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**A comparative study of the regulation of cytochrome P-450 and glutathione transferase gene expression in rat liver**

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**ABSTRACT**

A cDNA clone for the Ya subunit of glutathione transferase from rat liver was constructed in *E.coli*. The clone hybridized to Ya and Yc subunit messenger RNAs. On the basis of experiments involving cell-free translation and hybridization to the cloned probe, it was shown that prototype inducers of cytochrome P-450 such as phenobarbitone and 3-methylcholanthrene as well as inhibitors such as  $\text{CoCl}_2$  and 3-amino-1,2,4-triazole enhanced the glutathione transferase (Ya+Yc) messenger RNA contents in rat liver. A comparative study with the induction of cytochrome P-450 (b+e) by phenobarbitone revealed that the drug manifested a striking increase in the nuclear pre-messenger RNAs for the cytochrome at 12 hr, but did not significantly affect the same in the case of glutathione transferase (Ya+Yc). 3-Amino-1,2,4-triazole and  $\text{CoCl}_2$  blocked the phenobarbitone mediated increase in cytochrome P-450 (b+e) nuclear pre-messenger RNAs. These compounds did not significantly affect the glutathione transferase (Ya+Yc) nuclear pre-messenger RNA levels. The polysomal, poly (A)- containing messenger RNAs for cytochrome P-450 (b+e) increased by 12-15 fold after phenobarbitone administration, reached a maximum around 16hr and then decreased sharply. In comparison, the increase in the case of glutathione transferase (Ya+Yc) messenger RNAs was sluggish and steady and a value of 3-4 fold was reached around 24 hr. Run-off transcription rates for cytochrome P-450 (b+e) increased by nearly 15 fold in 4 hr after phenobarbitone administration, whereas the increase for glutathione transferase (Ya+Yc) was only 2.0 fold. At 12 hr after the drug administration, the glutathione transferase (Ya+Yc) transcription rates were near normal. Administration of 3-amino-1,2,4-triazole and  $\text{CoCl}_2$  blocked the phenobarbitone-mediated increase in the transcription of cytochrome P-450 (b+e) messenger RNAs. These compounds at best had only marginal effects on the transcription of glutathione transferase (Ya+Yc) messenger RNAs. The half-life of cytochrome P-450 (b+e) messenger RNA was estimated to be 3-4 hr, whereas that for glutathione transferase (Ya+Yc) was found to be 8-9 hr. Administration of phenobarbitone enhanced the half-life of glutathione transferase (Ya+Yc) messenger RNA by nearly two fold. It is suggested that while transcription activation may play a primary role in the induction of cytochrome P-450 (b+e), the induction of glutathione transferase (Ya+Yc) may essentially involve stabilization of the messenger RNAs.

**INTRODUCTION**

Cytochrome P-450 and glutathione transferase constitute two major protein constituents of the drug metabolizing system (1,2). The induction of specific species of cytochrome P-450 in response to drug administration is a well known phenomenon. For example, phenobarbitone induces predominantly cytochrome P-450 b and e

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species in rat liver, whereas 3-methylcholanthrene induces cytochrome P-450 c and d species (3-5). It has also been shown that these drugs act at the transcription level in this regard (6-9). Glutathione transferase represents a family of proteins consisting of two polypeptides recruited from Ya, Yb and Yc subunits in rat liver. These protein species can be homo or hetero-dimers and the possible existence of variants of the Ya, Yb and Yc subunits as well as the presence of additional new subunits have been recognized (10-13). Prototype inducers such as phenobarbitone and 3-methylcholanthrene have been reported to induce Ya and Yc subunits, with Ya being the major subunit induced. In addition, it is known that Ya and Yc have extensive homologies, but are derived from independent messenger RNAs (14-16).

