F₂-isoprostanes are free radical-catalyzed products of arachidonic acid. One of these compounds, 8-epiprostaglandin F₂ₐ, (8-epi-PGF₂ₐ), is a mitogen and vasoconstrictor. We have shown that 8-epi-PGF₂ₐ, unlike other F₂-isoprostanes, is a minor product of the prostaglandin endoperoxide synthase-1 (PG G/H S-1) expressed in human platelets (Praticò, D., Lawson, J. A., and FitzGerald, G. A. (1995) J. Biol. Chem. 270, 9800-9808). Human monocytes express PG G/H S-1 constitutively and exhibit regulated expression of PG G/H S-2. Induction of PG G/H S-2 by concanavalin A, the phorbol ester, phorbol 12-myristate 13-acetate, and bacterial lipopolysaccharide was confirmed with a specific antibody in monocytes pretreated with aspirin to inhibit PG G/H S-1. Induction of PG G/H S-2 by all three stimuli coincided with increased formation of prostaglandin E₂ (PGE₂), thromboxane B₂ (TxB₂), and 8-epi-PGF₂ₐ, but not of other F₂-isoprostanes. Confirmation of PG G/H S-2 as the source of 8-epi-PGF₂ₐ, formation was obtained by down-regulating the enzyme with dexamethasone; preventing protein synthesis with cycloheximide; and preventing synthesis of PGE₂, TxB₂, and 8-epi-PGF₂ₐ with the specific PG G/H S-2 inhibitor, L 745,337.

Monocytes also exhibit the facility to generate 8-epi-PGF₂ₐ in a free radical-dependent manner. Thus, stimulation with opsonized zymosan or coinoculation with low density lipoprotein was unassociated with product formation. However, coinoculation with low density lipoprotein with zymosan-stimulated human monocytes resulted in marked formation of 8-epi-PGF₂ₐ, but not of PGE₂, or TxB₂. Production of 8-epi-PGF₂ₐ coincided with that of thiobarbituric acid-reactive substances and lipid hydroperoxides, but was unaccompanied by PG G/H S-1 induction. Pretreatment of monocytes with the antioxidant, butylated hydroxytoluene or with superoxide dismutase, but not with L 745,337, suppressed formation of 8-epi-PGF₂ₐ, thiobarbituric acid-reactive substances, and lipid hydroperoxides.

In conclusion, human monocytes may form bioactive 8-epi-PGF₂ₐ, either via free radical- or enzyme-catalyzed pathways. 8-Epi-PGF₂ₐ, is a more abundant product of monocyte PG G/H S-2 than of platelet PG G/H S-1. Formation by inducible PG G/H S-2 must be considered as a source of this compound in vivo.

Monocytes are thought to play a central role in atherogenesis (1). They adhere to and transmigrate between endothelial cells (2). Monocyte-derived macrophages have high affinity receptors for oxidized low density lipoprotein (LDL) and LDL ingestion results in their transformation into foam cells (3, 4). Discharge of the contents of foam cells, themselves marked constituents of fatty streaks (5), is thought to contribute to formation of atherosclerotic plaque (6). Monocytes and macrophages exhibit the ability to transform arachidonic acid to prostaglandins and related compounds via the enzyme prostaglandin endoperoxide synthase (PG G/H S). There are two forms of this enzyme (7, 8). Monocytes express PG G/H S-1 constitutively (9), and PG G/H S-2 is up-regulated in response to cytokines, growth factors, and bacterial lipopolysaccharide (LPS) (9-11). It is unknown what role, if any, prostanoids may play in atherogenesis, although they have been implicated in regulating cellular proliferation (12, 13), vascular tone and permeability (14, 15), and aspects of cholesterol metabolism (16). Additionally, to its susceptibility to enzyme-catalyzed metabolism to biologically active compounds, arachidonic acid may be subject to free radical attack, leading to formation of prostaglandin isomers in situ in the cell membrane phospholipid (17, 18). These isoprostanes may exert biological effects intra- or extracellularly. A prostaglandin F₂ₐ isomer, 8-epi-PGF₂ₐ, induces vasoconstriction and cellular proliferation, effects that are prevented by thromboxane A₂ receptor antagonists (19, 20). Recently, we have shown that this compound is also a minor product of PG G/H S-1 in human platelets (21). This study demonstrates that PG G/H S-2 may also form 8-epi-PGF₂ₐ. Interestingly, it is a more abundant product of this enzyme in monocytes than of the PG G/H S-1 isoform in platelets. Monocytes also retain the ability to form this compound in a free radical-catalyzed manner. Indeed, coinoculation of monocytes with LDL results in a time-dependent formation of 8-epi-PGF₂ₐ, coincident with LDL oxidation.

