat-killed bacteria to lymphocytes was 20/1.

(control) or with 1 ml. of a saline suspension containing 2×10^9 heat-killed (Salm) or viable (infected) *S. typhimurium*. Organisms were prepared as previously reported.[4] Rectal temperatures, blood counts, and cultures were monitored. To evaluate deiodination of T_4 by leukocytes obtained sequentially from these monkeys (Table I), heparinized femoral venous blood samples (20 ml.) were drawn percutaneously at 24 hours and immediately prior to inoculation, and then at 8, 24, and 48 hours after inoculation. In these experiments (Table I) monkeys were studied in two groups of 9, with each study group consisting of 3 control, 3 Salm, and 3 infected monkeys. Data from these two groups were pooled for analysis. In all other experiments (Tables II through IV), larger blood samples (100 to 130 ml.) were obtained by femoral arterial catheterization 24 hours after inoculation of monkeys with either saline or viable salmonella. These monkeys were anesthetized with pentobarbital (50 mg. per kilogram intramuscularly) immediately prior to instrumentation.

Preparation of peripheral leukocytes. Peripheral leukocytes were isolated from freshly drawn heparinized whole blood by dextran sedimentation at 4° C. Plastic syringes and laboratory ware were used throughout. Following sedimentation, residual erythrocytes in the leukocyte pellets were lysed by exposure to cold distilled water for 25 seconds and hemoglobin removed by washing the cells 3 times with KRPG. Final cell suspensions were essentially free of contaminating erythrocytes. Purified lymphocytes were prepared from dextran-sedimented mixed leukocytes by nylon chromatography.[7] Cell viability, assessed at the initiation of all incubations by trypan blue exclusion, generally exceeded 95 per cent. Cells were always studied on the day of isolation and maintained at 4° C. until incubation.

Determination of T_4 deiodination. Leukocytes were incubated in 2 ml. of Krebs Ringers phosphate buffer (pH 7.4, 0.5 mM Ca^{++}) with 4 mg. of glucose (KRPG) and 0.2 μg of ^{131}I-labeled-*l*-thyroxine (^{131}I-T_4) (Obtained from Abbott Laboratories, North Chicago, Ill.). Since heterogeneous populations of peripheral leukocytes (primarily neutrophils + lymphocytes) were routinely employed for study, a standard number of neutrophils (10^7) was added to each reaction mixture. In order to maintain a constant neutrophil concentration in all assays, the total number of leukocytes added to the reaction mixtures varied from 2 to 4×10^7 cells from the noninfected monkeys to no more than 1.6×10^7 cells from infected monkeys. This variation was owing to the lymphocytic predominance (25 to 50 per cent neutrophils) of the

Table IV. Oxidation of ^{14}C-1-glucose in vitro by peripheral leukocytes from rhesus monkeys

Reaction mixture	*Control*	*Infected*
	(c.p.m. $^{14}CO_2$/10^7 cells per hour)	
A. Mixed leukocytes	1,745 (38%) ± 162	1,868 (91%) ± 177
B. Mixed leukocytes + *S. typhimurium*	7,708 (38%) ± 644	8,230 (91%) ± 735
C. Lymphocytes	394 (2%) ± 36	443 (4%) ± 38
D. Lymphocytes + *S. typhimurium*	427 (2%) ± 39	506 (4%) ± 52

Values shown are mean ± S.E. of triplicate determinations of $^{14}CO_2$ generation from ^{14}C-1-glucose in a representative experiment (performed 3 times) by mixed peripheral leukocytes or purified lymphocytes obtained 24 hours after inoculation of one monkey with saline (control) and another monkey with 2×10^9 viable salmonella (infected). Reaction mixtures A and B were adjusted to contain a total of 10^7 neutrophils and results are expressed per 10^7 neutrophils, after correction for the calculated contribution from lymphocytes. C and D contained approximately 10^7 purified lymphocytes and results are expressed per 10^7 lymphocytes. The proportion of neutrophils present in each reaction mixture is indicated by the values in parentheses.

cell populations obtained from healthy monkeys and the neutrophilic predominance (> 70 per cent) of those from infected monkeys. When deiodination of T_4 by purified lymphocytes was assessed (Table III), 10^7 lymphocytes were employed in each reaction mixture. To examine the effects of bacteria on the rate of T_4 deiodination by monkey leukocytes in vitro, heat-killed (56° C. for 30 minutes, *S. typhimurium* or *Escherichia coli* were added to reaction mixtures containing mixed leukocytes or purified lymphocytes at a ratio of 20 bacteria per neutrophil or lymphocyte, respectively. Prior to addition, bacteria were opsonized by incubation with pooled normal monkey serum for 20 minutes at 37° C. and then washed twice with KRPG to remove excess serum protein.