In terms of the mechanism of action of foreign chemicals in inducing the cytochrome P-450 and glutathione transferase protein families, some interesting questions arise. In the case of cytochrome P-450, prototype drugs such as phenobarbitone and 3-methylcholanthrene induce distinctly different species at the transcription level. In the case of glutathione transferase, both the drugs seem to induce the same subunit species, although induction of additional drug-specific subunit species may also be involved. At this stage, it is not clear whether a transcription activation mechanism is responsible for the induction of the glutathione transferase subunits. Secondly, it has been shown in this laboratory that compounds such as  $\text{CoCl}_2$  and 3-amino-1,2,4-triazole block cytochrome P-450 gene transcription (9). The effects of such inhibitors of cytochrome P-450 gene expression on the glutathione transferase system are not known. It is therefore of interest to examine the mechanism of induction of glutathione transferase in relation to that of cytochrome P-450, so that a possible explanation for the diverse effects of chemicals on the two gene systems can be attempted. In the present study, we have examined the mechanism of action of some of these chemicals on the expression of the glutathione transferase gene in rat liver, in relation to that of cytochrome P-450, using specific cloned cDNA probes.

### **EXPERIMENTAL PROCEDURES**

#### **Treatment of animals:**

Male albino rats (100g body weight) of the Institute strain were given single intraperitoneal injections of phenobarbitone (8mg/100g), 3-methylcholanthrene (4mg/100g), 3-amino-1,2,4-triazole (300mg/100g), cordycepin (3mg/100g), actinomycin D (100 $\mu$ g/100g) and  $\text{CoCl}_2$  (6mg/100g, subcutaneous), where indicated. In some experiments, multiple injections of some of the drugs were also given. The animals were killed by decapitation and livers processed for a variety of experiments.

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Purification of glutathione transferase subunits:

Glutathione transferase species were purified essentially by a combination of the procedures described by Habig *et al* (17) and Hayes *et al* (11). Briefly, the water extract of the livers was loaded on a DEAE-cellulose column and the escape was concentrated with ammonium sulphate and then loaded on a CM-Sephadex column. Four peaks of glutathione transferase activity were eluted using 0-80 mM NaCl gradient. Each peak was pooled, lyophilized and analyzed by 15% acrylamide-urea gels (18). In this gel system there was distinct separation of Ya, Yb and Yc subunits of molecular weights 24K, 27K and 30K respectively with another band moving faster than Ya. The protein bands were transiently stained opaque with KCl (19) and then each band was sliced out to elicit antibodies in rabbits. Antibodies were raised to the Yc subunit as well as a mixture of Ya+Yb+Yc subunits.

Construction of a cDNA clone for glutathione transferase:

A rat liver poly (A)-containing messenger RNA library was constructed in *E.coli* (C600) using standard protocols of double stranded cDNA synthesis, GC-tailing and Pst I cut pBR 322 as vector (20). The poly (A)-containing RNA was isolated from magnesium precipitated polyribosomes (21) obtained from phenobarbitone treated rat livers. 439 tetracycline resistant, ampicillin sensitive clones were screened with [<sup>32</sup>P]-cDNA prepared against purified glutathione transferase messenger RNA. Glutathione transferase messenger RNA was purified by immunobinding rat liver polyribosomes with IgG prepared against Ya+Yb+Yc subunits and isolating the immunobound polyribosomes using Protein A-Sepharose chromatography (22). Translation of total polyribosomal poly (A)-containing RNA and the purified messenger RNA in the reticulocyte cell-free translation system (23) revealed a 50 fold enrichment of the messenger RNA. The colonies lighting up were further characterized by hybrid selection. The restriction map and partial sequence data are presented in Fig.1. The clone characterized and used in this study was designated as pGT 19. A clone covering 2/3 of the cytochrome p-450 e messenger RNA from the 3' end and designated as pP-450 91 (9) was used as a probe to analyse cytochrome P-450 (b+e) gene expression.

Quantification of messenger RNA for glutathione transferase and cytochrome P-450:

The translatable messenger RNA activity for glutathione transferase (Ya+Yc) was assessed using the reticulocyte cell-free system (23). Poly(A)-containing RNA (8µg), isolated from rat livers given different treatments, was translated in a total volume of 100 µl in the presence of [<sup>35</sup>S] methionine. The translated products were immunobound to glutathione transferase antibodies. The antigen-antibody complex was isolated using *S.aureus* cells and analyzed by urea-polyacrylamide gel (15%) electrophoresis and fluorography (18, 24).

