Biochemical Pharmacology, Vol. 17, pp. 1769–1778. Pergamon Press. 1968. Printed in Great Britain

# EFFECT OF PARTIAL HEPATECTOMY ON THE RESPONSIVENESS OF MICROSOMAL ENZYMES AND CYTOCHROME P-450 TO PHENOBARBITAL OR 3-METHYLCHOLANTHRENE

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(*Received* 19 *February* 1968; *accepted* 29 *March* 1968)

**Abstract**—The effects of phenobarbital and 3-methylcholanthrene (3-MC) pretreatment on liver weight, microsomal protein, cytochrome P-450 content, microsomal aniline hydroxylase, hexobarbital hydroxylase and *p*-nitroanisole *O*-demethylase activities have been studied in control, sham-operated and partially hepatectomized rats. In unoperated rats, phenobarbital pretreatment significantly increased liver weight, microsomal protein, P-450 content and enzyme activities toward all three substrates. 3-MC pretreatment significantly increased liver weight, P-450 content and *p*-nitroanisole *O*-demethylation without influencing the other parameters. When administered to sham-operated or partially hepatectomized rats, phenobarbital and 3-MC caused substantially the same effects, though the magnitude of the effects was less than in unoperated animals. Thus, significant increases were seen in the various parameters after injection of phenobarbital or 3-MC into hepatectomized rats. It is concluded that the regenerating liver, like the fetal or newborn liver and certain rodent hepatomas, although exhibiting low levels of microsomal enzymes, has the capacity to respond to the enzyme inducers, phenobarbital and 3-MC.

Differential comparison of the enzyme changes expressed in terms of microsomal protein and also in terms of microsomal P-450 content revealed that the activity of the enzymes catalyzing *p*-nitroanisole and aniline oxidations parallel the microsomal P-450 content more closely than does the enzyme catalyzing hexobarbital hydroxylation.

THE ACTIVITIES of hepatic microsomal NADPH-dependent (drug-metabolizing) enzymes are subject to a variety of biological control mechanisms. Thus, within a single animal species drug metabolism may be influenced by such factors as age and strain, hormonal and nutritional status and drug pretreatment.[1]

It has been established that under many conditions characterized by rapid proliferation of hepatocytes, microsomal drug-metabolizing enzyme activities are lower than normal.[2] Thus, decreased activities of drug-metabolizing enzymes have been shown with microsomal preparations from livers of fetal or newborn animals,[3] livers which are rapidly proliferating after partial hepatectomy[4] and several transplantable hepatomas.[5]

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‡ Sponsored by the Swiss Academy of Medical Sciences.

Despite the low or undetectable levels of microsomal enzymes in fetal or newborn rabbit livers, the activities of these enzymes can be markedly stimulated by pretreating the animals with phenobarbital[6] or chlordane.[7] These findings suggest that although the immature liver is relatively deficient in enzyme content, it possesses the ability to respond to enzyme inducers. Moreover, Hart *et al.*[8] and Rogers *et al.*[9] reported that the low activity of microsomal drug-metabolizing enzymes in a variety of minimal-deviation hepatomas could be markedly enhanced by pretreating the host animals with phenobarbital. Similarly, Conney and Burns[1] reported enhancement of enzyme activity in microsomes prepared from hepatomas after administration of 3-methylcholanthrene (3-MC) to tumor-bearing rats.

As shown in this paper, phenobarbital and 3-MC retain their ability to induce drug-metabolizing enzymes in rapidly regenerating liver after partial hepatectomy.

## METHODS

*Animals.* Previous investigations[4, 10] have established that in partially hepatectomized rats, microsomal enzyme activity gradually declines, reaching a nadir within 4–7 days, and returns essentially to control levels within 10–14 days. In the present experiments the hepatic responsiveness to enzyme induction was tested at 6 and at 14 days after hepatectomy to determine whether there was a difference in responsiveness to enzyme inducers at these times.

