Leukotriene B$_4$ (LTB$_4$), formed by the 5-lipoxygenase pathway in human polymorphonuclear leukocytes (PMN), may be an important mediator of inflammation (5). Recent studies suggest that human leukocytes can convert LTB$_4$ to products that are less biologically active. To examine the catabolism of LTB$_4$, we developed (using high performance liquid chromatography) a sensitive, reproducible assay for this mediator and some of its $\omega$-oxidation products (20-OH- and 20-COOH-LTB$_4$). With this assay, we have found that human PMN, but not human monocytes, lymphocytes, or platelets, convert exogenous LTB$_4$ almost exclusively to 20-OH- and 20-COOH-LTB$_4$ (identified by gas chromatography-mass spectrometry). Catabolism of exogenous LTB$_4$ by $\omega$-oxidation is rapid ($t_{1/2}$ approximately 4 min at 37°C in reaction mixtures containing 1.0 pmol LTB$_4$ and 20 $\times$ 10$^6$ PMN/ml), temperature-dependent (negligible at 0°C), and varies with cell number as well as with initial substrate concentration. The pathway for $\omega$-oxidation in PMN is specific for LTB$_4$ and 5,12-diHETE. Even PMN that are stimulated by phorbol myristate acetate to produce large amounts of superoxide anion radicals catabolize exogenous leukotriene B$_4$ primarily by $\omega$-oxidation. Finally, LTB$_4$ that is generated when PMN are stimulated with the calcium ionophore, A23187, is rapidly catabolized by $\omega$-oxidation. Thus, human PMN not only generate and respond to LTB$_4$, but also rapidly and specifically catabolize this mediator by $\omega$-oxidation.

Human PMN can convert arachidonic acid to several biologically active products (1, 2). One of these products, leukotriene B$_4$, formed by the 5-lipoxygenase pathway, has received a great deal of attention recently because of the role that it may play as a mediator of inflammation (3). In vitro, LTB$_4$ acts at nanomolar concentrations to provoke directed migration (chemotaxis) as well as enhanced random migration (chemokinesis) of human PMN (4–6). At higher concentrations, LTB$_4$ provokes selective release of granule-associated (lysosomal) enzymes from cytolysin B-treated PMN (4, 7) and causes these cells to aggregate (5, 8, 9). In vivo, LTB$_4$ induces PMN exudation in human skin (10), adherence of PMN to the walls of postcapillary venules in the hamster cheek pouch (11), and PMN-dependent increases in vascular permeability in rabbit skin (12).

Evidence has appeared recently suggesting that LTB$_4$ can be converted by mixed human leukocytes to products that are less biologically active (13–16). One pathway by which LTB$_4$ can be catabolized appears to involve $\omega$-oxidation and yields 20-OH-LTB$_4$ and 20-COOH-LTB$_4$ (13). Both 20-OH-LTB$_4$ and 20-COOH-LTB$_4$ are formed when LTB$_4$ is incubated with PMN-enriched leukocyte suspensions (13), and 20-COOH-LTB$_4$ is the major product of the 5-lipoxygenase pathway that is generated when human PMN are stimulated with the synthetic chemotactic peptide, N-formylmethionylleucylphenylalanine (15). Despite these observations, it has not been determined whether $\omega$-oxidation is quantitatively the major pathway by which human leukocytes catabolize LTB$_4$. It also has not been determined whether this pathway is active in leukocytes other than PMN, or whether it is specific for LTB$_4$. Finally, it is unclear whether the activity of the enzyme(s) responsible for $\omega$-oxidation is sufficient to catabolize all of the LTB$_4$ that is generated when PMN are stimulated with either the calcium ionophore, A23187, or other agents.

Using HPLC, we have developed a sensitive, reproducible assay which permits quantification of LTB$_4$ and its $\omega$-oxidation products. With this assay, we have found that human PMN (but not human monocytes, lymphocytes, or platelets) convert exogenous LTB$_4$ almost exclusively to 20-OH- and 20-COOH-LTB$_4$. We also found that the pathway for $\omega$-oxidation in PMN is specific for LTB$_4$ and 5,12-diHETE. Finally we found that PMN stimulated by A23187 rapidly catabolize LTB$_4$ by $\omega$-oxidation.

