The major dihydroxy metabolites of arachidonic acid formed by human polymorphonuclear leukocytes (PMNL) are leukotriene B₄ (LTB₄), 6-trans-LTB₄, and 12-epi-6-trans-LTB₄, and 5,12-dihydroxy-7,9,11,14-eicosatetraenoic acid (1). LTB₄ is an important mediator of neutrophil function. It is a potent chemotactic agent for these cells (2,3) and stimulates aggregation (2) and degranulation (4,5). When administered in vivo, LTB₄ promotes margination of leukocytes (6) and their accumulation at the site of injection (7).

Leukotriene B₄ is rapidly metabolized by a hydroxylase in PMNL to 20-hydroxy-LTB₄ (8-12). This enzyme is localized in the microsomal fraction of PMNL and is a cytochrome P-450 (13). It is barely detectable in mononuclear cells (9). Although LTB₄, 20-hydroxylase also metabolizes other dihydroxy isomers of LTB₄, these compounds are not metabolized as rapidly as LTB₄ itself (9). The Kₘ for LTB₄ is about 1 μM, whereas those for 12-epi-6-trans-8-cis-LTB₄, which is formed by the combined actions of 5-lipoxygenase and 12-lipoxygenase and 6-trans-LTB₄, are 2.4 and 4.8 μM, respectively (9).

The other 6-trans isomer of LTB₄, 12-epi-6-trans-LTB₄, is metabolized even more slowly by this enzyme (9). The 20-hydroxy metabolite of LTB₄ is further metabolized to ω-carboxy LTB₄ by PMNL (14-16).

Metabolism of LTB₄ to 20-hydroxy-LTB₄ appears to result in considerable losses of both chemotactic (15) and proaggregatory (17,18) activities with respect to PMNL. The 20-hydroxy metabolite is also much less active than LTB₄ in causing degranulation of PMNL (5,18). However, there are conflicting reports suggesting that 20-hydroxy-LTB₄ is as active as LTB₄, as a chemotactic agent (19) and that it has about the same affinity for LTB₄ binding sites in human PMNL as LTB₄ itself (20). In the guinea pig lung, 20-hydroxy-LTB₄ clearly appears to be at least as active as LTB₄ in contracting parenchymal strips (14,18). However, the mechanism of action of LTB₄ on guinea pig lung differs from that on PMNL in that it is mediated by release of thromboxane A₂ (21). ω-Carboxy-LTB₄ is much less potent than its 20-hydroxy precursor (5,18,22).

We have recently observed a second pathway for the metabolism of dihydroxyeicosanoids related to LTB₄, involving reduction of one of the three conjugated double bonds (23). We found that human PMNL convert 6-trans-LTB₄ to a 20-hydroxy product which has a single λₑₐₓ at 231 nm in its UV spectrum, indicating that there are only two conjugated double bonds between the 5- and 12-hydroxy groups (23). This product is presumably formed by the combined actions of LTB₄, 20-hydroxylase and a reductase.

In order to investigate the reductase reaction in more detail, it would be desirable to use a substrate such as 12-epi-6-trans-LTB₄, which is not readily metabolized by the ω-oxidation pathway (9). We have now shown that this isomer of LTB₄, is metabolized primarily to a dihydro product by human PMNL.

We have investigated the mechanism of formation of this
product and have examined some of the characteristics of the reductase pathway.

