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REPEATED DEREPRESSION CYCLES OF THE GLUCURONOLACTONE DEHYDROGENASE AND CYTOCHROME P-450 IN THE RAT LIVER INDUCED BY PHENOBARBITONE ADMINISTRATION

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Abstract—Treatment of adult male rats with five doses of phenobarbitone was repeated at 4-day and monthly intervals in order to study the memory of the derepression cycles of glucuronolactone dehydrogenase and cytochrome P-450 in the liver. When the interval was 1 month, further drug treatments resulted in a similar increase of these enzyme levels and the relative liver weight, both during the second and third treatments. If the next treatment was commenced after an interval of 4 days, when the enzyme levels were still high, significantly lower cytochrome P-450 levels were observed after the third, than after the first and second treatments, and furthermore, after the third treatment the decline of cytochrome P-450 was more rapid than after the first phenobarbitone treatment.

The increase and decrease of glucuronolactone dehydrogenase activity took place at a considerably slower rate than that of cytochrome P-450. After five phenobarbitone administrations there was a significant increase of cytochrome P-450 and also of glucuronolactone dehydrogenase for up to 48 hr, followed by a decrease to normal level of cytochrome P-450 in 8 days. On the eighth day glucuronolactone dehydrogenase activity was still nearly two-fold in comparison with the controls.

The results indicate that successive phenobarbitone treatments do not result in any permanent memory effects in the metabolism of glucuronolactone dehydrogenase and cytochrome P-450, although if repeated with 4-day intervals a diminished inductive response was observed on the third treatment.

RATS which are physically barbitone dependent show, after drug withdrawal, hypersensitivity towards hexobarbitone together with a lower activity of various drug metabolizing liver enzymes than the control animals.[1] After phenobarbitone administration these rats also elicit a decreased inductive hexobarbitone oxidation response[1] as do the previously phenobarbitone treated rats in respect of aminopyrine demethylation.[2] The barbitone induced effect on the glucuronic acid pathway and other drug metabolizing enzyme systems decreases with increasing age of the rats.[3] These findings suggest that earlier derepressions of the drug metabolizing enzymes may result in some kind of permanent memory in the liver.

In order to study this memory effect the derepression of glucuronolactone dehydrogenase and cytochrome P-450 in the rat liver was brought about by intraperitoneal (i.p.) administration of phenobarbitone, from one to three times at intervals of 4 days and 1 month. It has earlier been demonstrated that phenobarbitone administration results in a highly increased synthesis of glucuronolactone dehydrogenase[4] and

cytochrome P-450,[5] which can be blocked by actinomycin D administration.[4,5] The effect of phenobarbitone exhibits strict liver specificity in the rat as far as the glucuronic acid pathway is concerned,[4,6] and the highest inductive response is found in glucuronolactone dehydrogenase.[4]

METHODS

Adult male Wistar rats fed *ad libitum* were used. They were 6 or 7 months old at the time of tissue analysis and weighed 250–350 g. Their total number was 250. The test and the control groups were approximately of the same size, and they both were from the same litters. Phenobarbitone (80 mg/kg) was administered i.p. once a day, and the tissue analysis was carried out 24 hr after the last dose, if not stated otherwise.

The animals were stunned by a blow on the head and bled by cutting the renal vessels. The excised liver was homogenized in 0·15 M KCl-solution by using a Teflon-pestled glass homogenizer. The microsomal and soluble fractions were prepared, and the glucuronolactone dehydrogenase activity was determined as described earlier.[4] Cytochrome P-450 was measured by recording the eleven-fold expanded difference spectrum from 400 to 500 nm using a Perkin–Elmer UV-137 double beam spectrophotometer equipped with an external recorder; for this purpose the enzyme was reduced with sodium dithionite and gassed with carbon monoxide in 0·1 M sodium phosphate buffer, pH 7·4.[7] The amount of the microsomal fraction per ml corresponded to 25 mg of fresh liver. Each measurement was carried out in duplicate.

