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ACCUMULATION OF 2-[^{14}C]PROPYLTHIOURACIL IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES*

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Abstract—Phagocytizing polymorphonuclear leukocytes (PMN) accumulate radioactive propylthiouracil ([^{14}C]PTU), methimazole ([^{14}C]MMI) and iodide ($^{125}I^-$) at rates 8- to 10-fold those of resting cells. Iodide, at concentrations of less than 5 μM, had no effect on PTU or MMI uptake. Perchlorate had no effect on either PTU or iodide uptake, while thiocyanate inhibited only iodide uptake. Iodide organification is inhibited by PTU, azide and cyanide. PTU accumulation increased 666 per cent as PTU was increased from 0.005 to 0.5 μmole/ml, while iodide organification was decreased 87.7 per cent with 0.05 μmole/ml of PTU. Similar results were obtained with azide and cyanide. This stimulation was accompanied by a rise in available H_2O_2 which was increased 255–700 per cent over that in resting cells by PTU and MMI at 25 nmoles/ml. Azide and cyanide also produced similar results. These results demonstrate that PTU is accumulated in PMN and that its uptake is independent of iodide transport and of iodide metabolism which is in contrast to that observed in the thyroid. The association between increased PTU uptake and increased H_2O_2 accumulation in the cells suggests that PTU accumulation is related to oxidation of the drug.

In the course of studying thyroidal accumulation of the antithyroid drugs, thiouracil (TU) [1], propylthiouracil (PTU) [2] and methimazole (MMI) [3], several investigators reported that these drugs were also concentrated in blood cells. However, these phenomena have not been investigated thoroughly. Although polymorphonuclear leukocytes (PMN) are vastly different from thyroid cells in primary functions, both cells are able to concentrate and metabolize iodide to form iodoproteins, monoiodotyrosines, diiodotyrosines and a thyroxine-like material [4]. Thus, it seemed likely that the PMN is also capable of accumulating the antithyroid drugs, PTU and MMI.

Numerous investigators [5–8] have clearly demonstrated the accumulation of PTU in the thyroid gland. However, our knowledge concerning the factors governing its accumulation in the gland is fragmentary. Several hypotheses have been offered in this area. Marchant *et al.* [5] suggested that the concentration of PTU by the thyroid was partially dependent upon the anion trapping mechanism, after observing a significant reduction in the accumulation of total radioactivity and of unaltered PTU in rats pretreated with large amounts of perchlorate or iodide. However, they pointed out that another process must also be involved since PTU accumulation proceeded even when iodide was completely abolished by perchlorate. However, Lindsay *et al.* [8] reported that accumulation of PTU and MMI was influenced by the same factors that influence iodide metabolism, except for low iodide diet. On this basis, they suggested that PTU and MMI accumulation was more closely related to iodide organification than to iodide trapping. Recently, Tourog and Riesco [9], in studying *in vitro* binding of PTU to thyroglobulin, concluded that binding occurred only as a consequence of its inhibitory effect on peroxidase-catalyzed iodination, suggesting that accumulation of PTU occurs only after its oxidation.

This study was done to determine the accumulation of PTU in PMN and to investigate the possible role of iodide in the accumulating process.

EXPERIMENTAL PROCEDURE

Materials. Na^{125}I (carrier-free), [^{14}C]formate (3.0 mCi/m-mole) and [^{14}C]MMI (3.8 mCi/m-mole) were obtained from the New England Nuclear Corp. (Boston, MA). 2-[^{14}C]PTU (3.78 mCi/m-mole) was purchased from Mallinckrodt Nuclear (St. Louis, MO). Bacto-latex beads (0.81 μm in diameter) were obtained from Difco-Lab. (Detroit, MI).

Isolation of human PMN. This method involves a two-phase system for the removal of red cells with methylcellulose as an erythrocyte-aggregating agent. About 12 ml of heparinized human blood was layered on top of 7.5 ml of Hypaque–methylcellulose (50 ml Hypaque mixed with 125 ml of 2% methylcellulose diluted to a final volume of 200 ml) in glass tubes and allowed to settle. The red cells settled to the bottom of the tube leaving a top layer of PMN-rich plasma which was withdrawn and centrifuged at 150 *g* for 10 min at 4°. The contaminating red cells were osmotically lysed with 0.2% NaCl for 20 sec, followed by an equal volume of chilled hypertonic 1.6% NaCl to restore

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isotonicity. The cell suspension was centrifuged and the pellet obtained was washed once with Hanks' phosphate solution and recentrifuged. The final cell pellet was suspended in 5 ml of Hanks' phosphate solution which was prepared by adding 5 ml of Hanks' balanced salt solution (10 ×), without bicarbonate and phenol red, to 40 ml of distilled water followed by 5 ml of 0.1 M phosphate buffer, pH 7.6, to make the final pH of the solution 7.4. This preparation yielded approximately 60–80 × 10^6 leukocytes as determined by representative differential cell counts (1:20 dilution). Using Wright's stained smears, 95 per cent of the preparation was found to be PMN and the rest was lymphocytes, monocytes, placelets and a few red cells. Viability of the PMN was determined by their ability to exclude trypan blue which was found to be 93 per cent. In addition, 30 per cent of the viable cells carried out phagocytosis, as indicated by their ability to phagocytize polystyrene beads. These results were consistent with those of Takanaka and O'Brien [10].