**MATERIALS AND METHODS**

**Preparation of Substrates**—Unlabeled 6-trans-LTB₄ and 12-epi-6-trans-LTB₄ were prepared by incubation of arachidonic acid (NaChek Prep, Inc.) and the divalent cation ionophore, A23187 (10 μM, Behring Diagnostics), with porcine PMNL, which had been preincubated with 5,8,11,14-eicosatetraenoic acid (24) in order to inhibit 12-lipoxygenase (25). Under these conditions, the major dihydro products formed were LTB₄, and its two 6-trans isomers. The latter products were purified by reversed-phase high pressure liquid chromatography (RP-HPLC) on a 5 μm Ultrasphere octadecylsil (ODS) silica column with water/methanol/acetic acid (41:59:0.01) as the mobile phase. They were further purified by rechromatography with water/acetonitrile/acetic acid (40:60:0.01) as the mobile phase, which completely separated 12-epi-6-trans-LTB₄ from 5,15-dihydroxy-6,8,11,13-eicosatetraenoic acid, which was not entirely removed from the first chromatography. The purified products were quantitated by RP-HPLC with LTB₄, (kindly provided by Dr. J. Rokach, Merck-Frosst, Pointe Claire, Quebec) as an internal standard. 12-Epi-6-trans-[5,6,8,9,11,12,14,15-3H]LTB₄ was synthesized from 5,8,11,14-eicosatetraenoic acid as described above. Deuterium-labeled arachidonic acid was synthesized by reduction of 5,8,11,14-eicosatetraenoic acid (kindly supplied by Dr. J. R. Pauluard of the Hoffman LaRoche Company) with deuterium gas (99.5% pure, Merck Sharp and Dohme Canada Ltd., Pointe Claire, Quebec) in the presence of Lindlar’s catalyst as described in the literature (26). The deuterium-labeled product was purified by chromatography on Lipidex-5000 (Packard Instrument Co. Inc., Downer’s Grove, IL) using water/methanol/methylene chloride/acetic acid (150:700:1:001) as the mobile phase (27). Radioactively labeled products were synthesized by incubation of either [1,4C]arachidonic acid or [5,6,8,9,11,12,14,15-3H]arachidonic acid (Du Pont-New England Nuclear) with human PMNL (9). They were purified and quantitated as described above.

**Preparation of Human PMNL**—PMNL were prepared as described previously (9) by treatment of blood with Dextran T-500 (Pharmacia LKB Biotechnology Inc.) to remove red blood cells. Mononuclear cells were removed by centrifugation over Ficoll-Paque (Pharmacia LKB Biotechnology Inc.) to remove red blood cells. Mononuclear cells were harvested by treatment with 0.135 M NH₄Cl. After washing with 0.15 M NaCl, the cells were resuspended in Dulbecco’s phosphate-buffered saline, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, 0.5 mM MgCl₂, and 0.9 mM CaCl₂.

**Incubation Conditions**—Human PMNL (10⁶ cells/ml) were preincubated for 2 min at 37 °C and then incubated with the two 6-trans isomers of LTB₄, (0.4 μM for 30 min, unless otherwise indicated). For preparative experiments, the incubations were terminated by centrifugation at 4 °C at 400 g for 10 min. The pellet was then washed by centrifugation over Ficoll-Paque (Pharmacia LKB Biotechnology Inc.). Any red blood cells remaining in the pellet were lysed by treatment with 0.135 M NH₄Cl. After washing with 0.15 M NaCl, the cells were resuspended in Dulbecco’s phosphate-buffered saline, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, 0.5 mM MgCl₂, and 0.9 mM CaCl₂.

**Purification of Products from Preparative Experiments**—The metabolites formed when 6-trans-LTB₄, (0.4 μM) was incubated with human PMNL were analyzed by RP-HPLC (Fig. 1A). The major products were metabolite Ia (retention time (tₑ), 18.2 min), metabolite IIa (tₑ, 23.4 min), and metabolite IIIa (tₑ, 66.8 min). Each of these metabolites was converted to its methyl ester and further purified by RP-HPLC either before or after hydrogenation using water/methanol as the mobile phase. We previously identified metabolite Ia as 20-hydroxy-6-trans-LTB₄ (9), and metabolite IIa as a 5,12,20-trihydroxy product containing a conjugated diene structure (23). Metabolite IIIa is the only product with a retention time longer than that of 6-trans-LTB₄, suggesting that it is not the product of an ω-oxidation reaction. Its slightly longer retention time would be consistent with the presence of fewer double bonds than are present in 6-trans-LTB₄.

A similar pattern of products was observed after incubation of 12-epi-6-trans-LTB₄ with human PMNL (Fig. 1B). In this case, however, the major product was metabolite IIb (tₑ, 82.1 min), which had a retention time longer than that of the substrate and was therefore unlikely to be an ω-oxidation product. When rechromatographed using a mobile phase consisting of methanol/water/acetic acid (62:38:0.05) at a flow rate of 1.5 ml/min, metabolite IIb was observed as a single peak of radioactivity and UV absorbance at 235 nm, with a retention time of 36.5 min. We detected much smaller amounts of metabolites Ib (tₑ, 22 min) and IIb (tₑ, 27.5 min), which, by analogy with the metabolites of 6-trans-LTB₄, were probably ω-oxidation products. Metabolite Ib, which was a relatively minor product under the reaction conditions employed, was not homogeneous and appeared to consist of at least two components. Since these products absorb at 280 nm, they are unlikely to be dihydro metabolites, but may be ω-hydroxy and ω-carboxy metabolites of 12-epi-6-trans-LTB₄. Because of the small amounts of these products, they were not further characterized.

