

## Synthesis of Two Hydroxy Fatty Acids from 7,10,13,16,19-Docosapentaenoic Acid by Human Platelets\*

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María Mónica Careaga‡ and Howard Sprecher§

From the Department of Physiological Chemistry, College of Medicine, The Ohio State University, Columbus, Ohio 43210

Platelets metabolize 7,10,13,16,19-docosapentaenoic acid (22:5(*n*-3)) into 11-hydroxy-7,9,13,16,19- and 14-hydroxy-7,10,12,16,19-docosapentaenoic acid via an indomethacin-insensitive pathway. Time-dependent studies with 20  $\mu$ M substrate show a lag in the synthesis of both the 11- and 14-isomers which was not observed for the synthesis of thromboxane B<sub>2</sub> (TXB<sub>2</sub>), 5,8,10-heptadecatrienoic acid, and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE) from arachidonic acid. When platelets were incubated with increasing concentrations of 22:5(*n*-3), the 11- and 14-isomers were not produced until the substrate concentration exceeded 5  $\mu$ M unless arachidonic acid was also added to the incubations. The stimulatory effect of arachidonic acid was not blocked by indomethacin thus suggesting that 12-hydroperoxyeicosatetraenoic acid or 12-HETE derived from arachidonic acid may activate the platelet lipoxygenase(s) which metabolize 22:5(*n*-3). Incubations containing 20  $\mu$ M 22:5(*n*-3) and increasing levels of [1-<sup>14</sup>C]arachidonic acid show that the (*n*-3) acid inhibits the synthesis of both 5,8,10-heptadecatrienoic acid and TXB<sub>2</sub> from arachidonic acid. At the same time, 12-HETE synthesis increased due to substrate shunting to the lipoxygenase pathway.

compound is a poor promoter of platelet aggregation (5). Morita *et al.* (7) recently reported that 12-HPETE, produced from arachidonic acid, stimulated TXA<sub>3</sub> synthesis. This interaction between a lipoxygenase metabolite with cyclooxygenase may further help to explain the clinical findings with Greenland Eskimos.

The merits of supplementing the diet with 20:5(*n*-3) imply that this acid must mediate both platelet and endothelial cell metabolism to reduce platelet aggregation. Needleman *et al.* (5) demonstrated that the artery metabolized exogenous PGH<sub>3</sub> to the corresponding prostacyclin and that this compound, like PGI<sub>2</sub>, inhibited platelet aggregation. Considerable controversy exists in the literature as to whether endothelial cell cyclooxygenase is able to efficiently metabolize 20:5(*n*-3) to PGH<sub>3</sub> and thus to PGI<sub>3</sub> in sufficient amounts to exert a biological effect (8-12). Fisher and Weber (13) recently identified  $\Delta$ 17-2,3-dinor-6-keto-PGF<sub>1 $\alpha$</sub>  in the urine of human volunteers who had eaten diets elevated in fish oil and thus suggest that PGI<sub>3</sub> can efficiently be made by man.

Any proposal advocating a higher dietary intake of 20:5(*n*-3) fails to recognize that fish oils are a rich source of both 22:5(*n*-3) and 22:6(*n*-3) (14). In addition 22:5(*n*-3) and 22:6(*n*-3) are made from 20:5(*n*-3) and both of these 22-carbon fatty acids are found in platelet phospholipids (3, 9). 22:6(*n*-3) is a competitive inhibitor of both platelet (15) and vesicular gland (16) cyclooxygenase but does not inhibit the synthesis of leukotrienes from arachidonic acid in RBL-1 cells (16). Human platelets metabolize 22:6(*n*-3) via a lipoxygenase pathway into a pair of isomeric acids having their hydroxyl group at carbons 11 and 14 (17). In this study we report that platelets metabolize 22:5(*n*-3) into 11- and 14-hydroxy docosapentaenoic acids via an indomethacin-insensitive pathway and that 22:5(*n*-3) and arachidonic acid interact to regulate TXB<sub>2</sub> production as well as the types and amounts of hydroxy acids produced via the lipoxygenase pathway(s).

