The Degradative Effects of Porphyrins and Heme Compounds on Components of the Microsomal Mixed Function Oxidase System

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The effects of in vitro treatment of the hepatic microsomal fraction with various porphyrin compounds on the activity and the content of the heme-containing components of the mixed function oxidase system were studied. The compounds examined were hematin, methemalbumin (with heme to protein molar ratio of 13.1 or 1.1), mesohemalbumin, bilirubin, biliverdin, mesoporphyrin IX, and protoporphyrin IX. The activity of the system was monitored by measuring its oxidative activity for the type I and type II substrates, ethylmorphine and aniline, respectively; as well as the microsomal contents of cytochrome P-450 and b5, and 14C-labeled heme. Mesoporphyrin IX was found to be most effective in inhibiting the oxidative activity of the mixed function oxidase system as well as in decreasing the microsomal contents of cytochromes P-450, b5, and heme. Biliverdin exerted no effect on these parameters. Hematin and the other compounds studied exerted variable inhibitory effects on the system. The degradative and inhibitory effects of protoporphyrin IX and mesoporphyrin IX could be blocked significantly by conducting the studies in the dark. The presence of biliverdin decreased the inhibitory effects of the porphyrins on the system; conversely the effects could be magnified in the presence of deuterium oxide. It is suggested that the mechanism by which porphyrins inhibit the mixed function oxidase system is through porphyrin-sensitized photo-oxidation of various constituents of the hepatic microsomal fraction and that the formation of singlet oxygen molecules is most likely involved in this process. Moreover the destructive effects of heme compounds on the microsomal components and activities of the drug-metabolizing mixed function oxidase system raise questions concerning the hypothesis that the components of this system, and specifically cytochrome P-450, are involved in the activity of the heme oxygenase system.

EXPERIMENTAL PROCEDURE

Materials—The following chemicals were purchased from Sigma Chemical Co: hemin chloride, protoporphyrin IX dimethylster, mesoporphyrin IX dimethylster, bilirubin, biliverdin, NAD, NADP, glucose 6-phosphate, and human serum albumin (essentially fatty acid free from Fraction V). 15NH4Cl, 15N-leucine, and deuterium oxide (D2O) were obtained from the New England Nuclear...
Corp. Glucose-6-phosphate dehydrogenase was purchased from Boehringer. Microsomal pellets, 160 mg, were used.

Preparation of Mesohem in—Mesoporphyrin IX dimethyl ester was hydrolyzed by treatment with 25% (w/v) HCl as described by Falk (15) and isolated from ether as described previously (16). Iron was inserted into the porphyrin nucleus by the ferrous sulfate method of Lemberg as described by Falk (17). The mesohemin IX was taken up into ether and crystallized. The mesohemin thus obtained was recrystallized by dissolving in chloroform and precipitating out by the addition of excess petroleum ether (b.p. 30-60°C)

Preparation of Protoporphyrin IX and Mesoporphyrin IX—Protoporphyrin IX dimethyl ester and mesoporphyrin IX dimethyl ester was hydrolyzed and isolated as above (15, 16).

Preparation and Treatment of Micromolar Fractions—Rats were fasted overnight. The animals were decapitated and the livers then perfused with 0.9% NaCl. Thereafter the livers were removed, pooled (four rats), and homogenized (in 4 times the liver weight) in potassium phosphate buffer (0.1 M, pH 7.4). The liver homogenate was centrifuged at 9,000 x g for 20 min. The supernatant fraction was filtered and centrifuged at 163,000 x g for 40 min. The microsomal pellets were resuspended in buffer (4 to 5 mg of protein/ml), and heme was extracted by treatment of the microsomal suspension with ethyl acetate. Thereafter the microsomal hemoproteins was labeled as described earlier (21), using amino-[14C]levulinic acid (specific activity, 50 μCi/0.14 mg, 4 rCi/lOO g). The reduced-minus CO-difference spectrum of methemalbumin with a heme to protein molar ratio of 13:1, as reflected in the increase in total microsomal heme content. In contrast when the added heme was in a totally complexed form, as in methemalbumin with a 1:1 molar ratio, no increase in microsomal heme content was noted.