PMN accumulation of PTU, MMI and iodide. The basic medium for all incubations was Hanks' phosphate solution. All additions to the incubating systems were dissolved in Hanks' phosphate solution.

A typical incubation medium contained 5–10 × 10^6 PMN and 0.05 μmole (0.189 μCi) [^{14}C] PTU or [^{14}C]MMI in a final volume of 1.0 ml. Incubation was carried out in duplicate under 95% O_2–5% CO_2 in stoppered siliconized glass tubes for 1 hr in a 37° water bath. Controls were without beads and/or cells with beads but without drugs. Phagacytosis was induced by the injection of 0.1 ml of polystyrene heads through the rubber stopper. After incubation, the cells were centrifuged at 150 *g* for 10 min at 4°. The cell pellet was washed once with Hanks' phosphate solution and recentrifuged. After discarding the supernatant solution, the tubes were inverted to drain off the excess fluid and the sides of the tubes were wiped dry to remove any remaining fluid. The cell pellet was then solubilized in 0.2 ml H_2O and 1.0 ml Protosol overnight and quantitatively transferred into counting vials to determine total radioactive uptake in a liquid scintillation counter.

For total $^{125}I^-$ uptake, carrier-free $^{125}I^-$ (0.25 μCi) was used, and the total radioactivity was determined in a gamma well-type counter.

Accumulation of PTU and iodide with time. The incubation medium contained 5–10 × 10^6 PMN, 0.05 μmole [^{14}C]PTU (0.189 μCi) or carrier-free $^{125}I^-$ (0.23 μCi) and Hanks' phosphate solution to a final volume of 1.0 ml. Controls were cells without polystyrene beads. Phagocytosis was stimulated by the addition of 0.1 ml of polystyrene beads. The rest of the procedure was similar to that described above. Incubation was performed at different time intervals ranging from 0 to 60 min. After each period, the reaction was stopped immediately by transferring the contents onto a glass fiber filter disc placed in a small, porcelain, Buchner funnel attached to a suction pump. The PMN on the filter were washed six to seven times with Hanks' phosphate solution. The filters with $^{125}I^-$ were counted in a gamma well-type counter, while those with [^{14}C]PTU were solubilized overnight with 0.2 ml H_2O and 1.0 ml Protosol before they were counted to determine total uptake of radioactivity.

Cell to medium (C/M) ratio of PTU, MMI and iodide. The PMN were incubated as described previously. At the end of the incubation, the PMN suspensions were transferred to 15-ml calibrated conical test tubes, and centrifuged at 2000 *g* for 10 min at 4°. Five μl of the supernatant fraction was assayed for radioactivity. A 5-μl aliquot of the suspension of medium and cells was measured and assayed for total radioactivity, while a duplicate volume was used to determine the packed cell volume. The results were expressed as C/M ratios, as described by Tong *et al.* [11].

$$C/M = \frac{\text{dis./min/ml of packed cells}}{\text{dis./min/ml of medium}}$$

The fluid trapped between the packed cells was not taken into consideration in these measurements. Therefore, the true C/M ratios are underestimated.

Formate oxidation. This method was a modification of that described by Klebanoff and Hamon [12]. [^{14}C]formate oxidation by catalase and H_2O_2 with the liberation of $^{14}CO_2$ is a measure of the H_2O_2 generated [12]. The incubation mixture consisted of 4–10 × 10^6 PMN, 3 μmoles sodium [^{14}C]formate (0.17 mCi), 18,000 units of catalase, 0.1 ml of polystyrene beads and Hanks' phosphate, in a final volume of 1.0 ml. Incubation was carried out under O_2 and O_2 in duplicate stoppered flasks with a removable center well at 37° for 1 hr. The reaction was terminated by the injection of 0.2 ml of 2 N H_2SO_4 through the stopper, followed by 0.5 ml Hyamine hydroxide into the center cell. The flasks were shaken for another 45 min. The Hyamine hydroxide was quantitatively transferred with 10 ml of scintillation fluid into counting vials and the radioactivity determined.

Statistical analysis. The data were evaluated statistically with Student's *t*-test.