### MATERIALS AND METHODS

**Fatty Acids**—7,10,13,16,19-[1-<sup>14</sup>C]Docosapentaenoic acid (47 Ci/mol) was prepared as previously described (18). [1-<sup>14</sup>C]Arachidonic acid (56.9 Ci/mol) was obtained from New England Nuclear. Arachidonic acid was purchased from Nu-Chek Preparations, Elysian, MN while 7,10,13,16,19-docosapentaenoic acid was made by total synthesis.

**Platelet Incubations**—Blood was drawn from healthy volunteers who had not taken any medication for two weeks. The blood was collected in 7.5% (v/v) 77 mM disodium EDTA and centrifuged for 15 min at 200  $\times$  g. Platelets were recovered by centrifuging the plasma to 2000  $\times$  g for 20 min. The platelets were resuspended in 0.15 M NaCl, 0.15 M Tris (pH 7.4), 77 mM disodium EDTA 90:8:2 (v/v) (19) and centrifuged at 2000  $\times$  g for 15 min. They were then resuspended in the above medium at 3  $\times$  10<sup>8</sup> cells/ml.

Platelets (0.5 ml) were preincubated for 2 min at 37 °C by stirring in siliconized tubes in a water bath. Reactions were initiated by addition of the potassium salt of the fatty acid (specific activity = 15 Ci/mol). Where noted, indomethacin or 5,8,11,14-heneicosatetraenoic

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Arachidonic acid is metabolized in platelets to TXA<sub>2</sub>,<sup>1</sup> HHT, and 12-HETE (1). The low incidence of myocardial infarction and ischemic heart disease observed in Greenland Eskimos has been attributed to their high dietary intake of fish oils which are a rich source of 20:5(*n*-3) (2-4). This acid replaces some of the arachidonic acid in platelet phospholipids. When platelets are stimulated with appropriate agonists, both 20:5(*n*-3) and arachidonic acid are released. 20:5(*n*-3) competes with arachidonic acid for cyclooxygenase to depress the synthesis of TXA<sub>2</sub>, which is both a vasoconstrictor and potent stimulator of platelet aggregation (5). Although small amounts of TXA<sub>3</sub> are made from 20:5(*n*-3) (6), this

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§ To whom all correspondence should be addressed at: The Department of Physiological Chemistry, The Ohio State University, 333 West Tenth Avenue, Columbus, OH 43210.

<sup>1</sup> The abbreviations used are: TX, thromboxane; HHT, 5,8,10-heptadecatrienoic acid; 12-HETE, 12-hydroxy-5,8,10,14-eicosatetraenoic acid; HPLC, high-pressure liquid chromatography; PG, prostaglandin; ECL, equivalent chain length; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid;

acid were added in 10  $\mu$ l of ethanol. This amount of ethanol had no effect on reaction rates. Reactions were terminated by addition of 0.1 ml of 2 N formic acid and the products were recovered by extracting three times with 3 volumes of ethyl acetate. The pooled ethyl acetate extracts were washed with 1 ml of water and the ethyl acetate was removed under a stream of  $N_2$ .

**High-performance Liquid Chromatography**—Reverse-phase HPLC was carried out with a DuPont HPLC consisting of an 870 pump, 8800 series gradient controller, column oven, and a variable wavelength detector. Radioactivity was quantitated with an HP radioactive flow detector (Radiomatic Instruments and Chemical Co., Inc. Tampa, FL). Chromatography was carried out using a Zorbax 10- $\mu$ m ODS column (0.46  $\times$  25 cm) preceded by a guard column (5  $\times$  0.46 cm) packed with Permaphase ODS (DuPont, Wilmington, DE). Samples were injected in 50  $\mu$ l of methanol. Chromatography was carried out at 35  $^\circ$ C with a flow rate of 1.5 ml/min while the flow of Flo-Scint II (Radiomatic Instruments and Chemical Co., Inc., Tampa, FL) was 4.5 ml/min through the radioactive detector. Counting efficiency was approximately 70%. Arachidonate metabolites were separated by isocratic elution for 20 min with 30% acetonitrile in water which was adjusted to pH 2.2 with phosphoric acid (Fisher). After this time the concentration of acetonitrile was increased to 42% with a linear gradient over 5 min. At 25 min the concentration of acetonitrile increased to 55% over 30 min using the -2 exponential gradient. Unreacted arachidonic acid was then removed by linearly increasing the concentration of acetonitrile to 100% over 10 min.