Effect of Metalloporphyrin Compounds on Various Parameters of Hepatic Microsomal Mixed Function Oxidase System—Table I shows the effect of treatment of microsomal fractions (1.5 to 2.5 mg of protein/ml, at 37°C for 30 min, under atmosphere, in light) with methemalbumin (having a heme to protein molar ratio of 13:1 or 1:1) and mesohemalbumin, at final concentrations of 17 and 34 μM. As shown, in all treatments there was a significant decrease in the oxidation of the type I and type II substrates, ethylmorphine and aniline, respectively. Generally all treatments had a greater effect on the oxidation of the type I substrate than on the type II substrate. Similarly there was a greater decrease in the cytochrome P-450 content of the microsomal fraction than in the content of cytochrome b6, the nonspecific binding of heme to microsomal proteins was observed when the microsomal fraction was pretreated with methemalbumin with a heme to protein molar ratio of 13:1, as reflected in the increase in total microsomal heme content. In contrast when the added heme was in a totally complexed form, as in methemalbumin with a 1:1 molar ratio, no increase in microsomal heme content was noted.

Table II shows that when the hepatic microsomal fractions (1.8 to 2.5 mg of protein/ml) were preincubated with various concentrations of hematin (37°C, 30 min, air, light) ranging between 8.5 to 34 μM there was a decrease in the oxidative activity of the microsomal fraction. Again enzymatic activity for the type I substrate was affected more significantly than that for the type II substrate. However, there did not appear to be a direct relationship between the concentration of hematin utilized and its effect on enzyme activity or on the hemoprotein content of the microsomal fraction.
Effect of metalloporphyrin compounds on various parameters of the hepatic microsomal mixed function oxidase system

Rat hepatic microsomal fractions (1.8 to 2.5 mg of protein/ml) prepared from pooled livers were incubated (37°, 30 min, air, light) with the above heme compounds which were prepared as described under "Experimental Procedure." At the end of the incubation period the microsomal fractions were resedimented (163,000 x g, 40 min) and thereafter, the pellets were resuspended in buffer (4 to 5 mg of protein per ml). Ethylmorphine demethylation was assayed by incubating (37°, air, 15 min) 1.0 ml of enzyme with ethylmorphine (2.0 mM) in a mixture (5 ml) containing an NADPH-generating system (NADP, 0.4 mM; glucose 6-phosphate 4 mM; glucose-6-phosphate dehydrogenase, 2 units); semicarbazide HCl, 7.5 mM; MgCl₂, 2 mM; 1.5% KCl and phosphate buffer, 40 mM. Formaldehyd formed was determined by Nash's method (18). Aniline hydroxylase activity was determined according to the method of Imai et al. (20) by incubating (37°, air, 20 min) aniline (1.0 mM) in an incubation mixture containing 1.0 ml of microsomal suspension with an NADPH-generating system (NADP, 1.6 mM; glucose 6-phosphate, 16 mM; glucose-6-phosphate dehydrogenase, 2 units); MgCl₂, 4 mM; 1.15% KC₁ and phosphate buffer, 40 mM. NADPH-reduced minus CO-difference spectrum of the microsomal fraction was measured as described by Omura and Sato (24). The cytochrome bo content was determined from the NADPH-reduced minus CO-difference spectrum, and the content of cytochrome b₅ was measured using NADH as the reducing agent. The concentration of heme was determined by the pyridine times. The values for the oxidative activities are expressed as the mean ± standard deviation. The data for the spectral studies are reported as the average value of three experiments. The results of ethylmorphine demethylation are expressed as the nanomoles of product (p-aminophenol) formed per mg of protein per hour. Aniline hydroxylase activity is reported as the nanomoles of product (p-aminophenol) formed per mg of protein per hour.

