Metabolism of L-Thyroxine by Phagocytosing Human Leukocytes

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ABSTRACT Intact normal human leukocytes deiodinated L-thyroxine (T_4) with the generation of inorganic iodide, chromatographically immobile origin material, and small quantities of L-triiodothyronine (T_3). When phagocytosis was induced in the leukocytes through the addition of zymosan particles that had been opsonized by coating with plasma, T_4-deiodination was greatly stimulated. In addition to the stimulation of T_4-deiodination, the accumulation by the leukocytes of undegraded T_4 was increased. Anoxia, which has previously been shown not to interfere with phagocytosis, did not prevent the increased cellular accumulation of T_4 that phagocytosis induced, but virtually abolished T_4-deiodination. On the other hand, calcium, which has previously been shown to be required for optimal phagocytosis, was required for the increase in both the cellular accumulation and deiodination of T_4 that phagocytosis induced. Phospholipase-C, which has previously been shown to induce a metabolic burst that mimics that induced by phagocytosis, did not increase the cellular accumulation or deiodination of T_4. On the other hand, colchicine, which has previously been shown to depress the metabolic burst that accompanies phagocytosis, did not prevent the increase in either the cellular accumulation or deiodination of T_4 that phagocytosis induced. Thus, increased accumulation of T_4 by the leukocytes during phagocytosis appears to be the primary factor responsible for the stimulation of deiodination that phagocytosis induces. The increased accumulation of T_4 did not appear to be owing to engulfment of suspending medium surrounding the particles or to binding of T_4 to the particles themselves. In addition to the enhanced cellular accumulation, other factors related to the metabolic burst that accompanies phagocytosis might also be involved in the stimulation of T_4-deiodination. In leukocytes from two patients with chronic granulomatous disease, a disorder in which phagocytosis appears to occur normally but in which the metabolic burst and attendant increase in hydrogen peroxide generation do not occur, stimulation of T_4-deiodination was either greatly diminished or totally lacking. In myeloperoxidase-deficient leukocytes, on the other hand, stimulation of T_4-deiodination was at least as great as that in normal cells. Thus, we conclude that the primary factor responsible for the increased deiodination of T_4 that phagocytosis induces is the enhanced cellular uptake of hormone. The increased generation of hydrogen peroxide that accompanies phagocytosis may be necessary for the enhanced deiodination of the accumulated T_4, but the latter reaction does not require the mediation of myeloperoxidase.

INTRODUCTION

We have shown that leukocytes isolated from the blood of rhesus monkeys previously inoculated with viable *Diplococcus pneumoniae* display an enhanced ability to deiodinate L-thyroxine (T_4) in vitro (1). Subsequently, we and others have found that stimulation of T_4-deiodination by normal human leukocytes also occurs when phagocytosis is induced in vitro through the addition of inert particles (2, 3). These findings were thought to reflect enhanced peroxidase-mediated deiodination of T_4, since normal leukocytes are rich in peroxidase and generate increased quantities of hydrogen peroxide consequent to the metabolic burst that accompanies phagocytosis (4). Moreover, there is evidence that T_4-deiodination in other tissues is mediated by a peroxidase-hydrogen peroxide system (5).

We undertook the present study to extend our previous observations and to define more fully the mechanism whereby phagocytosis stimulates the metabolism of T_4 by human leukocytes.

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METHODS

Blood was collected, with heparin as the anticoagulant (500 U/25 ml), from healthy laboratory personnel, from a patient with myeloperoxidase-deficient neutrophils (6),[1] and from two patients with chronic granulomatous disease.[2] Plasticware or siliconized glassware was used throughout.

Isolation of leukocytes. The leukocytes were isolated from the blood by the method of Bertino et al. (7). Briefly, the blood was allowed to sediment at 6°C in a dextran-saline solution, and the supernatant layer containing the leukocytes was collected and centrifuged. The erythrocytes remaining in the leukocyte pellet were lysed by exposure to hypotonic conditions for exactly 30 s and removed by washing. About 50% of the leukocytes from the original blood were recovered; approximately 90% of these were neutrophils with virtually no contaminating erythrocytes. The leukocytes were suspended in cold Krebs-Ringer phosphate glucose buffer (KRPG),[3] pH 7.4, at a concentration of approximately 1×10^7 cells/0.5 ml.