Normal phase HPLC was carried out using a Beckman system consisting of two 110 pumps, a 420 controller, and a variable wavelength detector (Bio-Rad). Chromatography was carried out at room temperature using a Zorbax Sil column (0.46  $\times$  25 cm) (DuPont, Wilmington, DE). Hydroxy acids or methyl esters of hydroxy acids were injected in 50  $\mu$ l of hexane/isopropanol, 99.5:0.5 (v/v). Methyl esters were separated by isocratic elution with 0.6% isopropanol in hexane while the separation of free acids was carried out with hexane/isopropanol/acetic acid 99:1:0.1 (v/v). The flow rate through the column was 1.5 ml/min, while the flow of scintillation fluid was 3 ml/min.

**Gas Chromatography-Mass Spectrometry**—Hydroxy acids were converted to methyl esters by reaction with ethereal diazomethane. Trimethylsilyl ethers were prepared by reacting the methyl ester with 10  $\mu$ l of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co.) and an equal volume of pyridine at room temperature for 1 h. Methyl esters were hydrogenated by bubbling hydrogen for 30 s into a solution of the methyl ester in 0.5 ml of methanol which contained about 1 mg of platinum oxide. The reaction mixture was rapidly transferred to a silicic acid column and eluted with 2 ml of methanol. Compounds were dissolved in isoctane for analysis by gas-liquid chromatography.

ECLs were determined using a Varian Vista 6000 gas chromatograph equipped with a glass column (6 foot  $\times$  2 mm, inner diameter) packed with 1% SP-2100 on 100/120 mesh Supelcoport (Supelco, Bellefonte, PA). Helium was the carrier gas (30 ml/min) and the temperatures of the injector, oven, and detector were, respectively, 250, 210, and 280  $^\circ$ C. Mass spectrometry was carried out with a Hewlett Packard 5970A mass selective detector and a 5790 gas chromatograph. Separations were carried out on a J and W DB-1 capillary column (15 m  $\times$  0.25 mm inner diameter) obtained from Applied Science, State College, PA. Injections were made in the splitless mode with an initial temperature of 70  $^\circ$ C and a valve time of 1 min. The temperature of the injector was 250  $^\circ$ C and the transfer line was 280  $^\circ$ C. One min after injection the oven was programmed at 30  $^\circ$ C/min to 210 or 240  $^\circ$ C, respectively, for analysis of unsaturated and saturated hydroxy acids. The ionizing voltage was 70 eV.

## RESULTS

Analysis of metabolites produced from [1- $^{14}$ C]22:5(*n*-3) by reverse-phase HPLC revealed the presence of a single radioactive compound with strong absorption at 234 nm suggesting that it was a hydroxy fatty acid. Fig. 1 shows that [1- $^{14}$ C]22:5(*n*-3) was metabolized into two metabolites when analysis was carried out by straight-phase HPLC.

The ultraviolet spectrum of compound I had  $\lambda_{max}$  236 nm with  $\epsilon$  = 28,000 in methanol. The ECL of the ME-TMS ether was 23.3. The mass spectrum (Fig. 2) had ions at  $m/z$  432 ( $M^+$ ), 417 ( $M$  - 15, loss of  $\cdot$ CH $_3$ ), 401 ( $M$  - 31, loss of