**UV Absorption Spectra**—Metabolite Ia, which was derived from 6-trans-LTB₄, has a UV spectrum typical of leukotrienes, with three absorption maxima at 259, 269, and 280 nm. Metabolite IIa, on the other hand, has only one absorption maximum, at 232 nm. Metabolite IIIa, the minor product formed from 6-trans-LTB₄, also has a single absorption maximum in its UV spectrum, at 231 nm. This suggests that IIIa is a dihydro metabolite of 6-trans-LTB₄, containing two con-
jugated double bonds between the 5- and 12-hydroxyl groups.

The major metabolite of 6-trans-LTB4 (metabolite IIb) has a UV spectrum similar to that of metabolite Ila, with an absorption maximum at 231 nm (Fig. 2). Metabolite IIb has a similar UV spectrum, with a maximum at 232 nm. These results suggest that both metabolites IIb and IIIb have two conjugated double bonds and are formed by the reduction of the triene chromophore of 12-epi-6-trans-LTB4.

Gas Chromatography-Mass Spectrometry—The two major metabolites of 6-trans-LTB4 (metabolites Ia and IIa) were tentatively identified on the bases of both their UV and mass spectra (9, 23). However, the number and positions of the double bonds in the 20-hydroxy metabolite of 6-trans-LTB4 exhibiting maximal UV absorbance at 232 nm could not be determined, because it had been hydrogenated prior to analysis by GC-MS. After hydrogenation, the methyl ester, trimethylsilyl ether derivatives of metabolites Ia and IIa, had identical retention times and mass spectra (9, 23). However, the number and positions of the double bonds in the 20-hydroxy metabolite of 6-trans-LTB4 exhibiting maximal UV absorbance at 232 nm could not be determined, because it had been hydrogenated prior to analysis by GC-MS. After hydrogenation, the methyl ester, trimethylsilyl ether derivatives of metabolites Ia and IIa, had identical retention times and mass spectra (9, 23).

The methyl ester, trimethylsilyl ether derivative of the major product (metabolite IIb) formed from 12-epi-6-trans-LTB4 has a mass spectrum (Fig. 3A) with fragment ions at m/z 481 (M−15), 465 (M−31), 406 (M−90), 395 (loss of C1, to C10), 385 (loss of C11 to C20), 375 (M−2×90), 295 (base peak, 385−90), 279 (loss of C1, to C10), 269 (loss of C1, to C20), 217, 205 (295−90), 203 (Me3SiO+ = CH(CH3)2−CO2Me), 131, and 129. This mass spectrum resembles that of the corresponding derivative of 12-epi-6-trans-LTB4, except that comparable fragment ions containing carbons 6−11 occur at masses 2 units higher in the mass spectrum of metabolite IIb. These results, combined with the UV spectrum of metabolite IIb, clearly indicate that one of the three conjugated double bonds of 12-epi-6-trans-LTB4, has been reduced to give a product with two conjugated double bonds. These two double bonds could theoretically be in the 6 and 8, 7 and 9, or 8 and 10 positions. The two ions at m/z 269 and 279 may be due to fragmentations on either side of the conjugated double bonds, as shown in Fig. 3A, suggesting that they are present in the 7 and 9 positions. The structure of the dihydro metabolite of 12-epi-6-trans-LTB4 would therefore be 5,12-dihydroxy-7,9,14-eicosatrienoic acid.

Analysis of the Dihydro Metabolite of Deuterium-labeled 12-Epi-6-trans-LTB4 by GC-MS

In order to get more information about the fragmentation pattern of dihydro-12-epi-6-trans-LTB4, and to determine whether the formation of this compound was accompanied by the loss of any hydrogen atoms, 12-epi-6-trans-[5,6,8,9,11,12,14,15-2H]LTB4 was incubated with human PMNL, and the resulting deuterium-labeled dihydro metabolite was purified by RP-HPLC. The mass spectrum (Fig. 3C) of the trimethylsilyl ether derivative of the methyl ester of this substance closely resembles that of the corresponding derivative of dihydro-12-epi-6-trans-LTB4 (Fig. 3A). Fragment ions are present at m/z 488 (M−15), 472 (M−31), 413 (M−90), 398 (M−15−90), 390 (loss of C10 to C20), 382 (M−31−90), 323 (M−2×90), 312 (loss of C1, to C10, and trimethylsilyl), 306 (base peak, 390−90), 285 (loss of C1, to C10, 272 (loss of C11 to C20), 219, 210 (300−90), 203 (Me3SiO+ = CH(CH3)2−CO2Me), and 132.