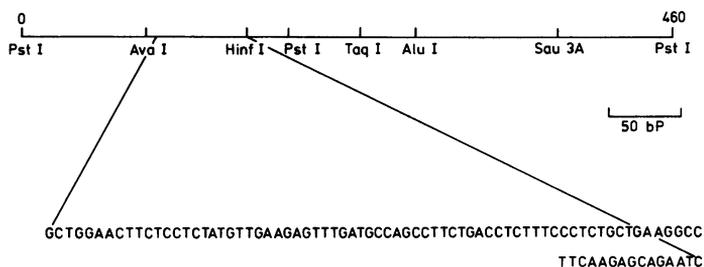


Fig.1. Restriction map and partial sequence analysis of pGT 19 DNA. The experimental details are given in the text.

Messenger RNA contents for glutathione transferase (Ya+Yc) as well as for cytochrome P-450 (b+e) were also quantified by hybridization to nick-translated probes employing dot blot and Northern blot hybridization procedures (25). For this purpose, poly (A)-containing RNA from polyribosomes as well as total RNA from nuclei were used. The isolation of nuclear RNA was by the procedure of Guertin *et al* (26). The amounts of radioactivity quantified in the hybrids in these experiments were found to be proportional to the concentration ranges of RNA used.

Cell-free nuclear transcription:

This was carried out again by the procedure of Guertin *et al* (26) and has been described (9). The RNA transcribed *in vitro* ( $10^7$  cpm) in the presence of [ $\alpha$ - $^{32}$ P] UTP was hybridized to filters containing pGT 19 or pP 450 91 DNA.

Kinetics of messenger RNA induction and half-life of messenger RNA:

Poly(A)-containing RNA was isolated from control as well as phenobarbitone treated rat liver polyribosomes at different time intervals and messenger RNA quantified by dot blot hybridization using nick-translated probes. To measure the half-lives of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) messenger RNAs, actinomycin D or cordycepin was administered 12 hr (cytochrome P-450 b + e) and 20 hr (glutathione transferase Ya+Yc) respectively after phenobarbitone administration to block fresh transcription and specific messenger RNAs quantified by hybridization at different time intervals upto 8hr after the administration of the inhibitors.

Other procedures

Glutathione transferase enzyme activity was measured using 1-chloro 2,4-dinitro benzene as substrate (17). The cDNA probes were nick-translated to give  $3-5 \times 10^7$  cpm/ $\mu$ g DNA. Quantitative values on the dot blots were obtained by punching out the radioactive spots and measuring radioactivity using 0.5% (w/v) 2,5

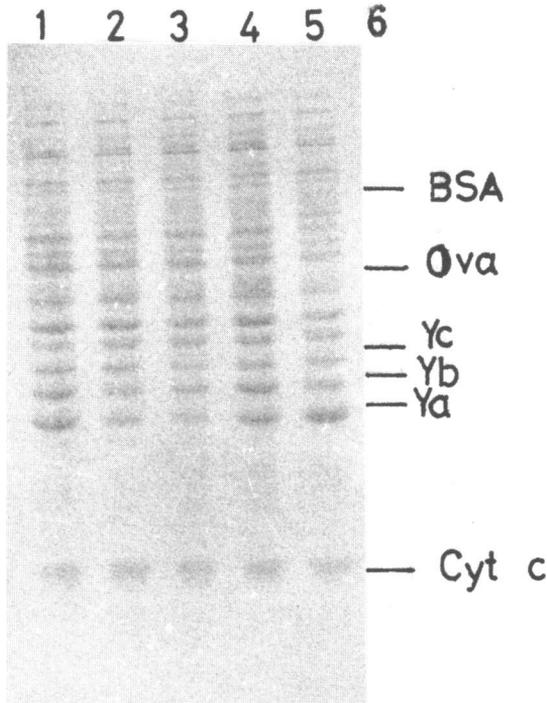


Fig.2. Urea-polyacrylamide (15%) gel analysis of rat liver cytosolic proteins. Cytosol was isolated from phenobarbitone (lane 1),  $\text{CoCl}_2$  (lane 2), control (lane 3), 3-amino-1,2,4-triazole (lane 4) and 3-methylcholanthrene (lane 5) treated rats.  $100 \mu\text{g}$  protein was loaded in each lane and stained with Coomassie Blue. The arrows indicate the positions of glutathione transferase subunits and the standard proteins (lane 6). BSA- Bovine Serum Albumin, Ova-Ovalbumin, Cyt c-Cytochrome c.

diphenyloxazole in toluene as scintillant. Similarly, where necessary the autoradiograms were scanned or the protein gel slices were digested with  $\text{H}_2\text{O}_2$  and the radioactivity measured using 0.5% (w/v) 2,5 diphenyloxazole in Triton-toluene based scintillant.