| Treatment | Concentration | Ethylmorphine demethylation | Aniline hydroxylation | Cytochrome P-450 | Cytochrome b₅ | Heme |
|-----------|---------------|----------------------------|-----------------------|-----------------|---------------|------|
| Control   | 0             | 316 ± 24                   | 34.9 ± 2.3            | 0.567           | 0.238         | 1.30 |
| Methemalbium (13:1) | 17            | 138 ± 26                   | 24.4 ± 2.9            | 0.284           | 0.238         | 2.57 |
| Methemalbium (1:1) | 34            | 162 ± 21                   | 23.2 ± 1.3            | 0.192           | 0.178         | 5.15 |
| Methemalbium (1:1) | 17            | 113 ± 34                   | 27.0 ± 3.6            | 0.280           | 0.230         | 1.30 |
| Methemalbium (1:1) | 34            | 136 ± 29                   | 20.6 ± 4.1            | 0.246           | 0.227         | 1.32 |
| Mesobilirubin (13:1) | 17            | 236 ± 10                   | 29.8 ± 3.1            | 0.129           | 0.216         | 0.29 |
| Mesobilirubin (13:1) | 34            | 228 ± 18                   | 26.8 ± 2.2            | 0.130           | 0.219         | -    |

* Could not be measured spectrally.

For determination of the cytochrome P-450 content, NADPH was used as the reducing agent in order to avoid the spectral interference of the reduced CO-complexed exogenous heme which was obtained when sodium dithionite was used as the reducing agent. Fig. 1 shows the absorption spectrum of the hematin-treated (17 µM) microsomal fractions. As shown, there was a great decrease in the microsomal content of cytochrome P-450 as well as an increase in the absorption at about 420 nm of the reduced CO-difference spectrum of the hematin-treated microsome.

Effect of Porphyrins, Biliverdin, and Bilirubin on Various Parameters of Hepatic Microsomal Mixed Function Oxidase System—Rat hepatic microsomal fractions were incubated

Table I

Effect of hematin concentration on hepatic microsomal mixed function oxidase system

Hepatic microsomal preparations (1.8 to 2.5 mg of protein/ml) from pooled livers of (fasted 15 hours) male rats (160 to 200 g) were incubated (37°, 30 min, air, light) with various concentrations of hematin which were prepared as described under "Experimental Procedure." At the end of incubation time the microsomal fractions were centrifuged (163,000 x g, 40 min) and resuspended in buffer (4 to 5 mg of protein/ml). Microsomal enzyme activities for the type I and type II substrates, ethylmorphine and aniline, respectively, were assayed as described in Table I. The microsomal content of cytochrome P-450 was determined from the NADPH-reduced minus CO-difference spectrum, and the content of cytochrome b₅ was measured using NADH as the reducing agent.

| Concentration | Ethylmorphine demethylation | Aniline hydroxylation | Cytochrome P-450 | Cytochrome b₅ | Heme |
|---------------|----------------------------|-----------------------|-----------------|---------------|------|
| 0             | 293 ± 18                   | 37.3 ± 2.1            | 0.517           | 0.249         | 1.28 |
| 8.5           | 119 ± 10                   | 26.0 ± 2.9            | 0.414           | 0.240         | 2.98 |
| 17            | 144 ± 19                   | 27.8 ± 1.7            | 0.274           | 0.207         | 3.01 |
| 34            | 156 ± 16                   | 22.8 ± 4.5            | 0.304           | 0.119         | 6.18 |

FIG. 1. The spectral profile of the microsomal fraction after treatment with hematin. Microsomal preparations (1.8 to 2.5 mg of protein/ml) were incubated (37°, 30 min, air, light) with hematin (17 µM). The microsomal fractions were resedimented and resuspended in buffer. Spectral studies were conducted as described under "Experimental Procedure"; NADPH was utilized as the reducing agent. ——, NADPH reduced CO-difference; ——, NADPH-reduced + CO-difference; ——, NADPH-reduced difference.
more effective against type I oxidation. In addition both compounds decreased the content of microsomal cytochrome \( b_5 \), and protoporphyrin IX diminished the cytochrome P-450 content by more than 50%. Fig. 2 illustrates the reduced CO-difference spectrum of the microsomal fraction following pretreatment with bilirubin or biliverdin. Although these compounds did not alter the microsomal content of cytochrome P-450, following bilirubin treatment no cytochrome P-420 was detectable in the microsomal preparation. The microsomal content of this hemoprotein was not changed when pretreated with biliverdin.