RESULTS

Already after a single phenobarbitone dose the cytochrome P-450 level increased to a four-fold level 72 hr after drug injection, although the glucuronolactone dehydrogenase showed only a minor increase and the relative liver weight remained at control level (Fig. 1 A). If the drug was injected daily and the analyses were carried out 24 hr after the last dose, the cytochrome P-450 plateau, at ca. 3·5-fold control level was reached after six drug injections (Fig. 1 B). When the drug was administered daily, glucuronolactone dehydrogenase activity began to increase after a short lag period of only 1 day, reaching a maximum value (ca. 3·2-fold) in 8 days as did the relative liver weight (1·5-fold) (Fig. 1 B).

When phenobarbitone was withdrawn after five doses, there was first a further significant increase of cytochrome P-450 and glucuronolactone dehydrogenase during 24 to 48 hr. Then the cytochrome P-450 levels returned to normal in 8 days (Fig. 1 C). Glucuronolactone dehydrogenase activity decreased more slowly, and 8 days after the last dose it was nearly twice normal (Fig. 1 C), decreasing in 1 month to control level (Fig. 2). The liver weight did not exhibit a postwithdrawal increase, and after 8 days it was nearly at the control level (Fig. 1 C).

When phenobarbitone treatment was repeated twice or three times at intervals of 1 month, the increases of the enzyme levels and the relative liver weights did not differ significantly from those observed in connection with the first drug treatment (Fig. 2). Also 1 month after the second treatment both enzyme levels and the relative liver weights were back at control values (Fig. 2).

If the intervals between phenobarbitone treatments were cut to 4 days, which meant that the next treatment was begun under the influence of the preceding treatment, the activity of glucuronolactone dehydrogenase was slightly and the level of cytochrome

