L-Triiodothyronine and L-Reverse-Triiodothyronine Generation in the Human Polymorphonuclear Leukocyte

KENNETH A. WOEBER with the technical assistance of BETTY A. MADDUX,
Department of Medicine, Mount Zion Hospital and Medical Center and University of California, San Francisco, California 94120

ABSTRACT Extrathyroidal monodeiodination of L-thyroxine (T_4) is the principal source of L-triiodothyronine (T_3) and L-reverse-triiodothyronine (rT_3) production. To define some of the cellular factors involved, we examined T_3 and rT_3 generation from added nonradioactive T_4 in human polymorphonuclear leukocytes, using radioimmunoassays to quantify the T_3 and rT_3 generated. Under optimum incubation conditions which included a pH of 6.5 in sucrose-acetate buffer, the presence of dithiothreitol as a sulfhydryl-group protector, and incubation in an hypoxic atmosphere, significant net generation of T_3 and rT_3 was observed. Of the several subcellular fractions studied, the particulate fraction obtained by centrifugation at 27,000 *g* was found to possess the highest T_3- and rT_3-generating activities per unit quantity of protein. With respect to T_3 generation from substrate T_4, the K_m was 5 μM and the V_{max} was 7.2 pmol/min per mg protein. Propylthiouracil, methimazole, and prior induction of phagocytosis inhibited both T_3 and rT_3 generation, but T_3 generation was inhibited to a greater extent. rT_3, in a concentration equimolar to that of substrate T_4, did not alter T_3 generation, but inhibited T_3 generation when the molar ratio of rT_3 to T_4 approached 10:1. Under the incubation conditions employed, particulate fractions of leukocytes obtained from five cord blood samples displayed an essentially normal relationship between T_3- and rT_3-generating activities, despite the distinctly divergent serum T_3 and rT_3 concentrations in these samples. From our findings, we draw the following conclusions: (*a*) the human polymorphonuclear leukocyte possesses the ability to generate T_3 and rT_3 from substrate T_4; (*b*) the T_3- and rT_3-generating activities are associated principally with the 27,000 *g* particulate fraction and display enzymic characteristics with a sulfhydryl-group requirement; (*c*) T_3-generating activity appears to be more susceptible to inhibitory influences than rT_3-generating activity; and (*d*) in cord blood leukocytes, the putative enzymes catalyzing T_3 and rT_3 generation appear to be functionally intact under the experimental conditions employed.

Received for publication 5 December 1977 and in revised form 10 May 1978.

INTRODUCTION

After the conclusive demonstration that the bulk of L-triiodothyronine (T_3)[1] in man arises through the extrathyroidal monodeiodination of the outer ring of L-thyroxine (T_4) (1–4), there has been a resurgence of interest in the use of tissue preparations for examining this phenomenon in vitro. Thus, conversion of T_4 to T_3 has been demonstrated to occur in cultured human liver and kidney cells (5), in cultured human fibroblasts (6), and in freshly isolated human polymorphonuclear leukocytes (7, 8). These studies involved the use of radioiodine-labeled T_4 as substrate, with detection and quantification of the T_3 generated by chromatographic analysis. More recently, the availability of radioimmunoassay methods for measuring T_3 has been exploited for examining T_4 to T_3 conversion in vitro. Radioimmunoassay, in addition to being more specific than chromatographic analysis, permits more precise quantification of the very small quantities of T_3 generated. Using this approach, several investigators have reported the generation of T_3 from added nonradioactive T_4 by rat tissues in vitro and have examined some of the characteristics of the T_3-generating system (9–15).

Recent work has demonstrated convincingly that extrathyroidal monodeiodination of T_4 is also responsible for almost all L-reverse T_3 (rT_3) production (16, 17), monodeiodination of T_4 in this instance occurring in the inner ring. rT_3 generation from T_4 has also been demonstrated in rat liver in vitro (12, 13).

[1] *Abbreviations used in this paper*: DC, disrupted cell preparation; DTT, dithiothreitol; MMI, methimazole; N, nuclear fraction; P, 27,000-*g* particulate fraction; PTU, propylthiouracil; rT_3, L-reverse-T3; S1, initial 400-*g* supernate; S2, 27,000-*g* supernate; SH, sulfhydryl; T3, L-triiodothyronine; T4, L-thyroxine.

We have previously demonstrated that intact human polymorphonuclear leukocytes, as well as the granule fraction isolated therefrom, are capable of deiodinating labeled T_4 in vitro, with the generation of labeled inorganic iodide and a labeled material that remains at the origin during chromatography (8, 18). In addition, we detected labeled T_3 as a product of T_4 deiodination in intact cells by chromatographic analysis. However, the net quantities of T_3 generated were very small, and the sensitivity of chromatographic analysis was insufficient to permit quantitative definition of the characteristics of the T_3-generating system. Accordingly, we undertook to study the generation of T_3 from added nonradioactive T_4 in the human polymorphonuclear leukocyte and its various subcellular fractions, using a specific radioimmunoassay to quantify the T_3 generated. In addition, we examined the generation of rT_3, using a specific radioimmunoassay for quantifying the rT_3 formed.

