On Agents Favoring Prostaglandin F Formation during Biosynthesis[†]

James Amigo Chan, M. Nagasawa, C. Takeguchi, and Charles J. Sih*

ABSTRACT: The microsomal fraction of bovine vesicular gland catalyzed the conversion of eicosapolyenoic acids exclusively to prostaglandin E in the presence of reduced glutathione, while hydroxy fatty acids, prostaglandins D and F, decreased to a negligible level. After solubilizing the microsomal fraction with cutscum, the prostaglandin synthetase activity was purified 11-fold by batchwise absorption and elution of the enzyme activity from DEAE-cellulose. This partially purified enzyme fraction did not respond to reduced glutathione in promoting prostaglandin E formation at the expense of other products. A number of glutathione analogs were examined, but none of these was as effective as reduced glutathione. Dithiol complexes of Cu^{2+}, Ni^{2+}, and Zn^{2+} exerted pronounced effects on relative amounts of the different prostaglandins biosynthesized. Both the Cu^{2+}-dithiothreitol (2:1) complex and stannous chloride markedly enhanced prostaglandin F synthesis at the expense of prostaglandin D and prostaglandin E. The following reagents chemically reduced the endoperoxide in ascaridole to *p*-menth-2-ene-*cis*-1,4-diol: Cu^{2+}-dithiothreitol, Cu^{2+}-epinephrine, and stannous chloride. It is concluded that the enhancement of prostaglandin F formation caused by copper-dithiols and L-epinephrine is due to nonenzymatic reduction of prostaglandin G or prostaglandin H.

The concentrations and proportions of prostaglandin E and F vary widely among different tissues (Nugteren et al., 1966; Hamberg and Samuelsson, 1967). E and F type prostaglandins often exert opposite effects within the body (Flower, 1974) suggesting that physiological processes could be regulated by enzymes that interconvert the two types of prostaglandins or by enzymes or cofactors that preferentially favor the formation of E or F type prostaglandin from a common endoperoxide intermediate (Hamberg and Samuelsson, 1973).

It is well documented that prostaglandin E 9-ketoreductase activity is widely distributed among mammalian tissues (Hensby, 1974; Lee and Levine, 1974). However, at least in ovine and bovine seminal vesicular tissues, prostaglandin E and F were shown to be noninterconvertible (Hamberg and Samuelsson, 1967; Sih and Takeguchi, 1973), and to be biosynthesized from eicosapolyenoic acid via the breakdown of a common endoperoxide intermediate (Hamberg and Samuelsson, 1973; Miyamoto et al., 1974).

When *all-cis*-8,11,14-eicosatrienoic acid was incubated with ovine vesicular gland homogenates, a wide array of oxygenated products were found (Nugteren et al., 1966; Hamberg and Samuelsson, 1966). These products were characterized as 11-hydroxy-8-*cis*,12-*trans*,14-*cis*-eicosatrienoic acid, 15-hydroxy-8-*cis*,11-*cis*,13-*trans*-eicosatrienoic acid, 12-hydroxy-8-*trans*,10-*trans*-heptadecadienoic acid, malonaldehyde, 15-hydroperoxy-11α-hydroxy-9-oxo-13-prostenoic acid, 11α-hydroxy-9,15-dioxo-13-prostenoic acid, PGD$_1$, PGE$_1$, and PGF$_{1\alpha}$.[1] A variety of chemical compounds were shown to alter the biosynthetic ratios of PGE to PGF when added to the incubation mixture. When both GSH and hydroquinone were included in the incubation mixture, not only was the yield of PGE$_1$ markedly enhanced, but also hydroxy fatty acids and other prostaglandins decreased to a negligible level. The same product profile and response to GSH have also occurred in the bovine seminal vesicle system (Sih and Takeguchi, 1973). A bicyclo[2.2.1]heptene derivative, structurally analogous to the cyclic endoperoxide, was shown to inhibit PGE$_1$ formation by 35-52% when included in the sheep vesicular gland enzyme system, but PGF$_{1\alpha}$ formation was unaffected (Wlodawer et al., 1971). Conversely, the anti-inflammatory drug, benzydamine, inhibited the formation of PGF$_{2\alpha}$ and PGD$_2$ by enzymes of bovine vesicular glands, but potentiated the biosynthesis of PGE$_2$ by 150% (Flower, 1974). A selective enhancement of the biosynthesis of PGF at the expense of other prostaglandin-like material by copper-dithiol complexes was reported by Lee and Lands (1972). Depending on the cofactors used in the incubation mixture, the ratio of PGE/PGF could also be dramatically altered in the absence of GSH. For example, in the bovine vesicular gland system, the ratio of PGE/PGF was 11:1 with hydroquinine as the cofactor, but 1:1 with epinephrine (Sih et al., 1970).