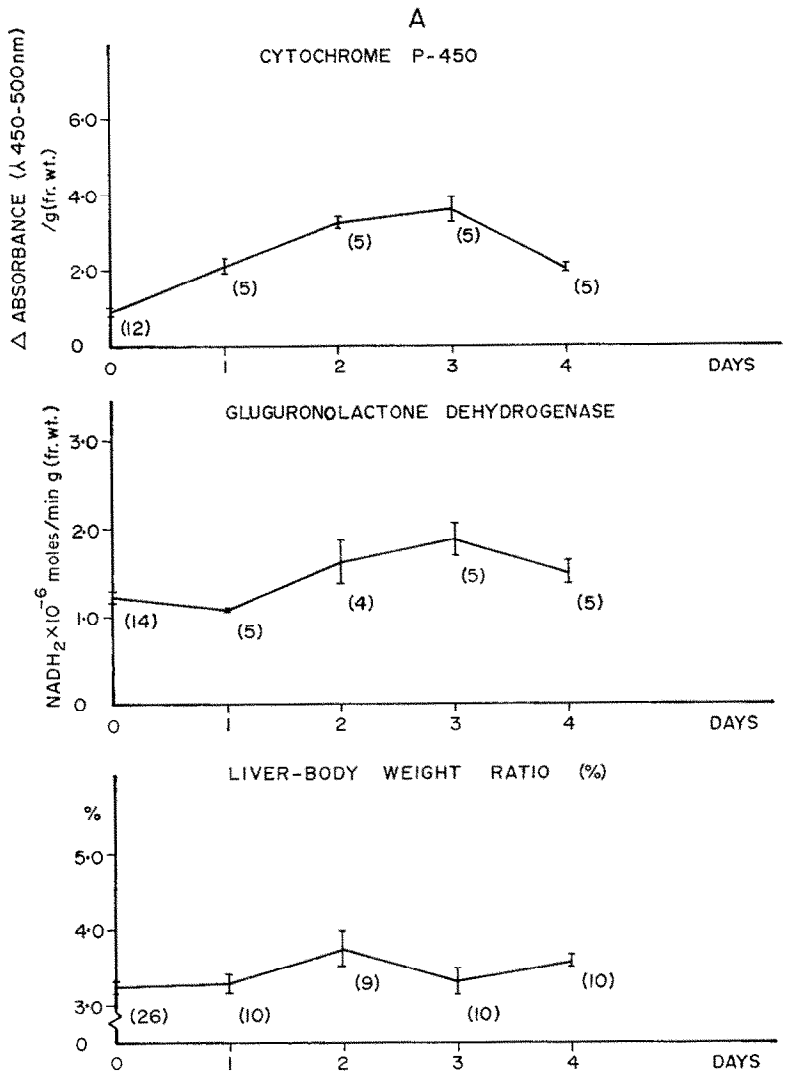

Fig. 1A

FIG. 1. Levels of liver cytochrome P-450 and glucuronolactone dehydrogenase together with the liver–body weight ratios in per cent after one (A) or repeated (B) intraperitoneal phenobarbitone injections and after withdrawal of drug treatment after five doses (C) in 7-months old male rats (December–January). Cytochrome P-450 levels are given as Δ-absorbance values and the activity of glucuronolactone dehydrogenase as the rate of NAD reduction on tissue fresh weight basis. The number of animals (in parentheses) and standard errors of the means are given together with the P-values for post-treatment peaking of cytochrome P-450 and glucuronolactone dehydrogenase.

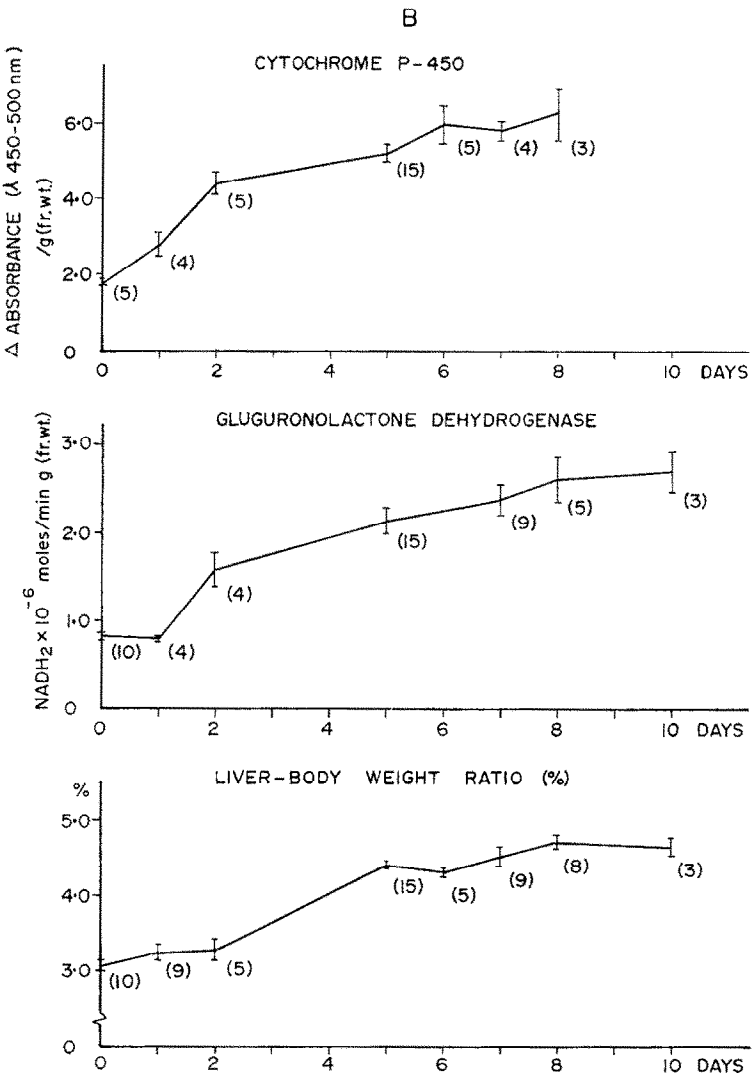
B
CYTOCHROME P-450
Δ ABSORBANCE (λ 450-500 nm) /g(fr.wt.)
6·0
4·0
2·0
0
(5)
(4)
(5)
(15)
(5)
(4)
(3)
0 2 4 6 8 10 DAYS
GLUGURONOLACTONE DEHYDROGENASE
NADH2 x 10^-6 moles/min g (fr.wt.)
3·0
2·0
1·0
0
(10)
(4)
(4)
(15)
(9)
(5)
(3)
0 2 4 6 8 10 DAYS
LIVER-BODY WEIGHT RATIO (%)
%
5·0
4·0
3·0
0
(10)
(9)
(5)
(15)
(5)
(9)
(8)
(3)
0 2 4 6 8 10 DAYS