METHODS

Blood was collected, with 100 U/25 ml heparin as the anticoagulant, from healthy laboratory personnel. Blood was also obtained from the umbilical cords of five full-term normal infants.

Plastic ware or siliconized glassware was used throughout. The leukocytes were isolated from the blood as described previously (8); at least 90% of the cells so obtained were polymorphonuclear, 3–8% were lymphocytes, <2% were monocytes, and erythrocytes were virtually absent. Freshly isolated leukocytes were employed for each experiment.

Leukocyte fractionation. The isolated leukocytes were disrupted by homogenization in cold 0.34 M sucrose. This disrupted cell preparation (DC) was centrifuged at 400 *g* for 15 min at 5°C to remove unbroken cells, nuclei, and cell debris, and the resulting supernate (S1) was saved. The pellet was washed three times with cold 0.25 M sucrose, 0.02 M Tris, pH 7.2, containing 0.5% (vol/vol) Triton X-100 (Beckman Instruments, Inc., Fullerton, Calif.) to yield a nuclear fraction (N) which was then washed and suspended in cold 0.25 M sucrose, 0.02 M sodium acetate, pH 6.5 (sucrose-acetate buffer). S1 and, when desired, DC in 0.34 M sucrose were diluted with cold sodium acetate to yield final concentrations of 0.25 M sucrose and 0.02 M sodium acetate, pH 6.5. In some experiments, S1 was diluted with cold Tris when final pH values of 7.0 and above were desired. S1 was further resolved by centrifugation at 27,000 *g* for 15 min at 5°C into a particulate fraction (P) and supernate S2. The P fraction, which has been shown to be composed of granules, plasma membrane fragments, and occasional mitochondria (19), was suspended in the sucrose-acetate buffer.

When the P fraction from phagocytosing cells was desired, the intact leukocytes were incubated at 37°C for 40 min before fractionation in Krebs-Ringer phosphate glucose buffer, pH 7.4, containing 0.5 mg/1 × 10^7 cells zymosan (K & K Laboratories Inc., Plainview, N. Y.) that had been opsonized by coating with fresh plasma, as described in detail previously (8). Intact leukocytes from the same isolate were preincubated concurrently in Krebs-Ringer phosphate glucose buffer alone to serve as control cells. The P fraction from the phagocytosing cells was free of zymosan particles, these sedimenting with the initial 400 *g* pellet during fractionation, and the yield, in terms of protein content, was very similar to that of the P fraction from the control cells.

Protein concentrations of all fractions were measured according to the method of Lowry et al. (20).

Measurement of T_4 conversion to T_3 and rT_3. The cell fractions in sucrose-acetate buffer (0.4 ml, representing the yield from ≅1 × 10^7 leukocytes) were added to plastic tubes containing 1 nmol of nonradioactive T_4 (Sigma Chemical Co., St. Louis, Mo.) and 50 nmol of dithiothreitol (DTT) (Sigma Chemical Co.); the final volume of the reaction mixture was 0.5 ml, yielding final concentrations of T_4 and DTT of 2 μM and 100 μM, respectively. For each fraction a control was prepared in which the cell fraction, and the T_4 plus DTT were incubated in separate tubes and then mixed at the end of incubation. This control was used to correct for T_3 and rT_3 contamination of the added nonradioactive T_4, for endogenous T_3 and rT_3 in the cell fraction, and for cross-reactivity of the added T_4 with the anti-T_3 and anti-rT_3 antisera used in the radioimmunoassays. In some experiments, the reaction mixtures were enriched with small quantities of nonradioactive T_3 (Sigma Chemical Co.) and rT_3 (obtained through the courtesy of Dr. Robert I. Meltzer from Warner-Lambert Research Institute, Morris Plains, N. J.) to assess the degradation of T_3 and rT_3; here, reaction mixtures enriched with identical quantities of T_3 and rT_3 at the end of incubation served as controls.

The tubes were incubated at 37°C in a metabolic shaker. An hypoxic atmosphere was attained by covering the tubes with a gassing hood and gassing with 100% nitrogen throughout the incubation period. At the end of incubation, 50 μl of human serum that had been stripped of its iodothyronine content by overnight exposure to an anion exchange resin (Amberlite IRA-400, Mallinckrodt Inc., St. Louis, Mo.) was added to each tube and the contents mixed. The stripped serum was added because it facilitated pelleting of the very small amount of tissue in the reaction mixture after the addition of ethanol. This was followed by the addition of 1 ml of cold 95% ethanol and further mixing. After standing at 5°C for 20 min, the tubes were centrifuged at 1,500 *g* for 20 min and the supernatant ethanol extracts were collected. In eight individual reaction mixtures, this extraction procedure resulted in recovery of 90±2% (mean±SE) of added nonradioactive T_3 and 80±2% of added nonradioactive rT_3.