Although it is generally surmised that PGF originates from the cyclic endoperoxide intermediate via reductive homolytic cleavage of the O-O bond, no definitive experimental evidence is yet available to indicate whether or not this reduction is enzyme catalyzed. The objective of this paper is to demonstrate that many factors reportedly favoring PGF formation during biosynthesis act by chemical rather than enzymic means.

Experimental Section

Materials. 5,8,11,14-Eicosatetraenoic acid (HP grade) was purchased from the Hormel Institute. *all-cis*-8,11,14-Eicosatrienoic acid (better than 85% chemical purity) was obtained from the Upjohn Co. through the courtesy of Dr. John Pike. Reduced glutathione (GSH), L-epinephrine (EPN), dithiothreitol, and Tris were products of Sigma. Ascaridole was purchased from K & K Labs. Arachidonic acid-*5,6,8,9,11,12,14,15-*^{3}*H* (11.4 Ci/mmol) and *all-cis*-

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[†] From the School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706. *Received October 15, 1974.* This investigation was supported in part by Research Grant AM-09688 of the National Institutes of Health.

[1] Abbreviations used are: PGD$_1$, 9α,15α-dihydroxy-11-oxo-13-*trans*-prostenoic acid; PGD$_2$, 9α,15α-dihydroxy-11-oxo-5-*cis*,13-*trans*-prostadienoic acid; PGE$_1$, 11α,15α-dihydroxy-9-oxo-13-*trans*-prostenoic acid; PGE$_2$, 11α,15α-dihydroxy-9-oxo-5-*cis*,13-*trans*-prostadienoic acid; PGF$_{1\alpha}$, 9α,11α,15α-trihydroxy-13-*trans*-prostenoic acid; PGF$_{2\alpha}$, 9α,11α,15α-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid; PGA$_1$, 15α-hydroxy-9-oxo-10,13-*trans*-prostadienoic acid; PGA$_2$, 15α-hydroxy-9-oxo-5-*cis*,10,13-*trans*-prostadienoic acid; PGG$_2$, 15-hydroperoxy-9α,11α-peroxidoprosta-5,13-dienoic acid; PGH$_2$, 15-hydroxy-9α,11α-peroxidoprosta-5,13-dienoic acid; GSH, reduced glutathione; EPN, L-epinephrine.

8,11,14-eicosatrienoic acid-*1*-^{14}C (58.8 Ci/mmol) were products of New England Nuclear Corp. Analytical precoated layers of silica gel F-254 glass plates for thin-layer chromatography (TLC) and silica gel (MN-Kiesegel, 70–270 mesh) were products of Brinkmann. Cutscum was a product of Fischer Scientific Co. All solvents were of reagent grade and were redistilled.