Male Sprague–Dawley rats (NIH colony), weighing 80–90 g at the time of hepatectomy, were used in all experiments. They were allowed free access to Purina laboratory chow and tap water at all times. Ether was employed as the anesthetic and partial hepatectomies were carried out essentially as described by Higgins and Anderson.[11] In the sham operations the animals were anesthetized and the livers were exteriorized and manipulated as with the hepatectomized animals. The duration of laparotomy was the same in both groups. The same investigator performed all the surgical manipulations and the day of operation was designated as day zero.

*Drug pretreatments.* Animals were divided into three sets of groups and received all injections i.p. Control animals received 0·5 ml of 0·9% NaCl/100 g body weight once daily for 4 days, starting on day 2 after surgery for the 6-day group and on day 10 after surgery for the 14-day group. The second set of 3 groups received phenobarbital sodium (80 mg/kg, 0·5 ml/100 g) once daily for 4 days, as described above for the saline-treated animals, and the last dose was administered 24 hr prior to sacrifice. The third set of 3 groups was injected with 3-MC in corn oil (40 mg/kg) in a single dose 36 hr prior to sacrifice. Control experiments revealed no differences between corn oil-injected animals and saline-injected animals in the parameters examined.

*Enzyme assays.* Animals were sacrificed by decapitation between 9 and 10 a.m. Livers were removed, weighed and homogenized with 2 vol. of ice-cold 1·15% KCl containing 0·02 M Tris-HCl (pH 7·4) in a Potter homogenizer having a motor-driven plastic pestle. All tissue manipulations were performed at 0–4°. The homogenate was centrifuged at 9000 *g* for 20 min. The supernatant was carefully decanted and centrifuged at 105,000 *g* for 60 min in a Spinco model L ultracentrifuge. The microsomal pellet was resuspended in fresh 1·15% KCl–Tris by gentle manual homogenization.

Incubation mixtures contained the following components: microsomal suspension equivalent to 300 mg liver, 600 μmole Tris–HCl buffer (pH 7·4), 1·9 μmole NADP,

20 μmole $MgCl_2$, 35 μmole sodium isocitrate, 2·5 units of isocitrate dehydrogenase substrate and enough 1·15% KCl to make a final volume of 5·0 ml. The amounts of the substrates present in the mixtures were: aniline, 20 μmole; *p*-nitroanisole, 20 μmole; and hexobarbital, 6 μmole. That cofactor and substrate concentrations were above saturating levels both in control and operated animals was verified prior to starting the present study. Incubations were carried out with shaking in a Dubnoff apparatus at 37° for 10 min under air.

*Analytical methods.* Microsomal protein was estimated by the method of Lowry *et al.*,[12] with crystalline bovine serum albumin as a standard. Aniline metabolism was measured by its conversion to *p*-aminophenol as described by Kato and Gillette.[13] Hexobarbital metabolism was estimated by the method of Cooper and Brodie.[14] The *O*-demethylation of *p*-nitroanisole to *p*-nitrophenol was estimated by a method devised by J. R. Fouts (personal communication) as follows: the enzymatic reaction was terminated by addition of 5 ml of 10% trichloroacetic acid, the precipitate was removed by centrifugation and an aliquot (5 ml) of the supernatant was shaken for 5 min with 5 ml $CHCl_3$ containing 10% isoamyl alcohol. After centrifugation, an aliquot (4 ml) of the organic phase was transferred to a clean shaking tube containing 3 ml of 1 N NaOH. The tubes were shaken for 5 min, centrifuged and the extinction of the aqueous phase was measured at 400 mμ. Standards (*p*-nitrophenol) and blanks were carried through the entire procedure and were used to quantify the *O*-demethylation reaction.