The concentrations of T_3 and rT_3 in the ethanol extracts were measured by double-antibody radioimmunoassay. For the T_3 radioimmunoassay, high specific activity [^{125}I]T_3 (≅300 μCi/nmol) was obtained from Abbott Diagnostics, Diagnostic Products, North Chicago, Ill., nonradioactive T_3 from Sigma Chemical Co., and rabbit anti-T_3 antiserum from Endocrine Sciences, Tarzana, Calif. Cross-reactivities of rT_3 and T_4 in this assay did not exceed 0.03% and 0.3%, respectively. For the rT_3 radioimmunoassay, L-[^{125}I]rT_3, nonradioactive L-rT_3, and rabbit anti-L-rT_3 antiserum were obtained from Serono Laboratories Inc., Boston, Mass. Cross-reactivities of T_3 and T_4 in this assay did not exceed 0.003 and 0.09%, respectively. The second antibody for both assays, goat anti-rabbit gamma globulin, was obtained from Antibodies Inc., Davis, Calif. In both assays, ethanol was routinely added to the standards to yield a final concentration identical to that in the samples; addition of ethanol yielded a standard curve that was virtually superimposable on that obtained with addition of an ethanol extract of iodothyronine-free serum, the coefficients of variation in two experiments being 8.1 and 7.3%. For each standard or sample a blank was prepared to contain all reagents except the anti-T_3 or -rT_3 antiserum. The value for percent of tracer bound in the blank was subtracted from the value in the corresponding standard or sample to correct for non-

specific binding. The net quantity of T_3 or rT_3 generated in a given reaction mixture was derived by subtracting from the concentration of T_3 or rT_3 in the ethanol extract thereof, the concentration of T_3 or rT_3 in the ethanol extract of the corresponding control in which the cell fraction and the T_4 plus DTT had been kept separated until the end of incubation. All values for T_3 and rT_3 were corrected for recovery, using the coefficients cited earlier.

RESULTS

Determination of optimum incubation conditions. In initial experiments carried out in air, significant net generation of T_3 from 1 nmol of added nonradioactive T_4 was observed after 3 h of incubation with DC and with S1 derived therefrom. T_3-generating activity was found to have a pH optimum of 6.5 in sucrose-acetate buffer. Activity was abolished by prior boiling of the tissue for 30 min or by incubation at 5°C, and was greater at 37°C than at room temperature. As depicted in Fig. 1, addition to the reaction mixture of DTT in a final concentration of 100 μM resulted in a moderate increase in net T_3 generation when incubation was carried out in air. However, when incubation was carried out in an hypoxic atmosphere attained by gassing with 100% N_2, the presence of 100 μM DTT resulted in a great increase in net T_3 generation and significant quantities of rT_3 were also detected. In the case of T_3, the gross quantity generated was 7.1±0.4 pmol, (mean±SE, n = 6), and the quantity present in the controls, representing T_3 contamination of the added T_4, endogenous T_3 in the tissue, and cross-reactivity of the added T_4 with the anti-T_3 antiserum, was 3.0±0.3 pmol. Thus, the net quantity of T_3 generated was 4.1±0.3 pmol. In the case of rT_3, the values for gross, control, and net quantities generated were 3.3±0.2, 1.1±0.1, and 2.2±0.1 pmol, respectively. In the absence of DTT, incubation in N_2 yielded values that were very similar to those obtained in air. As depicted in Fig. 2, a linear log concentration-response relationship was demonstrable between 10 μM and 1 mM DTT, and in this concentration range, the ratios of T_3- to rT_3-generating activities remained quite constant, ranging only between 2.3 and 2.6:1. At concentrations ≧1 mM DTT, significant generation of T_3 from T_4 was sometimes observed in the absence of tissue, and at >1-mM concentrations no further increase in generation by tissue was observed. Replacement of sucrose-acetate buffer with 0.14 M KCl-0.02 M sodium acetate buffer, pH 6.5, as the suspending medium, resulted in 54% reduction of T_3 generation and 59% reduction of rT_3 generation. Accordingly, sucrose-acetate buffer, pH 6.5, enriched with 100 μM DTT was routinely employed as the suspending medium, and incubation was carried out in 100% N_2 at 37°C.

Comparison of activity in various cell fractions. Fig. 3 depicts the net generation of T_3 and rT_3 from 1 nmol of added T_4 after a 3-h incubation with the fractions obtained from ≅1 × 10^7 cells, using the optimum incubation conditions. The bulk of activity in the DC preparation was recovered in S1, although the nuclear fraction also possessed some activity. As depicted in Fig. 4, the bulk of activity in the S1 fraction was recovered in P derived therefrom by centrifugation at 27,000 g, with little activity remaining in the resulting supernate (S2). The T_3- and rT_3-generating activities of P fraction per unit quantity of protein were approximately four- to fivefold greater than those of the DC preparation.

Characteristics of activity of particulate fraction. The net generation of T_3 and rT_3 from 1 nmol of added T_4 after a 3-h incubation with P fraction obtained from

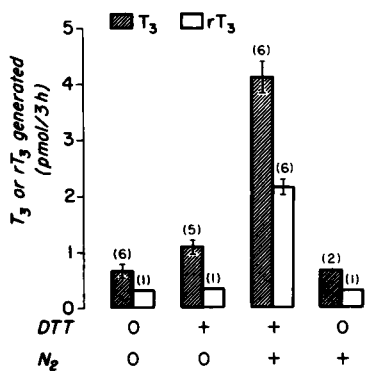

FIGURE 1 Influence of DTT and hypoxia attained by gassing with 100% N_2 on net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 by S1 derived from ≅1 × 10^7 human polymorphonuclear leukocytes and suspended in sucrose-acetate buffer, pH 6.5. Incubation was carried out at 37°C. Mean±SE and number of individual reaction mixtures studied are indicated.