Melting points, determined on a Thomas-Hoover melting point apparatus, are uncorrected. Infrared spectra were taken on a Perkin-Elmer 257 grating infrared spectrophotometer. Mass spectra were obtained with a Model 1015 Finnigan quadrupole mass spectrometer using direct probe introduction with an ion source temperature of 30°, electron potential of 70 eV, and an ionizing current of 250 μA. Proton magnetic resonance spectra were determined on a Varian EM 360 spectrometer at 60 Mcycles in deuterated chloroform using Me_4Si as an internal standard. TLC plates were developed in a solvent system consisting of ethyl acetate–acetic acid–isooctane–water (110:20:50:100, v/v), and the relative mobilities were: $PGF_{1\alpha}$ and $PGF_{2\alpha}$ = 0.22; PGE_1 and PGE_2 = 0.33; PGD_1 and PGD_2 = 0.40; PGA_1 and PGA_2 = 0.59. The prostaglandins were visualized by spraying with 3% ceric sulfate in 3 *N* H_2SO_4 and heating to 110° for a few minutes. Silicic acid powder (Mallinckrodt 2847; 100 mesh), mixed with 15% Celite, was used for column chromatography. Bovine seminal vesicle microsomes were prepared according to the method of Takeguchi et al. (1971). The tritiated arachidonic acid was purified on a 1 × 30 cm silicic acid–Celite column using a 7% diethyl ether–Skelly B elution system (Sih and Takeguchi, 1973). The dipeptide, γ-glutamylcysteine (γ-Glu-Cys), was a gift from Professor E. P. Abraham. Desulfurized glutathione (GH) was prepared by incubating GSH in 0.3 *N* $Ba(OH)_2$ in the presence of neutral $Pb(OAc)_2$ (Hopkins, 1929). Acetylglutathione (GS-AC) was prepared by reaction of GSH with acylthiophenol in methanol (Stadtman, 1957). Protein concentrations were estimated by the biuret method (Gornall et al., 1949) and the Lowry method (Lowry et al., 1951) using crystalline bovine serum albumin as standard.

Radioactive Assay. Unless otherwise stated, the incubation system contained 0.33 m*M* arachidonic acid (2 μCi of tritium), 1 m*M* of EPN as cofactor, 1 m*M* GSH (when specified), and 10 mg of microsomes in 10 ml of 0.05 *M* Tris buffer (pH 8.3). The reaction mixture was incubated for 30 or 60 min at 37°. After acidification to pH 2.0, the reaction mixture was extracted five times with ethyl acetate. The ethyl acetate layer was dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed over a silicic acid–Celite (85:15) column (1 × 17 cm). The radioactive PG peaks were combined, and their radioactivities were quantitatively determined in a Packard scintillation counter. Recovery of radioactivity was generally on the order of 70%. The identity of the PGS was confirmed by cochromatography with known standards on TLC plates and developed in the ethyl acetate–acetic acid–isooctane–water (110:20:50:100, v/v) solvent system.

Enzyme Purification. All operations were performed at 4°. Three grams of lyophilized microsomes were triturated with 150 ml of 0.05 *M* Tris buffer (pH 8.3) containing 1% cutscum and was stirred for 30 min. The suspension was then centrifuged for 105,000*g* for 30 min. Sufficient ammonium sulfate was added to the supernatant solution (140 ml) to bring the concentration to 30% saturation with constant stirring. The pH was maintained to 7.0 by the addition of 5 *N* NH_4OH. After standing for 20 min, this mixture was centrifuged for 5 min at 4000*g*, and the precipitate was discarded. Sufficient ammonium sulfate was then added to increase the concentration to 60% saturation. After it had stood for 20 min, the suspension was again centrifuged for 5 min at 4000*g*. The residue was dissolved in 30 ml of 0.05 *M* Tris buffer (pH 8.3) and was desalted by passage through a Sephadex G-25 column (2.5 × 30 cm). The enzyme activity, resided in the first 75 ml of the effluent (0.05 *M* Tris buffer, pH 8.3), was adsorbed on 5 g of DEAE-cellulose (0.92 mequiv/g) equilibrated in 0.05 *M* Tris buffer (pH 8.3). After centrifugation at 1000*g* for 5 min, the DEAE-cellulose was collected and washed with 30 ml of 0.05 *M* Tris buffer (pH 8.3) and the suspension was centrifuged again at 1000*g* for 5 min. The enzyme activity was then eluted from the DEAE-cellulose with 100 ml of 0.05 *M* Tris buffer (pH 8.3) containing 0.1 *M* KCl.