Preparation of coated zymosan. Zymosan,[4] the insoluble polysaccharide cell wall residue of yeast, was used as the particulate material for phagocytosis. Since plasma factors (opsonins) are required for optimal phagocytosis, the zymosan particles for each experiment were coated with plasma derived from the same blood from which the leukocytes were isolated. In those experiments in which the abnormal leukocytes were studied, the zymosan particles were coated with plasma derived from the abnormal blood and used with both the abnormal and concurrently studied normal leukocytes. 5 mg of zymosan were homogenized in 1 ml of plasma and allowed to incubate at room temperature for 30 min. The zymosan was then collected by centrifugation, washed twice with KRPG to remove residual plasma, and finally suspended in KRPG at a concentration of 0.5 mg/0.1 ml. When calcium-free KRPG was used as the suspending medium, the zymosan was washed and suspended in this buffer.

Measurement of T_4-deiodination. The leukocyte suspension (0.5 ml; approximately 1×10^7 cells) was added to Erlenmeyer flasks containing a tracer amount (approximately 200 pmol) of ^{131}I-labeled T_4[5] ($[^{131}I]T_4$) and either 1 ml of KRPG alone or 0.9 ml of KRPG and 0.1 ml (0.5 mg) of the coated zymosan suspension. Thus, the final concentration of added T_4 was approximately 0.1 μg/ml. In some experiments, a similar quantity of ^{125}I-labeled L-triiodothyronine[5] ($[^{125}I]T_3$) was added to the flasks, either with or without labeled T_4. In other experiments, the suspending media were enriched with colchicine[6] or phospholipase-C.[7] When a broken cell preparation was desired, the leukocyte suspension was sonicated for 10 s at 6°C. Tissue-free flasks were prepared in an identical manner, except that 0.5 ml of KRPG was added in place of the leukocyte suspension. All flasks were prepared in duplicate and were incubated at 37°C in air in a metabolic shaker. When an anoxic environment was desired, a second set of duplicate flasks in the same incubator was covered with a gassing hood and gassed with 100% nitrogen throughout the experiment. Samples (50 μl) were withdrawn from the reaction mixtures after 10, 30, 60, and 120 min of incubation and were transferred to tubes containing 50 μl of a 15% (wt/vol) solution of human serum albumin enriched with carrier T_4, carrier T_3, carrier iodide, and 0.10 M sodium metabisulfite to terminate the reaction. The mixtures were then subjected to ascending chromatography on filter paper strips in a butanol-acetic acid-water solvent system (8). The only products of $[^{131}I]T_4$ or $[^{125}I]T_3$ deiodination that are separated by this system are radioiodide and chromatographically immobile radioiodine-labeled origin material. The proportions of these two products were quantitated by cutting out the radioactive zones on the filter paper strips, as localized in autoradiograms, and counting them in a well-type scintillation counter. The values obtained in the presence of the leukocytes were corrected for spontaneous deiodination by subtracting from them the values obtained in the corresponding tissue-free samples. The latter values for the percent of origin material and sometimes iodide were slightly higher in the presence of coated zymosan, but together did not exceed 8% of the added labeled hormone after 120 min of incubation.

In some experiments, the generation of $[^{131}I]T_3$ as a product of the deiodination of $[^{131}I]T_4$ was sought. No $[^{131}I]T_3$ could be detected by analysis of samples of the entire reaction mixture. Accordingly, after the 120 min of incubation, 0.5 ml of 15% (wt/vol) human serum albumin enriched with carrier T_4, carrier T_3, carrier iodide, and 0.10 M sodium metabisulfite was added to the reaction mixtures remaining in the flasks to terminate the reaction, and the leukocytes were collected by centrifugation. The leukocyte pellet was washed twice with saline, dispersed in 0.3 ml of 4% (wt/vol) human serum albumin enriched with carrier T_4, carrier T_3, carrier iodide, and 0.10 M sodium metabisulfite, and sonicated. To correct for possible spontaneous conversion of T_4 to T_3, the tissue-free control samples were also sonicated. The sonicates were then subjected along with $[^{125}I]T_3$ as a marker to two-dimension chromatography on filter paper sheets in hexane-tertiary amyl alcohol-ammonia (9) and butanol-dioxane-ammonia (8) solvent systems. The radioactive zones on the filter paper sheets were localized by radioautography, cut out, and counted for ^{131}I and ^{125}I. The proportion of the total ^{131}I appearing in the marker $[^{125}I]T_3$ zone was calculated and corrected for the values obtained in the tissue-free samples.

