The Free Radical Formed during the Hydroperoxide-mediated Deactivation of Ram Seminal Vesicles Is Hemoprotein-derived*  

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Prostaglandin synthase is a multi-enzyme complex which catalyzes the oxygenation of arachidonic acid to the various prostaglandins. During the oxygenation, the enzyme is self-deactivated and, on the basis of ESR data, it has been proposed to form a self-destructive free radical. The free radical was suggested to form from the oxygen lost from prostaglandin G₂ during its reduction to prostaglandin H₂, and the destructive species was thought to be an oxygen-centered free radical, tentatively identified as the hydroxy radical. We have reinvestigated this ESR signal (g = 2.005) and have concluded, with the aid of the known ESR parameters for the hydroxy and other oxygen-centered free radicals, that the free radical formed during the oxygenation is neither a hydroxy nor any known oxygen-centered radical.

Prostaglandin synthase is thought to be a hemoprotein, so this unknown ESR signal was compared with the previously observed free radical formed by the reaction of H₂O₂ with methemoglobin. This comparison indicates that the free radical formed by the reaction of prostaglandin G₂ with ram seminal vesicles is hemoprotein-derived and may be formed by the oxidation of an amino acid(s) located near the iron of the heme.

A wide variety of compounds are co-oxidized during the biosynthesis of prostaglandins (1–4). Efforts to identify the oxidant initiating the co-oxidation of substrates have not been definitive. A free radical mechanism of co-oxidation proposes that a free radical generated as a result of the reaction of prostaglandin synthase with PGG₂ could be responsible for the co-oxidation (1, 4–6). The involvement of the superoxide anion as the oxidizing species appeared unlikely, because superoxide dismutase had no effect on the co-oxidation reactions (1) or on prostaglandin synthesis (1, 7). Neither does singlet oxygen appear to play a role in co-oxidation (1, 7).

Inhibition studies of luminol chemiluminescence in vesicular gland incubations indicate the possible involvement of the hydroxyl radical (1, 8).

The only ESR evidence concerning the nature of the oxidant originates from studies reported in 1976 (9). Using ESR spectroscopy these investigators detected a free radical signal in incubations containing ram seminal vesicle microsomes and arachidonic acid in air, but not in a nitrogen atmosphere; the same radical was detected in incubations containing micromoles and PGG₂, in both air and nitrogen atmospheres. Both phenol and methional (presumed oxygen-centered radical scavengers) inhibited the ESR signal intensity. It was concluded that the free radical obtained during arachidonic acid and PGG₂ metabolism by ram seminal vesicles is an oxygen-centered species, and that this oxygen-centered free radical caused the irreversible deactivation of cyclo-oxygenase; phenol and methional increased the lifetime of the cyclooxygenase by scavenging the oxygen-centered radical. The observation of the ESR signal from the reaction of PGG₂ with ram seminal vesicle microsomes originated the concept that the oxygen-centered radical [O₂] was released during the reduction of the 15-hydroperoxy moiety present in PGG₂ to the hydroxy moiety in PGH₂ (9).

The same ESR signal, with varying intensity, was observed during the reduction of PGG₂ to PGH₂, 15-hydroperoxy PGE₂, to PGE₆, and 15-hydroxy-5,8,11,13-eicosatetraenoic acid to 15-hydroxy-5,8,11,13-eicosatetraenoic acid by the ram seminal vesicle microsomes (5, 6). The same peroxidase activity would even reduce H₂O₂, concomitantly producing the ESR signal. The HO· radical may be formed from H₂O₂, so this result supports the identification of the HO· radical as the oxidant [O₂]. A wide variety of co-substrates stimulated the hydroperoxide reduction. All the substrates that stimulated the hydroperoxide reduction depressed the ESR signal (5, 6).

It was concluded that the oxygen-centered radical released during the hydroperoxide reduction is responsible for substrate co-oxidation, and substrates such as phenol, aminopyrine, and iodide protect the enzyme from deactivation by preferentially scavenging the oxygen-centered radicals (5, 6).