Lithium Aluminum Hydride Reduction of Ascaridole. Ascaridole (500 mg, 2.98 mmol) in 5 ml of diethyl ether (dried over $LiAlH_4$) was added to a suspension of $LiAlH_4$ (76 mg, 2 mmol) in 10 ml of dry ether and refluxed for 16 hr. The excess $LiAlH_4$ was destroyed by the addition of water and the aqueous layer was extracted with ether. Evaporation of the combined ether layers gave 250 mg of crystalline solids, which upon recrystallization from chloroform–petroleum ether gave 200 mg (39.4% yield) of white *p*-menth-2-ene-*cis*-1,4-diol crystals, mp 82–83° (reported value 82°, Paget, 1938); NMR ($CDCl_3$, 60 MHz) δ 0.9 (m, 6 H, isopropyl), 1.2 (s, 3 H, CH_3), 1.7 (m, 5 H, methylenes and methines), 5.6 ppm (q, 2 H, olefinic); ir ($CHCl_3$) 3400 cm^{-1} (OH). Mass spectrum gave *m/e* 155 ($M - CH_3$), 137 ($M - CH_3 + H_2O$), and 127 ($M - (CH_3)_2CH-$).

Reduction of Ascaridole with $SnCl_2$ and EPN. $SnCl_2$ (1.9 g, 8.3 mmol) was dissolved in 250 ml of Tris buffer (0.05 *M*, pH 8.3); EPN (1.5 g, 8.3 mmol) was dissolved in a minimal amount of 2 *N* HCl and then diluted with 500 ml of 0.05 *M* Tris buffer (pH 8.3). The $SnCl_2$ and EPN solutions were added to a suspension of ascaridole (500 mg, 2.98 mmol) in 250 ml of 0.05 *M* Tris buffer (pH 8.3) and the reaction mixture was incubated at 37° in air for 2 hr. The reaction mixture was then extracted three times with 200-ml portions of ethyl acetate; the ethyl acetate layer was washed with water and dried over Na_2SO_4. Evaporation of the solvent afforded 245 mg of an oily mixture. The oily residue was applied onto a silica gel (MN-Kieselgel) column (2.2 × 15 cm) and was washed with 200 ml of benzene–ethyl acetate (7:3). The column was then eluted with a gradient system consisting of 200 ml of benzene–ethyl acetate (7:3) in the mixing flask and 200 ml of benzene–ethyl acetate (4:6) in the reservoir flask and 5-ml fractions were collected. Fractions 60–80 were pooled to give 175 mg of crystalline *p*-menth-2-ene-*cis*-1,4-diol. Recrystallization from chloroform–petroleum ether gave a sample, mp 82–83°, whose infrared, NMR, and mass spectra were identical with a sample of *p*-menth-2-ene-*cis*-1,4-diol, prepared by $LiAlH_4$ reduction; mixture melting point gave no depression (82–83°).

In separate experiments, ascaridole (1.49 mmol, 250 mg) was incubated in the same manner with: (a) dithiothreitol (4.15 mmol, 635 mg), $CuSO_4 \cdot 5H_2O$ (2.15 mmol, 520 mg), Tris buffer (500 ml); (b) ascaridole (0.745 mmol, 125 mg), dithiothreitol (2.07 mmol, 317 mg), $CuSO_4 \cdot 5H_2O$ (1.07 mmol, 260 mg), EPN (2.07 mmol, 379 mg), Tris buffer (500 ml); and (c) $CuSO_4 \cdot 5H_2O$ (1.07 mmol, 260 mg), EPN (2.07 mmol, 379 mg), Tris buffer (500 ml). The yield of *p*-menth-2-ene-*cis*-1,4-diol is summarized in Table V.

Table I: Partial Purification of Prostaglandin Synthetase.[a]

Fraction	Protein (mg)	Total Activity (units)	Specific Activity (units/mg)	Recovery
Microsome	2040	825	0.40	100
Sephadex G-25 eluate	512	335	0.65	41
Batchwise DEAE eluate	78	330	4.30	40

[a] The incubation and radioactive assay were described under the Experimental Section. One unit of enzyme activity is defined as the amount of enzyme producing 1 mμmol of PG/min.

