

It has been reported that many drugs stimulate their own metabolism as the result of enhancement of liver drug metabolizing enzyme activities (1, 2) and that chloramphenicol (CP) inhibits liver drug metabolizing enzyme activities in rats (3, 4) and humans (5).

Recently, Della Bella et al. (6) observed in rats that pretreatment with phenobarbital enhanced decreases of the drug concentration in serum and chemotherapeutic activity of CP but did not affect those of thiamphenicol (TP).

On the other hand, although a study of the serum concentration of single dose of thiamphenicol glycinate hydrochloride (TPG) in rabbits has been reported (7), studies are not available as to the effects of multiple dosing of TPG on its own fluctuation of blood level and on liver drug metabolizing enzymes in rabbits.
The aim of the present work is to investigate the effect of TPG on its own fluctuation of serum concentrations and on liver drug metabolizing enzyme as compared to chloramphenicol succinate sodium (CPS) when given consecutively to rabbits.

Materials and Methods: Ten male white rabbits weighing approx. 2.0 kg were individually caged, and given a commercial diet with water ad libitum. The animals were then divided into two groups of five one group receiving TPG intravenous administration via ear vein and the other group given CPS by the same route. 100 mg/kg/day of each drug, as the base, was infused to the animals as 5% (W/V) solution using distilled water as the diluent, in a volume of 2 ml/kg and at a rate of 7 ml/min once daily for sixty days.

The measurement of drug concentrations in serum was conducted on day 1, 10, 20, 30, 45 and 60 of the treatment. Blood samples were obtained 30, 60 and 120 min after injection. Drug concentration in serum was determined based on standard microbiological assay techniques using the thin layer disc method and schigella flexneri 2a as the test organism.

All animals were sacrificed by decapitation on day 60. The liver of each animal was perfused with 1.15% KCl solution, immediately frozen and kept in dry ice for about 5 hrs. The liver (about 15 g) was homogenized with 30 ml of 1.15% KCl solution using Teflon homogenizer, centrifuged for 20 min at 9000×g and the supernatant fractions used for the enzymatic studies. Aminopyrine N-demethylation, aniline hydroxylation and hexobarbital hydroxylation were used for determining activities of microsomal drug metabolizing enzymes, the method being essentially the same as described by Kitagawa et al. (8)

The supernatant fractions obtained at 9000×g were further centrifuged for 60 min at 105000×g. The sediment suspended in 0.1 M phosphate buffer (pH 7.5) was used for the measurement of the cytochrome P-450 content in microsomal fractions. The content of cytochrome P-450 was determined by the difference spectrum of the carbon monoxide complex as described by Omura and Sato (9).

Results: 1) Fluctuations of serum concentrations of TPG and CPS: Fig. 1 shows the fluctuation of serum concentrations of drugs followed up and based on the time sequence.

![Fig. 1. Fluctuations of TPG and CPS serum concentrations in rabbits treated with these drugs for sixty consecutive days.](image-url)
Serum concentration of TP, an active form of TPG was 49.0 μg/ml 30 min after administration, then gradually decreased, however a fairly high serum concentration of TP (2.6–17.5 μg/ml) lasted up to 120 min after dosing. In contrast, serum concentration of CP, an active form of CPS 30 min after dosing was 15.6 μg/ml, which corresponds to about 1/3 that of TP. Sixty min after dosing, the serum concentration of CP markedly decreased and was hardly detectable after 120 min. Even with 2 months consecutive administration, fluctuation of serum concentrations of these drugs on the final day was almost comparable to that of the first day.

2) Effects of TPG and CPS on activities of liver microsomal drug metabolizing enzymes and the content of cytochrome P-450: Activities of drug metabolizing enzymes and the content of cytochrome P-450 in liver microsome are shown in Table 1.

| Drugs | Aminopyrine N-demethylation | Anilin hydroxylation formation of p-aminophenol | Hexobarbital hydroxylation disappearance of hexobarbital | Content of cytochrome P-450 |
|-------|-----------------------------|-----------------------------------------------|------------------------------------------------------|-----------------------------|
|       | Formation of 4-aminantipyrine | Formation of formaldehyde | formation of p-aminophenol | disappearance of hexobarbital | |
| TPG   | 0.582 ±0.353                | 2.118 ±1.306                   | 0.285 ±0.142                           | 2.961 ±1.036                         | 0.479 ±0.152               |
| CPS   | 0.583±0.334                 | 2.264±0.765                   | 0.499±0.159                           | 4.815±1.609                          | 0.500±0.048               |
| Control | 0.705±0.455               | 1.043 ±0.106                 | 0.210 ±0.129                           | 3.871±2.505                          | 0.457±0.074               |

a) : μmole/g, liver/hr                                      b) : ΔE 450 mμ–490 mμ
* : significant difference from control at p = 0.05
Results are given as means ± confidence limit (p = 0.05)

In the aminopyrine N-demethylation system, formation of 4-aminantipyrine was not significantly affected in either the TPG- or CPS-treated groups. Formation of formaldehyde, however, was significantly accelerated in CPS-treated groups, but showed only a tendency to increase in the TPG-treated group.