FIG. 1B

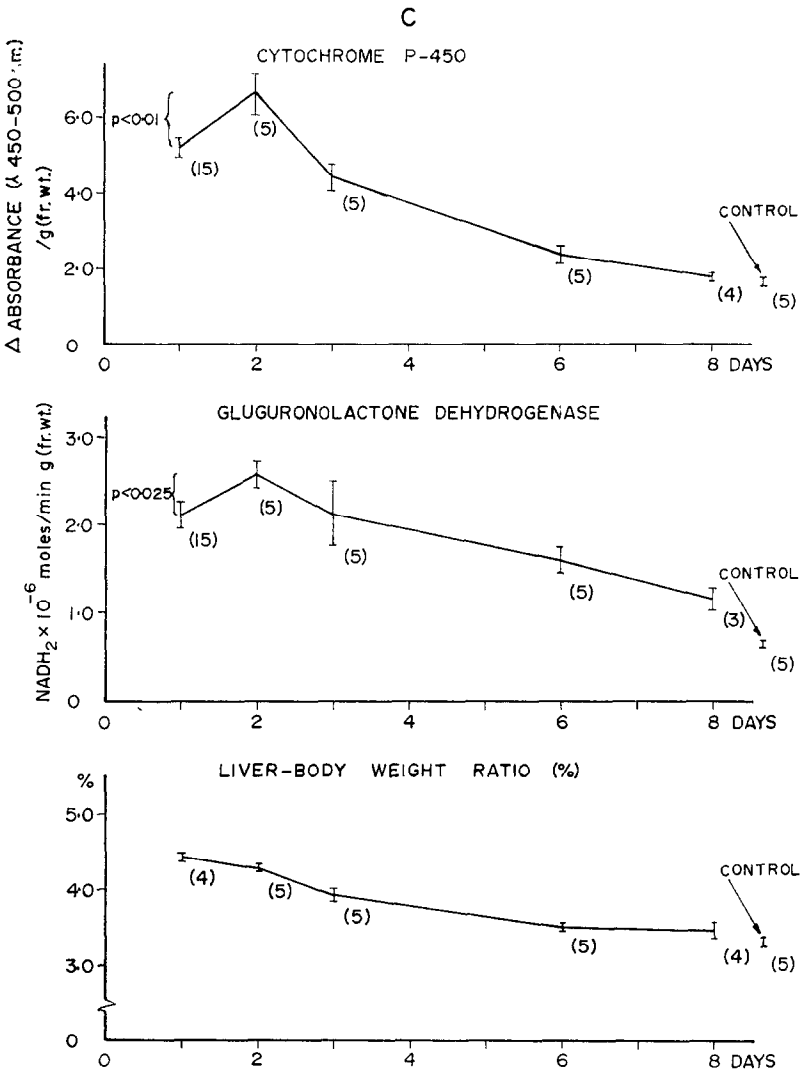
C
CYTOCHROME P-450
Δ ABSORBANCE (λ 450-500 m.)
/g(fr. wt.)
p<0·01
(15)
(5)
(5)
(5)
(4)
(5)
CONTROL
6·0
4·0
2·0
0
0
2
4
6
8 DAYS
GLUGURONOLACTONE DEHYDROGENASE
NADH2 x 10-6 moles/min g (fr.wt.)
p<0·025
(15)
(5)
(5)
(5)
(3)
(5)
CONTROL
3·0
2·0
1·0
0
0
2
4
6
8 DAYS
LIVER-BODY WEIGHT RATIO (%)
%
5·0
4·0
3·0
0
(4)
(5)
(5)
(5)
(4)
(5)
CONTROL
0
2
4
6
8 DAYS