**EXPERIMENTAL PROCEDURES**

Materials—Unlabeled arachidonic acid and [1H]arachidonic acid (87.4 Ci/mmol) were obtained from NuChek Prep and New England.

Portions of this paper (including part of "Experimental Procedures," part of "Results," and Figs. 1–4) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-0294, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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Nuclear, respectively. Synthetic LTB4, 20-0H-LTB4, 20-COOH-LTB4, and leukotriene A4 methyl ester were generously provided by Dr. J. Rokach (Merck Frosst). Stock solutions of LTB4, 20-0H-LTB4, and 20-COOH-LTB4 were stored in methanol under nitrogen at −70 °C. Concentrations of the leukotrienes were determined by ultraviolet absorption (molar extinction coefficient ε = 30,000) (13). Leukotriene A4 methyl ester was hydrolyzed to generate all-trans-conjugated isomers of LTB4, (6-trans-LTB4, and 12-epi-6-trans-LTB4), which were purified by HPLC as described previously (18). Soybean lipoygenase (EC 1.13.11.12) (Sigma) was used to generate 5,12-dihETE and 6,15-dihETE from arachidonic acid (18). Two individual cartridges were eluted sequentially with 5.0 ml of 10% acetonitrile. The 8.1- and 8.9-min peaks were observed after 5 min of incubation. As the duration of incubation increased, the peak with a retention time of 8.9 min diminished in area, while the peak with a retention time of 8.1 min increased in area. The peaks at 8.1 and 8.9 min were not products of autoxidation, since they were not observed when LTB4 was incubated for periods up to 24 h in the absence of PMN.

The products that eluted with retention times of 8.1 and 8.9 min were identified in three ways as 20-COOH-LTB4 and 20-OH-LTB4, respectively. First, the material in the 8.1- and 8.9-min regions gave a positive color reaction with either 5% potassium ferricyanide in 0.1 N NaOH or 2% aqueous potassium permanganate, and contained the expected UV absorption patterns. Second, a gas chromatograph-mass spectrometer (GC-MS) analysis of the material in the 8.1- and 8.9-min regions showed that the ions at m/z 436 and 454 corresponded to the molecular ion and molecular ion plus one mass unit, respectively, of the authentic standards of 20-COOH-LTB4 and 20-OH-LTB4, respectively. Third, the GC retention times of the compounds in the 8.1- and 8.9-min regions were identical to those of authentic 20-COOH-LTB4 and 20-OH-LTB4 standards. In later experiments, the amount of material eluting from the column at 8.9 min was smaller than that eluting at 8.1 min, and it was not possible to obtain sufficient quantities of the material eluting at 8.9 min to allow a full structure determination. However, in two experiments, the mass spectra of the material eluting from the column at 8.9 min showed a peak at m/z 437 corresponding to the molecular ion of 20-OH-LTB4.
8.9-min peaks co-chromatographed on reverse phase HPLC with synthetic 20-COOH-LTB₄ and 20-OH-LTB₄. Second, when methylated, synthetic 20-COOH-LTB₄ and 20-OH-LTB₄ were chromatographed using both HPLC systems. Finally, the products in the 8.1- and 8.9-min peaks were identified as 20-COOH-LTB₄ and 20-OH-LTB₄ by gas chromatography-mass spectrometry (see Miniprint).

**Time Course of the Catabolism of Exogenous LTB₄ by Human PMN**—Synthetic LTB₄ (1.0 μM) was incubated with PMN (20 x 10⁶/ml) at 37 °C for 0–60 min (Fig. 2). Under these conditions, the disappearance of LTB₄ was rapid (tₚ₀ of approximately 4 min). The accumulation of 20-OH-LTB₄ and 20-COOH-LTB₄ was rapid, particularly during the first 5 min of incubation. After 5 min of incubation, the concentration of 20-OH-LTB₄ exceeded the concentrations of both LTB₄ and 20-COOH-LTB₄. After 10 min of incubation, however, the concentration of 20-COOH-LTB₄ increased further, and the concentrations of 20-OH-LTB₄ and LTB₄ decreased proportionately. Whereas greater than 90% of the LTB₄ added initially could be accounted for during the first 10 min of incubation, less could be accounted for after longer periods of incubation (e.g. 75% at 60 min).