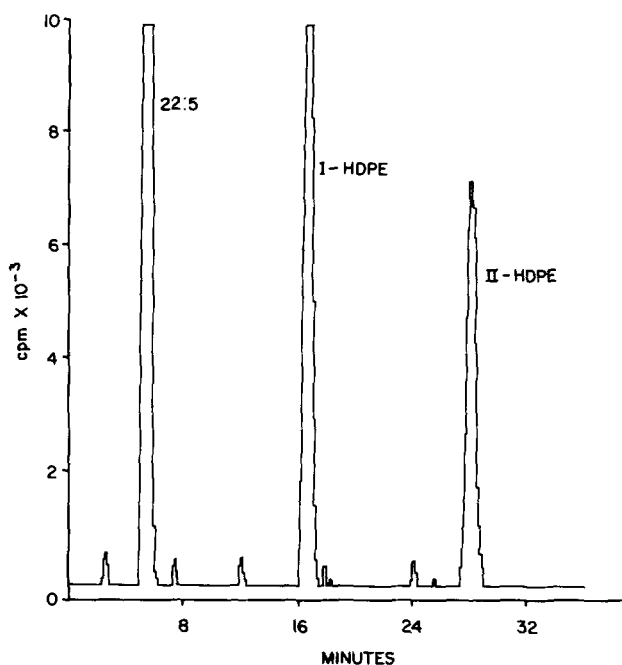


FIG. 1. Normal phase HPLC radiochromatogram of the metabolites produced from 7,10,13,16,19-[1- $^{14}$ C]docosapentaenoic acid. Platelets ( $1.5 \times 10^8/0.5$  ml) were incubated with 20  $\mu$ M substrate. After 3 min the metabolites were extracted with ethyl acetate and separated using hexane/isopropanol/acetic acid 99:1:0.1 (v/v).

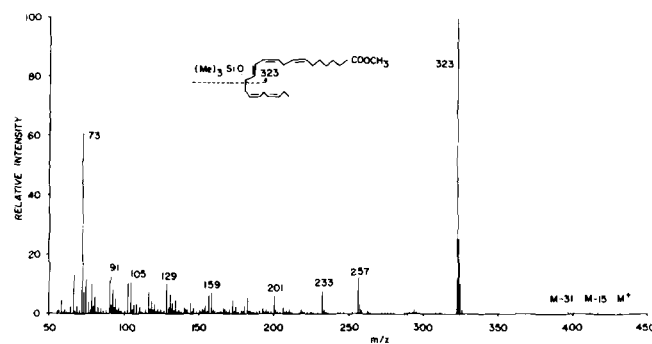


FIG. 2. Mass spectrum of the ME-TMS ether of compound I.

$\cdot$ OCH $_3$ ), 323 ( $M$  - 109, loss of  $\cdot$ CH $_2$ -(CH=CH-CH $_2$ ) $_2$ CH $_3$ ), 257, 233 (323 - 90, loss of Me $_3$ SiOH), 201 (323 - (90 + 32)) as well as at 159, 129, 105, 103, 91, and 73. The mass spectrum of the hydrogenated ME-TMS ether of compound I (ECL = 24.0) had ions at  $m/z$  427 ( $M$  - 15; 0.9%), 411 ( $M$  - 31; 2.0%), 395 ( $M$  - (15 + 32); 6.0%), 329 ( $M$  - 113, loss of  $\cdot$ CH $_2$ (CH $_2$ ) $_6$ CH $_3$ ; 92%), 300 ( $M$  - 142, loss of CH $_3$ (CH $_2$ ) $_7$ CHO followed by migration of the trimethylsilyl group to the carbomethoxy group 22.3% (20)), 215 ( $M$  - 227, loss of  $\cdot$ (CH $_2$ ) $_12$ COOCH $_3$  base peak). Compound I is thus identified as 14-hydroxy-7,10,12,16,19-docosapentaenoic acid and has a pair of conjugated double bonds in the *cis/trans* configuration (21).

Compound II had  $\lambda_{max}$  234 nm with  $\epsilon$  = 28,000 in methanol. The mass spectrum (Fig. 3) of the ME-TMS ether (ECL = 23.3) had ions at  $m/z$  417 ( $M$  - 15), 283 ( $M$  - 149, loss of  $\cdot$ CH $_2$ (CH=CH-CH $_2$ ) $_3$ CH $_3$ ); 193 (283 - 90), 161 (283 - (90 + 32)) and ions in the low mass region at 151, 133, 119, 91, and 73. The mass spectrum of the hydrogenated ME-TMS ether of compound II (ECL = 24.0) had ions at  $m/z$  427 ( $M$  - 15; 0.7%), 411 ( $M$  - 31; 1.6%), 395 ( $M$  - (15 + 32); 3.6%), 287

or 14-HDPE. Conversely, when incubations contained arachidonic acid, the synthesis of both 11- and 14-HDPE was stimulated at concentrations of [1-<sup>14</sup>C]22:5(*n*-3) below 10 μM. The results in Table I show that 10 μM indomethacin com-