## RESULTS

In preliminary studies two injections of phenobarbitone and 3-methylcholanthrene administration were found to result in enhanced glutathione transferase enzyme activity. The polyacrylamide gel electrophoretic analysis of the cytosolic proteins clearly revealed a distinct increase in the Coomassie Blue stain intensity of the bands comigrating with the Ya, Yb and Yc subunits of glutathione transferase under conditions of all the drug treatments. There were individual variations, but

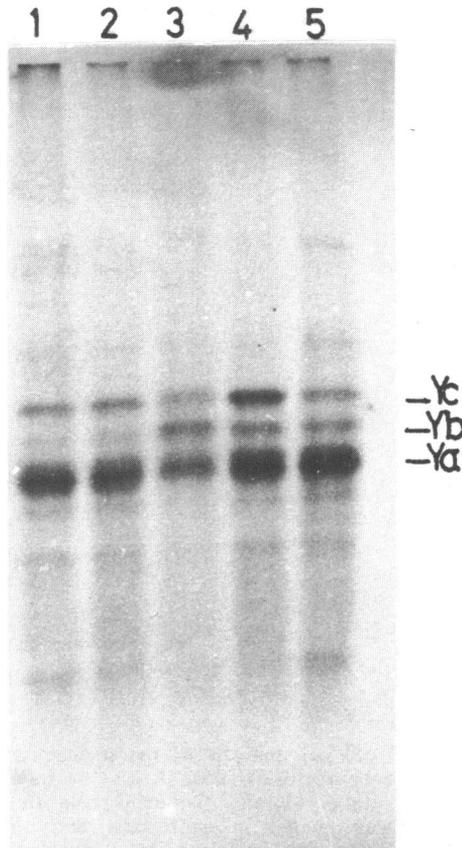


Fig.3. Effect of drug treatments on the translatable activity of glutathione transferase messenger RNA. Poly (A)- containing RNA was isolated from livers, 24 hr after the drug injection except in the case of CoCl<sub>2</sub> treatment where RNA was isolated 12 hr after the injection. 8 $\mu$ g of RNA was translated in 100 $\mu$ l of the reticulocyte lysate cell-free system and 5 x 10<sup>5</sup>cpm of total protein radioactivity was used for immunobinding and electrophoretic analysis. The treatments given were: Lane 1, phenobarbitone; Lane 2, 3-methylcholanthrene; Lane 3, saline (control); Lane 4, CoCl<sub>2</sub>; Lane 5, 3-amino-1,2,4-triazole.

the Ya subunit in general showed a maximum response (Fig. 2). It was significant that the inducers (phenobarbitone and 3-methylcholanthrene) as well as the inhibitors (3-amino-1,2,4-triazole and CoCl<sub>2</sub>) of cytochrome P-450 synthesis appeared to increase the glutathione transferase protein content. We also quantified the translatable messenger RNA activity for the Ya and Yc subunits using the reticulocyte cell-free system 24hr after a single injection of the drugs except in the case of CoCl<sub>2</sub> where the effects were studied 12hr after the injection. The latter time

TABLE 1

Messenger RNA quantification for glutathione transferase subunits by cell-free translation and northern blot hybridization.

Treatment	Cell-free translation Ya subunit (cpm)	Northern blot hybridization Ya+Yc subunits (cpm)
Control	123	715
Phenobarbitone	372	2563
3-methylcholanthrene	385	2772
CoCl <sub>2</sub>	421	1876
3-Amino-1,2,4-triazole	417	2473

The quantitative values were derived from data presented in Figs. 3 and 4. The procedures are described in the text.

period was chosen, since the inhibitory effect of CoCl<sub>2</sub> administration on cytochrome P-450 synthesis decreased with time and was much more pronounced at 12hr. There was nearly a 3-4 fold increase in the Ya subunit messenger RNA activity under all conditions of drug treatments. In addition, CoCl<sub>2</sub> treatment resulted in a significant increase in the messenger RNA activity for Yc subunit as well (Fig 3, Table 1).