**EXPERIMENTAL PROCEDURES**

Materials—LPS derived from Escherichia coli 026:B6, dexamethasone, cycloheximide, aspirin, zymosan, concanavalin A, phorbol 12-myristate 13-acetate (PMA), superoxide dismutase, butylated hydroxytoluene (BHT), leupeptin, Nonidet P-40, soybean trypsin inhibitor, and aprotinin were purchased from Sigma. Hanks’ balanced salt solution, Dulbecco’s phosphate-buffered saline, RPMI 1640 medium, fetal calf serum, L-glutamine, penicillin, and streptomycin were purchased from Life Technologies, Inc. [³H]TxB₂ and [³H]PGE₂ were obtained from Cayman Chemical Co., Inc. (Ann Arbor, MI). The internal standard used for 8-epi-PGF₂ₐ was the [¹⁴C]-labeled compound derived from arachidonic acid.

The abbreviations used are: LDL, low density lipoprotein; PG G/H S-1 and S-2, prostaglandin endoperoxide synthase-1 and -2, respectively; LPS, lipopolysaccharide; 8-epi-PGF₂ₐ, 8-epiprostaglandin F₂ₐ; PMA, phorbol 12-myristate 13-acetate; BHT, butylated hydroxytoluene; PGE₂, prostaglandin E₂; TxB₂, thromboxane B₂; GC/MS, gas chromatography/mass spectrometry; TBARS, thiobarbituric acid-reactive substance(s).

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thentic 8-epi-PGF$_{2\alpha}$ (Cayman Chemical Co., Inc.) using the technique described by Pickett and Murphy (22). L 745,337 was kindly donated by Dr. Ian W. Rodger (Merck Frosst Canada Inc., Dorval, Quebec, Canada). Mouse polyclonal antibodies against PG G/H S-1 were a gift from Dr. W. L. Smith (Michigan State University). Mouse polyclonal antibodies prepared against the sequence of the carboxyl terminus (amino acids 580–598) of human PG G/H S-2 (COOH-NASSRSLGD-DINPTVLLK) were obtained as described previously (23) and kindly provided by Dr. J. Maclouf (INSERM, Unité 348, Paris, France).

Isolation and Stimulation of Human Monocytes—Mononuclear cells were obtained from fresh peripheral blood of healthy volunteers, who did not take any medication during the previous 2 weeks. Blood was subjected to Ficoll-Hypaque density gradient centrifugation, as described by Boyum (24). The mononuclear cell layer was recovered and washed with Hank's balanced salt solution. The cells were suspended in RPMI 1640 medium containing 10% inactivated fetal calf serum and EDTA for 15 min at 37°C to remove platelets specifically adherent to the monocytes. They were washed twice with Hank's balanced salt solution and resuspended at $1 \times 10^6$ ml in RPMI 1640 tissue culture medium supplemented with $\gamma$-glutamine (220 mg/ml) and antibiotics (streptomycin and penicillin). More than 94% of the cells were estimated to be viable based on trypan blue dye exclusion.

The cells were plated in 24-well multiplates and maintained at 37°C in a temperature-controlled, humidified 95% air, 5% CO$_2$ incubator. The nonadherent cells were removed by washing the plates twice with Dulbecco's phosphate-buffered saline after 2 h of incubation, and the adherent cells were maintained in RPMI 1640 medium enriched with 5% inactivated fetal calf serum, $\gamma$-glutamine, and antibiotics. The resultant harvested adherent cells routinely contained >92% monocytes as determined by morphology and staining for nonspecific esterase (25).