that cyclooxygenase-derived metabolites are not involved in regulating 11- and 14-HDPE synthesis but that 12-HPETE or 12-HETE may activate the lipoxygenase(s) acting on 22:5(*n*-3). As shown in Fig. 6 when the total fatty acid con-

centration exceeded about  $45 \mu\text{M}$  ( $20 \mu\text{M}$  arachidonic plus  $25 \mu\text{M}$  [ $1\text{-}^{14}\text{C}$ ]22:5( $n$ -3)) there was an apparent inhibition in the synthesis of both 11- and 14-HDPE. Whether this represents true substrate competition for a common enzyme or some nonspecific inhibition due to the detergency effect of high concentrations of fatty acids is not known.

The results in Fig. 7 were obtained when platelets were incubated with and without  $20 \mu\text{M}$  22:5( $n$ -3) in the presence of increasing concentrations of [ $1\text{-}^{14}\text{C}$ ]arachidonic acid. These results show that 22:5( $n$ -3) inhibited the synthesis of both TXB<sub>2</sub> and HHT thus suggesting that 22:5( $n$ -3) inhibits cyclooxygenase even though it is not metabolized by this enzyme. The apparent stimulation in 12-HETE synthesis, at low [ $1\text{-}^{14}\text{C}$ ]arachidonic acid concentrations, in the presence of  $20 \mu\text{M}$  22:5( $n$ -3) is probably due to shunting of arachidonic acid to the lipoxygenase pathway. When cyclooxygenase was inhibited by 22:5( $n$ -3), the amount of arachidonic acid available for lipoxygenase increased. Again, when the total fatty acid concentration exceeded  $50 \mu\text{M}$ , there was inhibition of 12-HETE synthesis. As noted previously, we do not know if this is a general type of nonspecific inhibition.

Previously we reported that 5,8,11,14-heneicosatetraenoic acid was a selective lipoxygenase inhibitor which did not affect cyclooxygenase activity (22). The results in Fig. 8 confirm our previous results showing that 12-HETE synthesis was inhibited 50% by  $0.5 \mu\text{M}$  levels of this acetylenic acid. Surprisingly, only  $0.05 \mu\text{M}$  levels of this acid were acquired for 50% inhibition of both 11- and 14-HDPE. Virtually identical inhibition curves were obtained when the concentration of fatty acids was  $50 \mu\text{M}$ .

#### DISCUSSION

Our results show that both 22:5( $n$ -3) and 22:6( $n$ -3) (17) are metabolized by human platelets into 11- and 14-hydroxy acids

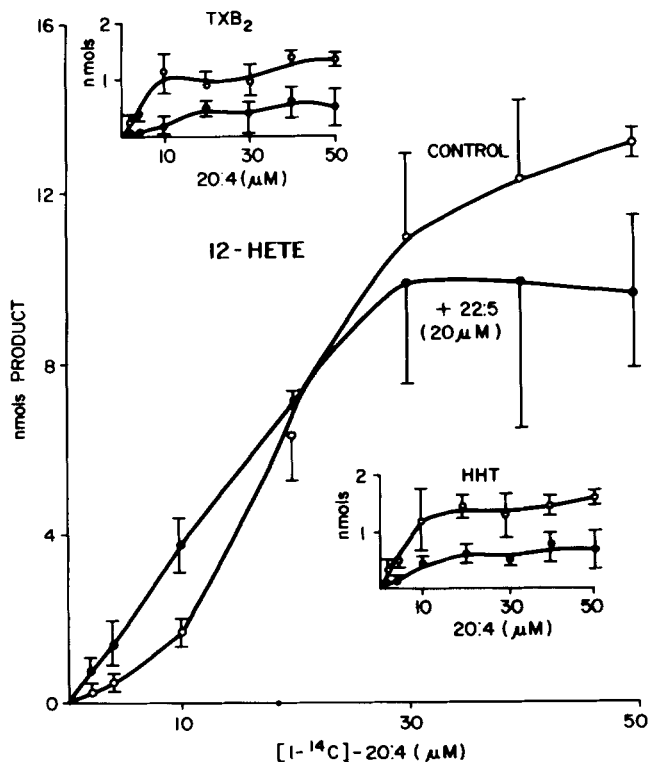


FIG. 7. Platelets ( $1.5 \times 10^8/0.5 \text{ ml}$ ) were incubated with increasing concentrations of [ $1\text{-}^{14}\text{C}$ ]arachidonic acid without ( $\circ$ ) and with ( $\bullet$ )  $20 \mu\text{M}$  7,10,13,16,19-docosapentaenoic acid for 3 min. Metabolites were separated as shown in Fig. 5. Results are the average of three experiments  $\pm$  S.E.

