# LACK OF CORRELATION BETWEEN CYANIDE-BINDING SPECTRUM AND FATTY ACID DESATURASE ACTIVITY IN LIVER MICROSOMES

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Summary. Spectrophotometric titration of rat liver microsomes with cyanide suggested the occurrence of two cyanide-binding components. No correlation was, however, found between the contents of these components and the microsomal fatty acid desaturase activity during the course of dietary induction. Adipose tissue microsomes, though capable of cyanide-sensitive desaturation, showed no cyanide-binding spectrum. It is concluded that the cyanide-binding spectrum of liver microsomes is due to cytochrome P-450 but not related to the cyanide-sensitive factor involved in the desaturase system.

Desaturation of fatty acyl CoA's to corresponding monoenoic acids in liver microsomes is catalyzed by an enzyme system requiring both oxygen and NADH or NADPH (1-3). Previous work from this laboratory indicated the involvement in this system of a cyanide-sensitive factor, or "CSF", which in the reduced form appears to react with oxygen and the substrate (4). Subsequent studies revealed that the electrons needed for the desaturation process are transferred from NADH or NADPH to the CSF via cytochrome $b_5$ (5,6). The CSF has been shown to interact with various phenols in a characteristic way (7), but its nature still remains to be elucidated.

It is well known that a difference spectrum with an absorption peak at 444 nm is induced on addition of cyanide to liver microsomes (8,9). From a study of this difference spectrum, Gaylor and coworkers (10) have recently concluded that rat liver microsomes contain two cyanide-binding components; one is cytochrome P-450 which

combines with high concentrations of cyanide ($K \cong 2.5$ mM), whereas the other has a higher affinity for cyanide ($K \cong 0.5$ mM). The latter component has been separated from cytochrome P-450 and reported to be a hemoprotein. They have also presented evidence that this high-affinity component is the CSF which is functional in both fatty acid desaturation and methyl sterol oxidation.

These findings of Gaylor et al. (10) prompted us to undertake a study on the effect of dietary induction of fatty acid desaturase on the cyanide-binding spectrum of rat liver microsomes. This communication reports that there was no correlation between the desaturase activity and the levels of the spectrophotometrically detectable cyanide-binding components. It is also reported that rat adipose tissue microsomes, though capable of cyanide-sensitive desaturation (6,7), failed to exhibit any spectral change on addition of cyanide.

## METHODS

Male Sprague-Dawley rats, weighing about 150 g, were treated essentially as described previously (7) to induce a high desaturase activity. After the second fasting, the animals were refed a high-carbohydrate diet (6) and killed 0, 5, 12.5 and 20 hours after the start of refeeding. Liver microsomes were prepared as described by Oshino et al. (4). Microsomes were also isolated from epididymal adipose tissues of rats maintained under uncontrolled dieatry conditions. Cyanide-induced difference spectra of microsomes were measured exactly as described by Gaylor et al. (10); as recommended, the base line was established with 33 μM NaCN in both cuvettes to eliminate interferences due to residual hemoglobin. Cytochrome P-450 was assayed according to Omura and Sato (11). Fatty acid desaturase activity was measured with 1-$^{14}$C-stearyl CoA as substrate and NADH as electron donor by the previously described method (6).

## RESULTS AND DISCUSSION

As reported previously (8-10), the addition of cyanide to liver

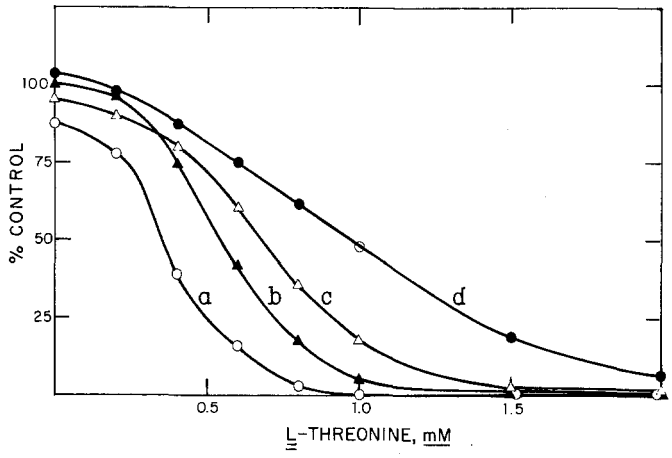


Fig. 1. Difference spectra caused by the addition of cyanide to liver microsomes. Liver microsomes were obtained from rats that were fed a laboratory chow (Oriental Yeast Company, Osaka). The microsomes (1.5 mg protein/ml) were suspended in 0.1 M potassium phosphate buffer, pH 7.4, and the difference spectra were measured under exactly the same conditions as described by Gaylor et al. (10). Curve A, base line; Curve B, 0.25 mM KCN; Curve C, 4 mM KCN.

microsomes, prepared from rats maintained on an ordinary laboratory chow, caused a difference spectrum having a peak at 444 nm and a trough in the 410 nm region (Fig. 1). In agreement with Gaylor et al. (10), the double reciprocal plot of the absorbance increment between 444 and 410 nm against the cyanide concentration added gave a biphasic curve, which indicated the apparent presence of two cyanide-binding components possessing spectral dissociation constants of 2.2 and 0.67 mM (Fig. 2). Assuming the same molar extinction coefficient for the two components, it was estimated that the ratio of the low- to high-affinity components was roughly 2:1, as compared with 4:1 reported by Gaylor et al. (10).