There are two interesting points about the mass spectrum shown in Fig. 3C. Fragment ions (e.g. those with m/z values of 488, 472, 413, and 323) containing the com-
Metabolism of Leukotriene B<sub>4</sub> Isomers

The two 6-trans isomers of LTB<sub>4</sub>, (0.4 μM) were incubated with human PMNL (10<sup>5</sup> cells/ml) for various times and the products analyzed by RP-HPLC. The time course for the formation of metabolite IIa from 6-trans-LTB<sub>4</sub>, shows that there is an initial lag phase in the formation of this product (Fig. 4A). This was not true for either metabolite Ia or IIIa. Initially, the amount of metabolite IIIa exceeded that of metabolite IIa, but the concentration of the former product reached a maximum and then declined, so that after 30 min, only a relatively small amount was present. Unlike the situation with metabolite IIIa, the amount of metabolite Ia in the incubation mixture increased steadily as a function of time. These results suggest that the precursor of the dihydro-20-hydroxy product IIa is dihydro-6-trans-LTB<sub>4</sub> (metabolite IIa) rather than 20-hydroxy-6-trans-LTB<sub>4</sub> (metabolite Ia). We did not have sufficient amounts of tritium-labeled metabolite IIIa to test this hypothesis directly. However, we found that 20-hydroxy-6-trans-LTB<sub>4</sub>, was not converted to any detectable dihydro metabolites by PMNL (data not shown). These results suggest that although dihydro products are good substrates for LTB<sub>4</sub>, 20-hydroxylase, 20-hydroxy products are not metabolized by the triene reductase.

The time course for the formation of products from 12-epi-6-trans-LTB<sub>4</sub>, (Fig. 4B) bears some resemblance to that shown in Fig. 4A for 6-trans-LTB<sub>4</sub>, except that there are considerable quantitative differences. Metabolite Iib, like dihydro-20-hydroxy-6-trans-LTB<sub>4</sub>, is formed only after a lag phase. This product was barely detectable after 4 min, but after 30 min, its concentration was over 40% that of dihydro-12-epi-6-trans-LTB<sub>4</sub> (metabolite Iib), which was the major product at all time points investigated. The amount of the latter product increased up to 15 min, after which time it did not change. On the other hand, the amounts of both 20-hydroxy-12-epi-6-trans-LTB<sub>4</sub>, (metabolite Ib) and especially dihydro-20-hy-
droxy-12-epi-6-trans-LTB₄, (metabolite IIb), increased between 15 and 30 min. These results are consistent with the formation of metabolite IIb from metabolite IIIb, analogous to the situation for the metabolites of 6-trans-LTB₄.

Effects of LTB₄ on the Metabolism of 12-Epi-6-trans-LTB₄ by PMNL

LTB₄ is converted very rapidly to 20-hydroxy-LTB₄ by human PMNL, and we have been unable to detect substantial amounts of dihydro metabolites of this substance in incubations with these cells. Another approach to investigating a possible interaction of LTB₄ with the triene reductase in PMNL would be to examine its effects on the metabolism of 6-trans isomers of LTB₄. We incubated PMNL with 12-epi-6-trans-[³H]LTB₄ (0.4 μM) in the presence of LTB₄ (2 μM) and measured the amounts of products formed after different times (Fig. 4C). The concentration of LTB₄, used in this experiment was higher than that of 12-epi-6-trans-LTB₄, because of the rapid metabolism of the former compound by LTB₄, 20-hydroxylation under these conditions (9). LTB₄, inhibited the formation of metabolites Ib, IIb, and IIIb from 12-epi-6-trans-[³H]LTB₄, by over 90% for the first 8 min of the reaction (Fig. 4C, inset). Between 15 and 30 min, by which time most of the LTB₄ had been metabolized, there was a rapid increase in the amount of dihydro-12-epi-6-trans-LTB₄, so that by 30 min, the amount of this product was nearly 70% of that in control incubations (Fig. 4B) carried out in the absence of LTB₄. The formation of ω-oxidation products from 12-epi-6-trans-LTB₄, was inhibited even more by LTB₄, than the formation of dihydro-12-epi-6-trans-LTB₄. Metabolites Ib and IIb could not be detected under these conditions (9). LTB₄, inhibited the formation of metabolite IIb by 85% and that of metabolite IIIb by 98%. It is not surprising that the formation of metabolite IIb was inhibited more than that of the other products, since it requires the actions of both LTB₄, 20-hydroxylase and the triene reductase.