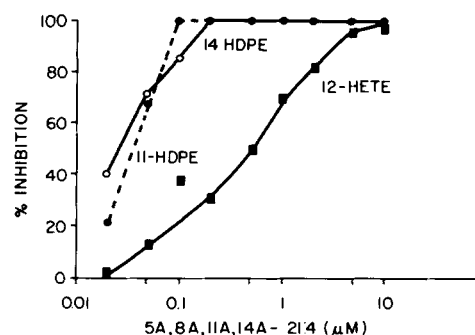


FIG. 8. Inhibition of hydroxy fatty acid biosynthesis by 5,8,11,14-heneicosatetraenoic acid. Platelets ( $1.5 \times 10^8/0.5 \text{ ml}$ ) were preincubated for 2 min at  $37^\circ\text{C}$  with various levels of 5,8,11,14-heneicosatetraenoic acid. Reactions were initiated by addition of  $20 \mu\text{M}$  [ $1\text{-}^{14}\text{C}$ ]arachidonic acid or 7,10,13,16,19-[ $1\text{-}^{14}\text{C}$ ]docosapentaenoic acid and incubations were continued for 3 min. Metabolites from 22:5( $n$ -3) were separated by normal-phase HPLC while those from arachidonic acid were separated by reverse-phase HPLC.

via an indomethacin-insensitive pathway. Platelet lipoxygenase metabolizes arachidonic acid only to 12-HETE (1). The initial step in this reaction involves proton abstraction from carbon 10 (the  $\omega$  11 carbon), followed by double-bond shift and allylic attack by the hydroperoxy radical at carbon 12 (23).

If lipoxygenase specificity is dictated by the terminal end of the fatty acid, then both 22:5( $n$ -3) and 22:6( $n$ -3) would be metabolized to a 14-hydroxy fatty acid since initial proton abstraction would take place at carbon 12, which is the  $\omega$  11 carbon. Previously we suggested that the 11-hydroxy isomer might be made by a second lipoxygenase involving initial proton abstraction from position-9 ( $\omega$  14) (17). Our present findings are not totally consistent with this hypothesis for the following reasons. There was a lag in the synthesis of both the 11- and 14- isomers in the absence of arachidonate. Secondly, arachidonic acid stimulated the synthesis of both isomers to the same extent. Finally, the synthesis of both isomers was inhibited 50% by the same concentration of 5,8,11,14-heneicosatetraenoic acid and this concentration was 10-fold less than required for 50% inhibition of 12-HETE synthesis. Hamberg (24) recently reported that 6,9,12-18:3 was metabolized by platelets via an indomethacin-insensitive pathway to 10- and 13-hydroxy octadecatrienoic acids. He suggests that platelets may contain a lipoxygenase with dual specificity for proton abstraction. If a single lipoxygenase acts on 22:5( $n$ -3) and 22:6( $n$ -3) to give the 11- and 14-isomers, presumably it must be different from the lipoxygenase which metabolizes arachidonic acid to 12-HETE. In addition there must be some inherent, as yet unrecognized, structural feature in a fatty acid which will determine whether it is metabolized by platelets into isomeric hydroxy acids. The acids, 22:5( $n$ -3) and 22:4( $n$ -6), are structurally similar in that their double bonds are located, respectively, at positions 7,10,13,16,19 and 7,10,13,16. Platelets metabolize the latter acid into dihomotxB<sub>2</sub>, dihomo-HHT, and 14-hydroxy-7,10,12,16-docosetetraenoic acid.<sup>2</sup> This latter acid constituted about 90% of the hydroxy acids made via an indomethacin-insensitive pathway and only 1-2% of an 11-hydroxy isomer was detected.