Microsomal cytochrome P-450 content was estimated essentially as described by Omura and Sato;[15] a sample cuvette containing the microsomal suspension (3 mg protein/ml) was bubbled with oxygen-free carbon monoxide for 5 min. To both sample and reference cuvettes a few milligrams of crystalline sodium hydrosulfite (dithionite) was then added, the contents were mixed, and the difference spectrum (350–500 mμ) was recorded in a Shimadzu model MPS-50L recording spectrophotometer. Cytochrome P-450 content was taken as ΔE between 450 and 490 mμ; an extinction coefficient of 91 $cm^{-1}mM^{-1}$ was employed in the calculations.[15]

Statistical comparisons were made by using the Student *t*-test;[16] P values of $< 0{\cdot}05$ were considered to represent significant differences between means.

## RESULTS

Phenobarbital or 3-MC caused significant increases in liver weight (g liver/100 g body wt.) in unoperated, sham-operated and partially hepatectomized animals (Table 1), except that 3-MC failed to increase liver weight in partially hepatectomized rats when administered early in the regenerative process (day 6). These findings thus suggest that the regenerating liver may be deficient in the capacity to increase its weight in response to 3-MC, but retains its responsiveness to phenobarbital at this time.

Table 2 presents data on microsomal protein content in control and operated animals. It can be seen that phenobarbital administration significantly increased microsomal protein in unoperated, sham-operated and partially hepatectomized animals at both day 6 and day 14 after surgery. It is of interest that saline-treated hepatectomized animals had lower microsomal protein content at both 6 and 14 days than did saline-treated unoperated or saline-treated sham-operated animals (Table 2; cf. reference 17). Except in the 14-day hepatectomized rats, 3-MC administration did

not significantly alter microsomal protein content. In contrast, phenobarbital administration increased the amount of microsomal protein. Recent reports[18, 19] have shown similar effects of phenobarbital and 3-MC both on rat liver weight and microsomal protein.

TABLE 1. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRENE (3-MC)* ON LIVER WEIGHT IN UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | Pretreatment | | |
|---|---|---|---|
| | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 4·7 ± 0·3 (5)† | 6·1 ± 0·3 (5)‡ | 5·1 ± 0·3 (5)‡ |
| Sham-operated (day 6) | 4·4 ± 0·4 (5) | 5·9 ± 0·4 (5)‡ | 5·4 ± 0·5 (5)‡ |
| Partially hepatectomized (day 6) | 4·6 ± 0·4 (5) | 5·8 ± 0·2 (5)‡ | 4·8 ± 0·5 (5) |
| Sham-operated (day 14) | 4·8 ± 0·4 (9) | 5·6 ± 0·3 (6)‡ | 5·4 ± 0·5 (10)‡ |
| Partially hepatectomized (day 14) | 5·0 ± 0·6 (8) | 5·6 ± 0·4 (7)‡ | 5·5 ± 0·2 (8)‡ |

* Dosage and dosage schedules were as described in Methods.

† Values expressed as g liver/100 g body wt. (mean ± S.D.). Number of animals is in parentheses.

‡ Significantly different ($P < 0·05$) from corresponding saline-treated group.

TABLE 2. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRENE (3-MC)* ON HEPATIC MICROSOMAL PROTEIN CONTENT IN UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | Pretreatment | | |
|---|---|---|---|
| | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 28·9 ± 4·9† | 35·7 ± 2·4‡ | 25·3 ± 5·1 |
| Sham-operated (day 6) | 30·1 ± 2·4 | 43·8 ± 3·9‡ | 31·3 ± 1·6 |
| Partially hepatectomized (day 6) | 22·9 ± 5·0 | 38·0 ± 7·2‡ | 23·9 ± 3·9 |
| Sham-operated (day 14) | 26·0 ± 2·1 | 36·9 ± 5·4‡ | 27·9 ± 2·4 |
| Partially hepatectomized (day 14) | 20·5 ± 5·5 | 37·1 ± 6·1‡ | 28·0 ± 2·2‡ |

* Dosage and dosage schedules were as described in Methods.

† Values expressed as mg protein/g liver. Each mean (± S.D.) is for 5 animals.

