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# ENHANCED ACTIVITY OF LIVER DRUG-METABOLIZING ENZYMES FOLLOWING CHRONIC ETHANOL CONSUMPTION AND STARVATION

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**ABSTRACT**

The activities of liver drug-metabolizing enzymes for some aromatic or chlorinated hydrocarbons were remarkably enhanced in one-day starved or chronically ethanol-fed rats, although the starvation or ethanol consumption produced no significant increase in the microsomal protein and cytochrome P-450 contents. In spite of causing a marked loss of liver weight, food deprivation increased significantly even the whole liver activity. The effect of chronic ethanol consumption on the enzyme activity almost disappeared after one-day withdrawal of ethanol. The enzyme activation by food deprivation or chronic ethanol intake was found to be related with changes occurring not in the soluble but in the microsomal fraction.

Starvation and chronic ethanol feeding have been known to increase the susceptibility of rats to some toxic agents such as carbon tetrachloride by affecting their metabolic fates[2,3]. In order to assess the effects of food deprivation and ethanol consumption on the activity of liver drug-metabolizing enzymes, the metabolic rates *in vitro* of some aromatic or chlorinated hydrocarbons were measured and a comparison was made between those in fed and fasted rats and also in rats fed diet with and without ethanol.

## METHODS

Rats of Wistar strain were fed a nutritionally adequate liquid diet[1] containing either ethanol to the extent of 30% total calorie intake or isocaloric carbohydrate for at least 3 weeks. The diet (1 cal/ml) was given daily at 4 p.m., 80 ml to male and 70 ml to female rats. The diet given at 4 p.m. was consumed by about 12 p.m. on the same day. Food deprivation was caused by substituting an equivolume of tap water for the liquid diet.

For the enzyme assay *in vitro*, rats were usually sacrificed at 10 a.m. The rat liver was minced and a 10% (w/v) crude homogenate was made with 1.15% KCl-phosphate buffer in a glass homogenizer. The homogenate was then centrifuged

at 10 000 g for 10 min. A part of the supernatant was further centrifuged at 105 000 g for 60 min to separate the microsomal fraction. One or half a milliliter of the 10 000 g supernatant was normally used as the enzyme source. An aliquot of hydrocarbon was dissolved in distilled water, normally 5–10 μl/100 ml. One tenth of a milliliter of this solution was used as the substrate. The enzyme and substrate were incubated with a cofactor solution in an air-tight vessel for 10 min. The rate of metabolism was assessed by measuring the rate of substrate disappearance by gas chromatography[6].

Microsomal protein level was measured according to the method of Lowry and co-workers[4], and microsomal cytochrome P-450 content by the method of Omura and Sato[5].

## RESULTS AND DISCUSSION

### Activation of drug-metabolizing enzymes following food deprivation

When the carbohydrate-containing diet was replaced by tap water the day before sacrifice, i.e., when the rats were fasted overnight, the enzyme activities to metabolize all the hydrocarbons studied increased markedly compared with the activities in the fed rats (Table 1). Overnight fasting produced no increase in the microsomal protein and cytochrome P-450 contents. On the contrary, the total

TABLE 1
Activation of drug-metabolizing enzymes in male rats after overnight food deprivation.