In order to quantify the messenger RNA content and the transcription rates of the glutathione transferase gene, we constructed a cloned cDNA probe. Clone pGT 19, on the basis of hybrid selection (data not presented) restriction mapping and partial sequencing (Fig 1) was characterized to be a Ya subunit clone from the complete sequencing data provided by Pickett *et al* (16) and Tu *et al* (27). pGT 19 covered nearly 2/3 of the Ya subunit messenger RNA from the 3' end, having an extensive homology with that of the Yc subunit, and therefore the cloned DNA would hybridize to Ya and Yc subunit messenger RNAs. Northern blot analysis of the polyribosomal poly (A)-containing RNA isolated from rats treated with the different drugs under conditions described in Fig.3 revealed that a 3-4 fold increase in Ya+Yc messenger RNA contents was achieved. The size of the messenger RNAs was around 900 nucleotides (Fig.4, Table 1). A dot blot analysis of the nuclear pre-m-RNAs, 12hr after a single injection of phenobarbitone also revealed a phenomenal increase in the case of cytochrome P-450(b+e) and this increase was blocked by the simultaneous administration of 3-amino-1,2,4-triazole or CoCl<sub>2</sub>. However, none of the drug treatments either alone or in the combination significantly changed the nuclear pre-mRNA contents for glutathione transferase (Ya+Yc) subunits (Fig.5).

A detailed analysis of the kinetics of induction of glutathione transferase (Ya+Yc) subunit messenger RNAs and cytochrome P-450 (b+e) messenger RNAs after a single injection of phenobarbitone was then undertaken. Nick-translated

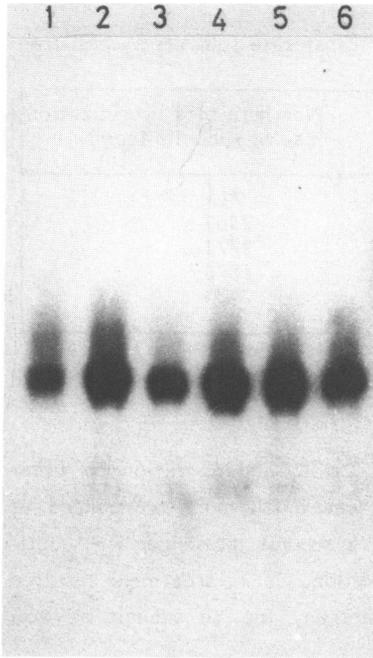


Fig.4. Northern blot analysis of poly (A)-containing RNAs isolated from drug treated rats. The RNA preparations (4  $\mu$ g) (described in Fig.3) were subjected to Northern blot analysis using nick-translated pGT 19. Lane 1, control; Lane 2, 3-methylcholanthrene; Lane 3, control (6  $\mu$ g RNA); Lane 4, phenobarbitone; Lane 5, 3-amino-1,2,4-triazole; Lane 6, CoCl<sub>2</sub> pBR 322 DNA cut with Hinf I was used for size calibration.

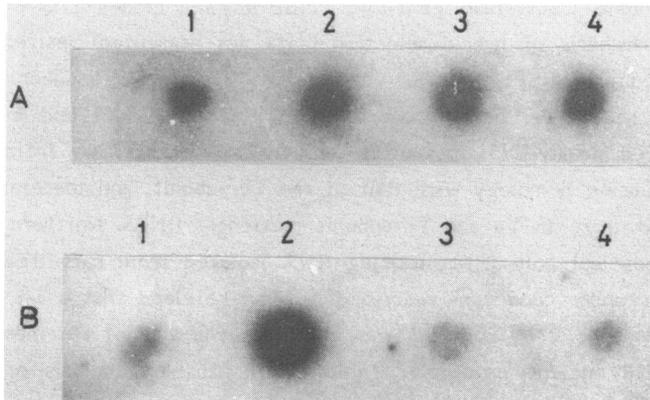


Fig.5. Dot blot analysis of nuclear RNA isolated from drug treated rats. Nuclear RNAs were isolated 12 hr after the drug administration and 10  $\mu$ g of each preparation was analysed by dot blot hybridization using nick-translated pGT 19 (A) and pP-450 91 (B). (1) control (2) phenobarbitone (3) 3-amino-1,2,4-triazole (4) CoCl<sub>2</sub>. In the case of cytochrome P-450 (b+e) studies, 3-amino-1,2,4-triazole and CoCl<sub>2</sub> were given along with phenobarbitone since their effects were studied on the induction process. In the case of glutathione transferase (Ya+Yc) studies, 3-amino-1,2,4-triazole and CoCl<sub>2</sub> were given in the absence of phenobarbitone. Even in the presence of phenobarbitone, the results obtained were essentially the same.