Measurement of radioiodine-accumulation. The reaction mixtures were prepared and incubated as outlined in the preceding section. After 60 min of incubation, 0.5 ml of 15% (wt/vol) human serum albumin enriched with carrier T_4, carrier iodide, and 0.10 M sodium metabisulfite was added to each flask to terminate the reaction. A sample (10 μl) was removed from each flask to serve as a counting standard. A second sample (10 μl) was also removed and subjected to ascending filter paper chromatography in the butanol-acetic acid-water solvent system for measurement of T_4-deiodination. The leukocytes and coated zymosan particles (only the latter in the tissue-free flasks) were then collected by centrifugation of the reaction mixtures;

[1] Through the courtesy of Dr. Robert I. Lehrer, University of California Medical Center, San Francisco, Calif.

[2] The blood of both patients was obtained through the courtesy of Dr. Thomas P. Stossel, Children's Hospital Medical Center, Boston, Mass. As judged from clinical and laboratory criteria, both patients had typical chronic granulomatous disease.

[3] Composition of KRPG: NaCl, 120 mM; KCl, 4.8 mM; $CaCl_2$, 0.8 mM; $MgSO_4$, 1.2 mM; Na_2HPO_4, 12.7 mM; NaH_2PO_4, 2.9 mM; glucose, 10 mM.

[4] Obtained from K & K Laboratories, Inc., Plainview, N. Y.

[5] Obtained from Abbott Laboratories, Chemical Marketing Division, North Chicago, Ill.

[6] Colchicine injection, Eli Lilly & Co., Indianapolis, Ind.

[7] Obtained from Sigma Chemical Co., St. Louis, Mo.

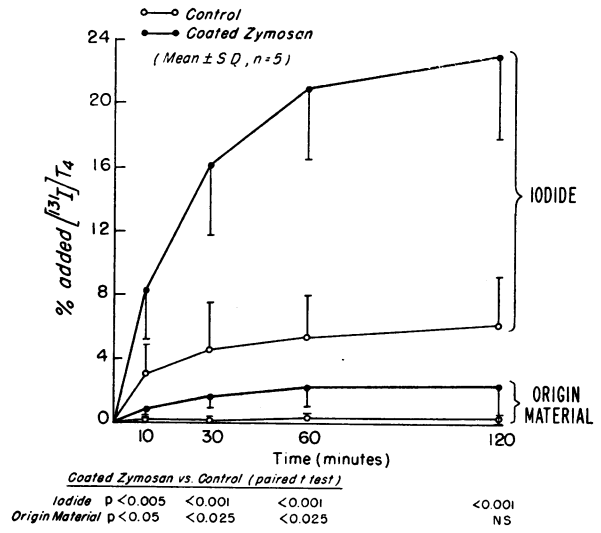

FIGURE 1 Metabolism of ^{131}I-labeled L-thyroxine ([^{131}I]T_4) by intact normal human leukocytes, as assessed from the generation of [^{131}I]iodide and chromatographically immobile ^{131}I-labeled origin material. The effects thereon of phagocytosis induced through the addition of zymosan particles coated with plasma (coated zymosan).

the pellets were washed twice with isotonic saline and dispersed in 0.3 ml of 4% (wt/vol) human serum albumin enriched with carrier T_4, carrier iodide, and 0.10 M sodium metabisulfite. The suspensions were then sonicated, their radioactivity compared to that in the standards prepared from the corresponding reaction mixtures, and chromatographed in the butanol-acetic acid-water solvent system. The values obtained for the accumulation of ^{131}I derived from [^{131}I]T_4 by the leukocytes in the presence of the coated zymosan particles were corrected by subtracting the values obtained in the tissue-free samples that contained coated zymosan alone, and the apportionment of the accumulated ^{131}I among origin material, iodide, and undegraded T_4 calculated. In one experiment, the accumulation of radioiodine derived from a tracer quantity of ^{125}I-labeled human serum albumin[8] added to the suspending medium was assessed by the foregoing procedure.

