# HYDROXYLATION IN ISOLATED LIVER CELLS

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Isolated cells were obtained by using EDTA and mechanical action to dissociate rat liver tissue. The presence of oxidative phosphorylation reactions in the hepatocytes was taken as an indication of their integrity. The hepatocytes were able to hydroxylate dimethylaniline, ethylmorphine, and aminopyrine. The maximal velocity of hydroxylation was higher in the cells than in microsomes, when calculated per nmole cytochrome P-450. The NAD·$H_2$ formed by oxidation of glutamate and malate in isolated cells can be used for the hydroxylation reactions.

KEY WORDS: isolated cells; hydroxylation; oxidative phosphorylation; oxidation.

The classical object for the study of hydroxylation reactions is the microsomal fraction of the liver [4]. There are only a few communications on the investigation of these processes in isolated cells [5, 6].

This paper describes a study of hydroxylase activity and possible interaction between the microsomal oxidative system and the mitochondrial system in cells with an intact mechanism coupling oxidation with phosphorylation.

## EXPERIMENTAL METHOD

Isolated rat liver cells were obtained by the use of complexone (EDTA) and mechanical action to dissociate the liver tissue. The composition of the isolation medium (in mmoles) was as follows: source 250, KCl 5, $KH_2PO_4$ 0.4, $Na_2HPO_4$ 0.4, dithiotreitol 2, $MgCl_2$ 0.8, EDTA 1; albumin 1%, pH 7.4. The presence of oxidative phosphorylation reactions in the cells was taken as an indication of their integrity.

## EXPERIMENTAL RESULTS AND DISCUSSION

Isolated hepatocytes were able to oxidize dimethylaniline (DMA), ethylmorphine, and aminopyrine at velocities of 5.1±0.41, 1.2±0.19, and 2.9±0.18 nmole formaldehyde (FA)·$mg^{-1}$·$min^{-1}$ respectively. The velocity of the hydroxylation reaction depended on the composition of the incubation medium and was maximal when a glucose-6-phosphate or isocitrate NADP·$H_2$-generating system was used instead of NADP·$H_2$ as the donor of reducing equivalents. This system in the absence of $NADP^+$ can maintain the velocity of hydroxylation reactions in isolated cells at the level of 30-50% of maximal.

It follows from the results obtained that NADP·$H_2$ cannot readily penetrate into isolated cells and cannot be effectively utilized as cosubstrate in hydroxylation processes.

Comparison of the velocities of hydroxylation of DMA in the microsomal fraction and isolated cells, calculated per nanomole cytochrome F-450 showed that $V_{max}$ and $K_m$ were higher in the cells than in the microsomes (21.2 and 12.7 nmoles FA·$min^{-1}$·$nmole^{-1}$ respectively). The decrease in the velocity of hydroxylation in the microsomal fraction compared with in the hepatocytes could be due to inactivation of the enzyme systems caused by homogenization of the tissue during isolation of the fraction.

In the study of interaction between the mitochondrial oxidation system and the microsomal system the results did not confirm the stimulating effect of many substrates of the Krebs' cycle on the velocity of the hydroxylation reactions obtained by Cinti et al. [2] on liver slices. At the same time it was shown

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TABLE 1. Effect of Substrates of the Krebs' Cycle on Velocity of Hydroxylation of DMA by Isolated Cells (M±m)

| Experimental conditions | Velocity of hydroxylation (in nmoles FA · $mg^{-1} \cdot min^{-1}$) |
|---|---|
| NADP · $H_2$-generating system | 3,9±0,22 |
| NADP · $H_2$-generating system without $NADP^+$ | 1,3±0,09 |
| NADP · $H_2$-generating system without $NADP^+$ + 10 nM glutamate + 10 mM malate | 2,3±0,10 |
| The same + 2 mM $NaN_3$ | 1,8±0,22 |
| The same + $10^{-5}$ M rotenone | 1,7±0,21 |
| 10 mM glutamate + 10 mM malate | 0,2±0,03 |

that glutamate and malate can increase the rate of demethylation of DMA by isolated cells if NADP · $H_2$ or low concentrations of oxidized $NADP^+$ in an NADP · $H_2$-generating system are used as substrate. The accelerating effect of glutamate + malate was not completely abolished by rotenone and $NaN_3$ in concentrations blocking mitochondrial oxidation (Table 1). The results suggest that the stimulant effect of glutamate + malate is the result of NAD · $H_2$ formation in reactions of mitochondrial and extramitochondrial oxidation of these substrates. The stimulant action of NAD · $H_2$ on the velocity of DMA hydroxylation in the microsomal fraction with low concentrations of NADP · $H_2$ has been demonstrated in the writers' laboratory [1] and also by Cohen and Estabrook [3].

It can thus be concluded from these findings that isolated cells with an undisturbed mechanism of coupling of oxidation and phosphorylation have high hydroxylase activity but cannot effectively utilize NADP · $H_2$ as cosubstrate.

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