Leukocytes from 3 healthy monkeys were incubated in the presence or absence of endotoxin (lipopolysaccharide B, *S. typhimurium*; Difco Laboratories, Detroit, Mich.). The latter was tested in concentrations of 0.1, 1.0, 10, and 100 μg per milliliter of incubation medium.

Reaction mixtures were routinely incubated in plastic vials for 2 hours in a Dubnoff metabolic shaker at 37° C., with duplicate vials run for each condition studied. This incubation time was chosen because preliminary investigations indicated that the percentage of added ^{131}I-T_4 deiodinated by monkey leukocytes reached a plateau value between 1 and 2 hours. In each experiment, leukocyte-free vials were employed to correct for nonspecific T_4 degradation (3 to 5 per cent of total ^{131}I-T_4 added) and, where appropriate, the effects of heat-killed bacteria alone were assessed. Reactions were stopped by adding to each vial 500 μl of 25 per cent human serum albumin containing propylthiouracil, carrier T_4 and iodide. The proportion of ^{131}I-T_4 deiodinated was then determined by subjecting 10 μl aliquots of this mixture to ascending chromatography on filter paper strips in a butanol-acetic acid-water solvent system. In this system, T_4 migrates most rapidly from the origin and is clearly separated from the more slowly moving inorganic iodide (I-) and from immobile origin material (OM).[8] The labeled areas of the strips were identified by autoradiography and counted in a well-type scintillation counter. The percentage of added ^{131}I-T_4 deiodinated was then calculated as I- + OM × 100/I- + OM + T_4. The precise nature of OM, the chromatographically immobile iodinated product formed during metabolic degradation of T_4 by mammalian tissues, is unknown. However, there is considerable evidence to indicate that it is comprised predominantly of iodoproteins, generated from the transfer of hormonally derived iodine to protein moieties.[9]

^{14}C-1-glucose oxidation. In these experiments, 10^7 neutrophils (as mixed leukocytes) or 10^7 purified lymphocytes were incubated in a metabolic shaker for 1 hour at 37° C. in 2 ml. of KRPG containing 2 mg. of unlabeled glucose and 1 μCi of ^{14}C-1-glucose (Amersham/

Searle, Inc., Arlington Heights, Ill.) (2.9 mCi per millimole). Incubations were conducted in siliconized, 25 ml. glass Erlenmeyer flasks which were sealed with a serum cap containing a center well. At the completion of the incubation, $^{14}CO_2$ formed was liberated by the addition of 0.2 ml. of 6 N H_2SO_4 to the reaction mixture, and collected in 0.2 ml. of Hyamine hydroxide (Packard Instrument Co., Downers Grove, Ill.) in the center well. After a 45-minute equilibration period, the entire center well was transferred to counting vials containing 15 ml. of toluene-based scintillation solution (Scintisol Complete) (Isolab, Inc., Akron, Ohio) and counted in a well-type liquid scintillation counter.

Differences between mean values were analyzed statistically using Student's t-test for unpaired values.

Results

Deiodination of T_4 by leukocytes from infected monkeys. During the 48-hour period of study after inoculation, monkeys receiving viable *S. typhimurium* experienced a septic, febrile illness with a neutrophilic leukocytosis (10,700 to 23,500 leukocytes per microliter with 65 per cent or more neutrophils) similar to that previously reported.[4] A transient neutrophilia and low-grade fever was noted (8 hours after inoculation only) in monkeys given heat-killed bacteria, whereas these parameters were not noticeably altered in saline-inoculated monkeys.

As shown in Table I, the in vitro deiodination of T_4 by leukocytes isolated from infected monkeys at 8, 24, and 48 hours after inoculation was significantly enhanced when compared both to the deiodinating activity of leukocytes obtained from these same monkeys prior to inoculation and to that of concomitantly isolated leukocytes from saline-inoculated monkeys. Deiodination of T_4 by leukocytes obtained from monkeys 8 hours after the inoculation of heat-killed bacteria was slightly increased (Table I) compared to the pre-inoculation value of this group, but this difference was not statistically significant. Deiodination of T_4 by leukocytes isolated sequentially from saline controls did not change appreciably with time. In all instances, the predominant product of T_4 metabolism identified chromatographically was inorganic iodide with no significant differences among the leukocyte groups in the proportion of degradation product appearing as iodide or immobile origin material. Although blood cultures were positive at 8, 24, and 48 hours after inoculation of viable salmonella, bacterial particles were not identified within peripheral leukocytes harvested from infected monkeys at these times by Giemsa stain.