Effects of Substrate Concentration on the Metabolism of 6-Trans Isomers of LTB₄

Human PMNL (10⁷ cells/ml) were incubated with various concentrations of 6-trans-LTB₄, or 12-epi-6-trans-LTB₄, for
Metabolism of Leukotriene B₄ Isomers

compared to only 20% initially metabolized by the hydroxylase pathway (i.e. metabolite Ia) (Fig. 5A). When the substrate concentration was raised to 2 μM, however, only 6% was initially metabolized by the reductase, whereas the proportion metabolized by the hydroxylase rose to 38% (Fig. 5B). These calculations are based on the assumption that the initial reaction in the formation of dihydro-20-hydroxy-6-trans-LTB₄ was catalyzed by the reductase.

The effects of various substrate concentrations on the formation of products from 6-trans-LTB₄ and 12-epi-6-trans-LTB₄ are shown in Fig. 6. It should be noted that the scale for the ordinate, which shows the amounts of products formed, is different for Fig. 6A (6-trans-LTB₄) and Fig. 6B (12-epi-6-trans-LTB₄). The formation of trihydroxy trienes from these two substrates is nearly linear with the substrate concentration up to at least 2 μM, suggesting that the ω-oxidation reaction has a fairly high Vₘₐₓ. The rate of formation of ω-oxidation products from 6-trans-LTB₄ is about four times greater than from 12-epi-6-trans-LTB₄.

The formation of reductase products is affected quite differently from that of hydroxylase products as the substrate concentration is raised. At low substrate concentrations (0.1 μM), the major pathway for the initial metabolism of both 6-trans-LTB₄ and 12-epi-6-trans-LTB₄ is the formation of dihydro products. However, the amounts of these products reach maximal levels at substrate concentrations between 0.4 and 0.8 μM and then decline at higher concentrations. These results suggest that both the Kₘ and the Vₘₐₓ of the reductase pathway are substantially lower than those of LTB₄ 20-hydroxylase.

**Effects of Concentration of PMNL on the Metabolism of 12-Epi-6-trans-LTB₄**

Various concentrations of PMNL were incubated with 12-epi-6-trans-LTB₄ (0.4 μM) for 30 min, and the amounts of products formed were analyzed by HPLC. At all cell concentrations, the reductase product, metabolite IIIb, was formed to the greatest extent (Fig. 7). As the concentration of PMNL was raised, the amounts of dihydro-12-epi-6-trans-LTB₄ (IIIb) and 20-hydroxy-12-epi-6-trans-LTB₄ (Ib) first increased in a nearly linear fashion and then reached nearly maximal levels due to limitations in substrate availability. The pattern for the formation of the combined reductase/hydroxylase product (Iib) was quite different. At low concentrations of PMNL, metabolite Iib was only a minor product, whereas at higher concentrations, quite substantial amounts were formed. This would suggest that there is no coupling between the reductase and hydroxylase reactions. The dihydro product is probably first formed by the PMNL and then released into the medium, where it can be converted by a second cell to the dihydro-20-hydroxy product. Thus, the formation of metabolite Iib requires two subsequent interactions of substrate with PMNL, whereas the formation of the other products requires only one such interaction.

**DISCUSSION**

There are two major pathways for the metabolism of 6-trans isomers of LTB₄ by human PMNL: formation of dihydro products by a triene reductase, and ω-oxidation (Fig. 8). The ω-hydroxylation of these products is presumably catalyzed by LTB₄ 20-hydroxylase, since LTB₄ strongly inhibits this process (Fig. 4C). Both 6-trans-LTB₄ and dihydro-6-trans-LTB₄ are relatively good substrates for the hydroxylase (although not nearly as good as LTB₄ itself), and we have identified 20-hydroxy products derived from both of these substances by UV spectrophotometry and GC-MS. On the other hand, 12-epi-6-trans-LTB₄ and its dihydro metabolite are much poorer.
substrates for LTB₄, 20-hydroxylase. We have detected ω-oxidized metabolites of these substances by HPLC, but have not yet isolated sufficient quantities for analysis by GC-MS. Dihydro-6-trans-LTB₄ (metabolite IIIa) is rapidly converted to its 20-hydroxy derivative, and we have not yet conclusively identified the former by GC-MS. However, this product has a λₑ at 231 nm as would be expected for a dihydro product with two conjugated double bonds. Its retention time upon analysis by RP-HPLC, which was slightly longer than that of 6-trans-LTB₄, would also be consistent with this structure. In order to investigate the formation of non-ω-hydroxylated dihydro metabolite from 6-trans-isomers of LTB₄, in more detail, we incubated PMNL with 12-epi-6-trans-LTB₄, since the latter is not a very good substrate for LTB₄, 20-hydroxylase. The major product (metabolite IIIb) formed from this substrate under most conditions was identified on the basis of its retention time, UV spectrum, and mass spectrum as dihydro-12-epi-6-trans-LTB₄.