RESULTS

Accumulation of PTU, MMI and I^-. The data presented in Table 1 demonstrate that resting PMN incubated with radioactive PTU, MMI and I^- *in vitro* accumulated radioactivity to achieve cell medium (C/M) ratios of 2–2.7. Accumulation of all three substances was dramatically increased in phagocytizing cells with C/M ratios of 12.6, 9.1 and 27.5 being achieved with [^{14}C]PTU, [^{14}C]MMI and $^{125}I^-$ respectively. In subsequent reports [13, 14], we demonstrated that approximately 45.0 per cent of the total accumulated radioactivity was extractable with ethanol, while the remainder was bound to cellular material. The extractable radioactivity was composed of unaltered PTU and PTU metabolites. Analyses of the medium after incubation with [^{14}C]PTU showed that detectable amounts of PTU metabolites were not present. Consequently, the accumulated radioactivity represents unaltered PTU or PTU metabolites formed within the cell.

Non-specific binding of I^-, PTU and MMI to polystyrene beads was presented in Table 2. As expected with the negatively charged iodide ion [15], no binding was reported. However, PTU and MMI, which are weak acids, have weak positive charges resulting in binding to the beads with bead to medium ratios of 3.0 respectively. If accumulation of PTU and MMI in the PMN was due exclusively to non-specific binding to the ingested polystyrene beads, the highest C/M ratio that could be obtained would be 3, and 100 per cent of the

Table 1. Accumulation of radioactive PTU, MMI or I^- by human PMN *in vitro**

Compound	State of PMN	Total radioactivity accumulated† (dis./min/1 × 10^6 PMN)	Cell/Medium
[^{14}C]PTU	Resting	66 ± 9.4	2.0
	Phagocytizing	995 ± 170	12.6
[^{14}C]MMI	Resting	192 ± 15.6	2.7
	Phagocytizing	1429 ± 218	9.1
$^{125}I^-$	Resting	610 ± 69	2.7
	Phagocytizing	7760 ± 602	27.5

* The incubation medium contained 5–10 × 10^6 PMN, 0.1 ml of polystyrene beads, and either carrier-free $^{125}I^-$ (0.23 μCi/ml), 0.05 μmole [^{14}C]PTU or [^{14}C]MMI (0.189 μCi/ml) in Hanks' phosphate solution, in a final volume of 1.0 ml. Incubation was for 1 hr at 37°. Refer to Experimental Procedure for details.

† Mean of four experiments ± S.E.

Table 2. Non-specific binding of I^-, PTU and MMI to polystyrene beads*

Compound	Bead/Medium ratio†
$^{125}I^-$	1.2 ± 0.01
[^{14}C]PTU	3.1 ± 0.1
[^{14}C]MMI	3.0 ± 1.0

* The incubation medium contained 0.1 ml of polystyrene beads, carrier-free $^{125}I^-$ (0.23 μCi), 0.05 μmole [^{14}C]PTU (0.189 μCi) or 0.05 μmole [^{14}C]MMI (0.189 μCi) and Hanks' phosphate, in a total volume of 1.0 ml. Controls were without radioactive agents. Incubation was for 1 hr at 37°. The rest of the procedure is described in Experimental Procedure.

† Mean of four experiments ± S.E.

beads would have to be ingested or the total volumme of the cell would have to be occupied by the beads in order to achieve a C/M ratio of 3. However, microscopic examination revealed that each cell ingested an average of only four to six beads (unpublished observations). Since C/M ratios of PTU and MMI in phagocytizing PMN were found to be 12.5 and 9.1, respectively, accumulation of these drugs could not be explained by non-specific binding to the ingested polystyrene beads. In addition, the C/M ratios of both drugs in resting PMN where polystyrene beads were absent were greater than 1 (Table 1), suggesting selective accumulation of the drugs in the cells.

Accumulation of $^{125}I^-$ and [^{14}C]PTU in phagocytizing cells, with respect to time, is shown in Fig. 1. Accumulation of both iodide and PTU increased with time for 30 min after which there was no net increase in uptake. The similarities in the PTU and iodide curves and the demonstration that both PTU and iodide are accumulated in resting and phagocytizing PMN suggest the possibility that PTU and iodide uptake in PMN may be related.

Effects of I^-, ClO_4^- *and* SCN^- *on PTU and MMI accumulation.* In Fig. 2, the effects of various concentrations of I^- on PTU accumulation in phagocytizing PMN are shown. Iodide concentrations of 1.0 nmole/ml or less had no effect on PTU accumulation, but concentrations of 50.0 nmoles/ml and above decreased PTU uptake by 20–30 per cent. Perchlorate competes with iodide for the thyroidal transport system and markedly inhibits iodide uptake. However, perchlorate at concentrations from 0.2 to 5.0 μmoles/ml had no measurable effect on either iodide or PTU uptake ($P > 0.1$) in phagocytizing PMN (Table 3). This is in marked contrast to observations of perchlorate effects on thyroidal iodide uptake *in vivo* in rats [16] and on iodide uptake in isolated thyroid cells [11].