Data from experiments using trace amounts of radiolabeled LTB₄ confirmed that ω-oxidation is the major pathway by which PMN catabolize this compound (Table I). After 5 min of incubation, 76.5% of the radioactivity added initially as [³H]LTB₄ was associated with 20-OH- and 20-COOH-LTB₄. In addition, the specific activity of each of the ω-oxidation products was identical to that of the [³H]LTB₄ added initially. Thus, the ω-oxidation products detected in these experiments were derived solely from the exogenously added LTB₄, and were not formed from newly synthesized LTB₄. The cell pellets contained very little radioactivity. This finding was confirmed in several experiments, in which no greater than 4.0% of the radioactivity added initially was found to be cell-associated. This result was not an artifact caused by the addition of ethanol to terminate the incubations. Similar results were obtained when the incubations were terminated by rapid centrifugation (radioactivity in cell pellets = 3.8 ± 1.2%, n = 6).

**Dependence of LTB₄ Catabolism by Human PMN on Substrate Concentration**—Increasing amounts of ω-oxidation products were generated by PMN when the cells were exposed to increasing concentrations of exogenous LTB₄ (up to 16.5 μM) (not shown). Although a double reciprocal plot of these data yielded a complex curve, a linear relationship (r = 0.999) was found between rate and substrate concentrations between 0.5 and 16.5 μM LTB₄.

**Comparison of PMN with Other Cell Types for the Ability to Catabolize Exogenous LTB₄**—Suspensions of human PMN prepared by Hypaque-Ficoll centrifugation and dextran sedimentation are contaminated with small numbers of mononuclear leukocytes and larger numbers of platelets. To deter-
mine whether these other blood cell types generate ω-oxidation products from LTB₄, paired experiments were performed using PMN and mononuclear leukocytes from the same donor (Table II). Suspensions of mononuclear leukocytes containing large numbers of platelets converted only small amounts of LTB₄ to 20-OH- and 20-COOH-LTB₄ (very likely attributable to contaminating PMN). Approximately 12-fold greater amounts of ω-oxidation products were generated after 15 min in PMN-enriched suspensions. Similar amounts of ω-oxidation products were observed when PMN were incubated with LTB₄ in the presence of mononuclear leukocytes and platelets (data not shown).

Although small amounts of ω-oxidation products were detected after incubation of LTB₄ with mononuclear leukocytes and platelets, only 34% of the LTB₄ added initially to these cells was recovered intact after 60 min. To determine whether this decrease could be explained by further catabolism of the ω-oxidation products by mononuclear leukocytes and/or platelets, experiments were performed in which these cells were compared with PMN for their ability to degrade 20-OH-LTB₄ and 20-COOH-LTB₄. After 15 and 60 min of incubation at 37 °C, recoveries of the ω-oxidation products in suspensions of PMN (96 and 91%) were nearly identical with recoveries in suspensions of mononuclear leukocytes and platelets (98 and 98%). Preliminary studies in which radiolabeled LTB₄ was incubated with mononuclear leukocytes and platelets for 60 min revealed only small amounts (<10%) of radioactivity in cell pellets but substantial amounts of radioactivity associated with very polar products in the supernatants. It appears, therefore, that mononuclear leukocytes and/or platelets can catabolize LTB₄ by one or more pathways other than ω-oxidation (albeit less efficiently than PMN).

Specificity of ω-Oxidation by Human PMN—The specificity of ω-oxidation was examined directly by incubating various dihydroxylated derivatives of arachidonic acid with human PMN (Table III). Whereas PMN converted large amounts of LTB₄ and 5,12-diHETE to ω-oxidation products, there was no catabolism by these cells of the 15-lipoxygenase products, 8,15-diHETE and 5,15-diHETE. The two all-trans-conjugated isomers of LTB₄ underwent minimal ω-oxidation.