There are three possible positions for the conjugated double bonds in dihydro-12-epi-6-trans-LTB₄: 6 and 8, 7 and 9, or 8 and 10. If these double bonds were present in the 6- and 8-positions, an intense ion would be expected in the mass spectrum at m/z 255 (CH = CH-CH = CH-CH(OSiMe₃)₂-(CH₃)₂-CO₂Me), due to cleavage between carbons 6 and 7, on the carboxyl side of the conjugated diene structure, and therefore consists of C₂₋C₂₀ (Fig. 3A). This is supported by the presence of an analogous fragment ion in the mass spectrum of the corresponding deuterium-labeled metabolite at m/z 285 (Fig. 3C). There are no significant ions in the mass spectra of unlabeled and deuterium-labeled 12-epi-6-trans-LTB₄ (Figs. 3, B and D) which correspond to the above ions. The ions at m/z 282 (Fig. 3B) and 288 (Fig. 3D) in the mass spectra of the latter compounds do not have the appropriate masses and were presumably formed due to subsequent cleavages between carbons 4 and 5 and carbons 12 and 13. The ions at m/z 289 (Fig. 3A) and 272 (Fig. 3C) in the mass spectra of the unlabeled and deuterium-labeled dihydro metabolites may be due to cleavage between carbons 10 and 11 on the alkyl side of the conjugated diene structure. However, it must be borne in mind that analogous ions are present at m/z 267 (Fig. 3B) and m/z 270 (Fig. 3D) in the mass spectra of unlabeled and deuterium-labeled 12-epi-6-trans-LTB₄. From the above considerations, the mass spectral evidence would strongly suggest that 12-epi-6-trans-LTB₄ is metabolized to 5,12-dihydroxy-7,9,14-eicosatrienoic acid by human PMNL.

The reduction of 5,12-dihydroxy trienes could occur by a number of different mechanisms. In most cases involving the enzymatic reduction of carbon-carbon double bonds, the double bond is activated by conjugation with an oxo group. For example, proglandins are biologically inactivated by conversion to 13,14-dihydro-15-oxo metabolites by the successive actions of 15-hydroxyprostaglandin dehydrogenase and prostaglandin A₁₀-reductase. Only 15-oxoproglandins, and not unmetabolized 15-hydroxyproglandins, are substrates for the reductase (31). Similarly, steroid 5α-reductases catalyze the reduction of the 4,5-double bond, which is conjugated with the 3-oxo group of steroids. The reductases involved in fatty acid chain elongation and β-oxidation also catalyze the reduction of double bonds which are activated by conjugation with the oxo groups of CoA esters. One of these enzymes catalyzes the reduction of a 2,4-dienoyl-CoA derived from linoleic acid to a 3-enoyl-CoA metabolite (i.e. reduction is coupled to migration of one of the double bonds, as appears to occur with 6-trans isomers of LTB₄) (32).

Conjugated dienes in which the double bonds are not activated by conjugation with an oxo group can also be reduced by reductases. For example, sterol 14-reductase converts Δ¹₄ sterols to the corresponding Δ³-sterols (33).

There are thus two probable mechanisms for the formation of 5,12-dihydroxy-7,9,14-eicosatetraenoic acid from 12-epi-6-trans-LTB₄. The simplest mechanism would be the direct reduction of the conjugated triene system, coupled with migration of the double bonds, to give the corresponding conjugated diene (Fig. 9). On the other hand, the 5- or the 12-hydroxyl group of 12-epi-6-trans-LTB₄ could first be oxidized to an oxo group by a dehydrogenase. Alternatively, and perhaps less likely, the oxo intermediate could also be formed by hydroxylation at C₅, to give a geminal diol, followed bydehydration. The formation of the oxo intermediate, which would result in activation of the double bonds, would be followed by two steps of reduction, coupled with migration of the double bonds, to give the dihydro product. Although this mechanism is more complicated than the direct reduction of one of the double bonds, it is analogous to the formation of 13,14-dihydroprostaglandins from prostaglandins. These products are formed by the initial actions of 15-hydroxyprostaglandin dehydrogenase and prostaglandin Δ¹₀ reductase as discussed above, followed by reduction of the 15-oxo group of the 13,14-dihydro-15-oxoprostaglandin to give a 13,14-dihydroprostaglandin (34).