Perhaps the strongest evidence that the oxidant released during the conversion of PGG₂ to PGH₂ is responsible for co-oxidation comes from experiment of the co-oxidization of sulindac sulfide (10). Sulindac sulfide is oxidized to its corresponding sulfoxide during the peroxidase-catalyzed reduction of a variety of hydroperoxides. By utilizing the¹⁸O-labeled 15-hydroperoxy-PG₂ as an oxidizing substrate, it was shown that the oxygen atom transferred to sulindac sulfide originated from the hydroperoxide, 15-hydroperoxy-PG₂ (10). Such an O–O scission was interpreted to favor the identification of the oxidant as a hydroxy radical (HO·) (6).

The same group of workers suggested that the oxidant, possibly HO· or HO₂, resulting from the hydroperoxide metabolism by ram seminal vesicle microsomes, is capable of destroying prostacyclin synthase (11). Thromboxane A₂ synthase is not affected by the oxidant. Since it is well known that the balance of the inhibitors, namely thromboxane A₂ and prostacyclin, plays a critical regulatory role in biological processes.
processes, the investigators proposed that the oxidant (HO* or RO*) released during the initial stage of arachidonic acid cascade may play a vital role in the understanding of inflammatory and other pathological processes (11). In essence, the Merck group has shown that the nascent oxidizing agent, an oxygen-centered species which is suggested to be the hydroxy radical (6, 9), is responsible for the deactivation of the cyclo-oxygenase (9), the peroxidase component of prostaglandin synthase (5), the co-oxidation of substrates (5, 6), and the deactivation of prostacyclin synthase (11). Thus, in order to understand fully these biochemical and biological processes, it is imperative to know the exact nature of the highly reactive oxidant.

In this paper, we have reinvestigated the free radical formed during the reaction of ram seminal vesicles with PGG<sub>2</sub>. Comparison of the g-value obtained from the ESR spectrum of this free radical with the known g-values of oxygen-centered radicals proves that the free radical released during arachidonic or PGG<sub>2</sub> metabolism by ram seminal vesicle microsomes is not a known oxygen-centered species and is certainly not a hydroxy or an alkoxy radical. We propose that the free radical is derived from the enzyme and in this respect is quite similar to the species obtained from the oxidation of methemoglobin (12, 13) or metmyoglobin (13-15) with hydrogen peroxide.

**MATERIALS AND METHODS**

Arachidonic acid was purchased from Nuchek, and indomethacin, methemoglobin, and tryptophan were obtained from Sigma. All other chemicals were of reagent grade and obtained from standard suppliers. Ram seminal vesicles were obtained from a local slaughterhouse and stored at −80°C. The microsomal protein was prepared from ram seminal vesicles as described previously (16), stored at −80°C and used within 1 week.

Oxygen uptake studies were carried out with an oxygen electrode (Yellow Springs Instrument). The stimulatory effects of the peroxidase substrates were determined from the increase in slopes of the oxygen uptake curves obtained upon the addition of arachidonic acid (400 μM) in ethanol (0.25% of total volume) to 1.2 ml of Tris buffer (air saturated, pH 7.5) containing 2.0 mg/ml of microsomal protein.

ESR measurements were made with a Varian Century series E-109 spectrometer equipped with a TM<sub>100</sub> cavity. The time-swept ESR signal was obtained by adjusting the magnetic field to the maximum resonance of the ESR signal and recording the signal with the magnetic field sweep switched off, using the instrument's time-based recorder. Low temperature ESR spectra were obtained with the standard Varian variable temperature accessory or liquid nitrogen dewar. For the low temperature work, the incubation mixtures were transferred to a 3-mm cylindrical quartz tube, freeze-quenched within 20 s, and microwave power 60 milliwatts.

**RESULTS**

The ESR spectrum of the free radical formed in an incubation mixture containing ram seminal vesicle microsomes and arachidonic acid was obtained according to published procedures (5, 9). If such an incubation is frozen in a 3-mm quartz sample tube containing a capillary of DPPH dispersed in KCl, the spectrum in Fig. 1 results. The g-value of the broad spectrum so obtained is clearly very near the g-value of DPPH (2.0037). More accurate g-values of this unknown free radical were obtained by means of a dual cavity using Fremy’s salt as a g-standard (17).