In Fig. 3, the effects of thiocyanate on iodide and PTU uptake in PMN are presented. Thiocyanate inhibits iodide uptake into the thyroid by enhancing the transport of iodide out of the cells before oxidation and organification of iodide occur. In PMN, iodide uptake

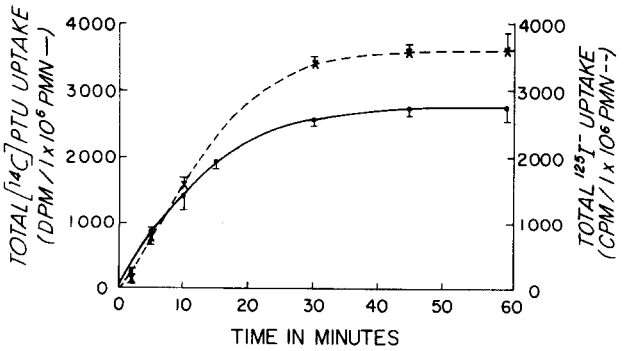

Fig. 1. Accumulation of [^{14}C]PTU and $^{125}I^-$ in phagocytizing PMN with time. The incubation medium was identical to that in Table 1. Each point is the mean of four experiments. Vertical bars represent standard errors.

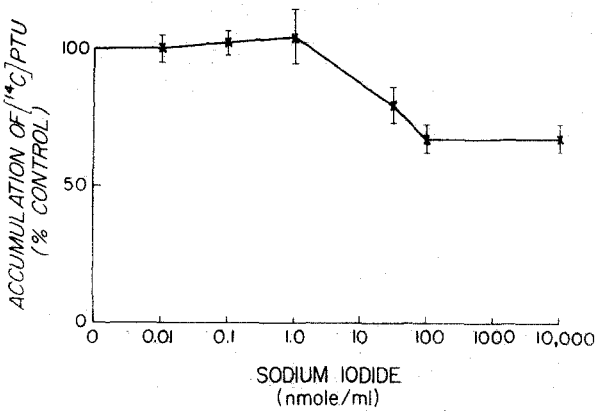

Fig. 2. Effects of various concentrations of sodium iodide on [^{14}C]PTU accumulation in phagocytizing PMN. The incubation medium contained 4–10 × 10^6 PMN, 0.1 ml of polystyrene beads, and 0.05 μmole [^{14}C]PTU (0.189 μCi) in Hanks' phosphate solution. See Fig. 1.

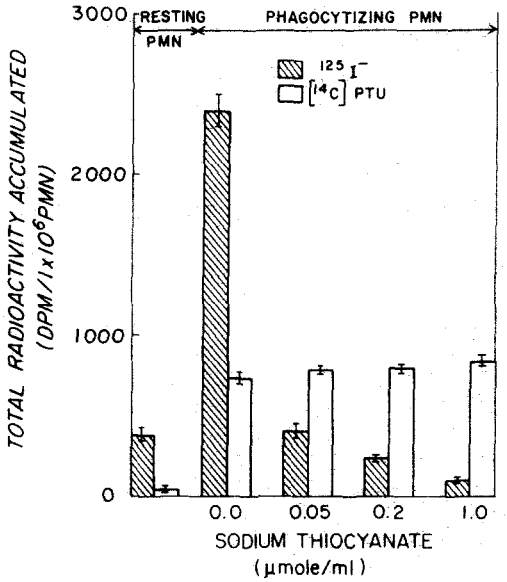

Fig. 3. Effects of various concentrations of sodium thiocyanate on accumulation of $^{125}I^-$ and [^{14}C]PTU in PMN. The procedure was similar to that described in Table 1, except for the addition of various concentrations of thiocyanate. Cross-hatched bars indicate $^{125}I^-$ experiments (n = 9); open bars indicate [^{14}C]PTU experiments (n = 4). See Fig. 1.

was very sensitive to thiocyanate, with 0.05 μmole/ml producing an 85 per cent ($P < 0.001$) and 1.0 μmole/ml producing a 95 per cent ($P < 0.001$) inhibition (Fig. 3). In contrast, PTU accumulation was unaffected by thiocyanate ($P > 0.1$). The results suggest the absence of a direct relationship between iodide uptake and PTU accumulation in PMN.

Effects of PTU, MMI, azide and cyanide on I^- and PTU accumulation. If PTU accumulation in PMN is related to iodide metabolism or myeloperoxidase (MPO) activity, inhibitors of thyroid peroxidase (TPO) and MPO would also inhibit PTU uptake, as seen in the thyroid. Therefore, effects of peroxidase inhibitors which are also potent inhibitors of MPO [12] were investigated. Total iodide accumulated was used as an indicator of iodide organification or metabolism, since both processes are related. In Fig. 4, the effects of various concentrations of PTU on PTU and iodide accumulation in phagocytizing PMN are compared. PTU accumulation increased more than 6-fold in the PMN as the concentration increased from 0.005 to 0.5 μmole/ml. In contrast, iodide accumulation progressively decreased with the higher concentrations of PTU and was less than 5 per cent of the control with 0.5 μmole/ml. The increase in PTU accumulation with increased PTU concentrations, shown in Fig. 4, is in contrast to results obtained in rats [5] and in isolated thyroid cells (R. H. Lindsay and B. S. Hulsey, unpublished observations) in which PTU uptake decreased when higher amounts of PTU were administered or present. PTU inhibition of the MPO system apparently occurred (Fig. 4) since iodide uptake was reduced drastically. Increased PTU in PMN is not related to iodide metabolism or MPO activity.