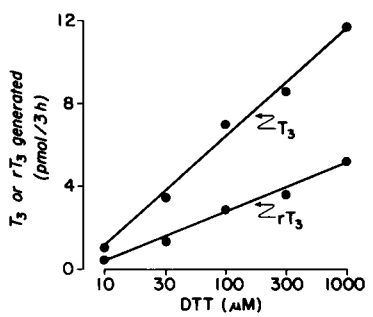

FIGURE 2 Net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 by S1 derived from ≅1 × 10^7 human polymorphonuclear leukocytes as a function of the concentration of added DTT. The suspending medium was sucrose-acetate buffer, pH 6.5, and incubation was carried out in 100% N_2 at 37°C. Each point represents the mean of duplicate determinations of an individual reaction mixture; very similar results were obtained in a second experiment.

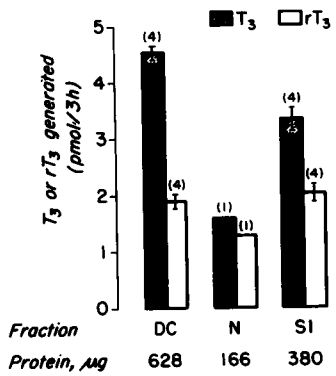

FIGURE 3 Comparison of net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 by various fractions obtained from ≅1 × 10^7 human polymorphonuclear leukocytes. In this and all the subsequent experiments depicted, the suspending medium was sucrose-acetate buffer, pH 6.5, enriched with 100 μM DTT, and incubation was carried out in 100 % N_2 at 37°C. Mean±SE and number of individual reaction mixtures studied are indicated.

≅1 × 10^7 cells, was much greater at pH 6.5 than at pH 7.4 or 9.0. The pmol of T_3 and rT_3 generated were, respectively, 3.7 and 2.4 at pH 6.5, 1.2 and 0.9 at pH 7.4, and 1.1 and 1.2 at pH 9.0. Using the optimum incubation conditions at pH 6.5, 5.0±1.3% (mean±SE) of added nonradioactive T_3 and 8.6±1.4% of added nonradioactive rT_3 were degraded after 3 h in 10 individual reaction mixtures. As depicted in Fig. 5, net T_3 generation appeared to increase as a linear function of time during the first 2 h of incubation, and then appeared to level off during the 3rd h. In the case of rT_3, net generation increased with time, but did not appear to level off during the 3rd h. In an experiment in which incubation was carried out for 20 h, the net quantity of T_3 generated after 20 h was only 37% greater than that after 3 h, whereas the net quantity of rT_3 generated was 116% greater than that after 3 h. Fig. 6 depicts the P fraction dependence of net T_3 and rT_3 generation from 1 nmol of added T_4 for a 3-h incubation period. Net generation of both T_3 and rT_3 increased with an increase in the concentration of P fraction in the reaction mixture, and at all concentrations, the ratios of T_3- to rT_3-generating activities remained constant at 2:1. Here, the highest concentration of P fraction of 326 μg/ml represented the mean yield of fraction (163 μg) from ≅1 × 10^7 cells suspended in a final volume of 0.5 ml. Fig. 7 depicts the T_4-concentration dependence of net T_3 generation. A double reciprocal plot of the net quantity of T_3 generated after 15 min incubation with 18 μg of P fraction as a function of the concentration of added T_4, suggested that T_3 generation conforms to Michaelis-Menten kinetics with a 5-μM K_m and a 7.2-pmol V_{max} of T_3 generated per minute per milligram protein. In the case of rT_3, generation was greater with higher concentrations of added T_4, but the data obtained in several experiments could not be plotted in the double reciprocal manner. Consequently, reliable values for K_m and V_{max} could not be derived.

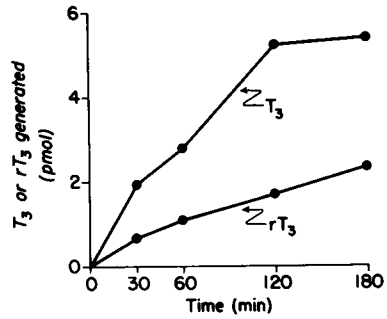

FIGURE 5 Time-course of net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 by the P fraction derived from ≅1 × 10^7 human polymorphonuclear leukocytes. Each point represents the mean of duplicate determinations of an individual reaction mixture.

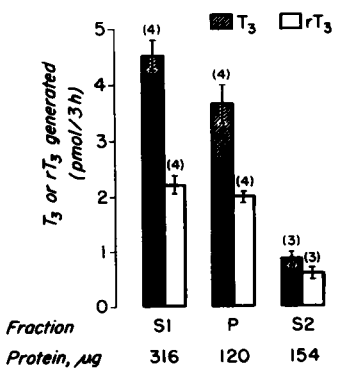

FIGURE 4 Comparison of net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 by S1 and its constituent P fraction and S2 derived from ≅1 × 10^7 human polymorphonuclear leukocytes. Mean±SE and number of individual reaction mixtures studied are indicated.