Deiodination of T_4 by leukocytes induced to phagocytize in vitro. As indicated in Table II, deiodination of T_4 by leukocytes from both control and infected monkeys was significantly enhanced when phagocytosis was induced in vitro by addition of opsonized, heat-killed bacteria to the reaction mixtures. However, upon stimulation of phagocytosis in vitro, leukocytes obtained from monkeys with salmonella sepsis deiodinated an appreciably greater proportion of added ^{131}I-T_4 than did concomitantly studied phagocytizing leukocytes from control monkeys (Table II). In vials containing heat-killed bacteria alone without leukocytes, degradation of T_4 was not detectably different from that observed in cell-free vials (3 to 5 per cent of added ^{131}I-T_4). Uptake of added bacterial particles by neutrophils from both control and infected monkeys was demonstrated by Giemsa stain of the leukocytes at the conclusion of the 2-hour incubations. In vitro uptake of bacteria by leukocytes from infected monkeys was

not obviously greater than that of control monkeys. Addition of either *S. typhimurium* or *E. coli* to reaction mixtures appeared equally effective in potentiating T_4 deiodination by leukocytes from monkeys with acute *S. typhimurium* sepsis.

As also shown in Table II, phagocytizing leukocytes from both control and infected monkeys formed greater quantities of chromatographically immobile origin material from T_4 degradation than did leukocytes not induced to phagocytize in vitro. Further, origin material constituted a greater fraction of the total metabolic products formed by phagocytizing leukocytes (approximately 20 per cent of OM + I^-) compared to nonphagocytizing cells (9 per cent of OM + I^-).

In contrast to the stimulatory effects of bacteria, the addition of endotoxin in vitro to reaction mixtures containing leukocytes from healthy monkeys had no detectable effects on the deiodination of T_4, expressed as mean per cent per 10^7 neutrophils per 2 hours ± S.E. (basal: 8.8 ± 0.9; endotoxin: 9.6 ± 1.0, 10.3 ± 1.0, 8.1 ± 0.5, and 7.5 ± 0.7 with test doses of 0.1, 1.0, 10, and 100 μg per milliliter, respectively).

Deiodination of T_4 in vitro by lymphocytes. As indicated in Tables I and II, leukocytic deiodination of T_4 was expressed per 10^7 neutrophils, although both neutrophils and large numbers of lymphocytes were present in the reaction mixtures. Moreover, leukocyte population isolated from control monkeys contained proportionately more lymphocytes than did leukocyte populations from septic monkeys. However, as shown in Table III, relatively purified preparations of lymphocytes isolated from either infected or control monkeys, deiodinated negligible quantities of ^{131}I-T_4 in vitro (0.4 to 1.1 per cent of added ^{131}I-T_4 per 10^7 lymphocytes) with little enhancement in the presence of bacteria (0.7 to 1.5 per cent). Lymphocytes were also added to reaction mixtures containing mixed leukocytes from infected monkeys in order to reduce the high proportion of neutrophils present in these cell populations to levels encountered in the leukocyte populations from control monkeys. Such additions did not appreciably alter the T_4 deiodinating activity of leukocytes from infected monkeys (Table III), implying that the lower activity of cell populations from noninfected monkeys was not attributable to their higher lymphocyte content.

^{14}C-1-glucose oxidation. Table IV shows ^{14}C-1-glucose oxidation by mixed leukocytes and purified lymphocytes isolated from control and infected monkeys. $^{14}CO_2$ formation by the neutrophils present was calculated by correcting that of the total leukocyte population by the value determined for lymphocytes alone. As estimated by this method, ^{14}C-1-glucose oxidation by neutrophils from control and infected monkeys appeared to be similar (Table IV). Moreover, $^{14}CO_2$ formation by neutrophils from both control and infected monkeys was comparably enhanced (approximately 4-fold) by the induction of phagocytosis in vitro (Table IV). By contrast, lymphocyte $^{14}CO_2$ formation was not appreciably increased by the addition of heat-killed bacteria to the reaction mixtures.

