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# PRELIMINARY COMMUNICATION

## EVIDENCE FOR THE PRESENCE OF CYTOCHROME P-450 IN RAT MAMMARY GLAND

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Most chemical carcinogens require metabolic activation to exert their tumorigenic effects. For the aromatic hydrocarbons and aromatic amines, the enzyme system responsible for their conversion to reactive metabolites appears to be the microsomal mono-oxygenase system. This system, consisting of the cytochromes P-450, a reductase, and phospholipid, is located in the endoplasmic reticulum and to a lesser extent in the nucleus of tissues in which these chemicals induce tumors. The tissues include liver, kidney, lung, skin, digestive tract, adrenals and placenta. The presence of cytochrome P-450 in mammary gland has not been reported although the susceptibility of the gland to carcinogenesis by aromatic hydrocarbons (1, 2), its ability to metabolize xenobiotics (3, 4), and the presence of an inducible aryl hydrocarbon hydroxylase (5 - 7) suggest that the cytochrome also occurs in mammary tissue.

### Materials and Methods

Virgin female Sprague-Dawley rats, 200-250 g, were housed in mesh-bottomed cages and given a commercial ration and water ad lib. Mammary glands from 6 to 12 animals were pooled for homogenization in 9 vol. of a solution containing 1.15% KCl and 0.05 M Tris-HCl, pH 7.5, with a Polytron (Brinkmann Inst., Westbury, NY). The homogenate was centrifuged at 10,000 g for 15 min and the resulting supernatant fraction centrifuged at 78,000 g for 90 min. The 78,000 g pellets were washed once with homogenization medium and the final microsomal pellets suspended in 0.1 M Tris-HCl, pH 7.5, to a protein concentration of 5-10 mg/ml. All solutions were kept at 0-4°. Rats pretreated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) received an i.p. injection of 100 μg/kg body weight 48 hr prior to removal of the mammary glands. TCDD was generously supplied by Dr. A. Poland, McArdle Laboratory, Madison, WI, U.S.A.

Cytochrome P-450 was determined using a method specifically developed to measure low concentrations of the cytochrome in microsomes heavily contaminated by hemoglobin (8). Ascorbic acid (0.25 mM) and phenazine ethosulfate (2.5 μM) are added to the microsomes prior to obtaining a difference spectrum of dithionite-reduced minus oxidized microsomes when both sample and reference have been bubbled with carbon monoxide. Cytochrome P-450 was quantified by measuring the difference in absorption between the maximum at 448 nm and the minimum at 465 nm; an extinction coefficient of 91 $cm^{-1}$ $mM^{-1}$ was used. The activity of NADPH-cytochrome c reductase was determined at 23° in a high ionic strength buffer solution according to Vermilion and Coon (9). Microsomes were kept on ice and the assays completed within 24 hr of preparation. Protein was determined in the presence of sodium dodecyl sulfate by a modification (10) of the Lowry procedure.

Results and Discussion

The reduced CO difference spectrum obtained with rat mammary microsomes is illustrated in Fig. 1. The absorption maximum of the 450 nm-region peak was routinely observed between 446 and 450 nm and was not influenced significantly by the relative size of the peak appearing at 425 nm. [The 425-nm peak was due, at least in part, to the presence of reduced cytochrome $b_5$ and was observed also in NADH-reduced microsomes. This cytochrome has been identified previously in rat mammary microsomes by spectrophotometric as well as immunologic methods (11).]

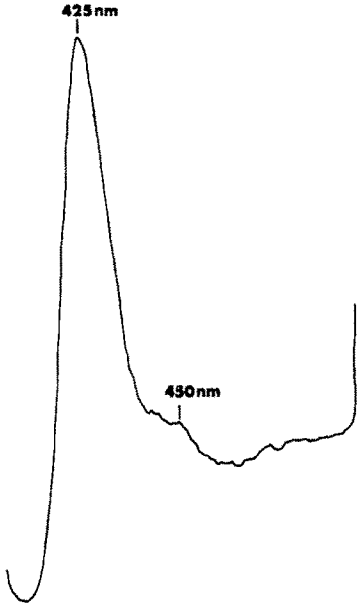


Fig. 1. Reduced CO difference spectrum of rat mammary microsomes. The spectrum was recorded using microsomes containing 4.4 mg protein/ml and 0.025 nmole cytochrome P-450/mg of protein, as described in Materials and Methods.

Results with several preparations of mammary microsomes from untreated virgin rats indicate that mammary tissue contains measurable quantities of cytochrome P-450, which range from 0.019 to 0.035 nmole/mg of microsomal protein. When animals were pretreated with TCDD, the cytochrome P-450 content approximately doubled (Table 1). It would appear that mammary cytochrome P-450 also can be induced by this potent inducer of hepatic and extrahepatic mono-oxygenases (12, 13). Although measurable, the cytochrome P-450 specific content was only about 3 percent of that found in hepatic microsomes. The low concentration together with difficulties in solubilizing the microsomes have hindered efforts to define the spectral properties of the mammary cytochrome. Moreover, attempts to investigate the multiplicity of the mammary P-450 cytochromes have been unsuccessful.

Table 1. Cytochrome P-450 and cytochrome c reductase activity in rat mammary microsomes

| | Cytochrome P-450 (nmoles/mg protein) | Cytochrome c reduction (nmoles/min/mg protein) |
|---|---|---|
| Control (4)* | 0.027 (0.019-0.035)+ | 15 (12-21) |
| TCDD-treated‡ (2) | 0.050, 0.065 | 8, 23 |

* Number of experiments.

\+ Means with range.

‡ 2,3,7,8-Tetrachlorodibenzo-p-dioxin, 100 μg/kg, 48 hr prior to sacrifice.

The specific activity of NADPH-cytochrome c (cytochrome P-450) reductase in the mammary microsomes was 12-21 nmoles cytochrome c reduced/min/mg of microsomal protein, about 10 percent of that present in liver microsomes. The activity was not increased by pretreatment with TCDD (Table 1). Low levels of NADPH-cytochrome c reductase activity have been detected also in microsomes isolated from bovine and rat lactating mammary glands (14).

Our results suggest that mammary microsomes contain electron transport components similar to those of the hepatic cytochrome P-450 system, but in much lower concentrations than in liver. The role of the mammary mono-oxygenase system in the activation and/or detoxification of chemical carcinogens remains to be established.

Acknowledgement

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