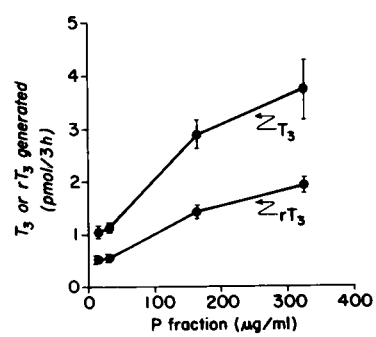

FIGURE 6 Net T_3 and rT_3 generation from 1 nmol of added nonradioactive T_4 as a function of the concentration of P fraction. Values represent mean±SE of four individual reaction mixtures.

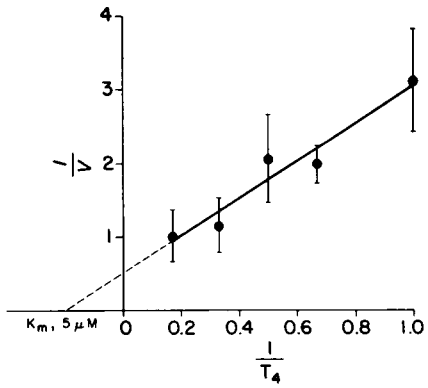

FIGURE 7 T_4-concentration dependence of net T_3 generation by 18 μg P fraction. *V* measured in picomoles of T_3 generated per 15 minute, and T_4 measured in micromolar concentrations. Values represent mean±SE of three individual reaction mixtures.

Factors affecting activity of particulate fraction. Table I summarizes the influence of several factors on the T_3- and rT_3-generating activities of P fraction obtained from ≅1 × 10^7 cells, employing 1 nmol of added T_4, a 3-h incubation period, and the optimum incubation conditions. Addition to the reaction mixture of either propylthiouracil (PTU) or methimazole (MMI) in concentrations of 10, 30, and 100 μM was accompanied by a progressive inhibition of net T_3 and rT_3 generation. Similarly, prior induction of phagocytosis in the leukocytes was accompanied by inhibition of the T_3- and rT_3-generating activities of the subsequently isolated P fraction. In the foregoing circumstances, T_3 generation appeared to be disproportionately depressed, as judged from the decreases in the ratio of T_3 to rT_3 generated. The presence of 100 μM PTU, 100 μM MMI, or P fraction from phagocytosing cells did not significantly affect the degradation of added nonradioactive T_3 or rT_3. Thus, the inhibition of net T_3 and rT_3 generation in the foregoing circumstances could not be attributed to increased degradation of product generated. Also presented in Table I are the results of an experiment in which the influence of added rT_3 on T_3 generation was examined. Addition of rT_3 in a concentration equimolar to that of the added substrate T_4 did not interfere with net T_3 generation, but inhibition was observed when a 10-fold greater concentration of rT_3 was employed.

Activity of particulate fraction from cord blood. Table II summarizes the results of studies in which T_3 and rT_3 generation from 1 nmol of added T_4 was examined with P fraction of leukocytes obtained from cord blood. Also presented are the values for concurrently studied P fraction from adult blood, as well as the values for serum T_3 and rT_3 concentrations. Incubation conditions were as outlined before. Because the yield of P fraction varied among the different blood samples, values for T_3 and rT_3 generation per 100 μg protein are also presented.

TABLE I

Influence of Various Factors on Net T_3- and rT_3-Generating and T_3- and rT_3-Degrading Activities of Leukocyte Particulate Fraction

Experiment	Factor	Net generating activity			Degrading activity	
		T_3	rT_3	T_3/rT_3	T_3	rT_3
		pmol/3 h			*% added*	
1	None	5.6	2.5	2.2	5.4	10.2
	PTU, 10 μM	3.5	1.8	1.9	—	—
	PTU, 30 μM	3.2	1.5	2.1	—	—
	PTU, 100 μM	1.3	1.2	1.1	7.3	11.0
	MMI, 10 μM	2.3	1.3	1.8	—	—
	MMI, 30 μM	1.5	1.0	1.5	—	—
	MMI, 100 μM	1.1	0.8	1.4	0	8.0
2	None	7.4	3.0	2.5	3.5	5.1
	Phagocytosing	3.2	1.9	1.7	2.9	10.3
3	None	3.8	—	—	—	—
	rT_3, 2 μM	4.5	—	—	—	—
	rT_3, 20 μM	2.6	—	—	—	—

In all five cord blood samples, the expected great increase in serum rT_3 concentration and modest decrease in serum T_3 concentration relative to adult blood were observed. For the group of five cord samples as a whole, T_3- and rT_3-generating activities per unit quantity of P fraction did not differ significantly from those for adult blood. In addition, the ratios of T_3-generating activity to rT_3-generating activity, which are independent of the quantity of P fraction employed, were very similar in the cord and adult samples.