Catabolism of Exogenous LTB₄ by Human PMN Stimulated with PMA—To determine whether stimulation of PMN oxidative metabolism influences the catabolism of LTB₄, we compared "resting" PMN with PMN stimulated by PMA for their ability to convert exogenous LTB₄ to 20-OH- and 20-COOH-LTB₄ (Table IV). PMA (500 ng/ml) did not stimulate PMN to produce either LTB₄ or its ω-oxidation products, but did cause these cells to generate superoxide anion radicals (20–30 nmol of cytochrome c reduced per 10⁶ PMN/5 min) (measured as superoxide dismutase-inhibitable ferricytochrome c reduction) (23). Nevertheless, like resting PMN, PMA-stimulated PMN converted exogenous LTB₄ primarily to 20-OH- and 20-COOH-LTB₄. Interestingly, PMN stimulated by PMA converted less LTB₄ to its ω-oxidation products and less 20-OH-LTB₄ to 20-COOH-LTB₄ than did resting PMN. This was observed in reaction mixtures incubated for 5–20 min (data not shown). Thus, even in PMN stimulated to produce abundant amounts of superoxide, ω-oxidation is the major pathway for LTB₄ catabolism.

Catabolism of exogenous LTB₄ by PMN stimulated with PMA

<table>
<thead>
<tr>
<th>PMA</th>
<th>20-COOH-LTB₄</th>
<th>20-OH-LTB₄</th>
<th>LTB₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37 ± 9</td>
<td>32 ± 13</td>
<td>15 ± 9</td>
</tr>
<tr>
<td>500</td>
<td>9 ± 4</td>
<td>26 ± 2</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>*</td>
<td>p &lt; 0.005</td>
<td>p &gt; 0.2</td>
<td>p &lt; 0.005*</td>
</tr>
</tbody>
</table>

* A paired Student's t test was used to compare results obtained in the presence and absence of PMA.

TABLE III

<table>
<thead>
<tr>
<th>ω-Oxidation products</th>
<th>% of initial LTB₄</th>
<th>LTB₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>83 ± 12</td>
<td>5,12-diHETE</td>
</tr>
<tr>
<td></td>
<td>63 ± 7</td>
<td>6-trans-LTB₄</td>
</tr>
<tr>
<td></td>
<td>11 ± 4</td>
<td>12-epi-6-tran.s-LTB₄</td>
</tr>
<tr>
<td></td>
<td>8 ± 1</td>
<td>8,15-diHETE</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5,15-diHETE</td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>LTBI (0.1 μM)</th>
<th>PMN-enriched suspension*</th>
<th>15-min incubation</th>
<th>60-min incubation</th>
<th>Mononuclear leukocyte/platelet-enriched suspension*</th>
<th>15-min incubation</th>
<th>60-min incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-min incubation</td>
<td>7 ± 5</td>
<td>81 ± 8</td>
<td>70 ± 9</td>
<td>7 ± 5</td>
<td>7 ± 5</td>
</tr>
<tr>
<td></td>
<td>60-min incubation</td>
<td>0</td>
<td>79 ± 9</td>
<td>7 ± 5</td>
<td>0</td>
<td>7 ± 5</td>
</tr>
<tr>
<td></td>
<td>Mononuclear leukocyte/platelet-enriched suspension*</td>
<td>71 ± 9</td>
<td>7 ± 5</td>
<td>60-min incubation</td>
<td>34 ± 4</td>
<td>12 ± 7</td>
</tr>
</tbody>
</table>

* PMN-enriched suspensions contained (per ml) 20 × 10⁶ PMN, 6–8 × 10⁶ eosinophils, <1 × 10⁶ mononuclear leukocytes, and 20–80 × 10⁶ platelets.