| | Metabolic rates (nmol/g liver/min) | | Fed / Fasted |
|---|---|---|---|
| | Fed ($\bar{X} \pm$ S.D.) | Fasted ($\bar{X} \pm$ S.D.) | |
| Benzene | 13.7 ± 5.4 | 36.4 ± 2.8 | 2.7 |
| Toluene | 18.1 ± 4.9 | 40.3 ± 4.8 | 2.2 |
| m-Xylene | 21.0 ± 5.5 | 41.0 ± 1.9 | 2.0 |
| Ethylbenzene | 22.9 ± 6.3 | 40.8 ± 3.5 | 1.8 |
| n-Propylbenzene | 46.5 ± 12.8 | 77.2 ± 17.4 | 1.7 |
| Cumene | 36.6 ± 4.3 | 75.9 ± 1.6 | 2.1 |
| Styrene | 28.5 ± 4.3 | 46.1 ± 1.6 | 1.6 |
| Dichloromethane | 28.5 ± 1.5 | 60.9 ± 1.9 | 2.1 |
| Chloroform | 19.7 ± 2.6 | 55.1 ± 7.5 | 2.8 |
| Carbon tetrachloride | 1.9 ± 0.2 | 5.9 ± 0.8 | 3.1 |
| 1,1-Dichloroethane | 19.1 ± 3.3 | 56.0 ± 2.4 | 2.9 |
| 1,2-Dichloroethane | 23.6 ± 1.1 | 59.8 ± 3.1 | 2.5 |
| 1,1,1-Trichloroethane | 0.5 ± 0.2 | 1.2 ± 0.2 | 2.4 |
| 1,1,2-Trichloroethane | 21.0 ± 1.9 | 56.0 ± 3.0 | 2.7 |
| 1,1,1,2-Tetrachloroethane | 8.1 ± 2.0 | 32.8 ± 8.4 | 4.0 |
| 1,1,2,2-Tetrachloroethane | 13.3 ± 0.6 | 40.0 ± 2.4 | 3.0 |
| 1,1-Dichloroethylene | 31.1 ± 6.6 | 67.3 ± 6.6 | 2.2 |
| Trichloroethylene | 18.9 ± 7.4 | 57.0 ± 1.9 | 3.0 |
| Tetrachloroethylene | 0.5 ± 0.3 | 1.9 ± 0.3 | 3.8 |
| 1-Chloropropane | 37.6 ± 5.9 | 100.0 ± 5.2 | 2.7 |
| Monochlorobenzene | 8.2 ± 1.6 | 35.5 ± 6.3 | 4.3 |

liver contents were drastically decreased due to a marked loss of liver weight (Table 2). However, the metabolism of these hydrocarbons was enhanced to such a great extent that the enhancement by overnight fasting was still at a significant level even when the activity was expressed as the total liver activity.

TABLE 2
Effects of overnight food deprivation on liver microsomal protein and cytochrome P-450 contents in male rats. The values represent the mean ± S.D. for five rats.

| | Liver weight (g) | Protein (mg/g liver) | P-450 (nmol/mg protein) |
|---|---|---|---|
| Fed | 9.74 ± 0.73 | 22.7 ± 2.7 | 0.84 ± 0.12 |
| Fasted | 7.58 ± 0.38 | 23.0 ± 2.7 | 0.82 ± 0.03 |

When food deprivation was continued over a period of two or three days, no further significant increase in the enzyme activity occurred in male rats, although in female rats the activity tended to further increase during this longer period of fasting (Fig. 1). These findings suggest that an important part of the change which is caused by starvation and thereby activates the enzymes occurs during a relatively short period of food deprivation.

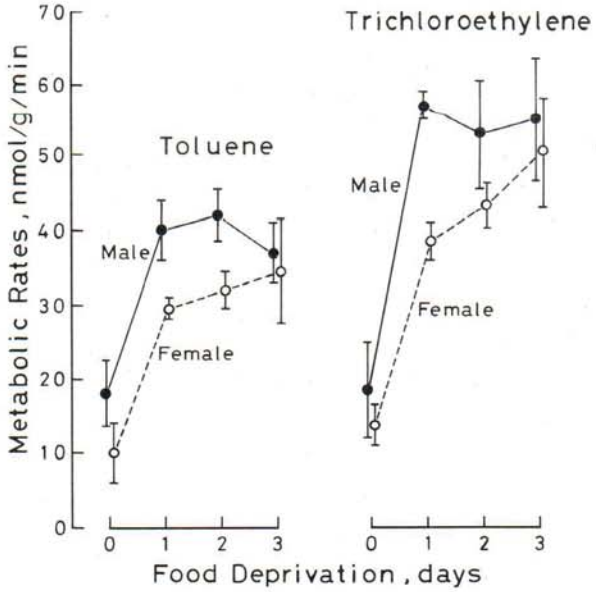


FIG. 1 – Activity of drug-metabolizing enzymes in relation to duration of food deprivation. Each point represents the mean ± S.D. for five rats.

The double reciprocal plots of metabolic rates of toluene and trichloroethylene against the substrate concentrations are shown in Fig. 2. Clearly, not only the maximum velocity but also the Michaelis constant changes with starvation. The Michaelis constant obtained from fasted rats was larger than that from fed rats.