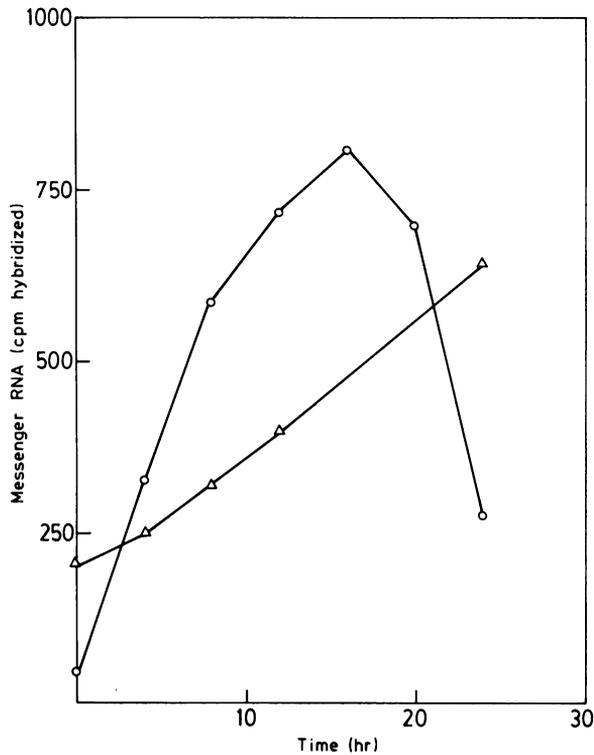


Fig.6. Time course of induction of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) messenger RNAs. The specific messenger RNAs were quantified by hybridization of total poly (A)-containing RNAs (1 $\mu$ g) with nick-translated probes in dot blot experiments. The values given are an average of two independent experiments. The other experimental details are given in text. (O) Cytochrome P-450 (b+e) messenger RNA content after phenobarbitone treatment; ( $\Delta$ ) glutathione transferase (Ya+Yc) messenger RNA content after phenobarbitone treatment.

pGT 19 and pP-450 91 were used as probes. It was found that the cytochrome P-450 (b+e) messenger RNAs increased sharply within 4hr, reached a 12-15 fold increase around 16hr and then showed a rapid decline. Glutathione transferase (Ya+Yc) subunit messenger RNAs showed a sluggish increase in comparison and reached a maximum of 3-4 fold around 24hr after a single injection of the drug (Fig.6).

The kinetics of messenger RNA induction as well as the status of nuclear pre-mRNAs are indicative of the transcription rates of the two gene systems. To confirm this, run-off transcription rates with nuclei isolated from rats given different drug treatments were assessed. The results presented in Table 2 indicate

TABLE 2

Comparative rates of transcription of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) genes with isolated nuclei *in vitro*.

Treatment	Cytochrome P-450 (b+e) (ppm/Kb of cloned probe hybridised)	Glutathione transferase (Ya+Yc)
Control	7	48
Phenobarbitone (4 hr)	102	95
Phenobarbitone (12 hr)	91	58
Phenobarbitone + CoCl <sub>2</sub> (4hr)	17	-
Phenobarbitone + CoCl <sub>2</sub> (12 hr)	21	-
Phenobarbitone +3-amino 1,2,4-triazole (4hr)	20	-
Phenobarbitone +3-amino 1,2,4-triazole (12hr)	25	-
CoCl <sub>2</sub> (4hr)	-	55
CoCl <sub>2</sub> (12hr)	-	50
3-Amino-1,2,4-triazole (4hr)	-	63
3-Amino-1,2,4-triazole (12hr)	-	58

The *in vitro* transcription was carried out with isolated nuclei in the presence of [ $\alpha$ -<sup>32</sup>P] UTP as described in the text (26). Nuclei were isolated from control and phenobarbitone treated rats. The *in vitro* transcripts (10<sup>7</sup> cpm) were hybridized to pGT 19 or pP-450 91 DNA loaded on nitrocellulose filters. pBR 322 DNA was used to compute non-specific hybridization, which was less than 10% of specific hybridization. The results are expressed as ppm (parts per million) of the transcripts hybridized per Kb of the cloned probe and represent the average from two different experiments. The other details are given in text.