The two all-trans-conjugated isomers of LTB₄ were detected after 30 min of incubation. In contrast, large amounts of polar products that eluted with retention times of 8.1 and 8.9 min accumulated in the supernatants. These retention times were identical to those of synthetic 20-COOH- and 20-OH-LTB₄. Furthermore, when subjected to methylation and normal phase HPLC, the material in the two major peaks co-chromatographed with the methyl esters of synthetic 20-OH- and 20-COOH-LTB₄ (not shown). Two of the minor peaks detected after normal phase HPLC were identified as ω-oxidation products of 5,12-diHETE. In Fig. 3, 20-OH-5,12-diHETE appears as the peak eluting with a retention time of 9.4 min. Negligible quantities of 20-COOH-5,12-diHETE were produced by PMN under the conditions used in these experiments.

Although there was some variability from experiment to
Catabolism of Leukotriene B₄

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FIG. 3. HPLC analysis of the products generated by human PMN stimulated with the calcium ionophore A23187. Shown are representative reverse phase HPLC chromatograms which were obtained after incubating PMN (20 × 10⁶ cells/ml) with 10 μM A23187 at 37 °C for 2 (A), 5 (B), 15 (C), and 30 min (D). Peaks represent 20-COOH-LTB₄ (a), 20-OH-LTB₄ (b), 20-OH-5,12-diHETE (c), PGB₂ (internal standard) (d), 6-trans-LTB₄ (e), 12-epi-6-trans-LTB₄ (f), LTB₄ (g), and 5,12-diHETE (h).

DISCUSSION

Results of the studies described in this report indicate that ω-oxidation is the major pathway by which human PMN catabolize exogenous LTB₄. Using a sensitive and reproducible HPLC assay, we have confirmed the findings of others (13) that exogenous LTB₄ is converted to 20-OH- and 20-COOH-LTB₄. Concentrations of LTBl were almost entirely recovered in reaction mixtures containing leukocytes after 15 min of incubation at 37 °C. In contrast, greater than 97% of LTB₄ was recovered intact after incubation for 15 min in buffer alone. Coincident with the disappearance of LTB₄ in reaction mixtures containing leukocytes was the appearance of two more polar products (Fig. 1). We identified these products as 20-OH-LTB₄ and 20-COOH-LTB₄, by co-chromatography with synthetic standards. After 15 min of incubation, 90% of the LTB₄ that disappeared from reaction mixtures containing human leukocytes was recovered as 20-OH- and 20-COOH-LTB₄ (Fig. 2). Results of experiments using trace amounts of [³H]LTBl indicated that this com-

experiment, we consistently found that PMN incubated with A23187 (0.1-20 μM) for 60 min generated much larger amounts of 20-OH- and 20-COOH-LTB₄ than of LTB₄ itself. Results of representative experiments demonstrating the time course of LTB₄ generation and ω-oxidation by human PMN stimulated by A23187 (± arachidonic acid) are shown in Fig. 4. After 60 min of incubation, much larger amounts of 20-OH- and 20-COOH-LTB₄ than of LTB₄ were detected in the medium surrounding PMN stimulated by 10 μM A23187 alone. In fact, only at the earliest time point sampled (i.e. 2 min) was more LTB₄ detected than the total of its ω-oxidation products. Concentrations of LTB₄ reached a maximum after 5 min of incubation, and then declined. In contrast, concentrations of the ω-oxidation products continued to increase. Qualitatively similar results were observed when PMN were incubated with 10 μM A23187 plus 30 μM arachidonic acid. Although larger amounts of LTB₄ than of ω-oxidation products were detected at the earlier time points, by 60 min, the reaction mixtures contained greater than 40-fold more ω-oxidation products than LTB₄. Finally, when PMN were stimulated by a low concentration of A23187 (0.5 μM) without exogenous arachidonic acid, only negligible amounts of LTB₄ were detected after 10 min of incubation (0.15 pmol/10⁶ PMN). In contrast, much larger amounts of 20-OH- and 20-COOH-LTB₄ accumulated in the reaction mixtures gradually over 60 min. Thus, almost all of the LTB₄ generated by PMN in response to stimulation with A23187 can be catabolized by ω-oxidation.