The contribution of PG G/H S-1 activity to eicosanoid production in response to the different stimuli was suppressed by pre-treating the blood with aspirin (10 $\mu$g/ml) in the sampling syringe and at time 0. This was confirmed by product analysis. Isolated monocytes were incubated for 1, 4, 8, and 24 h in the absence and presence of LPS (10 $\mu$g/ml), concanavalin A (10 $\mu$g/ml), or PMA (100 nM). Fresh medium containing 10 $\mu$g/ml arachidonic acid was added at each time point after removing the incubation medium; the supernatants were harvested after 30 min.

Concentrations of PGE$_2$, TxB$_2$, and 8-epi-PGF$_2\alpha$ were determined by gas chromatography/mass spectrometry (GC/MS) as described previously (21, 26). The contribution of induced PG G/H S-2 to formation of these products was elucidated by use of a protein synthesis inhibitor, cycloheximide (27); a specific inhibitor of this isoform of the enzyme, L 745,337 (28); or dexamethasone, which has previously been shown to down-regulate PG G/H S-2 (29).

GC/MS Analysis of PGE$_2$, TxB$_2$, and 8-Epi-PGF$_2\alpha$—Briefly, a 30-m DB-1 capillary column was used for analysis of all products. The temperature program was 190–320°C at 20°C/min. The ions monitored were $m/z$ 614 for TxB$_2$ and $m/z$ 618 for $[^{18}O]_{2}$TxB$_2$, $m/z$ 624 for PGE$_2$ and $m/z$ 628 for $[^{18}O]_{2}$PGE$_2$, and $m/z$ 695 for 8-epi-PGF$_2\alpha$ and $m/z$ 699 for 8-epi-$[^{18}O]_{2}$PGF$_2\alpha$.

Lipoprotein Preparation—LDL was prepared according to previously described methods that minimize oxidation and exposure to endotoxin (30). Each batch of LDL was assayed for endotoxin contamination by the Limulus amebocyte lysate assay. Final endotoxin contamination was always <0.02 unit/ml of LDL cholesterol. LDL was stored in 0.5 ml EDTA. Immediately before use, LDL was dialyzed at 4°C against phosphate-buffered saline without calcium or magnesium in the dark. LDL was used at final concentration of 0.4 mg of protein/ml.

LDL Incubation with Human Monocytes—Human monocytes were washed with RPMI 1640 medium without serum, plated into 12-well plates in RPMI 1640 medium containing 10% inactivated fetal calf serum and EDTA for 15 min at 37°C to remove platelets specifically adherent to the monocytes. They were washed twice with Hank's balanced salt solution and resuspended at $1 \times 10^6$ ml in RPMI 1640 tissue culture medium supplemented with $\gamma$-glutamine (220 mg/ml) and antibiotics (streptomycin and penicillin). More than 94% of the cells were estimated to be viable based on trypan blue dye exclusion.

The cells were plated in 24-well multiplates and maintained at 37°C in a temperature-controlled, humidified 95% air, 5% CO$_2$ incubator. The nonadherent cells were removed by washing the plates twice with Dulbecco's phosphate-buffered saline after 2 h of incubation, and the adherent cells were maintained in RPMI 1640 medium enriched with 5% inactivated fetal calf serum, $\gamma$-glutamine, and antibiotics. The resultant harvested adherent cells routinely contained >92% monocytes as determined by morphology and staining for nonspecific esterase (25).

The contribution of PG G/H S-1 activity to eicosanoid production in response to the different stimuli was suppressed by pre-treating the blood with aspirin (10 $\mu$g/ml) in the sampling syringe and at time 0. This was confirmed by product analysis. Isolated monocytes were incubated for 1, 4, 8, and 24 h in the absence and presence of LPS (10 $\mu$g/ml), concanavalin A (10 $\mu$g/ml), or PMA (100 nM). Fresh medium containing 10 $\mu$g/ml arachidonic acid was added at each time point after removing the incubation medium; the supernatants were harvested after 30 min.