RESULTS

Fig. 1 depicts the major features of the metabolism of [^{131}I]T_4 by intact normal human leukocytes. As assessed from chromatography of the reaction mixture in the butanol-acetic acid-water solvent system, two major products of [^{131}I]T_4 deiodination were formed, [^{131}I]-iodide and chromatographically immobile ^{131}I-labeled origin material. The deiodination reaction had the following characteristics: (*a*) it approached a plateau between 60 and 120 min of incubation; (*b*) it was abolished almost completely by prior boiling of the cells or by incubation in 100% nitrogen; and (*c*) it was not inhibited by an excess of stable iodide. Generally similar results were obtained with labeled T_3; however, in experiments in which the deiodination of tracer concentrations of the two hormones was directly compared, the percentage deiodination of T_3 was either equal to or less than that of T_4.

Analysis of the leukocytes recovered after incubation with [^{131}I]T_4 by two-dimension chromatography in the hexane-tertiary amyl alcohol-ammonia and butanol-dioxane-ammonia solvent systems also revealed the presence of [^{131}I]T_3 as a product of T_4-deiodination (Fig. 2). When corrected for the very small quantity of

[8] Obtained from Abbott Laboratories, Chemical Marketing Division, North Chicago, Ill.

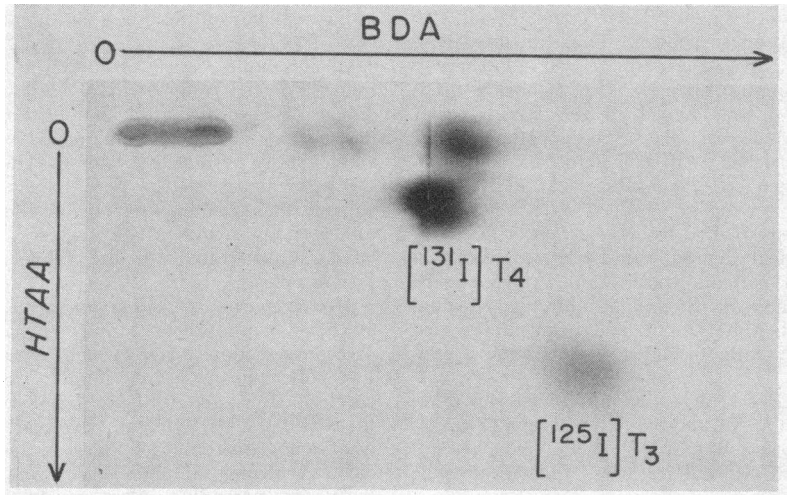

FIGURE 2 Two dimension paper chromatography in hexane-tertiary amyl alcohol-ammonia (HTAA) and butanol-dioxane-ammonia (BDA) of normal human leukocytes recovered after incubation with [^{131}I]T_4. ^{125}I-labeled L-triiodothyronine ([^{125}I]T_3) was also applied at the origin as a marker.

[^{131}I]T_3 found in the tissue-free samples, the net [^{131}I]T_3 generated comprised about 1% of the total ^{131}I that had been accumulated by the leukocytes.

The induction of phagocytosis through the addition of coated zymosan particles to the suspending medium resulted in a great stimulation of [^{131}I]T_4 deiodination by normal leukocytes. As depicted in Fig. 1, this was reflected in increased generation of both iodide and origin material. A similar stimulation was observed with respect to T_3-deiodination. A broken cell preparation deiodinated T_4 actively, and its activity was not altered by the addition of the coated zymosan particles.

The effects of induced phagocytosis on the accumulation by leukocytes of ^{131}I derived from [^{131}I]T_4 and the influence thereon of various factors are presented in Fig. 3 and Table I. In normal cells incubated in air, the addition of the coated zymosan particles resulted in the expected increase in T_4-deiodination, as assessed from analysis of the reaction mixture, and in an increased accumulation of ^{131}I by the cells themselves. The latter was comprised of an increase in undegraded [^{131}I]T_4, as well as increases in [^{131}I]iodide and ^{131}I-labeled origin material. In an anoxic environment, in two separate experiments (Fig. 3 and Table I), T_4-deiodination was greatly depressed, both in the control state and in the presence of the coated zymosan particles, and little origin material was evident on analysis of the leukocytes recovered after incubation. Nevertheless, even in this circumstance, the cells accumulated