Table II: The Effect of GSH on Partially Purified PG Synthetase.[a]

Enzyme	PGA_2	PGD_2	PGE_2	$PGF_{2\alpha}$	Total PG (μmol)
G-25	0.021	0.044	0.175	0.319	0.56 (17%)
G-25 + GSH		0.052	0.901	0.119	1.07 (32%)
DEAE		0.055	0.122	0.246	0.42 (13%)
DEAE + GSH		0.099	0.125	0.440	0.66 (20%)

[a] The incubation and radioactive assay of PGs are described under the Experimental Section except 62.2 and 15.2 mg of proteins of G-25 and DEAE fractions, respectively, were used. The reaction mixture (10 ml) was incubated for 30 min in air at 37°.

Results and Discussion

Direct evidence for the formation of an endoperoxide during prostaglandin biosynthesis was recently obtained by Hamberg and Samuelsson (1973) and Nugteren and Hazelhof (1973). Two prostaglandin endoperoxides were isolated and characterized as 15-hydroperoxy-9α,11α-peroxidoprosta-5,13-dienoic acid (PGG_2) and 15-hydroxy-9α,11α-peroxidoprosta-5,13-dienoic acid (PGH_2); both of these were readily reducible to $PGF_{2\alpha}$ by $SnCl_2$ (Hamberg et al., 1974). A series of enzymes was found to be required for the conversion of PGG_2 into the stable prostaglandins: glutathione peroxidase catalyzes the transformation of PGG_2 into PGH_2; PGD_2 isomerase catalyzes the conversion of PGH_2 into PGD_2 and PGE_2 isomerase, a GSH requiring enzyme catalyzes the conversion of PGH_2 into PGE_2. As yet it is uncertain whether or not the formation of $PGF_{2\alpha}$ from PGH_2 is enzyme catalyzed (Nugteren and Hazelhof, 1973). Thus, the levels of these enzymes may determine the type and relative amounts of the primary prostaglandins in various tissues.

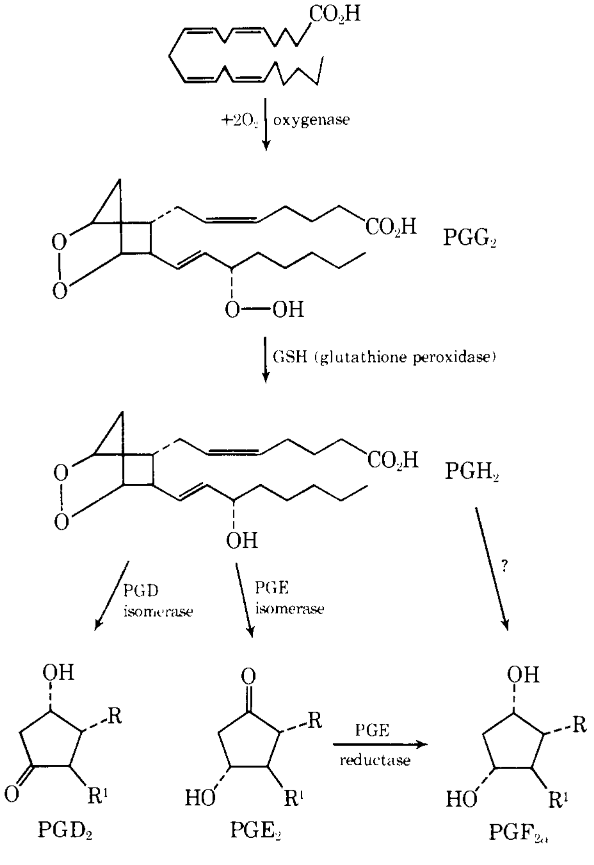


Table III: Effect of Glutathione Analogs on PG Synthetase Activity.[a]