FIG. 4. Time course of the generation of LTB₄ and its ω-oxidation products by stimulated PMN. Shown are results of representative experiments in which PMN (20 × 10⁶ cells/ml) were incubated at 37 °C with 10 μM A23187 (A), 10 μM A23187 + 30 μM arachidonic acid (B), and 0.5 μM A23187 (C). Aliquots were taken at the indicated time points and the quantities of LTB₄ (○) and total ω-oxidation products (20-OH-LTB₄ + 20-COOH-LTB₄) (●) were determined.
pound was converted almost exclusively by human leukocytes to \[^{[H]}\text{20-OH- and }[^{[H]}\text{20-COOH-LTB}_{4}\text{. (Table I).}

Conversion of exogenous \(\text{LTB}_{4}\) to its \(\omega\)-oxidation products in suspensions of human leukocytes (containing primarily \(\text{PMN}\)) was rapid (\(t\_\text{a} \approx 4\) min in reaction mixtures containing \(1.0 \times 10^6 \text{PMN/ml\text{,}} \times 20 \times 10^6 \text{PMN/m}\text{l, temperature-}

dependent (negligible at \(0^\circ\text{C}\)), and varied with cell number as well as with substrate concentration.

Even highly purified \(\text{PMN}\) suspensions prepared, as ours were, by combining Hypaque-Ficol centrifugation with dextran sedimentation are contaminated with small numbers of mononuclear leukocytes and larger numbers of platelets. Consequently, to determine which cell type was responsible for \(\omega\)-oxidation, we examined the ability of \(\text{PMN versus mononuclear}

leukocytes and platelets from the same donor to catabolize \(\text{LTB}_{4}\), Table II). In contrast to suspensions of \(\text{PMN, suspensions of monocytes, lymphocytes,}

and very large numbers of platelets converted only small amounts of \(\text{LTB}_{4}\) to \(20\)-OH- and \(20\)-COOH-\(\text{LTB}_{4}\). Although none of the cell types that we examined converted \(20\)-OH- and \(20\)-COOH-\(\text{LTB}_{4}\) very rapidly to other compounds, suspensions of mononuclear leukocytes and platelets did convert significant amounts of \(\text{LTB}_{4}\) to unidentified polar products. Nevertheless, it appears that among human peripheral blood cells, only \(\text{PMN rapidly and efficiently catabolize }\text{LTB}_{4}\) by \(\omega\)-oxidation.

The pathway of \(\omega\)-oxidation in \(\text{PMN}\) is quite specific. Of the various dihydroxylated derivatives of arachidonic acid that we tested, only \(\text{LTB}_{4}\) and \(5,12\)-diHETE were catabolized by \(\text{PMN to }\omega\)-oxidation products to any significant extent (Table III). In contrast, we observed no catabolism by \(\text{PMN of the 15}\)-lipoxigenase products, \(8,15\)-diHETE and \(5,15\)-diHETE, and only minimal \(\omega\)-oxidation of the two all-trans-conjugated isomers of \(\text{LTB}_{4}\).

Evidence has appeared recently suggesting that leukotrienes can be catabolized by human \(\text{PMN}\) as a consequence of the generation by these cells of oxygen-derived free radicals and \(\text{H}_2\text{O}_2\) (24–26). Henderson and Klebanoff (26), for example, found that more \(\text{LTB}_{4}\) and leukotriene \(\text{C}_{4}\) were recovered from supernatants of chronic granulomatous disease \(\text{PMN}\) (which are unable to generate superoxide anion radicals and \(\text{H}_2\text{O}_2\) (27) than from supernatants of normal \(\text{PMN}\) after stimulation by \(\text{A23187}\). Results of our studies suggest that 20-superoxide anion radicals produced primarily by \(\text{PMN stimulated by the synthetic chemotactic peptide }N\)-formylmethionylleucylphenylalanine, produced primarily small amounts of \(20\)-COOH\(-\text{LTB}_{4}\). They suggested that \(N\)-formylmethionylleucylphenylalanine “more specifically” stimulates the biosynthesis of \(20\)-COOH\(-\text{LTB}_{4}\) by \(\text{PMN}\) than the biosynthesis of \(\text{LTB}_{4}\). Results of our studies, however, suggest that it is more likely that \(N\)-formylmethionyl-leucylphenylalanine stimulates \(\text{LTB}_{4}\) synthesis in human \(\text{PMN only weakly (resembling }0.5 \mu\text{M A23187), and that the small amount of }\text{LTB}_{4}\text{ produced by the cells is converted rapidly and almost completely to its }\omega\text{-oxidation products.}