**Inhibition by Dark of Effects of Porphyrins on Hepatic Microsomal Mixed Function Oxidase Activity and on \(^{14}C\)-Labeled Microsomal Heme Content**—Rats were injected intravenously with amino-[\(^{14}\)C]levulinic acid (4 \( \mu \)Ci/100 g body weight, specific activity 50 \( \mu \)Ci/0.14 mg). Two hours after injection, the animals were killed and microsomal fractions with a protein concentration of 1.8 to 2.5 mg/ml were prepared. The microsomal fractions were incubated (37°, 30 min, air) in the presence, or absence of light with the compounds. Thereafter, the microsomal fractions were resedimented and resuspended in buffer (4 to 5 mg of protein/ml). Microsomal mixed function oxidase activity and the content of various components of this system were measured as described in Table I. Cytochrome P-450 content was determined using NADPH as the reducing agent. The non-heme compound solutions used in this study were prepared as described under "Experimental Procedure." A mixture. As Table IV shows, in the absence of light essentially the same amounts of \(^{14}\)C-labeled materials were extracted from the control as well as the porphyrin-treated microsomal preparations. However, in the presence of light there was an increase in the \(^{14}\)C-labeled extractable materials from the porphyrin-treated microsomes, with the greatest increase seen in porphyrin IX treated microsomes.

Table IV also shows that in the dark, the inhibitory effects of porphyrin compounds on the oxidative activity of hepatic microsomal enzymes was diminished significantly.

**Enhancement by Deuterium Oxide of Porphyrin Effect on Hepatic Microsomal Mixed Function Oxidase Activity and \(^{14}\)C-Labeled Microsomal Heme**—Hepatic microsomal fractions were prepared from rats treated with \( \delta \)-amino-[\(^{14}\)C]levulinic acid (4 \( \mu \)Ci/100 g body weight, specific activity 50 \( \mu \)Ci/0.14 mg, intravenously). The microsomal pellets were resuspended (1.8 to 2.5 mg of protein/ml) were incubated (37°, 30 min, air, light) with the compounds.

**TABLE III**

Effect of porphyrin compounds on various parameters of hepatic microsomal mixed function oxidase system

| Treatment | Ethylmorphine demethylation | Aniline hydroxylation | Cytochrome P-450 | Cytochrome \( b_5 \) | Heme |
|-----------|-----------------------------|----------------------|------------------|------------------|------|
| Control   | 333 ± 31                    | 34.9 ± 3.3           | 0.535            | 0.24             | 1.36 |
| Protoporphyrin | 192 ± 22                    | 30.6 ± 2.6           | 0.235            | 0.18             | 1.03 |
| Mesoporphyrin IX | 17 ± 8                     | 6.5 ± 2.8            | 0= 0             | 0.12             | 0.37 |
| Biliverdin | 327 ± 27                    | 36.0 ± 2.7           | 0.504            | 0.25             | 1.56 |
| Bilirubin  | 257 ± 16                    | 23.5 ± 3.1           | 0.520            | 0.16             | ----- |

* Could not be measured spectrally.