Additions	PGA_2	PGD_2	PGE_2	$PGF_{2\alpha}$	Total (μmol)
None	0.02	0.12	0.60	0.31	1.05 (32%)
GSH	0.07		1.4	0.09	1.56 (47%)
GH	0.09	0.16	0.46	0.53	1.24 (38%)
GSAc	0.04	0.13	0.39	0.29	0.85 (26%)
γ-Glu-Cys		0.23	0.94	0.20	1.37 (42%)

[a] The incubation and radioactive assay of PGs are described under the Experimental Section. The reaction mixtures (10 ml) were incubated for 30 min in air at 37°.

An 11-fold purification of prostaglandin synthetase activity may be readily achieved by the treatment of bovine seminal vesicular microsomes with cutscum; chromatography of the supernatant fraction over a Sephadex G-25 column; and batchwise adsorption and elution of the enzyme activity from DEAE-cellulose (Table I). The behavior of the G-25 enzyme fraction was similar to that of the intact microsomes, in that upon the addition of GSH there was an enhancement of PGE_2 synthesis; however, this occurred at the expense of other prostaglandins and other compounds. The DEAE-enzyme fraction, on the other hand, failed to respond to GSH in the above manner (Table II); instead, there was an enhancement of $PGF_{2\alpha}$ formation when GSH was added.

Recently, Miyamoto et al. (1974) reported the solubilization of the bovine prostaglandin synthetase system using Tween-20 in the presence of ethylene glycol. DEAE-cellulose column chromatography separated the prostaglandin synthetase into two fractions both of which were required for PGE_1 synthesis. When fraction 1 alone was incubated with 8,11,14-eicosatrienoic acid, an unstable compound presumed to be PGH_1 or PGG_1 accumulated. This unstable compound was converted into PGE_1 by the addition of fraction 2, or to $PGF_{1\alpha}$ with stannous chloride, or decomposed into PGD_1, PGE_1, and $PGF_{1\alpha}$ at room temperature.

One plausible explanation is that our DEAE-enzyme fraction has lost considerable amounts of PGE isomerase, a GSH-requiring enzyme; in its absence, nonenzymatic reduction of prostaglandin cyclic endoperoxide by metal complexes of GSH becomes competitive resulting in an increase in formation of PGF.

Table III shows that none of the synthetic glutathione analogs were as effective as GSH in favoring PGE_2 biosynthesis accompanied by a concomitant decrease in the formation of hydroxy fatty acids and other prostaglandins (Nugteren et al., 1966). GH appeared to yield more $PGF_{2\alpha}$ whereas γ-Glu-Cys afforded more PGE_2 and PGD_2. These experiments confirm the specificity of GSH and suggest

Table IV: Effect of Metal Chelates on the Ratios of PGs Biosynthesized.[a]

Additions	PGA$_2$	PGD$_2$	PGE$_2$	PGF$_{2\alpha}$	Total PG (μmol)
None	0.07	0.17	0.39	0.26	0.89 (27%)
DTT		0.11	0.14	0.11	0.36 (11%)
Cu^{2+}		0.04	0.15	0.14	0.33 (10%)
Cu^{2+}/DTT		0.02	0.04	0.86	0.92 (28%)
Ni^{2+}	0.04	0.08	0.16	0.12	0.40 (12%)
Ni^{2+}/DTT	0.01	0.01	0.02	0.22	0.26 (8%)
Zn^{2+}	0.03	0.03	0.08	0.23	0.37 (11%)
ZN^{2+}/DTT	0.05	0.16	0.32	0.12	0.65 (20%)

[a] The incubation and radioactive assay of PGs are described under the Experimental Section except metal ion concentrations were 5×10^{-4} *M* and dithiothreitol (DTT) concentration was 1×10^{-3} *M*. The reaction mixture (10 ml) was incubated in air for 30 min at 37°.