**Fig. 1.** The ESR spectrum obtained upon the addition of 400 μM (2.5 μl of a solution of 50 mg of arachidonic acid in 1 ml of ethanol) to 0.5 ml of 30 mg/ml of microsomal protein in Tris buffer (pH 7.5). The incubation mixture was saturated with O<sub>2</sub> for 2 min prior to the addition of arachidonic acid. A capillary containing DPPH was then inserted in the 3-mm cylindrical tube. The incubation mixture was freeze-quenched within 20 s and kept in liquid N<sub>2</sub>. The ESR spectrum was recorded at −196°C. The spectrometer conditions were as follows: gain = 1 x 10<sup>4</sup>, modulation amplitude = 3.2 G, time constant = 1 s, scan time = 8 min, and microwave power = 60 milliwatts.

From the arguments presented above, it is clear that the free radical generated during the reaction of hydroperoxides with ram seminal vesicles is not a freely rotating oxygen-centered radical, but must be derived from the ram seminal vesicle microsomes, presumably from their protein component. A g-value in the range of 2.004 to 2.005 suggests that an organic free radical, possibly from one or more aromatic amino acids, is responsible for the ESR signal of the unknown free radical. However, the absence of hyperfine structure or apparent g-anisotropy precludes the identification of the radical structure with ESR under these conditions. Incubations in D<sub>2</sub>O buffer did not improve the spectral resolution. Henceforth, the unknown free radical is referred to as the ram seminal vesicle free radical. Once it is realized that the ESR signal arises from the ram seminal vesicles and not from a
radicals, saturate much more easily than does the ram seminal vesicle radical. If a paramagnetic transition metal such as iron vesicles.
moproteins are near the heme iron higher powers (Fig. 2), but that it is more difficult to saturate 100 milliwatts at -196 °C 11 milliwatts at -196 °C 35 G at -196 °C 9 G* at 23 °C
but its free radicals, structurally related to aromatic amino acid free radicals, exclude the possibility that the ram seminal vesicle free radical is due to methemoglobin contamination of the ram seminal vesicle. On the other hand, aromatic organic free radicals becomes more difficult to saturate, as has been shown for aromatic organic free radicals of these proteins are nearly indistinguishable. This near parameters of the metmyoglobin free radical are also shown and/or g-value structure was observed.

Table I

| Radical Medium | Temperature °C | g-value |
|----------------|---------------|---------|
| HOO*           | H2O glass     | -196    | 2.016a |
| CH3COO*        | CH3COOH glass | 27      | 2.0137 |
| CH3COO*        | CH3COO(CH3)3 liquid | 2.0037 |

a This free radical was obtained upon the addition of arachidonic acid to ram seminal vesicles according to the literature (5, 9).

This free radical was obtained upon the addition of 1 mg/ml of methemoglobin to a solution of 100 μM H2O2 in Tris buffer (pH 7.5). The free radical was either observed at 23 °C where it decayed in a few minutes or frozen in liquid nitrogen after 30 s of reaction. This g-value is taken from Ref. 14, where the error was reported as ±0.0006. A similar error is estimated for the other g-values. The spectrum of this radical in Ref. 13 indicates that this g-value is g1, where g1 = 2.027. d Pmax is the microwave power at which the signal amplitude is the maximum. e P2/3 is the microwave power at which the signal amplitude is half of what it would be if no saturation occurred (22).

This line width increased from 10 G at 30 s to 16 G at 400 s of reaction time (13). The error in these line width measurements is about 1–2 G.

A modulation amplitude of 0.8 G was used for this line width measurement. A modulation amplitude of 3.2 G was used for the line width measurements at -196 °C. This line width is an estimate taken from Ref. 14 where hyperfine and/or g-value structure was observed.

Table II

| Radical | Medium          | Temperature °C | g(oo) |
|---------|----------------|---------------|-------|
| HO*     | Ice            | -196          | 2.025a|
| HOO*    | H2O glass      | -196          | 2.016a|
| CH3COO* | CH3COOH glass  | 27            | 2.0137|
| CH3COO* | CH3COO(CH3)3 liquid | 2.0037 |

a An isotropic hyperfine coupling of 26.4 G was reported for the hydrogen atom (18).