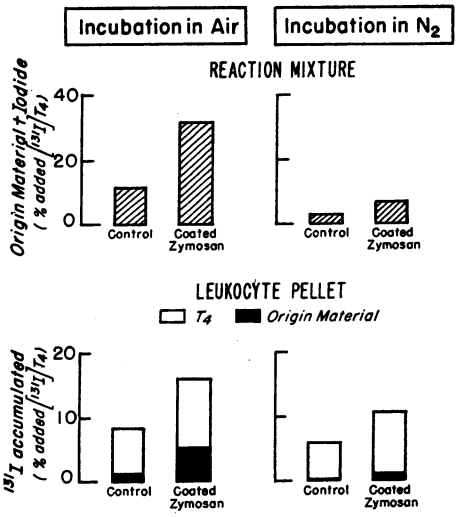

FIGURE 3 Comparison of the effects of incubating intact normal human leukocytes in air or in 100% nitrogen on the responses of deiodination and cellular accumulation of [^{131}I]T_4 to phagocytosis of coated zymosan particles. The results of a separate experiment are presented in Table I.

TABLE I

Influence of Various Factors on the Stimulation by Phagocytosis induced through the Addition of Coated Zymosan Particles of the Cellular Accumulation and Deiodination of ^{131}I-labeled L-Thyroxine ([^{131}I]T_4) by Human Leukocytes

Cells	State	Cellular accumulation at 60 min				Reaction mixtures at 60 min	
		Total	OM	I^-	T_4	OM	I^-
		% added [^{131}I]T_4				% added [^{131}I]T_4	
Normal*	Control	7.31	0.73	0.44	6.14	2.11	5.95
	Coated zymosan	15.76	5.10	3.64	7.02	8.61	32.78
	Control in 100% N_2	5.47	0.63	0.62	4.22	0.63	3.35
	Coated zymosan in 100% N_2	8.90	0.96	0.97	6.97	0.99	7.24
Normal	Control	7.48	0.57	0.26	6.65	0.89	7.42
	Coated zymosan	12.66	3.89	1.70	7.07	3.62	32.97
	Control + colchicine (2.5×10^{-4} M)	5.78	0.40	0.20	5.18	0.70	6.82
	Coated zymosan + colchicine (2.5×10^{-4} M)	11.07	3.45	1.55	6.07	8.89	37.88
Normal	Control	6.01	0.15	0.19	5.67	0.43	8.50
	Coated zymosan	19.54	4.86	4.56	10.12	5.08	27.26
	Phospholipase-C (0.5 mg, 2.5 U)	5.41	0.14	0.15	5.12	0.08	4.17
Normal	Control	7.99				0.38	9.18
	Coated zymosan	13.20				2.11	33.63
CGD‡	Control	6.76				1.74	20.48
	Coated zymosan	7.94				1.04	20.93

CGD, chronic granulomatous disease; OM, chromatographically immobile origin material; and I^-, iodide.

* The results of a separate experiment are depicted in Fig. 3.

‡ The results obtained with leukocytes from the other patient with CGD are depicted in Fig. 5.

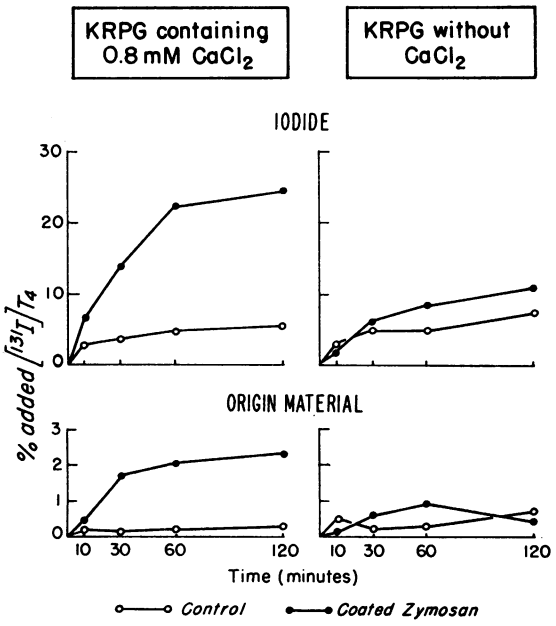

FIGURE 4 Comparison of the effects of phagocytosis of coated zymosan particles on the deiodination of [^{131}I]T_4 by intact normal human leukocytes incubated in Krebs-Ringer phosphate glucose buffer (KRPG) with and without calcium.