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Lipoprotein Preparation—LDL was prepared according to previously described methods that minimize oxidation and exposure to endotoxin (30). Each batch of LDL was assayed for endotoxin contamination by the Limulus amebocyte lysate assay. Final endotoxin contamination was always <0.02 unit/ml of LDL cholesterol. LDL was stored in 0.5 ml EDTA. Immediately before use, LDL was dialyzed at 4°C against phosphate-buffered saline without calcium or magnesium in the dark. LDL was used at final concentration of 0.4 mg of protein/ml.

LDL Incubation with Human Monocytes—Human monocytes were washed with RPMI 1640 medium without serum, plated into 12-well tissue culture plates (1 $\times$ 10$^6$/ml/well), and co-cultured for 24 h with LDL in phosphate-buffered saline at the presence or absence of concanavalin A (2 mg/ml) according to the method of Johnston (31). LDL was extracted by adding 2 volumes of dichloro reagent (chloroform and methanol at a 2:1 ratio) and base-hydrolyzed (1.0 M KOH) before quantitation as described above to determine the total 8-epi-PGF$_{2\alpha}$ level in the supernatant.

Measurement of Lipid Peroxidation—Briefly, the presence of lipid oxidation products on LDL was detected spectrophotometrically by measuring the thiorbarbituric acid-reactive substance (TBARS) levels monitored at 532 nm (32). The lipid hydroperoxide levels were measured using the FOX 2 assay at 560 nm (33).

Western Blot Analysis—Isolated human monocytes were lysed in ice-cold buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1% Nonidet P-40, 1 mM EDTA, 10 nM soybean trypsin inhibitor, and 10 $\mu$g/ml aprotonin). The protein content was determined using a microbicincho-
TABLE I

Effects of dexamethasone, cycloheximide, and L 745,337 on PG G/H S-2-induced prostanoid production by human monocytes stimulated with LPS

| Conditions       | 8-Epi-PGF$_{2a}$ | PGE$_2$ | TxB$_2$ |
|------------------|------------------|---------|---------|
|                  | pg/ml            | pg/ml   | ng/ml   |
| LPS              | 155 ± 10         | 820 ± 10| 31 ± 2.5|
| LPS + dexamethasone | 10 ± 4          | 85 ± 10 | 1.1 ± 0.3|
| LPS + cycloheximide | 15 ± 4          | 90 ± 8  | 1.0 ± 0.2|
| LPS + L 745,337  | 12 ± 5           | 75 ± 10 | 1.4 ± 0.2|

* Dexamethasone (2 μM), cycloheximide (5 μM/mL), and L 745,337 (100 μM) were added at the same time as LPS (10 μg/mL). Prostanoid production in the supernatant was determined at the end of a 24-h incubation period (n = 4).

**DISCUSSION**

Generation of 8-Epi-PGF$_{2a}$ by Human Monocytes

Fig. 2. Western blot analysis of PG G/H S-2 protein expression in monocytes treated with LPS (10 μg/mL). Isolated human monocytes were incubated with saline for 24 h (control (C)), with LPS alone for 1, 4, 8, and 24 h; with LPS + dexamethasone (D) for 24 h; and with LPS + cycloheximide (C) for 24 h. The figure is representative of three experiments.

Both concanavalin A (Fig. 6A) and PMA (Fig. 6B) caused induction of PG G/H S-2 protein, which was suppressed, together with product formation, by cycloheximide and dexamethasone (Tables II and III). L 745,337 suppressed formation of concanavalin A (IC$_{50}$ = 40 ± 6 nM), and PMA (IC$_{50}$ = 35 ± 8 nM) stimulated formation of 8-epi-PGF$_{2a}$ along with that of the other eicosanoids. The induced PG G/H S-2 protein was again unaffected by L 745,337.