An isotropic hyperfine coupling of 11 G was reported for the hydrogen atom (18).

b Isotropic hyperfine coupling constants for the 3 hydrogens were 5.2 G (19).

c No hyperfine coupling constants were observed (19).

small molecule, it is clear that our measured g-value will not necessarily correspond to an isotropic g-value even at room temperature, because motional averaging cannot be assumed. Microwave power saturation studies at -196 °C show that the ESR signal of the ram seminal vesicle radical saturated at higher powers (Fig. 2), but that it is more difficult to saturate than the methemoglobin free radical. This result is consistent with the ESR signal arising from an amino acid(s) free radical, because paramagnetic transition metal spectra usually cannot be saturated at -196 °C. On the other hand, aromatic organic free radicals, structurally related to aromatic amino acid free radicals, saturate much more easily than does the ram seminal vesicle radical. If a paramagnetic transition metal such as iron is near the organic free radical, then the free radical signal becomes more difficult to saturate, as has been shown for paramagnetic metal bound to melanin (22). These results probably indicate that the organic free radicals of these hemoproteins are near the heme iron (12).

Not only is the ram seminal vesicle free radical more difficult to saturate than the methemoglobin free radical, but its line width is significantly different (Table I). These results indicate that these hemoprotein free radicals are distinct, and exclude the possibility that the ram seminal vesicle free radical is due to methemoglobin contamination of the ram seminal vesicles.

There is a considerable amount of evidence in the literature that the cyclo-oxygenase and peroxidase activities of pure prostaglandin synthase are fully dependent on heme for their enzymatic activity (23–26). Hemoproteins such as methemoglobin or metmyoglobin substitute for hemin in restoring the synthase activity (23). Recently, Marnett and Reed (4) reported that hemin, methemoglobin, and metmyoglobin greatly increase the synthase-dependent peroxidatic benzo(a)pyrene co-oxidation. Another recent report suggests that destruction of the heme-binding site or removal of hemin may be the general mechanism of the irreversible inhibition of the prostaglandin synthase (26). In light of these findings, two models for the ram seminal vesicle free radical are proposed.

The first model was reported in 1958 by Gibson et al. who found that a free radical is formed during the oxidation of metmyoglobin with H2O2 (13–15). The second model is the methemoglobin radical, which also forms from the oxidation of the hemoprotein with H2O2 (12, 13). We have reinvestigated the latter species and determined its g-value and other spectral parameters (Table I). Literature values of the spectral parameters of the metmyoglobin free radical are also shown in Table I. Although significant differences in Pmax (the microwave power which maximizes the signal) and the peak-to-peak line width are apparent, the g-values of the three hemoprotein free radicals are nearly indistinguishable. This near identity in the g-values suggests that all three species are closely related. The metmyoglobin radical also forms as a result of reaction with either organic hydroperoxides or H2O2 (13).
The methemoglobin radical has been observed at room temperature (12), but the ram seminal vesicle radical had been reported only at temperatures near that of liquid nitrogen (5, 6, 9). Observation of the ram seminal vesicle free radical at room temperature shows it to increase for over 10 s after the addition of arachidonic acid, which corresponds to the time when oxygen is being consumed rapidly by the incubation. The signal then reaches a plateau and decays from this maximum over a period of 2 to 3 min. Under the conditions used to measure the line width of this spectrum, the doublet of the ascorbate semiquinone free radical is resolved ($\alpha^d = 1.8 G$); therefore the ram seminal vesicle free radical is not due to the ascorbate radical. Although the room temperature $g$-value of the ram seminal vesicle radical was little changed from its value at liquid nitrogen temperature, it is important to determine if the room temperature species responds to co-substrates in the same way as does the species observed at low temperatures (5, 6, 27).

With few exceptions, the compounds co-oxidized by the peroxidatic activity of prostaglandin synthase are known substrates of more thoroughly studied peroxidases. Phenol is one of the classical horseradish peroxidase substrates (28, 29), and is also a substrate for the peroxidatic activity of the methemoglobin/H$_2$O$_2$ system (30). Not surprisingly, the room temperature ram seminal vesicle radical signal is depressed by phenol (Table III) just as is the methemoglobin free radical signal (12). Iodide is almost unique in being a two-electron donor to horseradish peroxidase (28) and consequently does not form a free radical intermediate, unlike phenol and most other substrates of the horseradish peroxidase/H$_2$O$_2$ system (28, 29). Iodide destroys the methemoglobin free radical (14), and the effect of iodide on the prostaglandin synthase free radical ESR signal is shown in Table III.