undegraded [^{131}I]T_4 almost as actively as the cells incubated in air, and an increment in [^{131}I]T_4 accumulation that was as great as that in cells incubated in air followed the addition of the coated zymosan particles. Also presented in Table I are the results of adding colchicine and phospholipase-C. Colchicine did not prevent the increase in the cellular accumulation or deiodination of [^{131}I]T_4 that followed the addition of the coated zymosan particles. Phospholipase-C did not increase either the cellular accumulation or the deiodination of [^{131}I]T_4. Omission of calcium from the suspending medium inhibited the cellular accumulation of ^{131}I derived from [^{131}I]T_4 in the presence of the coated zymosan particles (6% vs. 12% in the presence of 0.8 mM $CaCl_2$ after 120 min of incubation), and, as depicted in Fig. 4, depressed the stimulation of T_4-deiodination that phagocytosis induced. In contrast to the results of the foregoing experiments with [^{131}I]T_4, leukocytes recovered after a 60 min period of incubation with ^{125}I-labeled human serum albumin accumulated only 0.023% of the added ^{125}I and no increase occurred following the addition of the coated zymosan particles.

Fig. 5 depicts the results of an experiment in which [^{131}I]T_4-deiodination by leukocytes from a patient with chronic granulomatous disease was compared with that effected by concurrently incubated normal cells. In the control state, the leukocytes of this patient deiodinated T_4 more actively than the normal cells. When phagocytosis was induced through the addition of the coated zymosan particles, T_4-deiodination was enhanced, but the increment in the generation of both iodide and origin material was much less than that observed in the normal cells. Leukocytes from a second patient with this disorder also deiodinated [^{131}I]T_4 more actively in the control state than concurrently incubated normal cells, but here the addition of the coated zymosan particles did not enhance T_4-deiodination and the increment in the cellular accumulation of ^{131}I was much less than that in the normal cells (Table I).

Fig. 6 depicts the results of an experiment in which [^{131}I]T_4-deiodination by myeloperoxidase-deficient leukocytes was compared with that effected by concurrently incubated normal cells. In the control state, T_4-deiodination by the normal and abnormal cells was similar. When phagocytosis was induced through the addition of the coated zymosan particles, both deiodinated T_4 more actively, but the generation of iodide was distinctly greater in the myeloperoxidase-deficient cells than in the normal cells.

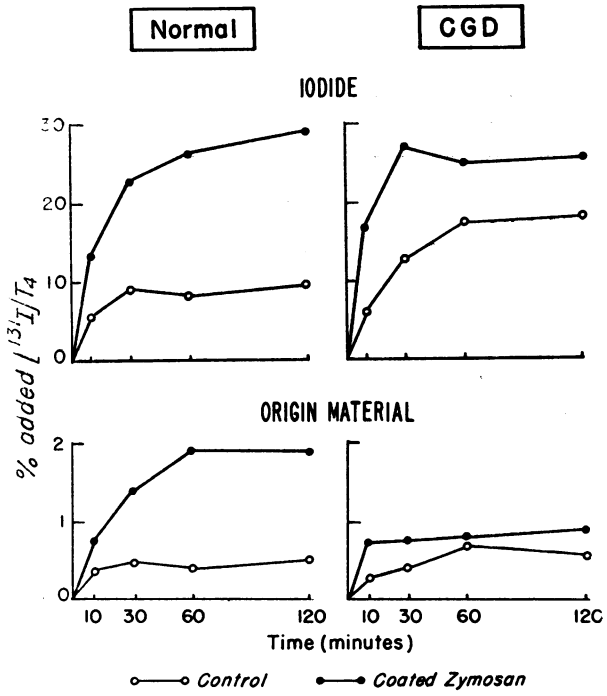

FIGURE 5 Comparison of the effects of phagocytosis of coated zymosan particles on the deiodination of [^{131}I]T_4 by intact leukocytes from a normal subject and from a patient with chronic granulomatous disease (CGD). The results obtained with the leukocytes from the other patient are presented in Table I.