\(\text{PMN are the most prominent cell type observed at sites of acute inflammation. The precise role that }\text{LTB}_{4}\text{ plays in provoking inflammation has not been determined. Results of our studies indicate that human }\text{PMN not only generate and respond to }\text{LTB}_{4}\text{, but also are capable of catabolizing this mediator. They do so rapidly, specifically, and primarily by }\omega\text{-oxidation. Although generation of }\text{LTB}_{4}\text{ by }\text{PMN might promote inflammation, catabolism of }\text{LTB}_{4}\text{ by }\text{PMN may be a mechanism whereby inflammatory reactions are modulated.}

Acknowledgment—We wish to thank Dr. Joshua Rockach (Merck Frost) for generously supplying us with synthetic leukotrienes.

REFERENCES

Catabolism of Leukotriene B4

Biol. Chem. 257, 6106-6110

Supplementary Material to "Catabolism of Leukotriene B4"

EXPERIMENTAL PROCEDURE

Gas chromatography-mass spectrometry (GC-MS) — In order to generate sufficient material for analysis, GC-MS, 50 mg of synthetic LTB4, was dissolved in 5 ml of acetonitrile, then diluted with 15 ml 1% acetone in 15 ml for 15 min at 37°C. Following extraction, GC-MS chromatography, reverse-phase HPLC, methylation, and normal-phase HPLC, the products were derivatized and subjected to GC-MS. Methylation was conducted with 30 ml of N,N-dimethylformamide/trimethylchlorosilane(1:1), with 0.05% trimethylchlorosilane (1% acetone in acetonitrile). The extraction was then performed, and the derivatized products were subjected to GC-MS. The results of these analyses are shown in Table 1.

Initial analysis of the methyl ester, trimethylsilyl derivatives was performed using a Hewlett-Packard 5890A gas chromatograph and flame ionization detector. The column was 1.88 m (30 mm i.d.) of Porapak Q. The temperature was held at 250°C, and the detector temperature was 300°C. The results of these analyses are shown in Table 2.

RESULTS

Gas chromatography of the trimethylsilyl ether, methyl ester derivatives of the material that eluted after reverse-phase HPLC with a retention time of 8.9 min showed a single peak with a corresponding C-value of 36.7 (3.08 SN:250:6). The mass spectrum (Fig. 1) contained a single ion of high intensity at m/z 481 (M+), loss of trimethylsilyl, 465 (M-31), and 441 (M-57) ions. In addition, ions of lower mass were also detected which were identical to those that we and others have observed in the mass spectrum of LTB4. These included m/z 353 (199, loss of 17H,20H-lysinoeicosatetraenoic acid), 337, 323, 299 (M-31), 285, 261, and 237 (probably dihydroxyeicosatetraenoic), from a rearrangement, 301 (M-19), 299, 285, 245, 225, and 211 ions are the mass spectral ions that are observed in the mass spectrum of LTB4. The ions at m/z 353 (199, loss of 17H,20H-lysinoeicosatetraenoic acid), 337, 323, 299 (M-31), 285, 261, and 237 (probably dihydroxyeicosatetraenoic), from a rearrangement, 301 (M-19), 299, 285, 245, 225, and 211 ions are the mass spectral ions that are observed in the mass spectrum of LTB4. These mass spectra agree with those published previously for 20-OH-ETE and 18-OH-ETE.

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Fig. 1. Mass spectrum of the material that eluted with a retention time of 8.9 min. Trimethylsilyl ether, trimethylsilyl ether derivative. The dashed lines show the major fragmentation pathways.