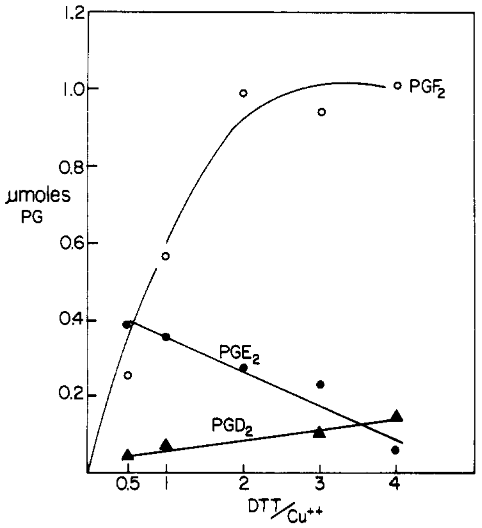


FIGURE 1: Effect of dithiothreitol (DDT)/Cu^{2+} ratios on PG biosynthesis. The reaction mixture contained 1×10^{-3}*M* cupric acetate and varying amounts of DTT. The PGs were estimated by the radioactive assay as described under the Experimental Section.

that both the SH group and the intact tripeptide structure are required for maximal stimulation.

Lee and Lands (1972) first demonstrated a selective enhancement of the biosynthesis of prostaglandin F at the expense of other prostaglandin-like materials when cupric ions and copper-dithiol complexes were added to sheep vesicular preparations. These authors raised the possibility of an enzyme-catalyzed reduction and that copper-dithiols serve as a means of enzyme regulation between E and F type hormones in vivo. Also, it was suggested that copper participated in a process which led to inactivation of PGE$_2$ synthetase with a concomitant activation of PGF$_{2\alpha}$ synthetase (Maddox, 1973).

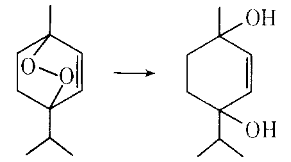
The effect of metal-dithiol chelates on the biosynthesis of prostaglandins in the bovine seminal vesicle microsomal system is illustrated in Table IV. Of the transition elements, it was found that only dithiol complexes of Cu^{2+}, Ni^{2+}, and Zn^{2+} exerted a profound effect on the relative amounts of prostaglandins and other products formed. Either the metal or dithiothreitol alone inhibited the biosynthesis of prostaglandins. The Ni^{2+}-dithiol chelate was inhibitory but afforded predominantly PGF$_{2\alpha}$, whereas the Zn-dithiothreitol complex stimulated the formation of PGE$_2$. The most pronounced effect was observed with the Cu^{2+}-dithiothreitol complex, which markedly stimulated the formation of PGF$_{2\alpha}$ at the expense of PGD$_2$ and PGE$_2$. Maximal PGF$_{2\alpha}$ formation was obtained when the ratio of dithiothreitol to cupric ions was 2:1 or greater (Figure 1).

Table V: Reduction of Ascaridole by Various Reagents.[a]

Reagents	Yield (%) *p*-menth-2-ene-*cis*-1,4-diol
SnCl$_2$	20
SnCl$_2$; EPN	34
DTT; CuSO$_4$	11
CuSO$_4$; EPN	10
DTT; CuSO$_4$; EPN	27

[a] The incubation conditions are described under the Experimental Section.

Other factors may also alter prostaglandin ratios during biosynthesis. For example, the ratio of PGD$_2$, PGE$_2$, and PGF$_{2\alpha}$ obtained with hydroquinone as cofactor was 3:11:1, respectively, but changed to 3:13:12 with EPN (Sih et al., 1970). To determine whether these agents which favor the production of PGF during biosynthesis act by chemical or enzymic means, we investigated the effect of the Cu^{2+} complexes of dithiothreitol and EPN (or its subsequent oxidation products) on the cyclic endoperoxide of ascaridole. The results in Table V clearly demonstrate that the Cu^{2+}-dithiothreitol and the Cu^{2+}-EPN (or its subsequent oxidation products) complexes are capable of catalyzing the reductive cleavage of the cyclic endoperoxide of ascaridole to the *cis*-1,4-diol; an additive effect was noted when both complexes were present together. EPN, dithiothreitol, or cupric ions alone were incapable of catalyzing this conversion. Either SnCl$_2$ alone or in combination with EPN was even more efficient in catalyzing this reductive transformation. Identification of the product, *p*-menth-2-ene-*cis*-1,4-diol, was made by comparison with an authentic sample obtained by LiAlH$_4$ reduction of ascaridole (Paget, 1938). The infrared,



nuclear magnetic resonance, and mass spectra of the two samples were identical and mixture melting point determination gave no depression (mp 82-83°).