**TABLE IV**

Inhibition by absence of light of effects of porphyrins on mixed function oxidase activity and \(^{14}\)C-labeled microsomal heme

Microsomal hemoproteins were labeled by treating rats intravenously with \( \delta \)-amino-[\(^{14}\)C]levulinic acid (4 \( \mu \)Ci/100 g body weight, 50 \( \mu \)Ci/0.14 mg). Two hours later the animals were killed, and the microsomal fractions were prepared and incubated (37°, 30 min, air, light) with bilirubin or biliverdin both at the final concentration of 34 \( \mu \)M. Thereafter, the microsomal fractions were resedimented and resuspended in buffer, and the reduced minus CO-difference spectra were determined as described under "Experimental Procedure." Bilirubin, biliverdin, and proteoporphyrin IX were used in light or dark. Following resedimentation, the \(^{14}\)C-labeled degradation products of microsomal heme were extracted with chloroform-methanol (2:1) mixture. As Table IV shows, in the absence of light essentially the same amounts of \(^{14}\)C-labeled materials were extracted from the control as well as the porphyrin-treated microsomal preparations. However, in the presence of light there was an increase in the \(^{14}\)C-labeled extractable materials from the porphyrin-treated microsomes, with the greatest increase seen in the mesoporphyrin IX treated microsomes.
to 2.5 mg of protein/ml in phosphate buffer made with water or deuterium oxide and were pretreated (37°, 30 min, air, light) with hematin or protoporphyrin IX both at a final concentration of 34 μM. Following resedimentation of the microsomal fraction, the 14C-labeled degradation products of microsomal hemoproteins were extracted with chloroform-methanol mixture (2:1). As Table V shows, when the microsomal fractions in normal buffer (H₂O) were incubated with both compounds there was a substantial increase in the 14C-labeled materials that were extracted from the microsomal preparation in comparison to that of the controls. This value was increased by about 15 and 20% when the microsomal fractions in D₂O buffer were treated with hematin and protoporphyrin IX, respectively.

Table V also shows the potentiation by D₂O of the inhibitory activity of porphyrin compounds on microsomal enzyme activity. When the microsomal enzymes were pretreated with hematin and protoporphyrin IX the demethylase activity was, in the presence of D₂O, reduced to about 30 to 50% of control and aniline hydroxylation was reduced to about 50 to 60% of control.

Protective Effect of Biliverdin against Hematin and Protoporphyrin IX Inhibition of Hepatic Microsomal Mixed Function Oxidase Activity—Table VI shows that when microsomal fractions (1.8 to 2.5 mg of protein/ml) were treated (37°, 30 min, air, light) with equimolar concentrations (34 μM) of hematin and biliverdin, as well as protoporphyrin IX and biliverdin, the inhibitory effects of these compounds on oxidative activity were largely nullified. In addition, their destructive effects on microsomal hemoproteins were greatly inhibited. In unreported studies, biliverdin also provided a significant protective effect against the deleterious effects of mesoporphyrin IX on the microsomal activity and components. However, the destructive effects of mesoporphyrin IX on microsomal enzymes were so profound that the equimolar concentration of biliverdin did not provide the full reversal of the mesoporphyrin IX effect as was observed with hematin and protoporphyrin IX.

### Table V

Enhancement by deuterium oxide of porphyrin effect on hepatic microsomal mixed function oxidase system

Microsomal pellets containing 14C-labeled hemoproteins were prepared as described in Table IV. One-half of the pellets were resuspended in phosphate buffer which was prepared using D₂O, the other half of the pellets were resuspended in normal buffer. The microsomal suspensions thus obtained were treated with 34 μM hematin or protoporphyrin IX (37°, 30 min, air, light). Thereafter the microsomal fractions were resedimented and resuspended in normal buffer. The 14C-labeled degradation products of microsomal hemoproteins were extracted with chloroform-methanol (2:1) as described under "Experimental Procedure." The oxidative activity of the enzyme system was determined as described in Table I.

| Treatment     | Ethylmorphine demethyl. | Aniline hydroxyl. | 14C-Extracted |
|---------------|-------------------------|-------------------|---------------|
|               | nmol/mg/hr              | dpm               |               |
| Control (H₂O) | 313 ± 17                | 35.2 ± 3.1        | 4955          |
| Control (D₂O)| 297 ± 27                | 34.1 ± 2.9        | 4676          |
| Hematin (H₂O)| 162 ± 23                | 22.5 ± 2.1        | 6588          |
| Hematin (D₂O)| 109 ± 12                | 18.8 ± 1.5        | 6932          |
| Protoporphyrin IX (H₂O) | 207 ± 20 | 29.0 ± 1.9        | 6451          |
| Protoporphyrin IX (D₂O) | 150 ± 18 | 21.4 ± 1.7        | 7846          |