that 4hr after phenobarbitone administration there was nearly two-fold increase in glutathione transferase (Ya+Yc) messenger RNA transcription rates. However, with 3-amino-1,2,4-triazole and CoCl<sub>2</sub> treatments the increase was only marginal. At 12hr after the drug treatments, phenobarbitone treatment at best elicited only about 30% increase in the glutathione transferase (Ya+Yc) messenger RNA transcription rates, whereas 3-amino-1,2,4-triazole and CoCl<sub>2</sub> treatments had no effect. In contrast, phenobarbitone treatment elicited high levels of transcription rates for cytochrome P-450 (b+e) messenger RNA at both time intervals and 3-amino 1,2,4-triazole and CoCl<sub>2</sub> treatments substantially blocked this increase. None of the treatments significantly affected the total transcription rates. Addition of  $\alpha$ -amanitin (1  $\mu$ g/ml) inhibited total transcription by around 50%, but the transcription of the two gene systems under consideration was inhibited by more than 95% (data not presented).

Next, an attempt was made to measure the half-lives of cytochrome P-450

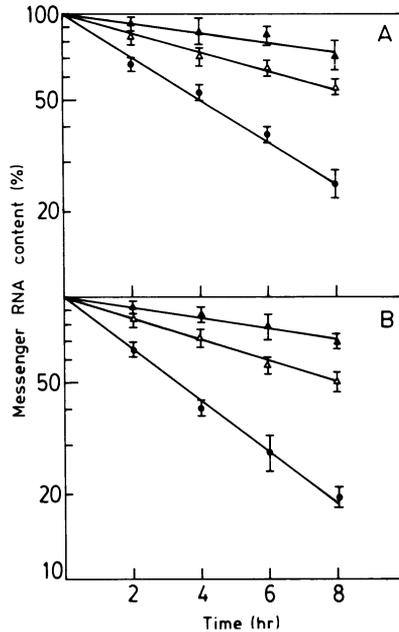


Fig.7. Half-life measurements of cytochrome P-450 (b+e) and glutathione transferase (Ya+Yc) messenger RNAs. Actinomycin D or cordycepin was administered 12 hr (cytochrome P-450 b+e) and 20 hr (glutathione transferase Ya+Yc) after phenobarbitone administration to block fresh transcription and poly (A)- containing RNAs isolated at different time intervals. The specific messenger RNAs were quantified by dot blot hybridization with nick-translated probes. The results represent the mean + S.D. obtained from three independent RNA preparations. A- Actinomycin D treatment. B- Cordycepin treatment. (●)- cytochrome P-450 (b+e) messenger RNA content in phenobarbitone-pretreated animals. (▲) Glutathione transferase (Ya+Yc) messenger RNA content in phenobarbitone pretreated and (Δ) saline treated animals.

(b+e) and glutathione transferase (Ya+Yc) messenger RNAs. For this purpose, actinomycin D or cordycepin was administered to control as well as phenobarbitone pre-treated rats and the specific messenger RNA contents were quantified by hybridization at different time intervals after the administration of the transcription inhibitors. In preliminary experiments, it was found that actinomycin D and cordycepin inhibited poly (A)-containing RNA synthesis by 95% and 80% respectively and that the inhibition lasted for atleast 8hr (data not presented). Therefore, the specific messenger RNA levels were quantified upto 8hr only. The results presented in Fig. 7 indicate that with both inhibitors glutathione transferase (Ya+Yc) messenger RNAs were found to have a half-life around 8-9hr and administration of phenobarbitone enhanced the half-life by 2-fold. In phenobarbitone treated rats, cyto-

chrome P-450 (b+e)messenger RNAs were found to have a half-life around 3-4 hr. It was not possible to make half-life measurements in the control animal, in view of the very low content of the cytochrome P-450 (b+e) messenger RNAs.