DISCUSSION

In a previous study (2), we have demonstrated that intact human leukocytes are capable of deiodinating radioiodine-labeled T_4 to yield radioiodide and a form of protein-bound radioiodine that is chromatographically immobile and is thus similar to that observed in other T_4-deiodinating systems (10). We have also found that the generation of these products is enhanced by induction of phagocytosis through the addition of zymosan particles coated with plasma. These findings have been confirmed by Klebanoff and Green (3) who have also found, as have we, that similar phenomena occur in the case of T_3.

Originally, we proposed that the increased T_4-deiodination induced by coated zymosan particles is a consequence of the metabolic burst that accompanies phagocytosis with its attendant increase in hydrogen peroxide generation. This interpretation seemed consonant with the presence in normal leukocytes of peroxidase and with the postulated role of a peroxidase-hydrogen peroxide system in T_4-deiodination in other tissues (5). A similar mechanism is suggested by the data of Klebanoff and Green (3). Our present observations would suggest, however, that although such factors might operate to some extent, the primary factor is an enhanced cellular accumulation of T_4 that accompanies the phagocytic event. Thus, in the several experiments in which these measurements were made, coated zymosan invariably induced an increase in the accumulation not only of origin material and iodide but also of undegraded T_4. Had phagocytosis primarily stimulated the presumed enzymic mechanism for T_4-deiodination, the accumulation of undegraded T_4 in the phagocytosing leukocytes should have been less than that in the control cells; however, the converse was true.

Other evidence in favor of the primacy of enhanced cellular accumulation is provided by the results of those experiments in which the influence of various factors on the cellular accumulation and deiodination of T_4 was assessed. First, the influence of anoxia was examined, since anoxia has been shown not to interfere with phagocytosis (11), but diminishes T_4-deiodination in many systems. In the present study, anoxia greatly diminished T_4-deiodination, but did not prevent either the cellular accumulation of T_4 in the control state or the stimulation thereof that phagocytosis induced. Second, the effect of colchicine was assessed, since colchicine in a concentration of 2.5×10^{-4} M has been shown to depress the metabolic burst that accompanies phagocytosis (12).[9] It is significant, therefore, that colchicine

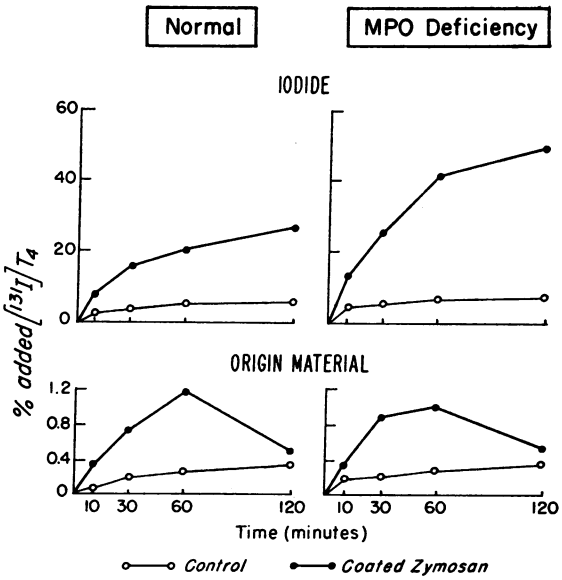

FIGURE 6 Comparison of the effects of phagocytosis of coated zymosan particles on the deiodination of [^{131}I]T_4 by intact leukocytes from a normal subject and from a patient with a genetic absence of myeloperoxidase (MPO).

did not inhibit the stimulation of either the cellular accumulation or the deiodination of T_4 that the coated zymosan particles induced. Third, the influence of phospholipase-C was examined, since this enzyme has been shown to induce a metabolic burst that mimics that induced by phagocytosis (14). In the presence of phospholipase-C, however, neither the cellular accumulation nor the deiodination of T_4 were enhanced. Finally, calcium, which has been shown to be required for optimal phagocytosis (13), was required for the increase in both the cellular accumulation and deiodination of T_4 that phagocytosis induced. These findings, together with the finding of increased accumulation of undegraded T_4 in the phagocytosing cells, strongly suggest that the primary factor responsible for the stimulation of T_4-deiodination is the enhanced uptake of T_4 that accompanies the phagocytic process.