Free radical-catalyzed formation of 8-Epi-PGF$_{2a}$ by human monocytes—Activation of human monocytes with opsonized zymosan contrasted with the other stimuli in that it did not result in formation of 8-epi-PGF$_{2a}$, PGE$_2$, or TxB$_2$. Similarly, coincubation of monocytes with LDL, a source of substrate for lipid peroxidation (30), did not induce release of any of these products into the supernatant. Moreover, no significant changes in the levels of TBARS or lipid hydroperoxides were observed in human monocytes incubated with LDL in the absence of zymosan. This is in agreement with previous observations that activation is required for monocytes to oxidize LDL (34).

Activation of human monocytes with opsonized zymosan in the presence of LDL caused a marked time-dependent increase in 8-epi-PGF$_{2a}$, but not in PGE$_2$ or TxB$_2$. The increase in 8-epi-PGF$_{2a}$ was associated with an increase in TBARS and hydroperoxide levels (Table IV). This phenomenon was prevented by the oxygen free radical scavengers superoxide dismutase (300 units/ml) and BHT (20 μM), but not by L 745,337 (100 nM) (Fig. 7) or by the nonselective inhibitor of PG G/H S, aspirin (data not shown). The quantities of 8-epi-PGF$_{2a}$ formed under these conditions, in the presence of an excess of lipid (LDL) substrate, are not comparable with those formed in isolated stimulated human monocytes in the earlier experiments. In contrast to the data presented in Fig. 3, an array of peaks corresponding to F$_2$-isoprostanes was evident in the supernatants of zymosan-stimulated human monocytes incubated with LDL (Fig. 8, center panel). One of these corresponds to the retention time of the 8-epi-PGF$_{2a}$ standard (m/z 699) (Fig. 8, upper panel). Pretreatment with antioxidants suppressed the peaks, reflecting free radical-catalyzed isoprostane formation under these experimental conditions (Fig. 8, lower panel). The retention time of authentic PGF$_{2a}$ is well away from that of the F$_2$-isoprostanes. Finally, coincubation of zymosan-activated human monocytes with LDL did not result in induction of PG G/H S-2 protein (data not shown).

**FIG. 2. Western blot analysis of PG G/H S-2 protein expression in monocytes treated with LPS (10 μg/mL)**. Isolated human monocytes were incubated with saline for 24 h (control (C)), with LPS alone for 1, 4, 8, and 24 h; with LPS + dexamethasone (D) for 24 h; and with LPS + cycloheximide (C) for 24 h. The figure is representative of three experiments.

**FIG. 3. Selected ion monitoring of 8-epi-PGF$_{2a}$**. The upper panel shows a peak at m/z 699 corresponding to authentic 18O$_2$-labeled internal standard. The center panel shows a peak (m/z 695) observed during LPS-stimulated human monocytes, corresponding to the retention time of authentic 8-epi-PGF$_{2a}$. No other isoprostanes were present. The lower panel shows the suppression of that peak (m/z 695) when the sample was treated with cycloheximide.

**FIG. 4. Time course of eicosanoid formation in human monocytes stimulated with concanavalin A (10 μg/mL)**. The supernatant was assayed for prostanoid production by GC/MS at the indicated times. Values are reported as mean ± S.D. from five experiments.

**FIG. 5. Time course of eicosanoid formation in human monocytes stimulated with concanavalin A (10 μg/mL)**. The supernatant was assayed for prostanoid production by GC/MS at the indicated times. Values are reported as mean ± S.D. from five experiments.
associated with increased oxidant stress in vivo, including paracetamol and paraquat poisoning (35), cigarette smoking (36), and vascular reperfusion (37). We have previously shown that it is a minor product of the PG G/H S-1 expressed in human platelets (21). However, this route of formation appears to contribute trivially to overall 8-epi-PGF$_{2\alpha}$ biosynthesis, even in a setting such as chronic cigarette smoking (36), in which platelet activation (38) is thought to coincide with increased oxidant stress.