### Table VI

Inhibition by biliverdin of porphyrin effect on hepatic microsomal mixed function oxidase system

Hepatic microsomal preparations (1.8 to 2.5 mg of protein/ml) were incubated (37°, 30 min, air, light) with hematin or protoporphyrin IX (both 34 μM) in the presence of 34 μM biliverdin. The microsomal fractions were resedimented and ethylmorphine demethylated, aniline hydroxylation, as well as the microsomal contents of cytochromes P-450 and b₅ were measured. All measurements were made as described in Table I.

| Treatment     | Ethylmor- | Aniline | Cytochrome P-450 | Cytochrome b₅ |
|---------------|-----------|---------|------------------|---------------|
|               | phine     | hydroxy- |                  |               |
|               | demethyl. | lation  |                  |               |
|               | nmol/mg/hr| dpm     |                  | nmol/mg       |
| Control       | 325 ± 20  | 38.5 ± 1.4 | 0.532           | 0.220         |
| Hematin       | 172 ± 27  | 27.5 ± 3.4 | 0.257           | 0.118         |
| Hematin + biliverdin | 311 ± 19 | 36.7 ± 2.7 | 0.407           | 0.210         |
| Protoporphyrin IX | 202 ± 17 | 30.8 ± 2.0 | 0.255           | 0.180         |
| Protoporphyrin IX + biliverdin | 292 ± 22 | 37.5 ± 2.8 | 0.410           | 0.185         |

**DISCUSSION**

Recently it has been reported that components of the mixed function oxidase system (12) which metabolizes drugs, and specifically the terminal oxidase of the system, cytochrome P-450, participate in the oxidation of various metalloporphyrin complexes (28, 29). Since porphyrin compounds have been shown to be effective photosensitizers and to cause severe photodynamic damage to cellular components, it seemed of interest to investigate the effects of these compounds on different parameters of the mixed function oxidase system.

It was found that preincubation of microsomal fractions with various metalloporphyrin compounds such as hematin, methemalbumin, and mesohemalbumin, as well as porphyrins such as protoporphyrin IX or mesoporphyrin IX greatly inhibited the enzymatic activity as well as contents of specific heme-containing microsomal constituents and total microsomal heme. Mesoporphyrin IX was the most active porphyrin compound found in this study and virtually abolished microsomal enzyme activity and content of hemoproteins. The degradative effects of the free porphyrins on all measured parameters of the mixed function oxidase system provide an alternative explanation for the observation made by others (13) that protoporphyrin IX and mesoporphyrin IX are not oxidized by the heme oxygenase system. Porphyrins, in the presence of oxygen and light, have deleterious effects on cellular constituents and their failure to be oxidized by microsomal preparations may not merely be due to their being ineffective substrates for the heme oxygenase system but may result from direct membrane damage produced in the in vitro preparations in which heme oxygenase activity was studied (13).

Bilirubin, although a singlet oxygen sensitizer, is a weak one (8, 30) and did not alter the activity of the mixed function oxidase system, nor did it change the microsomal content of cytochrome P-450 (Table III and Fig. 2). In contrast to bilirubin, biliverdin has been shown to be a singlet oxygen quencher (31) and as the data in Table III and Fig. 2 indicate, it failed to affect significantly the contents of microsomal cytochromes P-450, P-420, or b₅.

