# EFFECT OF ROSEOFLAVIN ON MICROSOMAL DRUG-METABOLIZING ENZYME SYSTEM

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## 1. Introduction

The $B_2$ vitamin antagonist 7-methyl-8-dimethyl-amino-10-D-ribityl-isoalloxazine, roseoflavin (ROF) product of *Streptomyces* strain no. 768, discovered [1,2] has been shown to be a substrate of flavokinase (EC 2.7.1.26). The false nucleotide formed might be built into the apo-old yellow enzyme and thereby inhibit the oxidation of NADH and NADPH [3].

ROF in vitro ($\leqslant$ 50 $\mu$g/ml) has no effect on the NADPH cytochrome *c* reductase, aniline hydroxylase and aminopyrine *N*-demethylase activity of isolated liver microsomes (data not shown). We have found that in rats kept on normal diet ($B_2$ vitamin conc. 60 ppm), 4 mg/kg ROF ($LD_{50}$ 3.000 mg/kg in mice [1]) administered orally transiently prolongs the hexobarbital sleeping time (fig.1) showing that ROF in vivo is antagonist. The repeated administration of ROF in similar doses increases the hexobarbital sleeping time both in non-induced and phenobarbital-induced animals. Now, we report the results of those experiments, where multiple doses of ROF were applied.

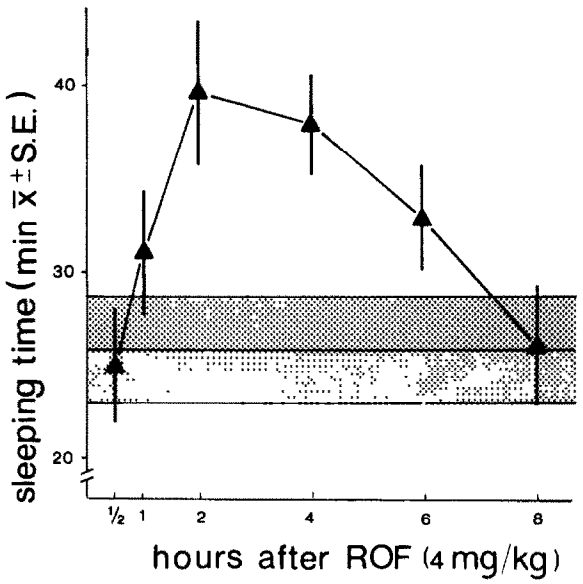


Fig.1. Effect of a single oral dose of roseoflavin (ROF) on hexobarbital sleeping time. Vertical bars: SE of 6–9 determinations. Shaded area: control ± SE.

## 2. Methods

### 2.1. *Animals*

Female Wistar (Wi:RG) rats (100–120 g) were kept on standard Lati food (containing 60 ppm vitamin $B_2$) and water ad libitum.

### 2.2. *Drug administration*

Various amounts (1–4 mg/kg) of ROF suspended in water were administered by stomach tube at about 8:00 a.m.; phenobarbital 60 mg/kg suspended in water was given orally at about 9:00 a.m. Appropriate vehicle controls were included. Animals were treated daily for 3 days. The experiments were performed 24 h after the last treatment.

### 2.3. *In vivo capacity of drug metabolism*

It was assessed by measuring the duration of sleeping time (from loss to retain righting reflex) after

an i.v. injection of hexobarbital (Evipan Natrium®) at a dose of 40 mg/kg.

2.4. *Assays on isolated microsomes*

The animals were sacrificed by decapitation and the livers were excised, chilled on ice and homogenized with a Teflon–glass homogenizer in 3 vol. 150 mM KCl, 50 mM Tris–HCl buffer (pH 7.4) at 0°C. The homogenate was centrifuged at 9000X*g* for 17 min. The supernatant was centrifuged at 105 000X*g* for 60 min. The pellet was resuspended in 0.1 M Tris–HCl buffer (pH 7.5).

Aniline hydroxylase activity was assayed in a medium containing 2 mg/ml microsomal protein suspended in 0.1 M Tris–HCl buffer (pH 7.5); 5 mM $MgCl_2$; 1.0 mM NADPH and 4 mM aniline. Incubation was carried out at 37°C for 15 min.

The *p*-aminophenol formed was measured as in [4].

*N*-demethylase activity was performed in a reaction mixture containing 1.5 mg/ml microsomal protein suspended in 0.1 M Tris–HCl buffer (pH 7.5); 5 mM $MgCl_2$; 1.0 mM NADPH and 8 mM aminopyrine for 10 min at 37°C. The formaldehyde formed was determined by the method in [5]. Cytochrome *P*-450 was measured by the method in [6]. NADPH-cytochrome *c* (*P*-450) reductase (EC 1.6.2.3) activity was assayed as in [7].