Oxygen consumption is a good index of cyclo-oxyngease activity (9), and the inverse relationship between prostaglandin and radical formation has been interpreted as co-substrate scavenging of the free radical oxidant [O$_2^*$], thereby protecting the enzymatic activity from free radical destruction (5, 6, 9). However, the simplest explanation is an electron transfer from the electron-donating substrates, thereby oxidizing the co-substrates and reducing the ram seminal vesicle free radical to its original state, which may be the active form of cyclooxygenase (24). Alternatively, since the metmyoglobin radical oxidizes its own aromatic amino acids, in the absence of alternate substrates (13), a similar process could account for the deactivation of prostaglandin synthase and its associated peroxidase activity.

Tryptophan also depresses the room temperature ram seminal vesicle ESR signal and concomitantly increases the arachidonic acid-induced oxygen consumption (Table III). Tryptophan protects prostaglandin peroxidase activity against inactivation by an interaction with heme without being oxidized stoichiometrically (31). It should be noted that the related compound indoleacetate is a much-studied horseradish peroxidase substrate (28).

In any case, the effect of co-substrates on the ram seminal vesicles at room temperature is very similar to the decreases in signal amplitude reported for phenol (5, 9) and iodide (6, 27) at $-185^\circ$C. The corresponding increases in oxygen consumption caused by these substances are also similar to those previously reported. Indomethacin, a classic cyclo-oxyngease inhibitor, completely inhibited the formation of the room temperature ram seminal vesicle radical, presumably by preventing PGG$_2$ formation (9).

**DISCUSSION**

Once the similarity between the ram seminal vesicle free radical and the methemoglobin and metmyoglobin radicals is realized, a role for this free radical in the peroxidase activity of prostaglandin synthase can be proposed. It is possible that the prostaglandin synthase radical is formed by the reaction of hydroperoxides, including hydrogen peroxide, with the hemoprotein.

$$E(X, Fe^{IV}) + ROOH \rightarrow E(X', Fe^{IV}) + ROH$$

The species $X'$ is the free radical described in this work. Note that $X'$ is the ultimate site of oxidation and may not be the primary free radical. The chemical reactivity of the hydroperoxide-induced enzyme free radical is essentially identical with that of the compound I intermediate of horseradish peroxidase (28), the metmyoglobin radical (32 and references therein), or the ES complex of cytochrome $c$ peroxidase (33 and references therein). A similar view of the enzyme origin of the prostaglandin synthase radical has been suggested (34). These enzyme states of horseradish peroxidase and cytochrome $c$ peroxidase contain an organic free radical in one-to-one stoichiometry with the heme (35, 36), and the chemical identity of these free radicals is an area of very active research. The cytochrome $c$ peroxidase radical cannot be power-saturated at liquid nitrogen temperature (33), and the horseradish peroxidase radical can be observed quantitatively only at liquid helium temperatures under conditions of rapid adiabatic passage (35), indicating that, in these cases, the organic free radical is closer to the paramagnetic iron of the heme than it is in the case of the ram seminal vesicle radical. The marked differences in line widths of the free radicals seen with myoglobin, methemoglobin, and ram seminal vesicles may be the result of small differences in the radical-heme distance.

In addition to phenol, aminopyrine and aromatic amines are good one-electron donors in the horseradish peroxidase/H$_2$O$_2$, the metmyoglobin/H$_2$O$_2$ (37), and the prostaglandin synthase/PGG$_2$ systems (11, 38). In the presence of these substrates, one electron is transferred from the substrate (AH$_2^+$) to the free radical enzyme intermediate.

$$E(X, Fe^{IV}) + AH_2 \rightarrow E(X, Fe^{IV}) + AH^+$$

A one-electron transfer plus a proton transfer is equivalent to the loss of a hydrogen atom depicted above, and no preference for either possibility is implied. The formation of $Fe^{IV}$ in this sequence of reactions is widely accepted in the peroxidase literature (35 and references therein), but it is not an integral part of this scheme. The incorporation of $O_2^*$ from PGG$_2$ into a substrate is clearly a reaction which our incomplete scheme cannot explain. Similar oxygen transfer reactions have been described as peroxyngease-catalyzed reactions, whereas peroxides oxidize the substrate without a transfer of oxygen (39). Other examples of the incorporation of molecular oxygen into substrates may well be the result of substrate free radicals reacting with molecular oxygen (34, 40).