Spectrophotometric titration with cyanide was then performed on liver microsomes obtained from fasted rats and those that had been refed the high-carbohydrate diet for various periods of time. The results thus obtained are summarized in Table I, together with the fatty acid desaturase activities and the contents of cytochrome

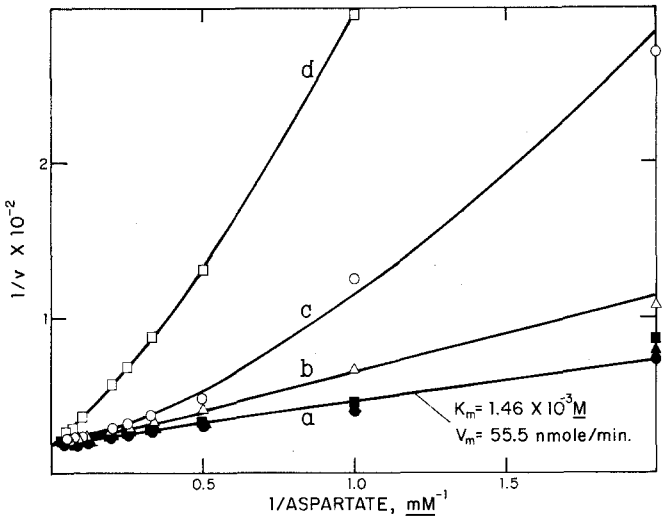


Fig. 2. Double reciprocal plot of $\Delta A_{444-410nm}$ and KCN concentration. Liver microsomes from laboratory chow-fed rats were used, and the measurement of cyanide-difference spectra was conducted as described in Fig. 1, except that the KCN concentration was varied.

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P-450 in the microsomes employed. All the microsomes examined contained both the low-affinity cyanide-binding component (K = 1.7-2.5 mM) and the high-affinity component (K = 0.32-0.67 mM). However, when the levels of both components in these preparations were estimated by extrapolating the double reciprocal plots to the ordinate ($\Delta A_{max}$/mg protein), no correlation could be detected between the values obtained and the desaturase activity. Thus, the levels of both components, especially that of the high-affinity component which has been suggested to be the CSF (10), were lowered at the initial phase of refeeding and remained constant thereafter, while there was a 17-fold increase in the desaturase activity after 20 hours induction. This lack of correlation indicates clearly that neither of the cyanide-binding components can be the CSF, since it has been shown that the dietary induction of desaturase activity results from a net increase in the amount of CSF (7). Previous studies have also shown that cyanide inhibition of the desaturation

TABLE I

Contents of Low- and High-affinity Cyanide-binding Components in Rat Liver Microsomes during Dietary Induction of Fatty Acid Desaturase

| Refeeding time (hr) | 0 | 5 | 12.5 | 20 |
|---|---|---|---|---|
| Desaturase activity (nmole/min/mg protein) | 0.51 | 1.11 | 6.34 | 8.62 |
| Low-affinity cyanide-binding component ($\Delta A_{max}$/mg protein*) | 0.111 | 0.063 | 0.053 | 0.059 |
| High-affinity cyanide-binding component ($\Delta A_{max}$/ mg protein) | 0.035 | 0.030 | 0.026 | 0.030 |
| Cytochrome P-450 (nmole/mg protein) | 1.69 | 0.96 | 0.81 | 0.91 |
| Low component/P-450 | 0.065 | 0.066 | 0.065 | 0.065 |
| High component/P-450 | 0.020 | 0.031 | 0.033 | 0.032 |
| Number of rats used | 5 | 3 | 7 | 20 |

* $\Delta A_{max}$ was obtained graphically from the double reciprocal plot of the magnitude of cyanide-induced spectral change (between 444 and 410 nm) against the cyanide concentration.

reaction can be established only when stearyl CoA is supplied to the system (6), suggesting that cyanide does not bind to the CSF in the absence of the substrate. This excludes further the relationship between the CSF and the cyanide-induced spectral change, since the latter could be measured in the absence of stearyl CoA.

Another point of importance salient from Table I is the beautiful correlations between the content of cytochrome P-450 and those of both cyanide-binding components. This is indicative of the possibility that cytochrome P-450 is responsible for both the low- and high-affinity binding of cyanide to microsomes, although the reason for the biphasic response of the cytochrome is to be elucidated. The fact that the cyanide-binding spectra of both the low- and high-

affinity components show the same absorption maxima and minima lends another support to the above interpretation.

Microsomes from rat adipose tissue have been shown to catalyze the cyanide-sensitive desaturation of stearyl CoA and to be devoid of cytochrome P-450 (6). The presence of CSF in these microsomes has also been demonstrated (7). Nevertheless, no spectral change could be induced in adipose tissue microsomes when 4 mM cyanide was added. The microsomes examined possessed the NADH-dependent activity of stearyl CoA desaturation (1.58 nmole oleate formed/min/mg protein), which was inhibited by cyanide to the extent similar to that observed with liver microsomes. The preparation contained cytochrome $b_5$ (0.095 nmole/mg protein), but no cytochrome P-450 could be detected. These findings reinforce the view that the cyanide-induced difference spectrum observed with liver microsomes is due to cytochrome P-450 but is not related to the CSF.

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