One way to distinguish between the above two possibilities would be to incubate 12-epi-6-trans-[5,6,8,9,11,12,14,15-²H]LTB₄ with PMNL and analyze the resulting dihydro product by GC-MS. If the triene group of the substrate were reduced directly, no deuterium atoms would be lost from the substrate. On the other hand, if there were an oxo intermediate, the deuterium atom in either the 5-position or the 12-position would be lost (Fig. 9). The results of this experiment clearly indicate that a deuterium atom was lost from the 5-position during the formation of heptadecueto-5,12-dihydroxy-7,9,14-

![Fig. 9. Possible mechanisms for the formation of dihydro metabolites of 6-trans isomers of LTB₄, showing both the direct reduction of the substrate by a reductase (top) and the indirect reduction, via oxo intermediates (bottom). When the substrate was 12-epi-6-trans-[5,6,8,9,11,12,14,15-²H]LTB₄, a deuterium atom was present in the 5-position of the substrate. This deuterium atom would be retained if the substrate were reduced directly or lost if there were a 5-oxo intermediate.](image-url)
eicosatrienoic acid, strongly suggesting the presence of an intermediate with a 5-oxo group. We have not detected any such compounds in incubations of human PMNL with either 6-trans-LTB₄ or 12-epi-6-trans-LTB₄. However, it is possible that such intermediates could be very short-lived and be rapidly converted to the dihydro metabolites IIIa and IIIb. There is some evidence for the formation of an oxo metabolite of LTB₄ by rat hepatocytes (35). Although this product was not isolated and identified, it could be an intermediate in the formation of isomers of LTB₄ metabolites formed by these cells (35).

Because of the limited availability of labeled and unlabeled substrates, the experiments on the effects of substrate concentration illustrated in Fig. 6, A and B, were not designed for the determination of the $K_m$ and $V_{max}$ values for the hydroxylase and reductase enzymes. However, these data suggest that the $V_{max}$ for the formation of dihydro products is considerably lower than that for the formation of ω-oxidation products. It would also appear that the reductase pathway is subject to substrate inhibition, since the amounts of dihydro products formed from both 6-trans-LTB₄ and 12-epi-6-trans-LTB₄ decrease as the substrate concentration is raised from 0.8 to 2 μM (Fig. 6). At low substrate concentrations, the reductase pathway is the major pathway for the initial metabolism of 6-trans-LTB₄ and 12-epi-6-trans-LTB₄, suggesting that the $K_m$ for the formation of dihydro products from these substrates is lower than that for their hydroxylation.

Since LTB₄ is rapidly metabolized by LTB₄-20-hydroxylase and since 20-hydroxylated products are not substrates for the reductase, we have not yet been able to detect dihydro metabolites of LTB₄ in human PMNL. There is indirect evidence that LTB₄ may be a substrate for the reductase; however, LTB₄, strongly inhibited the conversion of 12-epi-6-trans-LTB₄ to both dihydro and 20-hydroxylated products by PMNL (Fig. 4C), suggesting that LTB₄ competitively inhibited the metabolism of this substrate by both the triene reductase and the 20-hydroxylase. An alternative explanation of these data, which cannot be excluded, is that LTB₄ did not inhibit the reductase directly, but rather competitively inhibited the active uptake of 12-epi-6-trans-LTB₄ into the PMNL. We are currently investigating which of these hypotheses is correct.

Acknowledgments—We are grateful to Drs. M. Evans and M. Bertrand of the Department of Chemistry, University of Montreal and to Dr. O. Mamer of the Department of Medicine, McGill University, for assistance with the gas chromatography-mass spectrometry.