If 22:5( $n$ -3) is released along with arachidonic acid from platelet phospholipids, our results suggest that it will interact with arachidonate in two ways. It inhibits cyclooxygenase as do both 20:5( $n$ -3) (4) and 22:6( $n$ -3) (16) to depress the syn-

<sup>2</sup> M. VanRollins, L. Horrocks, and H. Sprecher, manuscript in preparation.

thesis of TXB<sub>2</sub> and HHT. In turn, more arachidonic acid is shunted to the lipoxygenase pathway resulting in an increase in 12-HPETE production. The 12-HPETE may then stimulate the synthesis of both the 11- and 14-hydroxy acid isomers from 22:5(*n*-3). This stimulatory effect of 12-HPETE on 11- and 14-HDPE synthesis might then be similar to activation to leukocyte 5-lipoxygenase by 12-HPETE (25). The overall effect would be to increase the amount of hydroxy fatty acids produced in the platelet.

## REFERENCES

1. Hamberg, M., and Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 3400-3404
2. Dyerberg, J., and Bang, H. O. (1978) *Lancet* **II**, 152-153
3. Dyerberg, J., and Bang, H. O. (1979) *Lancet* **II**, 433-435
4. Siess, W., Roth, P., Scherer, B., Kurzmann, I., Böhlig, G., and Weber, P. C. (1980) *Lancet* **I**, 441-444
5. Needleman, P., Raz, A., Minkes, M. S., Ferrendelli, J. A., and Sprecher, H. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 944-948
6. Hamberg, M. (1980) *Biochim. Biophys. Acta* **618**, 389-398
7. Morita, I., Takahashi, R., Saito, Y., and Murota, S. (1983) *J. Biol. Chem.* **258**, 10197-10199
8. Hornstra, G., and Hemker, H. C. (1979) *Hemostasis* **8**, 211-226
9. Hornstra, G., Hazelhof, E. C., Haddeman, E., ten Hoor, F., and Nugteren, D. H. (1981) *Prostaglandins* **21**, 727-738
10. Dyerberg, J., Jorgensen, K. A., and Arnfred, T. (1981) *Prostaglandins* **22**, 857-862
11. Hamazaki, T., Hirai, A., Terano, T., Sajiki, J., Kondo, S., Fujita, T., Tamura, Y., and Kumagai, A. (1982) *Prostaglandins* **23**, 557-567
12. Morita, I., Saito, Y., Chang, W. C., and Murota, S. (1983) *Lipids* **18**, 42-49
13. Fisher, S., and Weber, P. C. (1984) *Nature (Lond.)* **307**, 165-168
14. Goodnight, S. R., Harris, W. S., Conner, W. E., and Illingworth, R. D. (1982) *Arteriosclerosis* **2**, 87-113
15. Rao, G. H. R., Radha, E., and White, J. G. (1983) *Biochem. Biophys. Res. Commun.* **117**, 549-555
16. Corey, E. J., Shih, C., and Cashman, J. R. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 3581-3584
17. Avelaño, M. I., and Sprecher, H. (1983) *J. Biol. Chem.* **258**, 9339-9343
18. Sprecher, H., and Sankarappa, S. (1982) *Methods Enzymol.* **86**, 357-366
19. Minkes, M., Stanford, N., Chi, M. M.-Y., Roth, G. J., Raz, A., Needleman, P., and Majerus, P. W. (1977) *J. Clin. Invest.* **59**, 449-454
20. Eglington, G., Hunneman, D. H., and McCormick, A. (1968) *Org. Mass. Spectrom.* **1**, 593-611
21. Chan, H. W. S., and Levett, G. (1977) *Lipids* **12**, 99-104
22. Wilhelm, T. E., Sankarappa, S. K., VanRollins, M., and Sprecher, H. (1981) *Prostaglandins* **21**, 323-332
23. Hamberg, M., and Hamberg, G. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1090-1097
24. Hamberg, M. (1983) *Biochem. Biophys. Res. Commun.* **117**, 593-600
25. Maclouf, J., Laclous, B. F., and Borgeat, P. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79**, 6042-6046.