The present study does not elucidate the mechanism whereby phagocytosis increases the cellular accumulation of T_4, although two possibilities appear to be excluded. First, simple engulfment of suspending medium containing T_4 was excluded by the finding that phagocytosis did not increase the negligible cellular uptake of radioiodinated human serum albumin from the medium. Second, the possibility that significant quantities of T_4 were brought into the cell bound to the coated zymosan particles was excluded, since the values for the cellular

[9] Although this concentration of colchicine reportedly did not affect phagocytosis per se, recent work employing a more precise method for quantitating phagocytosis has revealed that a similar concentration might be inhibitory (13).

accumulation of T_4 were corrected for the T_4 bound to the coated zymosan in the absence of the leukocytes. Moreover, the addition of coated zymosan did not alter the deiodination of T_4 by a broken cell preparation; were coated zymosan capable of binding T_4 to a significant extent, a decrease in T_4-deiodination would have been expected.

It would appear, therefore, that enhanced cellular accumulation is the primary factor responsible for the increased hormonal deiodination that phagocytosis induces. However, other factors might also be operative. It is generally agreed that in leukocytes from patients with chronic granulomatous disease phagocytosis occurs normally, but the metabolic burst and attendant increase in hydrogen peroxide generation are lacking (15, 16). It seemed likely, therefore, that the greatly diminished or absent stimulation of T_4-deiodination that we and Klebanoff and Green (3) have observed in these cells following the addition of the coated zymosan particles was owing to deficient generation of hydrogen peroxide during phagocytosis. However, in the leukocytes of one of our two patients in which we measured the cellular accumulation of ^{131}I derived from [^{131}I]T_4, this function increased only minimally following the addition of the coated zymosan particles. In this regard, it is noteworthy that in a recent study the leukocytes of one of three patients with chronic granulomatous disease displayed a somewhat subnormal rate of particle uptake (17). However, whether the minimal increment in the cellular accumulation of ^{131}I in the leukocytes of our patient was a reflection of impaired phagocytosis is not known, since correlative measurements of the cellular accumulation of T_4 and of phagocytosis are lacking. Thus, it is uncertain whether the lack of stimulation of T_4-deiodination in these cells is owing to impaired cellular accumulation of T_4 or to lack of the normal increase in hydrogen peroxide generation during phagocytosis.

Our observations with myeloperoxidase-deficient leukocytes also bear on the question of the role of hydrogen peroxide in the stimulation of T_4-deiodination during phagocytosis. In our study, the stimulation of T_4-deiodination in these cells following the addition of the coated zymosan particles was seemingly greater than that in the normal cells. This finding might have been owing to the greater than normal availability of hydrogen peroxide as a result of the absence of myeloperoxidase-mediated reactions (18), and, therefore, would suggest a role for hydrogen peroxide in mediating the stimulation of T_4-deiodination during phagocytosis. Moreover, the brisk stimulation of T_4-deiodination that we and Klebanoff and Green (3) have observed in these cells during phagocytosis would indicate that, if hydrogen peroxide is involved in mediating the stimulation of T_4-deiodination, the latter reaction need not be catalyzed by myeloperoxidase.

While the foregoing discussion has concerned itself with the mechanism of the stimulation of hormonal deiodination induced by phagocytosis, our study also provides some information concerning the mechanism of T_4-deiodination by leukocytes in the control or resting state. The finding of normal T_4-deiodination by myeloperoxidase-deficient cells indicates that this enzyme is not required for deiodination in the resting state, just as it is not required for the stimulation of deiodination that occurs during phagocytosis. Whether hydrogen peroxide itself is required is uncertain, however. The leukocytes from the two patients with chronic granulomatous disease deiodinated T_4 normally in the resting state. This finding may be owing to the fact that these cells are not totally lacking in the capacity to generate hydrogen peroxide, but lack the ability to increase the generation of hydrogen peroxide during phagocytosis (16).

The present study also demonstrates that freshly-isolated human leukocytes in the resting state, like human liver and kidney cells and fibroblasts grown in tissue culture (19, 20), are capable of generating T_3 during the metabolism of T_4. Our failure to observe an increased proportion of T_3 following induction of phagocytosis may have reflected enhanced degradation of the newly-formed T_3 in this circumstance.

Finally, although its significance with respect to overall thyroid hormone economy has not been directly evaluated, it seems possible that the increased metabolism of T_4 by phagocytosing cells may contribute to the accelerated cellular uptake and metabolism of T_4 that occur in the host during acute bacterial sepsis (1, 21, 22).

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