Discussion

The results demonstrate that leukocytes isolated from monkeys with *S. typhimurium* sepsis deiodinate T_4 in vitro at an enhanced rate compared to

leukocytes harvested from the same monkeys prior to infection or to concomitantly studied leukocytes from noninfected monkeys. Induction of phagocytosis in vitro increased the deiodination of T_4 by monkey leukocytes, a response previously noted with human leukocytes.[5, 6] Moreover, upon stimulation of phagocytosis in vitro the deiodinating activity of leukocytes harvested from septic monkey donors was significantly greater than that of phagocytizing cells from healthy donors (Table II). Although the leukocyte populations studied contained appreciable numbers of lymphocytes as well as neutrophils, in vitro metabolism of T_4 was predominantly an action of neutrophils (Table III). These findings are consistent with a possible role for neutrophils in the acceleration of host peripheral metabolism of T_4 seen during bacterial sepsis.[2-4] The results further suggest that the contribution of neutrophils to the total T_4 deiodinating activity of the host may be particularly prominent in infections characterized by intense direct interaction between neutrophils and bacteria (Table II). Such an in vivo setting for neutrophil ingestion of invasive bacteria would be expected to occur in acute bacterial pneumonias, illnesses in which both acceleration of peripheral T_4 metabolism[3] and isotopic localization of labeled-T_4 in the lung lesions[10] have been observed in man. However, the extent to which the in vitro findings of enhanced neutrophil T_4-degradative activity correlate with the in vivo activity of these cells during acute infection must still be established.

Both the mechanisms mediating accelerated metabolism of T_4 by leukocytes following induction of phagocytosis in vitro, and those responsible for the increased deiodinating activity of leukocytes obtained from infected donors, are uncertain. In leukocytes,[5, 6, 11] as in other tissues,[12] peroxidative metabolism appears to be an important physiologic pathway of T_4 degradation and induction of phagocytosis has been shown to increase the activity of peroxidase-H_2O_2 systems in leukocytes.[13, 14] Although there is evidence to implicate potentiation of peroxidative metabolism in the increased deiodinating activity of phagocytizing leukocytes,[5] other studies have suggested that an enhanced rate of T_4 accumulation by phagocytizing cells may be a primary factor.[6] These questions are not specifically addressed in the present study. However, it is known that T_4 may be substituted for inorganic halide as an oxidizable cofactor in the myeloperoxidase-H_2O_2-halide antimicrobial system of leukocytes[15] and that this cell system utilizes iodide in the iodination and killing of ingested bacteria.[16, 17] As shown in Table II, following addition of bacteria to reaction mixtures, an increased proportion of radioiodine released from ^{125}I-T_4 appeared in the form of chromatographically immobile origin material. This change likely reflects accelerated transiodination processes with increased iodoprotein formation by phagocytizing cells,[6, 5] perhaps in part due to iodination of ingested bacteria or bacterial protein.

It is tempting to speculate that the enhanced in vitro deiodinating activity of leukocytes from infected monkeys might be related to their active participation in bacterial phagocytosis in vivo prior to isolation. However, there is no direct evidence to support this possibility. Bacterial particles were not identified within neutrophils from infected donors and upon addition of heat-killed bacteria to the reaction mixtures in vitro, neutrophils from infected monkeys did not appear to accumulate more bacteria than those from noninfected donors.

Further, leukocyte ^{14}C 1-glucose oxidation, a parameter known to be potentiated by phagocytosis,[18] was not detectably greater in cells obtained from bacteremic monkeys (Table IV). The absence of evidence of recent phagocytic activity in peripheral leukocytes obtained from bacteremic monkeys may, in part, be related to the fact that fixed tissue phagocytic cells of the liver and spleen, rather than circulating neutrophils, are the primary sites of clearance of blood-borne bacteria.[19] Accordingly, it seems possible that factors other than active phagocytosis, such as bacterial products, may mediate the enhanced T_4 deiodinating activity of neutrophils during sepsis. In this regard, direct in vitro addition of endotoxin to reaction mixtures had no demonstrable effect on T_4 degradation by neutrophils from healthy donors. In previous studies, intravenous administration of this agent similarly failed to increase the peripheral metabolism of T_4 in monkeys.[1] Further, leukocytes from monkeys inoculated with *Diplococcus pneumoniae*, an organism devoid of endotoxin, have been shown to degrade T_4 at an increased rate in vitro.[2] Thus, specific evidence to implicate endotoxin is lacking, and those factors responsible for the increase in T_4 deiodinating activity of leukocytes during infection remain to be delineated.

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