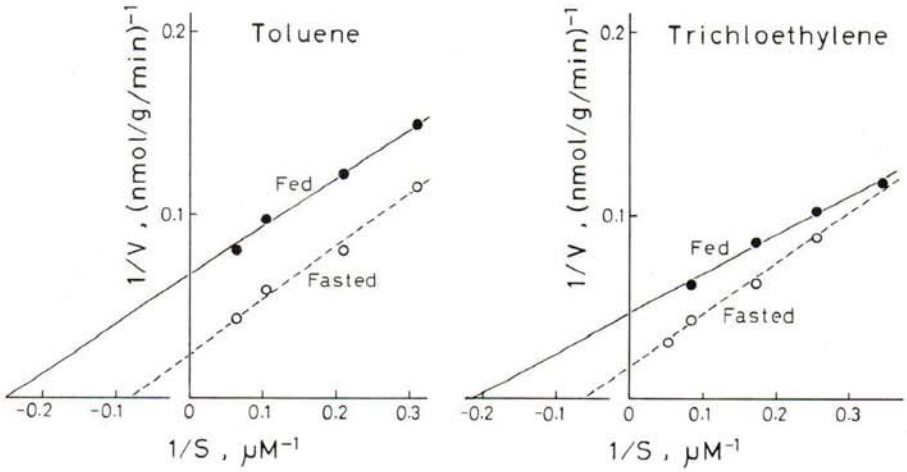


FIG. 2—Double reciprocal plots of metabolic rate (v) versus substrate concentration (S). Each point represents the mean for three fed or three overnight fasted male rats.

In order to elucidate whether the enhanced activity in fasted rats was due to changes in the soluble or in the microsomal fraction, the metabolic rates of toluene and trichloroethylene were measured by exchanging the microsome and the soluble fraction between fed and fasted rats. The soluble fraction (S) from a fasted rat was added to the microsome (M) from a fed rat, and conversely, (S) from the fed rat to (M) from the fasted rat. The metabolic rates of toluene and trichloroethylene were measured with these reconstituted 10 000 g supernatants. The supernatant composed of (M) from the fasted rat and (S) from the fed rat metabolized these hydrocarbons at exactly the same rate as the supernatant composed of (M) and (S) both from the fasted rat. Therefore, it can be said that the enhanced enzyme activity following overnight fasting is associated with changes occurring in the microsomal fraction and not in the soluble fraction.

**Activation of drug-metabolizing enzymes following chronic ethanol consumption**

The activity of enzymes in rats fed ethanol daily for 3 weeks was enhanced remarkably for all the hydrocarbons studied compared with that of rats fed ethanol-free control diet (Table 3). Microsomal cytochrome P-450 was also increased significantly by the ethanol feeding (Table 4). It can be said, however, that its increase was too slight to fully explain the marked enhancement of the microsomal enzyme activity. Moreover, when the ethanol-containing diet was

TABLE 3

Activation of drug-metabolizing enzymes in male rats after chronic ethanol consumption. The values in the columns Control, EtOH(+), and EtOH(−) represent the mean ± S.D. for five rats.

| | Metabolic rates (nmol/g liver/min) | | | EtOH(+) |
|---|---|---|---|---|
| | Control | EtOH(+) | EtOH(−) | Control |
| Benzene | 13.7 ± 5.4 | 87.5 ± 13.7 | 15.5 ± 3.3 | 6.4 |
| Toluene | 18.1 ± 4.9 | 88.5 ± 2.6 | 20.5 ± 5.4 | 4.9 |
| m-Xylene | 21.0 ± 5.5 | 76.6 ± 2.6 | 22.8 ± 5.1 | 3.6 |
| Chloroform | 19.7 ± 2.6 | 126.6 ± 10.5 | 20.2 ± 1.1 | 6.4 |
| Carbon tetrachloride | 1.9 ± 0.2 | 8.3 ± 1.5 | 1.7 ± 0.3 | 4.4 |
| 1,1-Dichloroethane | 19.1 ± 3.3 | 120.9 ± 7.5 | 20.3 ± 0.5 | 6.3 |
| 1,2-Dichloroethane | 23.6 ± 1.1 | 128.6 ± 7.9 | 22.7 ± 0.3 | 5.5 |
| 1,1,1-Trichloroethane | 0.5 ± 0.2 | 1.8 ± 0.5 | 0.6 ± 0.3 | 3.6 |
| 1,1,2-Trichloroethane | 21.0 ± 1.9 | 117.6 ± 2.1 | 21.5 ± 1.3 | 5.6 |
| 1,1-Dichloroethylene | 31.1 ± 6.6 | 100.6 ± 10.8 | 34.1 ± 4.9 | 3.2 |
| Trichloroethylene | 18.9 ± 7.4 | 105.3 ± 1.5 | 20.1 ± 5.3 | 5.6 |
| Tetrachloroethylene | 0.5 ± 0.3 | 2.6 ± 0.4 | 0.5 ± 0.4 | 5.2 |

Control: Rats fed control diet up to the day before sacrifice.
EtOH (+): Rats fed ethanol-containing diet up to the day before sacrifice.
EtOH (−): Ethanol-treated rats fed control diet in place of ethanol-containing diet only on the day before sacrifice.

replaced by the ethanol-free control diet the day before sacrifice, the enhanced activity in the ethanol-treated rats was reduced almost to the control level (Table 3). This indicates that the effect of chronic ethanol administration on the enzyme activity almost disappears after one-day withdrawal of ethanol.