FIG. 1C

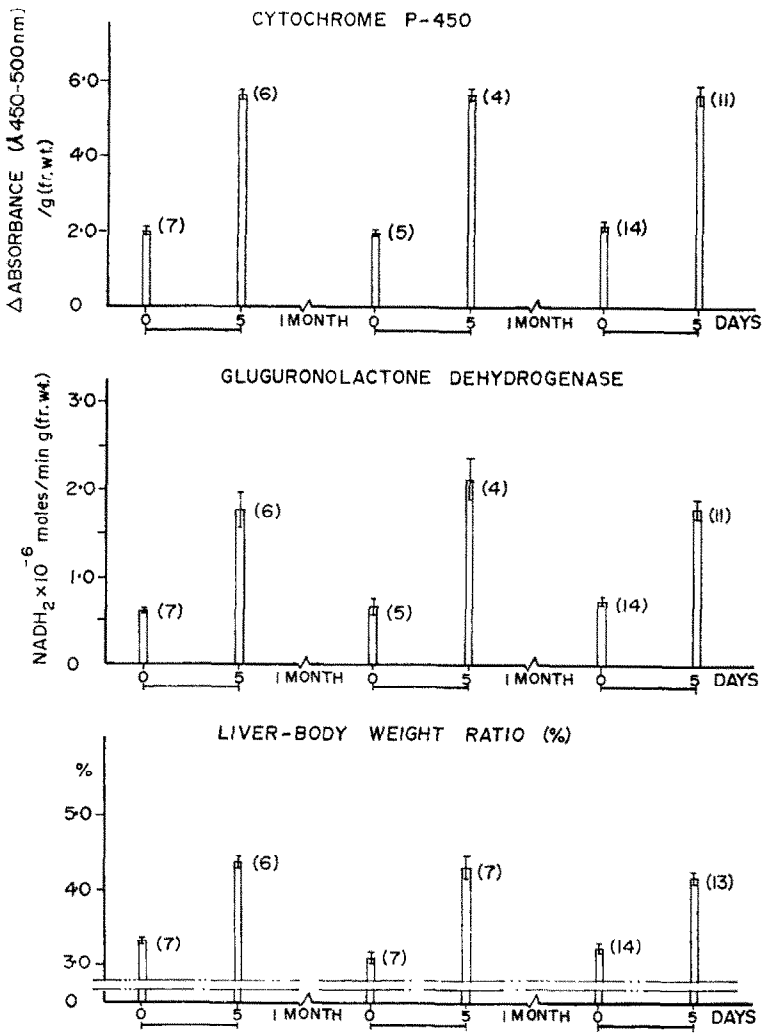

FIG. 2. The effect of up to three phenobarbitone treatments (five doses each) at one month intervals on the levels of cytochrome P-450 and glucuronolactone dehydrogenase and the liver–body weight ratios in per cent one day and one month after the last dose. The bars under the abscissa indicate the duration of the phenobarbitone administrations. The experiments were carried out in December using 6-months old male rats. Thus, each experimental group was of the same age during the tissue analysis eliminating seasonal and age effects.[3,9] For further explanations see Fig. 1.