DISCUSSION

In the present study, we have provided evidence that the human polymorphonuclear leukocyte possesses the ability to generate both T_3 and rT_3 from substrate T_4. The pH optimum for T_3 generation was found to be 6.5 which is very similar to that observed by others in rat liver homogenate (12) or rat liver microsomes (13). In contrast to these latter studies in which no rT_3 generation could be detected at pH 6.5, this only being detected at ≅pH 9.0, we found significant quantities of rT_3 at pH 6.5. In fact, the quantity of rT_3 generated at pH 6.5 exceeded that at pH 7.4 and 9.0.

We found that DTT, a protective reagent for sulfhydryl (SH) groups (21), and an hypoxic atmosphere were prerequisites for optimum generation of both T_3 and rT_3. In their absence, T_3 generation was greatly depressed and rT_3 generation was barely detectable. The stimulatory effect of SH-group protectors was first recognized by Nakagawa and Ruegamer (22) who

TABLE II

Comparison of Serum T_3 and rT_3 Concentrations and T_3- and rT_3-Generating Activities of Leukocyte Particulate Fraction from Cord Blood and from Normal Adult Blood

Experiment	State	Serum concentration		P fraction	Net generating activity				
		T_3	rT_3		T_3	rT_3	T_3	rT_3	T_3/rT_3
		ng/dl		*μg*	*pmol/3 h*		*pmol/3 h/100 μg*		
1	Cord blood	42	233	175	2.9	1.7	1.7	1.0	1.7
	Concurrent adult	133	12	201	3.6	1.9	1.8	0.9	2.0
2	Cord blood	88	168	105	0.6	0.4	0.6	0.4	1.5
	Concurrent adult	124	14	191	4.3	2.1	2.2	1.1	2.0
3	Cord blood	43	160	88	1.2	0.6	1.4	0.7	2.0
	Concurrent adult	116	11	105	2.6	1.0	2.5	1.0	2.5
4	Cord blood	64	135	179	4.0	1.6	2.2	0.9	2.4
	Concurrent adult	116	12	196	4.2	1.8	2.1	0.9	2.3
5	Cord blood	42	130	130	2.3	1.2	1.8	0.9	2.0
	Concurrent adult	110	19	217	3.2	1.9	1.5	0.9	1.7
				Cord blood, Mean			1.5	0.8	1.9
							±SE	±0.3	±0.1	±0.2
				Concurrent adult, Mean			2.0	1.0	2.1
							±SE	±0.2	±0.0	±0.1

demonstrated that thioglycolate caused a threefold or greater stimulation of T_4 deiodination to inorganic iodide in rat liver homogenate. Recently Hüfner et al. (12) have demonstrated that mercaptoethanol enhances T_4 conversion to T_3 in rat liver homogenate, and Visser et al. (23) and Chopra (24) have reported a similar effect of DTT. Although, in our study, the presence of DTT in a concentration of 100 μM resulted in a slight increase in T_3 generation when incubation was carried out in air, the stimulatory effect of DTT on T_3 and rT_3 generation was clearly evident when incubation was carried out in an hypoxic atmosphere. These findings suggest that SH groups either serve as cofactors or are a constituent of the putative enzymes catalyzing T_3 or rT_3 generation, and because such groups are readily oxidized in air to disulfides, an hypoxic atmosphere serves to maintain them in the reduced state.

The bulk of the T_3- and rT_3-generating activities in the human polymorphonuclear leukocyte was found to be associated with the particulate fraction obtained by centrifugation at 27,000 *g*. This finding is consonant with our earlier work demonstrating that the leukocyte granule fraction represents the subcellular locus for the degradation of T_4 to inorganic iodide and origin material (18). In this earlier work, the experimental conditions employed did not permit detection of either T_3 or rT_3 generation. In rat liver, on the other hand, the subcellular locus of T_3 and rT_3 generation has been reported to be associated with the microsomal fraction (9, 13), although in rat kidney, it appears to be associated with a particulate fraction comprising plasma membranes and mitochondria (15). Recently, Maciel et al. (25) have presented evidence suggesting that the plasma membrane fraction is the subcellular locus of T_3-generating activity in rat liver. Accordingly, entrapment of active plasma membrane components in various subcellular fractions could account for the apparent discordance in the reported subcellular localization of T_3-generating activity. Although our particulate fraction is comprised largely of neutrophil granules, the presence of plasma membrane components could be responsible for the activity observed.

The T_3- and rT_3-generating activities of the human leukocyte displayed enzymic characteristics, including pH and temperature optima and tissue-concentration and time dependence. The derived value for K_m of the putative enzyme responsible for T_3 generation was of the order of 5 μM. This is similar to the K_m of the T_3-generating system in rat liver and kidney homogenates (12, 14, 15). On the other hand, the V_{max} of 7.2 pmol of T_3 generated per minute per milligram of particulate fraction was much greater than that of rat liver homogenate (0.13 pmol/min per mg) (14) or that of rat kidney homogenate (0.03 pmol/min per mg) (15), reflecting at least in part a greater degree of purification of the putative enzyme. In the case of rT_3 generation, reliable values for K_m and V_{max} could not be derived from the data obtained.