‡ Significantly different ($P < 0·05$) from corresponding saline-treated group.

Cytochrome P-450 in hepatic microsomes is thought to be involved in drug metabolism.[20, 21] The data presented in Table 3 demonstrate that the amount of cytochrome P-450 is significantly increased by the administration of phenobarbital or 3-MC to control, sham-operated or partially hepatectomized rats. Similar results have been obtained in unoperated animals.[22, 23] It is noteworthy that on day 6 the increase of cytochrome P-450 by both inducers was lower in sham-operated and partially hepatectomized rats than in unoperated controls. A partial decrease in effectiveness of the inducers may persist in the hepatectomized animals through day 14.

TABLE 3. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRNE (3-MC)* ON CYTOCHROME P-450 CONTENT OF HEPATIC MICROSOMES FROM UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | Pretreatment | | |
|---|---|---|---|
| | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 0·44 ± 0·12† | 1·66 ± 0·09‡ | 0·91 ± 0·13‡ |
| Sham-operated (day 6) | 0·29 ± 0·06 | 1·15 ± 0·10‡ | 0·50 ± 0·12‡ |
| Partially hepatectomized (day 6) | 0·23 ± 0·04 | 1·07 ± 0·12‡ | 0·76 ± 0·19‡ |
| Sham-operated (day 14) | 0·41 ± 0·10 | 1·50 ± 0·19‡ | 0·67 ± 0·09‡ |
| Partially hepatectomized (day 14) | 0·36 ± 0·11 | 1·38 ± 0·22‡ | 0·64 ± 0·16‡ |

* Dosage and dosage schedules were as described in Methods.

† Values expressed as mμmoles P-450/mg microsomal protein. Each mean (± S.D.) is for 5 animals.

‡ Significantly different ($P < 0·05$) from corresponding saline-treated group.

When enzyme activities were expressed per milligram of microsomal protein, treatment of control or operated rats with either phenobarbital or 3-MC significantly enhanced the *O*-demethylation of *p*-nitroanisole by hepatic microsomes (Table 4).

TABLE 4. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRENE (3-MC)* ON THE *O*-DEMETHYLATION OF *p*-NITROANISOLE BY HEPATIC MICROSOMES FROM UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | *p*-Nitrophenol (mμmoles formed/mg protein/10 min) | | | *p*-Nitrophenol (mμmoles formed/mμmole P-450/10 min) | | |
|---|---|---|---|---|---|---|
| | Pretreatment | | | | | |
| | Saline | Phenobarbital | 3-MC | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 5·9 ± 2·8† | 29·3 ± 1·3‡ | 13·6 ± 3·1‡ | 13·0 ± 4·4 | 17·6 ± 1·9 | 14·5 ± 2·7 |
| Sham-operated (day 6) | 3·6 ± 0·6 | 25·0 ± 3·2‡ | 8·3 ± 2·3‡ | 13·1 ± 2·2 | 21·9 ± 3·5‡ | 16·9 ± 2·8‡ |
| Partially hepatectomized (day 6) | 2·7 ± 1·6 | 9·0 ± 2·7‡ | 6·0 ± 0·8‡ | 8·8 ± 3·8 | 8·7 ± 3·3 | 8·3 ± 2·5 |
| Sham-operated (day 14) | 5·5 ± 1·7 | 24·8 ± 4·5‡ | 11·1 ± 1·2‡ | 13·1 ± 1·4 | 16·6 ± 1·0‡ | 16·8 ± 2·2‡ |
| Partially hepatectomized (day 14) | 5·5 ± 1·9 | 23·1 ± 1·6‡ | 10·4 ± 2·4‡ | 15·3 ± 1·7 | 17·1 ± 3·0 | 16·4 ± 1·4 |

* Dosage and dosage schedules were as described in Methods.

† Values are expressed as mμmoles *p*-nitrophenol formed either per mg protein or per mμmole P-450/10 min. Each mean (± S.D.) is for 5 animals.