Further evidence supporting this interpretation is presented in Figs. 5 and 6. Sodium azide and potassium cyanide are non-thiocarbamides which are potent inhibitors of MPO activity [17]. As presented in Fig. 5, azide stimulated PTU and MMI accumulation by 189 per

Table 3. Effects of various concentrations of perchlorate on accumulation of $^{125}I^-$ and [^{14}]PTU in phagocytizing PMN*

Perchlorate (μmoles/ml)	Total radioactivity accumulated (1 × 10^6 PMN)	
	Iodide† (cpm)	PTU‡ (dis./min)
0	3177 ± 518	3233 ± 181
0.2	3056 ± 446	3066 ± 140
1.0	2832 ± 332	3135 ± 148
5.0	3067 ± 497	3375 ± 127

* The incubation medium and procedure were similar to those described in the legend of Table 1, except for the addition of perchlorate to achieve the various concentrations shown.

† Mean of four experiments ± S.E.

‡ Mean of six experiments ± S.E.

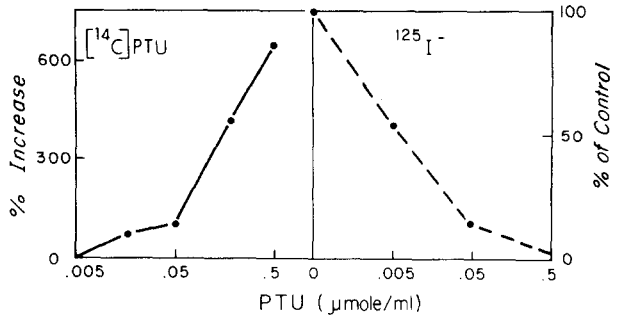

Fig. 4. Effects of various concentrations of PTU on accumulation of [^{14}C]PTU and $^{125}I^-$ in phagocytizing PMN. The incubation procedures are identical to those described for PTU and iodide accumulation in Table 1 except for the addition of various concentrations of PTU. Each point is the mean of a single experiment.

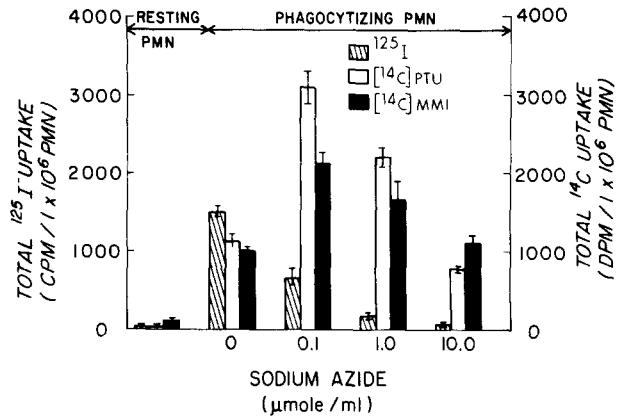

Fig. 5. Effects of various concentrations of sodium azide on accumulation of radioactive iodide, PTU and MMI in PMN. Cross-hatched bars indicate $^{125}I^-$ experiments (n = 9); open bars indicate [^{14}C]PTU experiments (n = 6); and solid bars indicate [^{14}C]MMI experiments (n = 6). See Fig. 1.

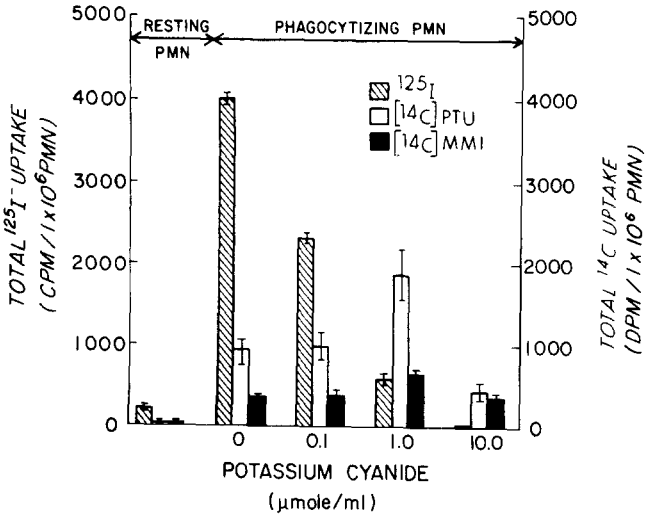

Fig. 6. Effects of various concentrations of potassium cyanide on accumulation of radioactive iodide, PTU and MMI in PMN. Cross-hatched bars indicate $^{125}I^-$ experiments (n = 4); open bars indicate [^{14}C]PTU experiments (n = 6); and solid bars indicate [^{14}C]MMI experiments (n = 4). See Fig. 1.