### DISCUSSION

Foreign chemicals induce enzymes in and outside the drug metabolizing pathway. The exact mechanism of this induction process has not been understood. However, in the case of cytochrome P-450, a specific transcriptional activation mechanism has been recognized (6-9). Strong evidences for the existence of a receptor for hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3-methylcholanthrene have been presented and the involvement of the inducer-receptor complex in activating specific cytochrome P-450 genes has been suggested (28-30). In that light, the mechanism of action of inducers on the other enzymes of the drug metabolizing complex assumes importance, since it would raise questions on the specificity and multiplicity of receptors for an inducer, if a transcriptional activation mechanism is favoured in every case.

Phenobarbitone also induces specific cytochrome P-450 species by transcriptional activation (6,7,9), although there is no definitive evidence for the mediation of a receptor. However, it is interesting to point out that whereas phenobarbitone and 3-methylcholanthrene induce distinctly different species of cytochrome P-450, they appear to induce the same subunit species (Ya+Yc) of glutathione transferase. In addition, inhibitors of cytochrome P-450 (b+e) messenger RNA synthesis such as  $\text{CoCl}_2$  and 3-amino-1,2,4-triazole actually enhance the glutathione transferase (Ya+Yc) messenger RNA levels.

The present studies reveal that while the dramatic increase in cytochrome P-450 (b+e) messenger RNA levels in response to a single injection of phenobarbitone can be explained on the basis of sustained high transcription rates of the genes concerned, the increase in glutathione transferase (Ya+Yc) messenger RNAs cannot be explained on this basis. Phenobarbitone does increase glutathione transferase (Ya+Yc) transcription rates by nearly 2-fold at early time intervals, but by 12hr the rates are only marginally higher than control, when the specific messenger RNA levels continue to increase. In addition,  $\text{CoCl}_2$  and 3-amino-1,2,4-triazole have only a marginal or no effect on transferase transcription rates, but nevertheless increase the specific messenger RNA contents.

Therefore, we have examined the possibility whether stabilization of glutathione transferase messenger RNAs by drug treatments can explain the increase in messenger RNA levels and enzyme protein content and activity. Measurement of half lives of protein and nucleic acids after blocking fresh translation and trans-

cription with suitable inhibitors is a standard procedure, although the possibility of the inhibitors by themselves having an effect on the degradative process cannot be ruled out (31,32). We have used actinomycin D, an inhibitor of transcription and cordycepin, an RNA synthesis chain terminator to block fresh RNA synthesis. Earlier, it has been shown that cordycepin blocks the phenobarbitone-mediated messenger RNA induction for enzymes such epoxide hydratase, NADPH-cytochrome P-450 reductase and cytochrome P-450b (33-35). On the basis that the transcription inhibitors exert similar effects in control and drug-treated rats, the results indicate that phenobarbitone administration enhances the half-life of glutathione transferase (Ya+Yc) messenger RNAs. Besides, the time course of induction of glutathione transferase (Ya+Yc) messenger RNAs after phenobarbitone administration (Fig.6) indicates that the messenger RNA half-life values obtained for the drug treated animals by the use of the transcription inhibitors (Fig.7) are approximately in the same range of values that can be calculated on the basis that the rate of approach to the new steady state is directly related to the degradation rate constant (36). However, it has been possible to make half-life measurements only upto 8hr, since the transcription inhibitors were found to inhibit poly (A) - containing RNA synthesis maximally only upto this time point. Besides, the half-life values are a composite of two subunit messenger RNAs since the probes used hybridize to both the messenger RNAs having extensive homologies. Despite these limitations, the data clearly indicate stabilization as a major mechanism for the induction of transferase messenger RNAs, whereas, the increase in cytochrome P-450 messenger RNAs is essentially due to sustained high transcription rates. The list of messenger RNAs known to be regulated atleast in part by turnover control includes hen ovalbumin, Xenopus vitellogenin, rat casein, dihydrofolate reductase and tubulin (36). It is not clear whether the initial increase in the glutathione transferase transcription after phenobarbitone administration 'triggers' a cascade of events. We have, however, found that a wide variety of chemicals induce glutathione transferase (Ya+Yc) subunits and perhaps a generalized second messenger type response rather than an inducer-specific receptor mechanism is involved. It may also be pointed out that glutathione transferase is a major cytosolic protein in the normal rat liver unlike the induced cytochrome P-450 species which are present at very low levels in the untreated animal. Therefore, the glutathione transferase gene is perhaps transcribed at a high efficiency in the normal animal and the induction process should rather involve stabilization mechanisms.

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