Further studies of the role of the ram seminal vesicle hemoprotein radical in the peroxidase activity of prostaglandin synthase are necessary. In particular, neither the stoichi-
omety of the heme and the free radical, nor the stoichiometry of the enzyme radical and substrate radical has been determined. In fact, with exactly the same kinds of evidence Shiga and Inaizumi (12) proposed that the hemoprotein radical of the methemoglobin/H₂O₂ system was the result of the decomposition of X, which was not observable with ESR. Furthermore, the species X was proposed to possess chemical reactivity similar to that of compound I and/or compound II of horseradish peroxidase.

\[
\text{MetHb + H}_2\text{O}_2 \rightleftharpoons X \rightleftharpoons \text{MetHb radical}
\]
\[
X + \text{AH}_2 \rightarrow \text{MetHb (?) + AH}^+
\]

This scheme, also, is consistent with all of the available evidence. It is even possible that some hemoprotein other than prostaglandin synthase is a substrate and reacts with X to form the ram seminal vesicle hemoprotein radical. In this case the enzyme free radical would be merely a consequence of the peroxidase activity of ram seminal vesicles. This last possibility is supported by the recent finding that purified prostaglandin cyclo-oxygenase/hydroperoxidase does not show any ESR signal at all when arachidonic acid is oxygenated (41). The interpretation of this result is complicated by the fact that heme must be added to the purified enzyme, which is an apoprotein, to obtain an active preparation. The significance of the hemoprotein-derived free radical observed in ram seminal vesicles is clearly unknown.

These ESR investigations have shown that the free radical observed in ram seminal vesicles and long described as a small oxygen-centered free radical is, in fact, a hemoprotein free radical. It is related to, but distinct from, a free radical formed by the reaction of methemoglobin with H₂O₂. The metmyoglobin free radical may be an oxidation product of tyrosine observed in ram seminal vesicles and long described as a small free radical which was not observable with ESR. Further-