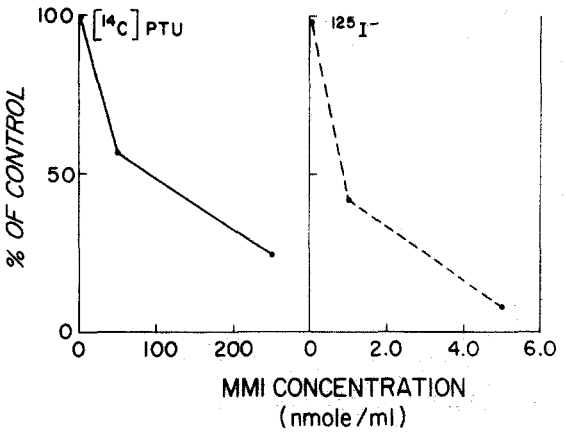

Fig. 7. Effects of various concentrations of MMI on [^{14}C]PTU and $^{125}I^-$ uptake in phagocytizing PMN. See Fig. 4.

cent ($P < 0.001$) and 100 per cent ($P < 0.001$), respectively, at 0.1 μmole/ml, while inhibiting iodide uptake by 50 per cent ($P < 0.001$) at the same azide concentration. Higher azide concentrations further inhibited iodide uptake by producing a lesser stimulation or little effect on PTU and MMI accumulation. Similar results were obtained with cyanide, as shown in Fig. 6.

MMI, which is a thioureylene drug like PTU, is also a potent inhibitor of MPO activity. As presented in Fig. 7, MMI progressively inhibited PTU uptake and iodide accumulation in phagocytizing PMN. At a concentration of 50.0 nmoles/ml of MMI, PTU accumulation was decreased by 43.8 per cent, whereas 5.0 nmoles/ml decreased iodide accumulation by 90 per cent. It appears that iodide accumulation is more sensitive to MMI than PTU.

Effects of PTU, MMI azide and cyanide on [^{14}C]formate oxidation to $^{14}CO_2$. H_2O_2 availability in PMN was measured by the oxidation of [^{14}C]formate to $^{14}CO_2$ by catalase and H_2O_2 [18], and the results are shown in Fig. 8. [^{14}C]formate oxidation to $^{14}CO_2$ was markedly greater in phagocytizing cells and was further stimulated in the presence of PTU. At a PTU concentration of 5000 nmoles/ml, the increase in formate oxidation represented a 382 per cent ($P < 0.001$) rise in available H_2O_2 over that in control resting cells. MMI also increased H_2O_2 availability ($P < 0.01$) in the phagocytizing PMN at concentrations up to 25.0 nmoles/ml. At higher concentrations, H_2O_2 availability was decreased. Presently, there is no explanation for this biphasic effect. Similarly, azide and cyanide, which are potent inhibitors of MPO activity, also stimulate H_2O_2 availability in phagocytizing cells. In Fig. 9, both azide and cyanide at concentrations of 100 nmoles/ml and 1000 nmoles/ml, respectively, increased [^{14}C]formate oxidation in phagocytizing PMN 70–75 per cent ($P < 0.05$) above that in the controls.

DISCUSSION

Accumulation of TU and PTU in blood tissue was first suggested by Williams and Kay [1, 2] who presented data indicating accumulation of antithyroid drugs in thyroid, bone marrows and both red and white blood cells. More recently, Marchant *et al.* [6] reported the concentration of MMI in whole blood cells. In the present investigation, it was demonstrated that radioactivity was accumulated in both resting and phagocytizing PMN incubated with [^{14}C]PTU to levels of 2 and 12.6 times that in the incubating medium respectively (Table 1). Chromatographic analyses of the total accumulated radioactivity demonstrate that 22.3 per cent was unaltered PTU, 21.0 per cent was PTU metabolites and the remainder was bound to protein [14]. Further study into the possible role of iodide in the accumulation of PTU was carried out. Our data suggest that accumulation is independent of the iodide trapping mechanism. Small amounts of iodide, which stimulate PTU uptake in rat thyroids *in vivo* [8] and in isolated thyroid cells (R. H. Lindsay and B. S. Hulsey, unpublished data), appeared to have no effect on PTU accumulation in phagocytizing PMN (Fig. 2). However,