‡ Significantly different ($P < 0·05$) from corresponding saline-treated group.

Similar results with phenobarbital have been recently reported.[24] It should be emphasized that since these data are expressed as specific activities (activity/μg protein), they are independent of differences in microsomal protein content per gram of liver. Data obtained on day 6 demonstrate that although hepatectomy tended to attenuate the response to phenobarbital and 3-MC when compared with either sham-operated or unoperated animals, significant responses to the two

inducers were obtained. By day 14, both the basal enzyme levels (saline-treated animals) and the levels induced by either phenobarbital or 3-MC had returned to the levels seen in unoperated controls. When the activity of *p*-nitroanisole *O*-demethylase was expressed per millimicromole of microsomal cytochrome P-450, it can be seen (Table 4) that values remain relatively constant after phenobarbital or 3-MC pretreatment. Thus, although slight but significant increases were seen in sham-operated animals on day 6 and day 14, most of the increased *O*-demethylase activity observed after phenobarbital or 3-MC administration paralleled apparent increases in microsomal cytochrome P-450.

Phenobarbital pretreatment of control, sham-operated or partially hepatectomized rats significantly enhanced the activity of hexobarbital hydroxylase per milligram of hepatic microsomal protein, while 3-MC administration had no significant effect (Table 5). Similar results have been obtained in hepatectomized[25] and unoperated

TABLE 5. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRENE (3-MC)* ON THE HYDROXYLATION OF HEXOBARBITAL BY HEPATIC MICROSOMES FROM UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | Hexobarbital (mμmoles metabolized/mg protein/10 min) | | | Hexobarbital (mμmoles metabolized/mμmole P-450/10 min) | | |
|---|---|---|---|---|---|---|
| | Pretreatment | | | | | |
| | Saline | Phenobarbital | 3-MC | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 18·6 ± 8·3† | 110·2 ± 15·2‡ | 18·3 ± 3·4 | 32·3 ± 7·4 | 66·1 ± 6·2‡ | 19·7 ± 2·5‡ |
| Sham-operated (day 6) | 27·6 ± 8·1 | 85·1 ± 20·4‡ | 19·7 ± 5·6 | 81·0 ± 20·0 | 75·4 ± 23·2 | 40·2 ± 6·7‡ |
| Partially hepatectomized (day 6) | 35·4 ± 10·2 | 110·8 ± 17·4‡ | 29·4 ± 9·0 | 163·0 ± 65·5 | 104·3 ± 9·2 | 40·6 ± 14·5‡ |
| Sham-operated (day 14) | 25·9 ± 10·7 | 91·9 ± 16·9‡ | 26·6 ± 10·5 | 63·8 ± 20·1 | 61·4 ± 1·4 | 41·0 ± 20·5 |
| Partially hepatectomized (day 14) | 29·9 ± 6·0 | 88·3 ± 19·1‡ | 25·1 ± 6·4 | 87·5 ± 25·2 | 65·3 ± 17·0 | 42·2 ± 19·2‡ |

* Dosage and dosage schedules were as described in Methods.

† Values are expressed as mμmoles hexobarbital metabolized either per mg protein or per mμmole P-450/10 min. Each mean (± S.D.) is for 5 animals.

‡ Significantly different ($P < 0.05$) from corresponding saline-treated group.

control rats.[26, 27] When the data on hexobarbital metabolism were expressed per unit of microsomal P-450 content, the inductive effect of phenobarbital was not seen in all groups (Table 5). Although phenobarbital pretreatment was associated with marked stimulation of microsomal hexobarbital hydroxylase activity, approximately proportional increases were observed in cytochrome P-450. Therefore, with the exception of the unoperated control animals, the effects of phenobarbital pretreatment on hexobarbital metabolism might be related to an increased cytochrome P-450 content. In contrast, 3-MC treatment caused significant reduction in activity per unit of cytochrome P-450.