Human monocytes contain PG G/H S-2 additional to the isoform expressed in platelets. This form of the enzyme is highly regulated by cytokines, LPS, hormones, and mitogenic factors (9–11). It is thought to be the source of prostanooid production in inflammatory states (39) and, perhaps, in cancer (40). The primary sequences of both human enzymes exhibit 60% similarity (8, 41); however, mutational analysis suggests that the active site of PG G/H S-2 is the more accommodating.
For example, although aspirin is a relatively nondiscriminate PG G/H S inhibitor (42, 43), inhibition of PG G/H S-2, but not of PG G/H S-1, by aspirin is associated with increased production of (15R)-hydroxyeicosatetraenoic acid, coincident with inhibition of prostanoid formation (44). The present studies demonstrate that 8-epi-PGF$_2\alpha$ may be formed in a PG G/H S-2-dependent manner. Using three independent stimuli, formation coincided in time with the kinetics of the de novo synthesis of PG G/H S-2. Furthermore, reduction in activity of the induced enzyme, by either cycloheximide or steroids, prevented 8-epi-PGF$_2\alpha$ formation coincident with that of conventional PG G/H S-2 products of these cells, PGE$_2$, and thromboxane A$_2$. A selective PG G/H S-2 inhibitor suppressed generation of all three products without preventing synthesis of the enzyme. PG G/H S-1 was not induced during stimulation of 8-epi-PGF$_2\alpha$ generation in these experiments; thus, the contribution of the two enzymes to its production is clearly segregated.

Unlike the case with platelet PG G/H S-1, 8-epi-PGF$_2\alpha$ is formed in greater abundance relative to the conventional products of PG G/H S-2 in monocytes. This, whereas maximal 8-epi-PGF$_2\alpha$ formation in platelets is roughly $1/10_0$ that of TXB$_2$, maximal formation by PG G/H S-2 in monocytes is $1/4$ and $1/10_0$ of the corresponding production of PGE$_2$ and TXB$_2$, respectively, the most abundant conventional products of PG G/H S-2 in these cells. This observation raises the possibility that the mitogenic properties of 8-epi-PGF$_2\alpha$ (20) may contribute to the role of PG G/H S-2 activation in syndromes of vascular proliferation (45, 46). Selective inhibitors of this enzyme might be used in clarifying the utility of urinary 8-epi-PGF$_2\alpha$ as an index of free radical generation in syndromes putatively associated with oxidant stress, in which PG G/H S-2 induction is possible. Although enzyme-catalyzed formation seems to be a feature of 8-epi-PGF$_2\alpha$, but not of other F$_2$-isoprostanes, similar caution might be applied to estimates of “F$_2$-isoprostanes” (47), of which 8-epi-PGF$_2\alpha$ is an abundant constituent (48).

Monocytes are the first example of cells in which both enzyme- and free radical-catalyzed formation of 8-epi-PGF$_2\alpha$ have been demonstrated. Activation of monocytes with zymosan, in contrast to LPS, concanavalin A, or PMA, fails to induce PG G/H S-2 expression or 8-epi-PGF$_2\alpha$ generation. However, when coinubcation with LDL affords the availability of an abundant lipid substrate, zymosan-activated monocytes catalyze the formation of a substantial amount of 8-epi-PGF$_2\alpha$. This occurs coincident with production of TBARS and hydroperoxides, both indices of lipid oxidation, but not with either induction of PG G/H S-2 or generation of either PGE$_2$ or TXB$_2$. Under these circumstances, selective PG G/H S-2 inhibitors are ineffective in preventing 8-epi-PGF$_2\alpha$ formation. Rather, antioxidants, such as superoxide dismutase or BHT, inhibit its production. Incubation of human monocytes with LDL in the absence of zymosan activation fails to stimulate 8-epi-PGF$_2\alpha$ production.

Previous work has demonstrated that copper-induced oxidation of LDL (49) is associated with increased 8-epi-PGF$_2\alpha$ generation. Whether it or indeed other arachidonic acid products contribute to the role of monocytes and their cellular derivatives in the process of atherogenesis remains to be determined.