## 3. Results

Repeated ROF administration prolongs hexobarbital sleeping time both in non-induced and phenobarbital-induced rats (table 1). This result indicates that the $B_2$ antagonist ROF slows down biotransformation of hexobarbital.

Repeated ROF treatment did not influence the cytochrome *P*-450 concentration in liver microsomes. Phenobarbital and ROF administered simultaneously increased the *P*-450 concentration to a higher level than phenobarbital alone (fig.2). ROF treatment decreased the activity of NADPH-cytochrome *c* (*P*-450) reductase. In the livers of animals simultaneously treated with phenobarbital and ROF the NADPH-cytochrome *c* (*P*-450) reductase activity was inhibited depending on the ROF doses. The diminution of reductase activity on the ROF treatment was identical in control and phenobarbital-treated groups (fig.3). The aminopyrine demethylase and aniline hydroxylase activity of isolated microsomes was not significantly decreased under the effect of ROF treatment (fig.4,5).

## 4. Discussion

In preliminary experiments we have found that ROF ($\leqslant 50$ μg/ml) in vitro has no effect on the liver microsomal drug-metabolizing enzymes, however, in vivo this new $B_2$ vitamin antagonist in low doses influences the microsomal drug-metabolizing enzyme activity of rats kept on normal diet. A single 4 mg/kg ROF dose per os transiently prolongs the hexobarbital sleeping time. The most probable explanation for this phenomenon is that a false coenzyme is produced in the organism.

Repeated administration of ROF both in non-induced and phenobarbital-induced groups increases the sleeping time 24 h after the last treatment,

Table 1
Effect of roseoflavin (ROF) on hexobarbital sleeping time in control and phenobarbital-induced rats

| | Hexobarbital sleeping time (min ± SE) | | | |
|---|---|---|---|---|
| | 0 mg ROF/kg | 1.0 mg ROF/kg | 2.0 mg ROF/kg | 4.0 mg ROF/kg |
| Control | 25.7 ± 2.21 (39) | N.D.[a] | N.D.[a] | 34.0 ± 2.14 (32)[b] |
| Induced | 5.9 ± 0.32 (39)[c] | 11.5 ± 1.18 (15)[c] | 13.2 ± 1.52 (15)[c] | 10.6 ± 0.90 (32)[c] |

[a] not determined
[b] $P < 0.05$
[c] $P < 0.01$ compared to induced and ROF non-treated group

no. animals are in parenthesis

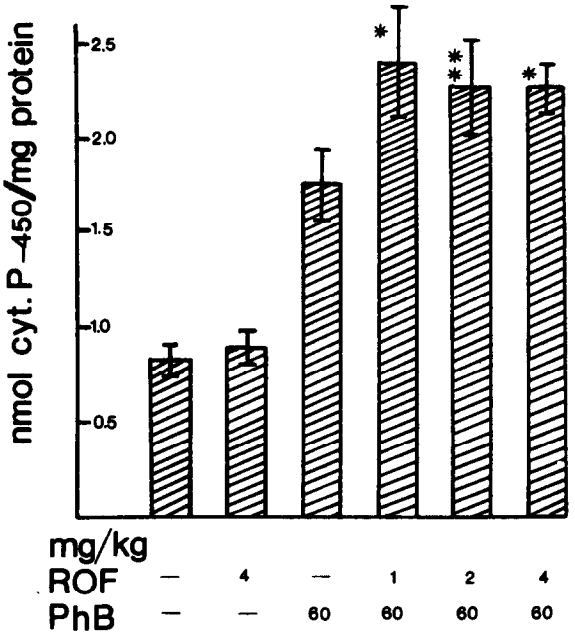


Fig.2. Cytochrome *P*-450 concentration in the liver of control and pretreated rats (mean ± SD, $n$=8): ROF, roseoflavin; PhB, phenobarbital. *$p < 0.01$, **$p < 0.05$.

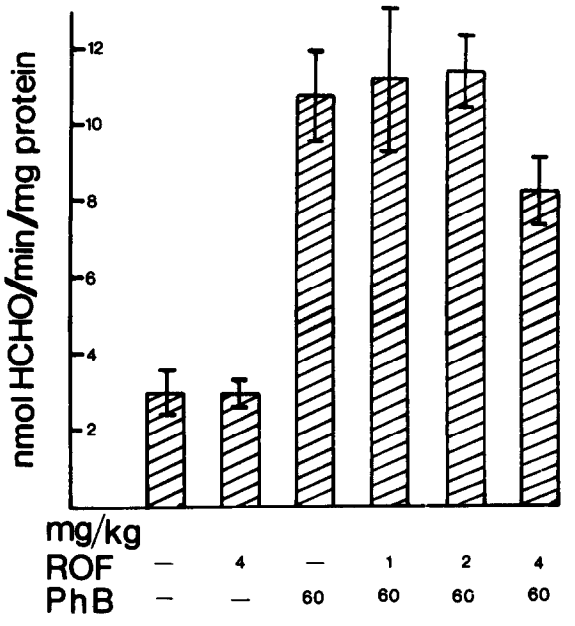


Fig.4. *N*-demethylase activity in the liver microsomes of control and pretreated rats (mean ± SD, $n$=8).