Our present results strongly indicate that the increase in PGF formation caused by EPN and copper-dithiols is not due to their inherent abilities to serve as cofactors but rather to nonenzymatic reduction of PG endoperoxide(s) by their respective cupric complexes. The 5,6-dioxabicyclo[2.2.2]octene-2 system in ascaridole is quite stable at room temperature and undergoes decomposition only at temperatures about 130-150° (Gollnick and Schenk, 1967). In contrast, the more strained 5,6-dioxabicyclo[2.1.2]heptene-2 system is considerably less stable as indicated by the half-lives of PGH$_2$, which is about 5 min in aqueous medium and 2.7 hr at 20° in nonpolar organic solvents. Thus, when these cupric complexes are included in the incubation mixture, they no doubt compete with PGE and PGD isomerases for PGG and PGH by reducing them to PGF. The relative amounts of PGD, PGE, and PGF would depend on the level of these isomerases present and the effectiveness of the particular cupric complex. This ex-

Table VI: Effect of $SnCl_2$ on PG Biosynthesis from 8,11,14-Eicosatrienoic Acid.[a]

	(μmol)				
Additions	PGA_1	PGD_1	PGE_1	$PGF_{1\alpha}$	Total PGs
EPN	0.05	0.13	0.35	0.24	0.77 (23%)
DTT; Cu^{2+}; EPN		0.01	0.06	0.78	0.85 (26%)
Sn^{2+}; EPN		0.01	0.04	0.88	0.93 (28%)

[a] The incubations and radioactive assay of PGs are described under the Experimental Section except 0.33 m*M* *all-cis*-8,11,14-eicosatrienoic acid (containing 1 μCi of *all-cis*-8,11,14-eicosatrienoic acid-1-^{14}C) was substituted for arachidonic acid.

planation is consistent with our inability to detect any accumulation of PGH or PGG in incubations with EPN as the cofactor. Miyamoto et al. (1974) was able to accumulate the unstable endoperoxide intermediate using L-tryptophan as the cofactor, which apparently did not decompose PGH or PGG. It is interesting to note that some years ago, we isolated substantial quantities of L-tryptophan from the supernatant fraction of bovine vesicular gland homogenates (P. Foss and C. J. Sih (1970), unpublished results). It is possible that L-tryptophan may be the natural cofactor in the bovine vesicular gland.

In the presence of GSH, the PGE isomerase operates at a rapid rate, giving an excess of PGE; in its absence, or in the presence of PGE isomerase inhibitors such as the bicyclo-[2.2.1]heptene derivatives (Wlodawer et al., 1971), the rate of PGE isomerase reaction is slowed down, permitting the cupric complexes to reduce PGG and PGH to PGF. This rationalization is further supported by the experiment with $SnCl_2$, a well-established endoperoxide reducing agent (Hamberg and Samuelsson, 1973), which very effectively enhanced $PGF_{1\alpha}$ formation at the expense of other products and prostaglandins when it was added in the incubation mixture (Table VI).

In the absence of definitive evidence for the presence of a reductase and the ease and efficiency with which cyclic endoperoxides are reduced by metal complexes of organic compounds, this raises the question as to whether or not any PGF formation from the cyclic endoperoxide is enzymatically catalyzed. To date, the only clearly defined pathway for PGF formation is via reduction of PGE, catalyzed by prostaglandin E 9-ketoreductase. Thus, the current conjecture of the mode of formation of PGF is somewhat akin to that of PGA.

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