It is of interest that on day 6, the activity of hexobarbital hydroxylase per unit of cytochrome P-450 underwent marked increases in operated animals (compare saline-treated controls with day 6 saline-treated sham-operated and saline-treated partially hepatectomized rats). Partial hepatectomy quintupled the activity while sham-operation nearly tripled it. Per milligram of microsomal protein, the activity varied only about 2-fold between saline-treated unoperated and saline-treated operated animals.

TABLE 6. EFFECT OF PRETREATMENT WITH PHENOBARBITAL* OR 3-METHYLCHOLANTHRENE (3-MC)* ON THE HYDROXYLATION OF ANILINE BY HEPATIC MICROSOMES FROM UNOPERATED, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RATS

| Animals | *p*-Aminophenol (mμmoles formed/mg protein/10 min) | | | *p*-Aminophenol (mμmoles formed/mμmole P-450/10 min) | | |
|---|---|---|---|---|---|---|
| | Pretreatment | | | | | |
| | Saline | Phenobarbital | 3-MC | Saline | Phenobarbital | 3-MC |
| Unoperated controls | 5·3 ± 2·4† | 19·5 ± 1·0‡ | 8·0 ± 2·7 | 11·4 ± 3·9 | 11·8 ± 1·2 | 6·8 ± 4·3 |
| Sham-operated (day 6) | 8·1 ± 1·6 | 17·3 ± 2·3‡ | 6·5 ± 0·9 | 28·4 ± 4·3 | 15·2 ± 2·5‡ | 14·1 ± 4·3‡ |
| Partially hepatectomized (day 6) | 4·0 ± 0·9 | 9·9 ± 2·6‡ | 6·1 ± 1·2‡ | 17·6 ± 2·5 | 9·5 ± 2·7‡ | 8·4 ± 2·5‡ |

*Dosage and dosage schedules were as described in Methods.

† Values are expressed as mμmoles *p*-aminophenol formed either per mg protein or per mμmole P-450/10 min. Each mean (± S.D.) is for 5 animals.

‡ Significantly different ($P < 0·05$) from corresponding saline-treated group.

Table 6 presents data on the induction of aniline hydroxylase. It is clear that phenobarbital administration significantly enchanced the metabolism of aniline per milligram of microsomal protein in day 6 hepatectomized and sham-operated rats and in control rats. In accord with the results obtained with *p*-nitroanisole, partial hepatectomy appeared to attenuate the inductive effect of phenobarbital on aniline hydroxylase activity. Although the induction was less pronounced in the hepatectomized animals than in the sham or unoperated groups, it was highly significant ($P < 0·005$) when expressed either as absolute change or as per cent of control. The dose of 3-MC employed in these experiments failed to stimulate aniline hydroxylase activity in unoperated or sham-operated animals. It did, however, cause a significant ($P < 0·025$) stimulation in the hepatectomized animals. Although the reason for this difference is not known, it is possible that during the process of liver regeneration, enhanced responsiveness to the inductive effects of 3-MC might occur. When the aniline hydroxylase data were expressed per millimicromole of microsomal cytochrome P-450 content, the inductive effects of phenobarbital pretreatment were not observed; indeed, significant reduction in specific activities per millimicromole cytochrome P-450 occurred (Table 6). Thus, phenobarbital increased cytochrome P-450 content to a greater extent than it did aniline hydroxylase activity.

## DISCUSSION

The results of the present investigation demonstrate that regenerating rat liver responds to injections of phenobarbital or 3-MC with increases in the activity of

several microsomal drug-metabolizing enzymes and content of cytochrome P-450. These results are in accord with those of other investigators who reported enzyme induction with phenobarbital or 3-MC in normal, unoperated animals,[19, 23, 25–27] in newborn animals,[6, 7] and in hepatomas.[1, 8–10] Increases in cytochrome P-450 content have also been reported in phenobarbital-[18] and 3-MC-treated[18, 22] animals. Microsomal enzymes were observed to respond to the inducers both 6 days after hepatectomy, when enzyme levels are at a low point, and 14 days after surgery, when enzyme activities have returned to control levels. It is thus apparent that those factors that account for the reduced microsomal enzyme activity in regenerating liver do not preclude enzyme induction by phenobarbital or 3-MC.