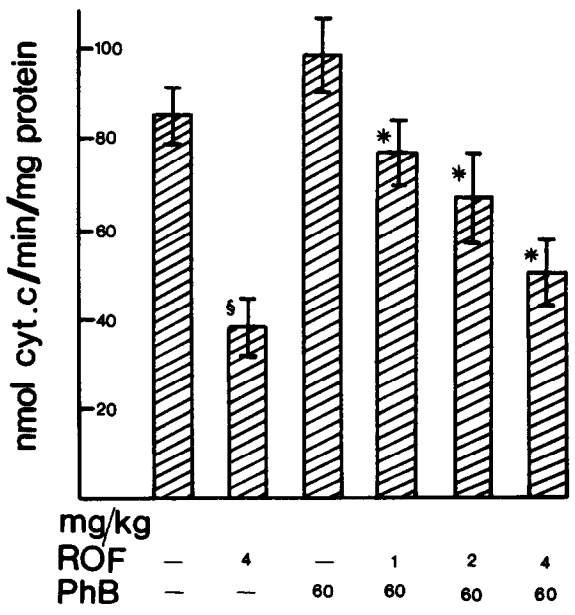


Fig.3. NADPH-cytochrome (*P*-450) activities in the liver of control and pretreated rats. (mean ± SD, $n$=8): §$p < 0.01$, control; *$p < 0.01$, PhB-treated group.

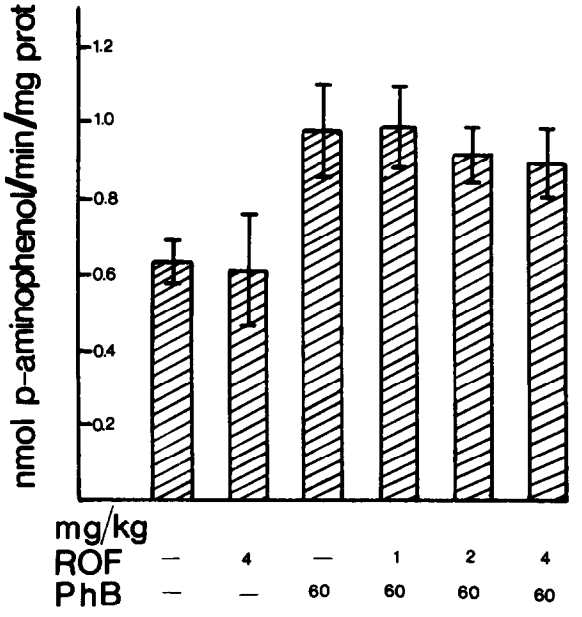


Fig.5. Aniline hydroxylase activity in the liver microsomes of control and pretreated rats (mean ± SD, $n$=8).

indicating the slower biotransformation of hexobarbital in vivo. The aniline hydroxylase and aminopyrine *N*-demethylase activities were not changed significantly in rats pretreated with ROF or ROF plus phenobarbital. Thus, in these experimental conditions the in vitro parameters of metabolism are inconsistent with the in vivo results. The NADPH-cytochrome *c* (*P*-450) reductase activity decreases in a dose-dependent fashion after repeated ROF administration. In the ROF-treated animals the liver cytochrome *P*-450 content was unchanged. If ROF is given together with phenobarbital, the elevation of cytochrome *P*-450 content is higher than in the phenobarbital-induced group. This result was unexpected since the applied phenobarbital dose alone causes maximal induction.

Our results indicate some probems concerning the microsomal drug metabolism. First, the in vivo and in vitro tests for drug metabolism do not parallel each other. Moreover, the in vitro assays themselves are contradictory. The aniline hydroxylase and *N*-demethylase activities did not change significantly after repeated treatments with ROF, and at the same time NADPH-cytochrome *c* (*P*-450) reductase activity and the cytochrome *P*-450 content – thought to be rate-limiting [8–11] – changed considerably in the opposite direction.

Thus, this $B_2$ vitamin antagonist seems to be a useful tool for the comparative study of the mechanism of microsomal drug enzyme induction in vivo and in vitro.

## References

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