A variety of physiologic and pathologic states are

accompanied by subnormal serum T_3 concentrations and by increased serum rT_3 concentrations (26), and this has led to the suggestion that inner and outer ring monodeiodination of T_4 may be regulated in a reciprocal manner. PTU has been shown to result in a decrease in serum T_3 concentration and an increase in serum rT_3 concentration in vivo (27) and to inhibit T_3 generation from T_4 by rat liver and kidney homogenates in vitro (10–12, 14, 15), but its effect on rT_3 generation has not been examined. In common with this earlier work, we found that PTU inhibited T_3 generation and, in addition, found that rT_3 generation was also inhibited, but to a lesser extent. MMI in a concentration equimolar to that of PTU was also found to inhibit both T_3 and, to a lesser extent, rT_3 generation by particulate fraction. This latter finding is discordant with previous reports of a lack of an effect of MMI on T_4 conversion to T_3 in vivo (27) or in vitro (11, 14, 15). This discordance may be due to the large concentrations of MMI and the small quantity of tissue employed in our study relative to the in vivo circumstance and to the previous in vitro studies. As was the case with PTU and MMI, prior induction of phagocytosis also resulted in inhibition of both T_3 and, to a lesser extent, rT_3 generation by particulate fraction. Thus, none of the foregoing factors resulted in a true dissociation of T_3- and rT_3-generating activities, but T_3 generation appeared to be more susceptible to inhibition than rT_3 generation. Because outer ring monodeiodination of T_4 is involved in the degradation of rT_3 as well as representing the pathway for T_3 generation whereas inner ring monodeiodination has the converse actions, a greater inhibition of outer ring monodeiodination might ultimately result in a net increase in rT_3 and a net decrease in T_3 in the in vivo circumstance.

rT_3 has been reported to inhibit T_4 conversion to T_3 in rat liver homogenate (11, 12, 14), although the physiologic relevance of this finding has been questioned (28). We also found that rT_3 inhibited T_3 generation from T_4, but only when the molar ratio of rT_3 to T_4 approached 10:1.

The very early newborn period represents a physiologic state in which serum T_3 and rT_3 concentrations are distinctly divergent. Despite this divergence, we found that the T_3- and rT_3-generating activities of particulate fraction from cord blood leukocytes did not differ significantly from those of normal adult blood. This discordance could be interpreted as suggesting that the leukocyte is an unimportant site of T_3 and rT_3 generation with little influence on overall T_3 and rT_3 economy in vivo. On the other hand, there is an alternative explanation for our findings. Chopra (24) has recently reported that DTT restores T_3-generating activity of liver homogenate from fetal sheep, and, accordingly, suggested that deficient generation of T_3 in fetal tissue was due not to a quantitative deficiency of the enzyme per se, but to the redox state of SH groups in the tissue. Because our incubation conditions required the presence of DTT for detection of significant net T_3 and rT_3 generation, our findings of normal activity with particulate fraction from cord blood leukocytes may reflect correction of the putative abnormality in the redox state of SH groups. Thus, if it is granted that the leukocyte is an important site of T_3 and rT_3 generation, the results would suggest that, as in the case of fetal liver, the putative enzymes catalyzing T_3 and rT_3 generation appear to be functionally intact in the very early newborn period.

The human polymorphonuclear leukocyte resembles other thyroid-hormone-responsive tissues in displaying a calorigenic response to thyroid hormone and in possessing saturable nuclear binding sites for T_3 (29). The present study demonstrates that this cell also has the capability of generating T_3 and rT_3 from substrate T_4.

ACKNOWLEDGMENTS

This study was supported in part by research grant AM-19081 from the National Institutes of Health, Bethesda, Maryland, and in part by Mount Zion Hospital and Medical Center, San Francisco, California.

REFERENCES

1. Braverman, L. E., S. H. Ingbar, and K. Sterling. 1970. Conversion of thyroxine (T_4) to triiodothyronine (T_3) in athyreotic human subjects. *J. Clin. Invest.* **49**: 855–864.
2. Pittman, C. S., J. B. Chambers, Jr., and V. H. Read. 1971. The extrathyroidal conversion rate of thyroxine to triiodothyronine in normal man. *J. Clin. Invest.* **50**: 1187–1196.
3. Surks, M. I., A. R. Schadlow, J. M. Stock, and J. H. Oppenheimer. 1973. Determination of iodothyronine absorption and conversion of L-thyroxine (T_4) to L-triiodothyronine (T_3) using turnover rate techniques. *J. Clin. Invest.* **52**: 805–811.
4. Braverman, L. E., A. Vagenakis, P. Downs, A. E. Foster, K. Sterling, and S. H. Ingbar. 1973. Effect of replacement doses of sodium-L-thyroxine on the peripheral metabolism of thyroxine and triiodothyronine in man. *J. Clin. Invest.* **52**: 1010–1017.
5. Sterling, K., M. A. Brenner, and V. F. Saldanha. 1973. Conversion of thyroxine to triiodothyronine by cultured human cells. *Science (Wash. D. C.).* **179**: 1000–1001.
6. Refetoff, S., R. Matalon, and M. Bigazzi. 1972. Metabolism of L-thyroxine (T_4) and L-triiodothyronine (T_3) by human fibroblasts in tissue culture: evidence for cellular binding proteins and conversion of T_4 to T_3. *Endocrinology.* **91**: 934–947.
7. Klebanoff, S. J., and W. L. Green. 1973. Degradation of thyroid hormones by phagocytosing human leukocytes. *J. Clin. Invest.* **52**: 60–72.
8. Woeber, K. A., and S. H. Ingbar. 1973. Metabolism of L-thyroxine by phagocytosing human leukocytes. *J. Clin. Invest.* **52**: 1796–1803.
9. Hesch, R. D., G. Brunner, and H. D. Söling. 1975. Conversion of thyroxine (T_4) and triiodothyronine (T_3) and the subcellular localization of the converting enzyme. *Clin. Chim. Acta.* **59**: 209–213.
10. Visser, T. J., I. Van Der Does-Tobé, R. Docter, and G. Hennemann. 1975. Conversion of thyroxine into tri-