As previously noted, photodynamic actions of chemicals require the presence of light, which provides energy for the conversions of the sensitizer to the first excited singlet state...
and subsequently the transition to the triplet excited state. This is the form which is involved directly in the formation of singlet oxygen or radicals which result in photodynamic damage to cellular components. The present study indicates (Table IV) that in the absence of light the extent of photodynamic activity of porphyrin compounds, represented in terms of inhibition of enzyme activity and degradation of microsomal heme, is reduced or nullified. The observation that the inhibitory effects of hematin were not as effectively nullified as were those of protoporphyrin IX and mesoporphyrin IX suggests that the mechanism of the photodynamic action of these sensitizers may be somewhat different. This could be partly due to differences in the quantum requirement for these photosensitizers, since although the treatments with these compounds were carried out in the almost total absence of light, exposure of the preparations to some light was unavoidable during the course of the experiments.

On the basis of the finding that the lifetime of singlet oxygen is extremely sensitive to the nature of solvents (32), Merkel et al. (33) demonstrated that the lifetime of singlet oxygen in D$_2$O was greatly prolonged over that in H$_2$O. It has proved possible to use this property to infer the intermediacy of singlet oxygen in most photochemical reactions. The present study shows (Table V) that D$_2$O did indeed potentiate the degradative effects of hematin and protoporphyrin IX on microsomal enzyme activity and on microsomal heme content. This potentiation of sensitizer effects was not attributable to the actions of D$_2$O on microsomal enzymes since control microsomal fractions incubated in D$_2$O alone showed no significant alterations in enzymatic activity (Table V).

Throughout this study, a general agreement was found between the decrease in enzymatic activity and the microsomal content of cytochrome P-450; but in the experiments in which this question was explored specifically, there was no stoichiometric correlation between the inhibition of drug metabolism and the amount of $^1$C-labeled heme degradation products produced. Similarly, in other experiments no such correlation was found between the decrease in the oxidative activity of the microsomal fraction and the amount of extractable $^1$C-labeled intact microsomal heme. In such experiments when the microsomal fractions containing $^1$C-labeled heme were treated with hematin in the light, and the intact heme then extracted, a decrease of only 15% in the microsomal content of labeled heme was observed, e.g. an average (four trials) value of 25,942 dpm/ml for the control versus 22,982 dpm/ml for the hematin-treated microsomes was obtained. These findings indicate that the destruction of microsomal heme is only one of several alterations which exogenous hematin and porphyrins produce on cytochrome P-450 and other components of the mixed function oxidase system.

The observation that hematin exerted virtually no concentration-dependent effects on the microsomal enzymes also suggests that only a limited number of exposed hematin-sensitive sites are available on the endoplasmic reticulum. Thus some constituents of the mixed function oxidase complex would be expected to come into closer contact with, or be more susceptible to, the effects of added porphyrins while others might be protected. This would be a plausible explanation of our findings, particularly in light of the fact that photodynamic actions of porphyrin compounds (because of the very short lifetime of singlet oxygen), are almost exclusively local and intramolecular rather than intersystemic. The importance of localization effects may be critical since even heme prosthetic groups have been shown to act as photosensitizers for the labile amino acids in their proximity (34, 35).

The involvement of singlet oxygen formation in chemical reactions is strongly suggested by the quenching of singlet oxygen production by appropriate acceptors. Several effective singlet oxygen quenchers are known, with biliverdin being one of the few which are soluble in water (30). Accordingly, good evidence for the intermediacy of singlet oxygen in the destruction of hepatic microsomal mixed function oxidase components is provided in this study by the observations (Table VI) that in the presence of equimolar concentrations of biliverdin the effects of hematin and protoporphyrin IX on the complex were diminished significantly.

Finally, it should be noted that all of the heme compounds which were found in this study to have degradative effects on microsomal components have been shown to be effective in vitro substrates for the hepatic heme oxygenase system (13).

The specific involvement of cytochrome P 450 and components of the drug-metabolizing mixed function oxidase complex in the heme oxygenase system has been proposed (28). It would seem paradoxical that the substrates of an enzymatic system should be so destructive to the constituents of the system itself and in light of the findings of this study the putative role of cytochrome P-450 in the heme oxygenase system (29) should be re-evaluated.

Acknowledgments—We are indebted to Mr. Eric Feldman for technical assistance and Miss Ann Marie Quatela for preparation of the manuscript.

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