In aniline hydroxylation, formation of p-aminophenol was significantly accelerated in the CPS-treated groups, but not in the TPG-treated group.

In hexobarbital hydroxylation, the enzyme activity showed a tendency to decrease in the TPG-treated group and to increase in CPS-treated group. These results were not however significantly different from the control.

Contents of cytochrome P-450 in liver microsome were not affected in either treated group.

Discussion: It has been reported that many drugs stimulate their own metabolism and tolerance to drugs develops as a result of the increased drug metabolizing enzyme activities in the liver (1, 2, 10).

Our present experiment in rabbits showed that even with daily intravenous administration of drugs for sixty consecutive days, the peak of the serum concentrations and fluctuations of TP and CP based on the time sequence did not change throughout the period of treatment. These results suggest that metabolic rates of either of the drugs are not
affected by continuous administration. Katayama et al (7), reported that when TPG and CPS were intravenously injected into rabbits in a dose of 100 mg/kg, the peak of serum concentration of TP was about 3 times as high as that of CP. As this is in agreement with results obtained herein, TPG is considered to be more potent than CPS in the field of chemotherapeutics.

In studies on liver drug metabolizing enzyme activities in TPG-treated rabbits, significant influences were not observed in any of the enzyme systems examined herein. In contrast, the CPS-treated group showed significant increases of formaldehyde formation in the aminopyrine N-demethylation system as well as formation of p-aminophenol in aniline hydroxylation. No increases were seen in 4-aminoantipyrine and hexobarbital hydroxylation. The fact that in the aminopyrine N-demethylation system, TPG and CPS had no effect on the formation of 4-aminoantipyrine, while CPS significantly accelerated the formation of formaldehyde with TPG showing a tendency to increase it, suggests that these drugs may affect the first stage of the demethylation system, but not the second stage as follows;

\[
\begin{align*}
\text{HCHO} & \quad \text{HCHO} \\
\text{Aminopyrine} & \xrightarrow{\text{first stage}} \text{Monomethyl} & \xrightarrow{\text{second stage}} \text{4-aminoantipyrine}
\end{align*}
\]

Gram (11) has suggested the existence of different enzyme systems which are concerned with each of two stages of aminopyrine N-demethylation system, respectively and it is considered that the effects of TPG and CPS on these stages are different.

Although CPS produced induction of some enzyme activities, the content of cytochrome P-450 was not affected. This is as expected since it has been established that induction of enzymes is not always accompanied by an increase of cytochrome P-450. For example, Kato et al (12), reported that starvation produced induction of some drug metabolizing enzymes in rabbits with no increase in the content of cytochrome P-450.

Yamashita (4) reported that metabolism of pentobarbital in liver microsome of rat was markedly inhibited 1-4 hrs after intramuscular administration of CP and accelerated significantly after 24-48 hrs. The present results with CPS partially coincide with Yamashita’s observations.

Although the measurements of enzyme activities herein were carried out only 24 hr after dosing, it is noteworthy that liver drug metabolizing enzymes were not affected by TPG despite multiple dosing for sixty days in rabbits.

**Summary:** In a 60 consecutive day intravenous administration to rabbits of thiamphenicol glycinate hydrochloride (TPG) and chloramphenicol succinate sodium (CPS), the fluctuation of serum concentrations did not change on the basis of the time sequence observations. The highest serum concentration of thiamphenicol, an active form of TPG, was about 3 times as high as that of CPS.

Aminopyrine N-demethylation, aniline hydroxylation, hexobarbital hydroxylation and content of microsomal cytochrome P-450 were scrutinized as to the effects of TPG and
CPS on liver drug metabolizing enzyme systems. TPG had no effect on either the enzyme activities or cytochrome P-450 content. In contrast, CPS significantly increased activities of aniline hydroxylase and accelerated the formation of formaldehyde in aminopyrine N-demethylation system, but did not affect the activities of other enzymes and the cytochrome P-450 content.

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PHARMACOLOGY OF A NEW HYPNOTIC DRUG: HQ-355

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In 1955 Gujral et al. reported that 2-methyl-3-(o-tolyl)-quinazoline-4 (methaqua-
alone) showed a potent hypnotic activity (1). Moreover, Ravina also reported the
clinical usefulness of methaqualone in insomnia (2). Recently, many derivatives of
2-alkyl-3-(o-tolyl)-quinazoline-4 were synthesized by K. Okumura et al. and it was
found that 2-monofluoromethyl-3-(o-tolyl)-quinazoline-4 (HQ-355) possesses the most

[Chemical structure of HQ-355]