REFERENCES

1. Borgeat, P., and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3213-3217
2. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., and Smith, M. J. H. (1980) Nature 286, 264-265
3. Palnblad, J., Malmsten, C. L., Uden, A.-M., Rådmark, O., Engstedt, L., and Samuelsson, B. (1981) Blood 58, 658-661
4. Showell, J. H., Naccache, P. H., Borgeat, P., Picard, S., Vallarand, P., Becker, E. L., and Sha'afi, R. I. (1982) J. Immunol. 128, 511-816
5. Feinmark, S. J., Lindgren, J. A., Claesson, H.-E., Malmsten, C., and Samuelsson, B. (1981) FEBS Lett. 136, 141-144
6. Dahlen, S.-E., Bjork, J., Hedqvist, P., Arfors, K.-E., Hammarstrom, S., Lindgren, J. A., and Samuelsson, B. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3887-3891
7. Higgs, G. A., Sainon, J. A., and Spayne, J. A. (1981) Br. J. Pharmacol. 74, 429-433
8. Lindgren, J. A., Hansson, G., and Samuelsson, B. (1981) FEBS Lett. 128, 229-335
9. Powell, W. S. (1984) J. Biol. Chem. 259, 3082-3089
10. Shak, S., and Goldstein, I. M. (1984) J. Biol. Chem. 259, 10181-10187
11. Nadeau, M., Fruteau de Laclots, B., Picard, S., Braquet, P., Corey, E. J., and Borgeat, P. (1984) Can. J. Biochem. Cell. Biol. 62, 1321-1328
12. Soberman, R. J., Harper, T. W., Murphy, R. C., and Austen, K. F. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2292-2295
13. Shak, S., and Goldstein, I. M. (1985) Clin. Invest. 76, 1218-1226
14. Hansson, G., Lindgren, J. A., Dahlen, S.-E., Hedqvist, P., and Samuelsson, B. (1981) FEBS Lett. 130, 107-112
15. Juhlin, W., Rådmark, O., Malmsten, C., Hansson, G., Lindgren, J. A., Palmblad, J., Uden, A.-M., and Samuelsson, B. (1982) J. Biol. Chem. 257, 6106-6110
16. Sumimoto, H., Takeshigi, K., and Minakami, S. (1985) Biochem. Biophys. Res. Commun. 132, 846-870
17. Camp, R. D. R., Woolard, P. M., Mallet, A. I., Fincham, N. J., Ford-Hutchinson, A. W., and Bray, M. A. (1982) Prostaglandins 23, 631-641
18. Ford-Hutchinson, A. W., Ruckham, A., Zamboni, R., Rakach, J., and Roy, S. (1983) Prostaglandins 22, 29-37
19. Dahinden, C. A., Clancy, R. M., and Hugli, T. E. (1984) J. Immunol. 133, 1477-1482
20. Clancy, R. M., Dahinden, C. A., and Hugli, T. E. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 5723-5728
21. Sirois, P., Roy, S., Borgeat, P., Picard, S., and Vallarand, P. (1982) Prostaglandins Leukotrienes Med. 8, 157-170
22. Naccache, P. H., Molski, T. F. P., Becker, E. L., Borgeat, P., Picard, S., Vallarand, F., and Sha'afi, R. I. (1982) J. Biol. Chem. 257, 6028-6031
23. Powell, W. S. (1986) Biochem. Biophis. Res. Commun. 136, 707-712
24. Powell, W. S. (1983) Anal. Biochem. 128, 93-102
25. Borgeat, P., Picard, S., Vallarand, P., and Sirois, P. (1981) Prostaglandins Med. 6, 537-547
26. Hamberg, M., Niehaus, W. G., and Samuelsson, B. (1968) Anal. Biochem. 22, 145-153
27. Woolard, P. M., Hensby, C. N., and Lascelles, P. T. (1978) J. Chromatogr. 166, 411-422
28. Powell, W. S. (1982) Methods Enzymol. 86, 467-477
29. Powell, W. S. (1987) Anal. Biochem. 164, 117-131
30. Borgeat, P., Hamberg, M., and Samuelsson, B. (1976) J. Biol. Chem. 251, 7816-7820
31. Westbrook, C., and Jarabak, J. (1978) Arch. Biochem. Biophys. 185, 429-445
32. Cuevas, D., and Schulz, H. (1982) J. Biol. Chem. 257, 14140-14144
33. Paik, Y.-B., Trzaskos, J. M., Shaffee, A., and Gaylor, J. L. (1984) J. Biol. Chem. 259, 13413-13423
34. Hamberg, M., and Samuelsson, B. (1971) J. Biol. Chem. 246, 1073-1077
35. Harper, T. W., Gattay, M. J., and Murphy, R. C. (1986) J. Biol. Chem. 261, 5414-5418