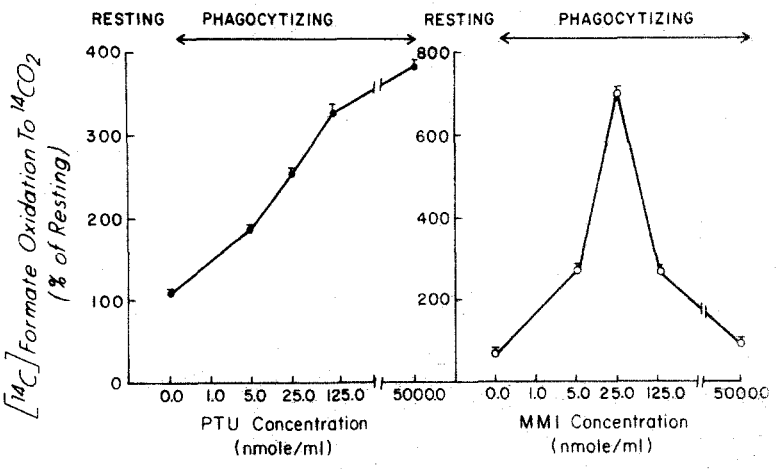

Fig. 8. Effects of various concentrations of PTU and MMI on oxidation of [^{14}C]formate to $^{14}CO_2$ in PMN. The incubation medium contained 4–10 × 10^6 PMN, 3 μmoles of sodium [^{14}C]formate (0.17 μCi), and 18,000 units of catalase in Hanks' phosphate, in a final volume of 1.0 ml. Left panel: formate oxidation produced by PTU (n = 5); right panel: formate oxidation produced by MMI (n = 4). See Fig. 1.

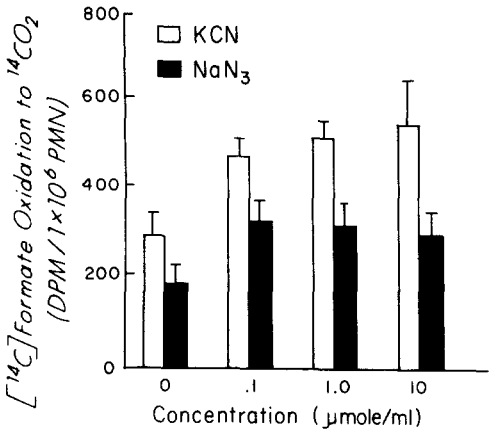

Fig. 9. Effects of various concentrations of potassium cyanide (KCN) and sodium azide (NaN_3) on [^{14}C]formate oxidation in phagocytizing PMN. Experimental conditions were identical to those described in the legend of Fig. 8. Open bars indicate formate oxidation produced by KCN (n = 3); solid bars indicate formate oxidation produced by NaH_3 (n = 4); and vertical bars are standard errors of the means.

high iodide concentrations inhibited PTU uptake in PMN, which is similar to the earlier reports of Marchant *et al.* [5] with normal rat thyroids, suggesting that large amounts of iodide either compete with PTU for the same uptake site or oxidative agent in PMN or inhibit MPO. Iodide has been shown to inhibit its own accumulation and organification in thyroid tissue [19, 20]. The demonstration that iodide stimulates PTU accumulation in thyroid tissue but does not have a similar effect in PMN suggest that the PTU accumulating mechanisms in PMN and in thyroid tissue are different.

Further evidence supporting the conclusion that the accumulation of iodide, as well as PTU, in PMN is different from that of the thyroid was obtained with perchlorate. Perchlorate, which competes with iodide for the thyroidal transport system and markedly inhibits iodide uptake (Refs. 11 and 16; R. H. Lindsay and B. S. Hulsey, unpublished data), has no effect on iodide or PTU accumulation in phagocytizing cells (Table 3). Although several investigators have suggested that the iodide accumulated in the PMN is through an active transport process [4, 21], this study is the first demonstration that iodide uptake in PMN is unaffected by perchlorate. This demonstrates that iodide uptake in PMN is different from that in the thyroid. Rall *et al.* [22] reported that perchlorate inhibited iodide uptake in all extrathyroidal tissues examined, suggesting that the iodide uptake system in PMN is not an active transport process. However, Stole [4] demonstrated that iodide accumulates in PMN to achieve C/M ratios of 2.7–4.2 when iodide organification was blocked by MMI. This provides the only direct evidence supporting the conclusion that iodide transport in PMN is an active process. If iodide accumulation in PMN is an active process, it is a relatively weak one since C/M ratios achieved with isolated thyroid cells under conditions identical to those above are ten times higher than those in PMN. It is quite possible that iodide becomes bound to protein in the PMN in the absence of MPO. Klebanoff [23] reported that some binding of iodide to *Escherichia coli in vitro* occurred in the presence of H_2O_2 and the absence of MPO. Our data show that H_2O_2 production increases during phagocytosis and that further increased in H_2O_2 availability occur in the presence of PTU or MMI (Fig. 8). Thus, the increased cell content of H_2O_2 could oxidize the iodide, resulting in binding to cellular protein in the PMN even with MPO completely inhibited. This would result in C/M ratios of iodine greater than 1.