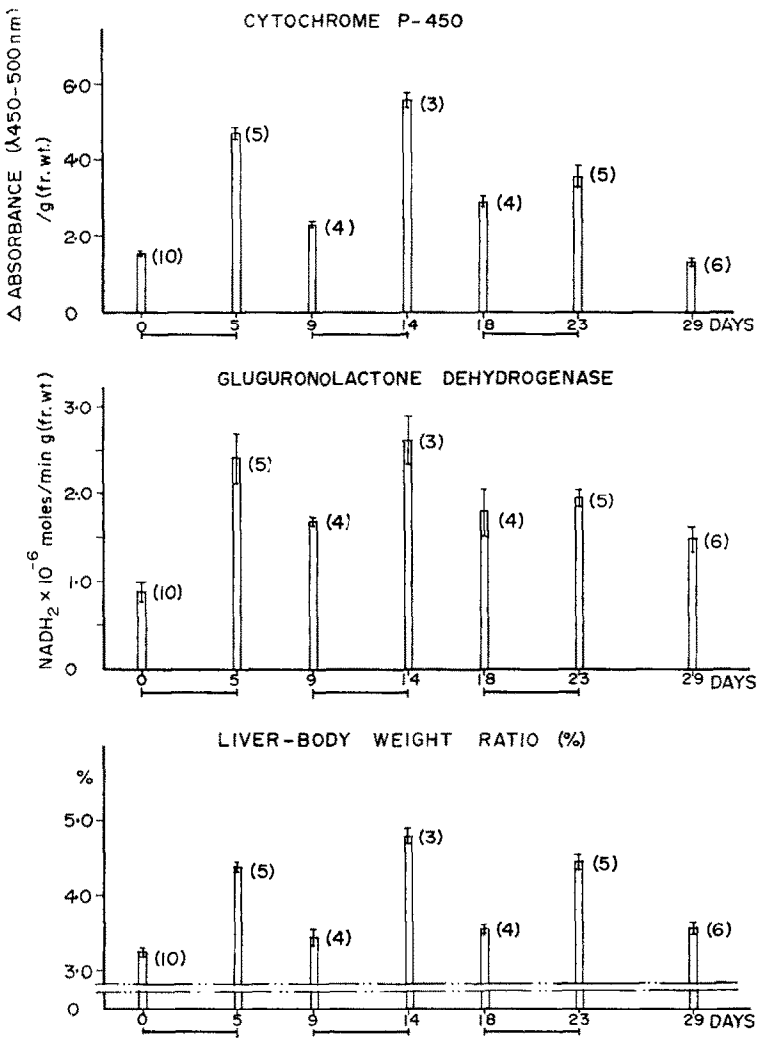

FIG. 3. The levels of liver cytochrome P-450 and glucuronolactone dehydrogenase and the liver–body weight ratio in per cent during a phenobarbitone treatment with five successive drug administrations intermingled with 4-day drug free periods. The bars under the x-axis indicate the duration of phenobarbitone administration. The experiments were carried out in February using 7 months old rats. The P-value for the difference between the first and third phenobarbitone period is $P < 0{\cdot}005$ for cytochrome P-450 and $P < 0{\cdot}10$ for glucuronolactone dehydrogenase. For further explanations see Fig. 1 and Fig. 2.

P-450 significantly lower after the third than after the first treatment (Fig. 3). Six days after the third phenobarbitone treatment the cytochrome P-450 level was lower than in the control group (Fig. 3) although after the first treatment it was still slightly above control level (Fig. 1 B). The P-value for the difference of the cytochrome P-450 levels was $P < 0{\cdot}005$ on the sixth day after the first and third phenobarbitone periods. The relative liver weight exhibited the same response after the first and the third phenobarbitone treatment (Fig. 3).

DISCUSSION

After each of at least 3 monthly phenobarbitone treatments similar increases were elicited both in the liver mass and in the levels of glucuronolactone dehydrogenase and cytochrome P-450, and normalization took place within 1 month after such drug treatments. This indicates that a phenobarbitone treatment of five doses does not cause any permanent effects on the liver metabolism reflected in these parameters. Neither does repeated treatment lead to a defect in the ability of the liver to respond to the phenobarbitone administration in the normal way. The present results thus differ from those obtained in connection with barbitone dependent rats.[1] A lowered response was also seen in the present study, when the phenobarbitone treatment was started anew before normalization had taken place. Orrenius and Ericsson[2] found that if a second series of phenobarbitone injections was started 10 days after the last dose of the initial drug treatment, the increase of aminopyrine demethylase activity was significantly lower than during the first treatment. They proposed the presence of a repressor as an explanation.

The peaking of glucuronolactone dehydrogenase activity on the second post-treatment day might be explained by release of enzyme inhibition during the decay of phenobarbitone concentration in the liver extracts, since phenobarbitone has been found to be a powerful inhibitor of this enzyme *in vitro*.[4] The enzyme activity is also still slowly increasing from the fifth dose onwards during prolonged drug treatment. The similar behaviour of cytochrome P-450 remains unexplained, since maximal levels of this enzyme have been obtained during the injection period. It is possible that the binding of a substrate (phenobarbitone) to the microsomal detoxicating enzyme system prevents the complete reduction of cytochrome P-450 and/or the binding of carbon monoxide. A slight post-treatment increase has previously been demonstrated after phenobarbitone administration in the case of aminopyrine demethylase and $NADPH_2$ cytochrome *c* reductase.[5] Phenobarbitone administration has been shown to have a strong anticatabolic effect on the microsomal enzymes in the liver[8] and it may have a similar effect on the soluble proteins as well.

The fact that the increases and decreases of glucuronolactone dehydrogenase and cytochrome P-450 levels were very different suggests that these enzymes are synthesized and degraded independently and that the mechanism of action of phenobarbitone on glucuronolactone dehydrogenase synthesis most probably is less direct than on cytochrome P-450 synthesis. The slower decay observed might indicate that glucuronolactone dehydrogenase has a longer $T_{\frac{1}{2}}$ than cytochrome P-450.

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