When added to the incubation mixture ethanol itself inhibited the metabolism of toluene and trichloroethylene competitively. Figure 3 shows the reciprocal plots of toluene and trichloroethylene metabolism with addition of varying amounts of ethanol. Clearly, ethanol acts as if it were a competitive metabolic inhibitor despite the fact that when given to a living rat it acted as a stimulator of the enzymes.

TABLE 4

Effects of chronic ethanol consumption on liver microsomal protein and cytochrome P-450 contents. The values represent the mean ± S.D. for five rats.

| | Protein (mg/g liver) | Cytochrome P-450 (nmol/mg protein) |
|---|---|---|
| Control | 22.7 ± 2.7 | 0.87 ± 0.12 |
| EtOH(+) | 24.5 ± 2.0 | 1.22 ± 0.16 |
| EtOH(−) | 21.1 ± 1.5 | 1.00 ± 0.15 |

Control: Rats fed control diet up to the day before sacrifice.
EtOH (+): Rats fed ethanol-containing diet up to the day before sacrifice.
EtOH (−): Ethanol-treated rats fed control diet in place of ethanol-containing diet only on the day before sacrifice.

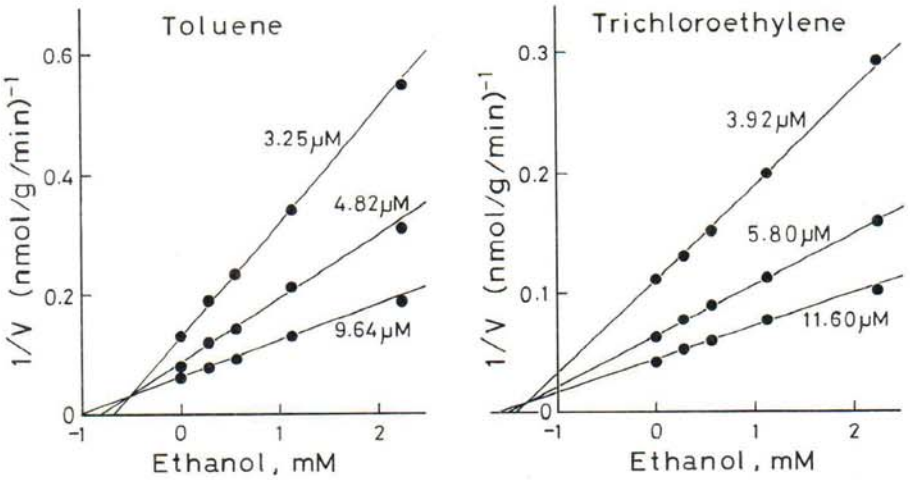


FIG. 3 – Reciprocal plots of metabolic rate (v) of toluene or trichloroethylene in male rats with addition of varying amounts of ethanol (Dixon plot).

The reconstituted 10 000 g supernatant composed of (M) from an ethanol--treated rat and (S) from a control rat metabolized toluene and trichloroethylene at the same rate as the supernatant composed of (M) and (S) both from the control rat. As in the case of food deprivation, therefore, the elevated activity in the ethanol-treated rat was not related to any factors in the soluble fraction but to changes caused in the microsomal fraction.

Judging from the slight but significant increase in the cytochrome P-450 content in the ethanol-treated rats, there is no doubt that chronic ethanol feeding induces liver drug-metabolizing enzymes. However, the extent of the induction is insufficient to fully explain the elevated activity. Our present findings indicate that the effect of chronic ethanol feeding more closely resembles changes observed after food deprivation rather than the enzyme induction produced by well-known inducers such as phenobarbital.

In conclusion, the metabolism of aromatic and chlorinated hydrocarbons in rats is remarkably enhanced by food deprivation or by chronic ethanol consumption. It is another big problem to be studied from hydrocarbon to hydrocarbon whether the enhanced hepatic enzyme activity will lead to protection against toxic effects or conversely to potentiation of toxicity.

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