REFERENCES
1. Marnett, L. J., Wlodawer, P., and Samuelsson, B. (1975) J. Biol. Chem. 250, 8510-8517
2. Sivarajah, K., Anderson, M. W., and Eling, T. E. (1978) Life Sci. 23, 2571-2578
3. Zenser, T. V., Mattammal, M. B., and Davis, B. B. (1979) Arch. Biochem. Biophys. 186, 292-299
4. Egan, R. W., Gale, P. H., and Kuehl, F. A., Jr. (1979) Arch. Biochem. Biophys. 187, 489-479
5. King, N. K., and Winfield, M. E. (1963) J. Biol. Chem. 238, 1520-1528
6. Gibson, J. F., Ingram, D. J. E., and Nicholls, P. (1958) Nature (Lond.) 181, 1398-1399
7. Yonetani, T., and Schleyer, H. (1967) J. Biol. Chem. 242, 1974-1980
8. Parkes, D. G., and Eling, T. E. (1974) Biochim. Biophys. Acta 377, 193-200
9. Kalyanaraman, B., Perez-Reyes, E., and Mason, R. P. (1980) Biochim. Biophys. Acta 650, 119-130
10. Egan, R. W., Gale, P. H., VandenHeuvel, W. J. A., Walker, R. W., Fagerness, P. E., and VandenHeuvel, W. J. A., Walker, R. W., Fagerness, P. E., and Kuehl, F. A., Jr. (1980) Arch. Biochem. Biophys. 196, 219-2194
11. Shiga, T., and Inaizumi, K. (1975) Arch. Biochem. Biophys. 167, 469-479
12. Gibson, J. F., Ingram, D. J. E., and Nicholls, P. (1958) Nature (Lond.) 181, 1398-1399
13. Yonetani, T., and Schleyer, H. (1967) J. Biol. Chem. 242, 1974-1980
14. Parkes, D. G., and Eling, T. E. (1974) Biochim. Biophys. Acta 377, 193-200
15. Kalyanaraman, B., Perez-Reyes, E., and Mason, R. P. (1980) Biochim. Biophys. Acta 650, 119-130
16. Egan, R. W., Gale, P. H., VandenHeuvel, W. J. A., Walker, R. W., Fagerness, P. E., and Kuehl, F. A., Jr. (1980) Arch. Biochem. Biophys. 196, 219-2194
17. Shiga, T., and Inaizumi, K. (1975) Arch. Biochem. Biophys. 167, 469-479
18. Gibson, J. F., Ingram, D. J. E., and Nicholls, P. (1958) Nature (Lond.) 181, 1398-1399
19. Yonetani, T., and Schleyer, H. (1967) J. Biol. Chem. 242, 1974-1980
20. Parkes, D. G., and Eling, T. E. (1974) Biochim. Biophys. Acta 377, 193-200
21. Kalyanaraman, B., Perez-Reyes, E., and Mason, R. P. (1980) Biochim. Biophys. Acta 650, 119-130
22. Sarna, T., Hyde, J. S., and Swartz, H. M. (1976) Science 192, 1132-1134
23. O'Brien, P. J., and Hulett, L. G. (1980) J. Biol. Chem. 255, 1061-1068
24. Marnett, L. J., and Lands, W. E. M. (1980) J. Biol. Chem. 255, 6253-6261
25. Van Der Ouderaa, F. J., Buytenhek, M., Nugteren, D. H., and Van Dorp, D. A. (1977) Biochim. Biophys. Acta 487, 318-331
26. Van Der Ouderaa, F. J., Buytenhek, M., Slukkerveer, F. L., and Van Dorp, D. A. (1979) Biochim. Biophys. Acta 572, 29-42
27. Egan, R. W., Gale, P. H., Beveridge, G. C., Phillips, G. B., and Marnett, L. J. (1978) Prostaglandins 16, 861-869
28. Yamazaki, I. (1977) in Free Radicals in Biology (Pryor, W. A., ed) Vol. III, pp. 183-218, Academic Press, New York
29. Mason, R. P. (1979) in Reviews in Biochemical Toxicology (Hodgson, E., Bend, J. R., and Philpot, R. M., eds) Vol. I, pp. 151-200, Elsevier/North-Holland, New York
30. Shiga, T., and Inaizumi, K. (1973) Arch. Biochem. Biophys. 154, 540-547
31. Onio, N., Ohki, S., Yamamoto, S., and Hayashi, O. (1979) J. Biol. Chem. 254, 829-836
32. Cleare, G. M., Lane, A. N., and Hollaway, M. R. (1980) Inorganyen Chimica Acta 46, 139-146
33. Hoffman, B. M., Roberts, J. E., Brown, T. G., Kang, C. H., and Margoliash, E. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6126-6136
34. Marnett, L. J., Bienkowski, M. J., and Page, W. R. (1979) J. Biol. Chem. 254, 5077-5082
35. Schulz, C. E., Devaney, P. W., Winkler, H., Debrunner, P. G., and Hager, L. P. (1977) FEBS Lett. 103, 102-105
36. Yonetani, T., Schleyer, H., and Ehrenberg, A. (1966) J. Biol. Chem. 241, 3240-3243
37. Griffin, B. W. (1978) Arch. Biochem. Biophys. 190, 850-853
38. Lasker, J. M., Sivarajah, K., Mason, R. P., Kalyanaraman, B., Aboiu-Doria, M. B., and Eling, T. E. (1981) J. Biol. Chem. 256, 7764-7776
39. Ishimaru, A. (1980) Bioorg. Chem. 9, 472-481
40. Marnett, L. J., and Bienkowski, M. J. (1980) Biochem. Biophys. Res. Commun. 66, 630-647
41. Egan, R. W., Gale, P. H., Baptista, F. M., Kennicott, K. L., VandenHeuvel, W. J. A., Walker, R. W., Fagerness, P. E., and Kuehl, F. A., Jr. (1981) J. Biol. Chem. 256, 7352-7361
42. King, N. K., Looney, F. D., and Winfield, M. E. (1964) Biochim. Biophys. Acta 88, 235-236
43. La Mart, G. N., de Ropp, J. S., Smith, K. M., and Langry, K. C. (1979) J. Biol. Chem. 254, 237-243
44. Roberts, J. E., Hoffman, B. M., Rutter, R., and Hager, L. P. (1981) J. Biol. Chem. 256, 2118-2121
45. Piesach, J., Mims, W. B., and Davis, J. L. (1979) J. Biol. Chem. 254, 12379-12389
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