It is apparent that the perchlorate studies (Table 3) suggest the absence of an iodide transport system in PMN. However, the effects of thiocyanate on iodide and PTU uptake are a little more complicated. The results (Fig. 3) suggest that iodide and thiocyanate are competitive substrates for an MPO-mediated peroxidative step, while PTU does not compete with either of them. Thiocyanate may also increase the efflux of iodide out of the PMN, as it does in the thyroid cells, before organification of iodide could occur, resulting in an inhibition of iodide uptake in these cells.

Although large amounts of iodide inhibit PTU uptake, the results obtained with perchlorate and thiocyanate, inhibitors of iodide transport, and with PTU, azide and cyanide, inhibitors of MPO and iodide organification, suggest that accumulation of PTU in PMN is partially independent of iodide metabolism. This observation is in marked contrast to that observed in thyroid tissue. It appears that PTU accumulation in the thyroid occurs as a consequence of its metabolism (oxidation) and that PTU metabolism occurs primarily by reaction with oxidized iodide which is produced by the action of TPO on H_2O_2 (Ref. 24; R. H. Lindsay, unpublished data).

Several hypotheses have been proposed regarding the actual mechanism involved in the metabolism of PTU. Morris and Hager [25] observed that chloroperoxidase, a mold enzyme, catalyzed the oxidation of TU only in the presence of iodide (or other halogens) and concluded that tyrosine and TU are competitive substrates for a common reactant. On this basis, they proposed that the intermediary formation of a peroxidase–iodinium complex could act either as an iodinating agent toward tyrosine or as an oxidizing agent toward TU. Similarly, Maloof and Soodak [26] observed that thiourea is oxidized by crude TPO only in the presence of iodide.

Recently, Taurog and Riesco [9] reported that bind-

ing of PTU to protein *in vitro* occurred only when TPO and iodide were present. In a later report, Taurog [24] observed that, under conditions favoring the formation of oxidized iodide, extensive drug oxidation and protein binding occurred *in vitro*. No oxidation or protein binding of the drug was reported in the absence of iodide. These results were confirmed in a recent detailed study by Davidson *et al.* [27]. These authors demonstrated that oxidation of MMI occurred only when iodide was present in a mixture of TPO, MMI and H_2O_2. These results strongly suggest that the oxidized iodide formed in the thyroid oxidizes the accumulated PTU, resulting in further drug uptake into the gland (R. H. Lindsay, personal communication). Therefore, in the thyroid, oxidized iodide is the oxidizing species responsible for the oxidation and subsequent accumulation of PTU.

In contrast to the conclusion that oxidized iodide is responsible for the oxidation of PTU in the thyroid, oxidation of PTU in PMN appears to be unrelated to iodide. The oxidizing agent in PMN appears to be H_2O_2 or related oxidizing agents. Our data show that the availability of H_2O_2 increases in the PMN in the presence of PMO inhibitors (Figs. 8 and 9). Under these conditions, PTU accumulation also increases in the PMN. Generally, production of H_2O_2 implies that superoxide is also generated. Furthermore, increased accumulation of H_2O_2 and probably superoxide may lead to the formation of hydroxyl radicals. Since these substances, H_2O_2, superoxide and hydroxyl radical, are strong oxidants [28], the accumulation of PTU in PMN may be due to oxidation of PTU in these cells by the aforementioned oxidants. Analyses of the accumulated radioactivity in PMN revealed primarily PTU metabolites and unaltered PTU [13, 14]. Oxidation of sulfhydryl compounds by H_2O_2 (Ref. 29; R. H. Lindsay, unpublished data) and by superoxide has been demonstrated.

The decrease in PTU accumulation with increasing MMI concentrations (Fig. 7) is consistent with reports observed with thyroid fractions. Jirousek and Cunningham [30] reported a decrease in [^{14}C]TU uptake in thyroid microsomes pretreated with thiols and thioureylenes. Similarly, Marchant *et al.* [31] observed a decrease in [^{35}S]MMI oxidation and binding to thyroidal protein in the presence of TU and suggested that TU and MMI compete for the same metabolizing or oxidative pathway. Since PTU and MMI possess a similar thiourea moiety in their structures, the inhibition of PTU uptake in the presence of MMI (Fig. 7) may be due to MMI competing with PTU for the same oxidizing agent, H_2O_2 in PMN.

In summary, we have demonstrated that phagocytizing PMN accumulate [^{14}C]PTU and [^{14}C]MMI. The accumulation is found to be independent of iodide transport and of iodide metabolism except for large amounts of iodide, which is in contrast to that found in the thyroid. In addition, PTU accumulation is accompanied by an increase in H_2O_2 availability in phagocytizing PMN, suggesting that PTU accumulation is related to its metabolism (oxidation). Since the collective mass of leukocytes is several-fold greater than the thyroid, these findings suggest that the leukocytes would certainly constitute another important site for the metabolism and disposition of PTU as well as other drugs. Furthermore, these cells may also represent an important site for these drugs to exert their biological effects.

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