Fig. 8. Selected ion monitoring of 8-epi-PGF$_2\alpha$. The upper panel shows a peak at m/z 699 corresponding to authentic $^{13}$O$_2$-labeled internal standard. The center panel shows a peak (m/z 695) corresponding to the retention time of authentic 8-epi-PGF$_2\alpha$. The array of peaks seen in the chromatogram most likely corresponds to F$_2$-isoprostanes. The lower panel shows the reduction of the peaks (m/z 695) when the sample was incubated with butylated hydroxytoluene supporting the free radical-catalyzed origin of them.

REFERENCES

1. Ross, R. (1993) Nature 362, 801–809
2. Gerrity, R. G. (1983) Am. J. Physiol. 245, 181–190
3. Brown, M. S., and Goldstein, J. L. (1983) Annu. Rev. Biochem. 52, 223–261
4. Navab, M., Hough, G. P., Stevenson, L. W., Drinkwater, D. C., Laks, H., and Fogelman, A. M. (1988) J. Clin. Invest. 82, 1853–1863
5. Brown, M. S., and Goldstein, J. L. (1990) Nature 343, 508–509
6. Davies, M. J., Richardson, P. D., Woof, N., Katz, D. R., and Mann, J. (1993) Br. Heart J. 69, 377–381
7. Laneuville, O., Brucker, D. K., Xu, N., Huang, Z. H., Gage, D. A., Watson, J. T., Lagarde, M., DeWitt, D. L., and Smith, W. L. (1995) J. Biol. Chem. 270, 19330–19336
8. Funk, C. D., Funk, L. B., Kennedy, M. E., Pong, A. S., and FitzGerald, G. A. (1991) FASEB J. 5, 2304–2312
9. Fu, J. Y., Masferrer, J. L., Selbert, K., Raz, A., and Needleman, P. (1990) J. Biol. Chem. 265, 16737–16740
10. Lee, S. H., Soyoda, E., Channugum, P., Hart, S., Sun, W., Zhong, H., Liu, S., Simmons, D., and Hwang, D. (1992) J. Biol. Chem. 267, 25934–25938
11. O’ Sullivan, G. M., Chilton, F. H., Huggins, E. M., Jr., and McCaill, C. (1992) J. Biol. Chem. 267, 14547–14550
12. Chepfer, K. P., Diaz, A., and Jimenez, S. A. (1994) J. Biol. Chem. 269, 21786–21792
13. Ma, T. S., Zhang, L. M., Newman, W. H., and Meier, K. E. (1994) J. Biol. Chem. 269, 5693–5698
14. Sessa, W. C., and Nasjletti, A. (1990) Circ. Res. 66, 383–388
15. Seeger, W., Walter, H., Suttorp, N., Muhly, M., and Bhakdi, S. (1989) J. Clin. Invest. 84, 220–227
16. Sinzinger, H., Virgolini, I., Li, S. R., Gerakakis, A., Fitsch, P., and O’Grady, J. (1993) J. Cardiovasc. Pharmacol. 21, 503–506
17. O’Connor, D. E., Mihelich, E. D., and Coleman, M. C. (1984) J. Am. Chem. Soc. 106, 3577–3584
18. Morrow, J. D., Hill, K. E., Burkh, R. F., Nammour, T. M., Badr, K. F., and Roberts, L. J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3983–3987
19. Takahashi, K., Nannmour, T. M., Fukunaga, M., Ebert, J., Morrow, J. D., Roberts, L. J., Hoover, R. L., and Badr, K. F. (1992) J. Clin. Invest. 90, 136–141
20. Fukunaga, M., Makita, N., Roberts, L. J., Morrow, L. D., Takahashi, K., and Badr, K. F. (1993) Am. J. Physiol. 265, C1619–C1624
21. Praticò, D., Lawson, J. A., and FitzGerald, G. A. (1995) J. Biol. Chem. 270, 9980–9988
22. Pickert, W. C., and Murphy, R. C. (1981) Anal. Biochem. 111, 115–121
23. Habib, A., Cremoinon, C., Frobert, Y., Grassi, J., Padelles, P., and Madouf, J. (1993) J. Biol. Chem. 268, 23449–23454
24. Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, (suppl.) 97–98