iodothyronine by rat liver homogenate. *Biochem. J.* **150:** 489–493.
11. Chopra, I. J. 1977. A study of extrathyroidal conversion of thyroxine (T_4) to 3,3′,5-triiodothyronine (T_3) in vitro. *Endocrinology.* **101:** 453–463.
12. Hüfner, M., M. Grussendorf, and M. Ntokalou. 1977. Properties of the thyroxine (T_4) monodeiodinating system in rat liver homogenate. *Clin. Chim. Acta.* **78:** 251–259.
13. Höfken, B., R. Ködding, and R-D Hesch. 1977. Conversion of T_4 to T_3 and rT_3 and their cytoplasmic binding: pH dependency. *Clin. Chim. Acta.* **78:** 261–266.
14. Kaplan, M. M., and R. D. Utiger. 1978. Iodothyronine metabolism in rat liver homogenates. *J. Clin. Invest.* **61:** 459–471.
15. Chiraseveenuprapund, P., U. Buergi, A. Goswami, and I. N. Rosenberg. 1978. Conversion of L-thyroxine to triiodothyronine in rat kidney homogenate. *Endocrinology.* **102:** 612–622.
16. Chopra, I. J. 1976. An assessment of daily production and significance of thyroidal secretion of 3,3′,5′-triiodothyronine (reverse T_3) in man. *J. Clin. Invest.* **58:** 32–40.
17. Gavin, L., J. Castle, F. McMahon, P. Martin, M. Hammond, and R. R. Cavalieri. 1977. Extrathyroidal conversion of thyroxine to 3,3′,5′-triiodothyronine (reverse-T_3) and to 3,3′,5-triiodothyronine (T_3) in humans. *J. Clin. Endocrinol. Metab.* **44:** 733–742.
18. Woeber, K. A. 1976. A granule-associated L-thyroxine deiodinating system in the human leukocyte. *Endocrinology* **98:** 802–806.
19. Babior, B. M., J. T. Curnutte, and B. J. McMurrich. 1976. The particulate superoxide-forming system from human neutrophils. *J. Clin. Invest.* **58:** 989–996.
20. Lowry, O. H., N. J. Rosebrough, A. F. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
21. Cleland, W. W. 1964. Dithiothreitol, a new protective reagent for SH groups. *Biochemistry.* **3:** 480–482.
22. Nakagawa, S., and W. R. Ruegamer. 1967. Properties of a rat tissue iodothyronine deiodinase and its natural inhibitor. *Biochemistry.* **6:** 1249–1261.
23. Visser, T. J., I. Van Der Does-Tobé, R. Docter, and G. Hennemann. 1976. Subcellular localization of a rat liver enzyme converting thyroxine into tri-iodothyronine and possible involvement of essential thiol groups. *Biochem. J.* **157:** 479–482.
24. Chopra, I. J. 1978. Sulfhydryl groups and the monodeiodination of thyroxine to triiodothyronine. *Science (Wash. D. C.).* **199:** 904–906.
25. Maciel, R. M. B., Y. Ozawa, and I. J. Chopra. 1978. Subcellular localization of T_4 and reverse T_3 (rT_3) outer ring monodeiodinase activities. *Clin. Res.* **26:** 107A. (Abstr.)
26. Cavalieri, R. R., and B. Rapoport. 1977. Impaired peripheral conversion of thyroxine to triiodothyronine. *Annu. Rev. Med.* **28:** 57–65.
27. Kaplan, M. M., M. Schimmel, and R. D. Utiger. 1977. Changes in serum 3,3′,5′-triiodothyronine (reverse T_3) concentrations with altered thyroid hormone secretion and metabolism *J. Clin. Endocrinol. Metab.* **45:** 447–456.
28. Nicod, P., A. Burger, G. Strauch, A. G. Vagenakis, and L. E. Braverman. 1976. The failure of physiologic doses of reverse T_3 to effect thyroid-pituitary function in man. *J. Clin. Endocrinol. Metab.* **43:** 478–481.
29. Woeber, K. A. 1977. Observations concerning the binding of L-triiodothyronine in the human polymorphonuclear leukocyte. *J. Clin. Endocrinol. Metab.* **44:** 62–68.