25. Koski, I. R., Poplack, D. G., and Blaese, R. M. (1976) in In Vitro Methods in Cell-mediated and Tumor Immunity (Bloom, B. R., and David, J. R., eds) pp. 359–362, Academic Press, New York.
26. Knapp, H. R., Healy, C., Lawson, J. A., and FitzGerald, G. A. (1980) Thromb. Res. 10, 377–386
27. Hila, T., and Nelson, K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7384–7388
28. Chan, C. C., Boyco, S., Brideau, C., Ford-Hutchinson, A. W., Gordon, R., Guay,
29. O’Sullivan, M. G., Huggins, E. M., Jr., and McCaill, C. E. (1993) Biochem. Biophys. Res. Commun. 191, 1294–1300
30. Cathcart, M. K., Chisolm, G. M., McNally, A. K., and Morel, D. W. (1988) In Vitro Cell. & Dev. Bid 24, 1001–1008
31. Johnston, R. B. (1981) in Methods for Studying Mononuclear Phagocytes (Adams, D. O., Edelson, P. J., and Koren, H., eds) pp. 489–497, Academic Press, New York
32. Iuliano, L., Praticò, D., Ghiselli, L., Bonavita, M. S., and Violi, F. (1992) Lipids 27, 349–353
33. Nourooz-Zadeh, J., Taajaddini-Sarmandi, J., and Wolff, S. P. (1994) Anal. Biochem. 220, 403–409
34. Cathcart, M. K., McNally, A. K., Morel, D. W., and Chisolm, G. M. (1989) J. Immunol. 142, 1963–1989
35. Delanty, N., Reilly, M., Lawson, J. A., FitzGerald, D. J., and FitzGerald, G. A. (1996) Br. J. Clin. Pharmacol., in press
36. Reilly, M., Delanty, N., Lawson, J. A., and FitzGerald, G. A. (1996) Circulation, in press
37. Delanty, N., Reilly, M., Lawson, J. A., McCarthy, J., FitzGerald, D. J., and FitzGerald, G. A. (1996) Circulation, in press
38. Nowak, J., Murray, J. J., Oates, J. A., and FitzGerald, G. A. (1987) Circulation 76, 6–14
39. Madferrer, J. L., Zweifel, B. S., Manning, P. T., Hauser, S. D., Leahy, K. M., Smith, W. G., Isakson, P. C., and Seibert, K. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3228–3232
40. Ebener, C. E., Coffey, R. J., Radhika, A., Giardello, F. M., Ferrenbach, S., and Dubois, R. N. (1994) Gastroenterology 107, 1183–1188
41. Jones, D. A., Carlton, D. P., Mcintyre, T. M., Zimmerman, G. A., and Prescott, S. M. (1993) J. Biol. Chem. 268, 9049–9054
42. Laneuville, O., Breuer, D. K., DeWitt, D. L., Hla, T., Funk, C. D., and Smith, W. L. (1994) J. Pharmacol. Exp. Ther. 271, 927–934
43. Mitchell, J. A., Akarasereenont, P., Thiemermann, C., Flower, R. J., and Vane, J. R. (1994) Proc. Natl. Acad. Sci. U.S.A. 90, 11693–11697
44. Leconte, M., Laneuville, O., Ji, C., DeWitt, D. L., and Smith, W. L. (1994) J. Biol. Chem. 269, 13207–13215
45. Maier, J. A. M., Hla, T., and Maciag, T. (1990) J. Biol. Chem. 265, 10805–10808
46. Han, J. W., Sadowski, H., Young, D. A., and Macara, I. G. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 3373–3377
47. Morrow, J. D., Hill, K. E., Frei, B., Longmire, A. W., Gaziano, J. M., Lynch, S. M., Shyr, Y., Strauss, W. E., Oates, J. A., and Roberts, L. J. (1995) N. Engl. J. Med. 332, 1128–1203
48. Morrow, J. D., Minton, T. A., Badr, K. F., and Roberts, L. J., II (1994) Biochim. Biophys. Acta 1210, 244–248
49. Lynch, S. M., Morrow, J. D., Roberts, L. J., and Frei, B. (1994) J. Clin. Invest. 93, 988–1004