In accord with the report of Bengmark *et al.*,[17] partial hepatectomy appeared to reduce microsomal protein content for at least 14 days (Table 2), suggesting that the reduction in microsomal enzyme activity per gram of liver, which occurs after hepatectomy,[4] can be explained in part by a decreased amount of enzyme protein. In this regard, it was found that the sp. act. of hexobarbital hydroxylase from saline-treated, partially hepatectomized animals was higher than that from either unoperated control or sham-operated animals (Table 5). If the decreased protein content of microsomes from hepatectomized animals were the result of either reduced synthesis or increased catabolism, then hexobarbital hydroxylase would seem to have a different turnover time than the bulk of microsomal protein.

Evaluation of the enzyme data both in terms of microsomal protein content and in terms of cytochrome P-450 content disclosed some interesting quantitative differences (Tables 4–6). For example, considering the responsiveness of all groups of animals to phenobarbital, there was a 200–600 per cent increase in *p*-nitroanisole demethylase (Table 3) in terms of microsomal protein, but when expressed in terms of P-450 content there was no group that showed even a 100 per cent increase in the activity of this enzyme. This suggests that the oxidative demethylation of *p*-nitroanisole is very closely associated with the level of P-450 as measured in the presence of dithionite and carbon monoxide.

The aniline hydroxylase data (Table 6) indicated that the effects of phenobarbital and 3-MC are manifested by an increased activity of the enzyme per milligram of microsomal protein but by significantly reduced activity when expressed per millimicromole of cytochrome P-450 in the 6-day operated animals. Thus, the inducers caused larger increases in cytochrome P-450 content than in enzyme activity. There were large increases in the rates of metabolism of hexobarbital (Table 5) in the phenobarbital-treated rats compared with those receiving saline. When expressed as activity per milligram of protein, hexobarbital was metabolized about 3 times as fast in phenobarbital-treated as in saline-treated operated rats, but on the basis of P-450 content, none of the operated animals had significantly elevated hexobarbital hydroxylase activity. On the other hand, operated animals treated with 3-MC had significantly decreased hexobarbital hydroxylase activity when compared with the corresponding saline-treated rats. Hence it would seem that the reported failure of 3-MC to stimulate hexobarbital metabolism[28] (see also Table 5) is associated with its inability to induce microsomal protein synthesis (Table 2) and not with its ability to induce increases in microsomal cytochrome P-450 content (Table 3). Furthermore, the finding that phenobarbital treatment of the operated animals increased both the microsomal protein and the cytochrome P-450 content suggest the possibility that 3-MC and

phenobarbital may induce the synthesis of different forms of cytochrome P-450. Others have postulated that more than one form of this cytochrome[28–30] may exist.

In conclusion, it should be emphasized that, after phenobarbital or 3-MC administration, there was no consistent proportional relationship between enhanced enzyme activities and increased cytochrome P-450 content in both unoperated and operated animals. Thus, enzyme activity expressed per unit of cytochrome P-450 content was increased significantly in some instances and was unchanged or decreased in others. When enzyme activity and P-450 content were both increased by a given treatment, the magnitude of enhancement of the two parameters was often markedly different (Tables 4–6). Accordingly, not all of the changes in enzyme activity reported in this paper can be explained solely on the basis of cytochrome P-450 content of the microsomes. Other investigators have similarly obtained differential changes in enzyme activity and total cytochrome P-450 content, both in hepatic microsomes[31, 32] and in adrenal cortical mitochondria.[33] This would suggest the possibility that cytochrome P-450 content, as measured in the presence of dithionite and carbon monoxide, may not accurately reflect the form of cytochrome P-450 which functions in the metabolism of drugs.

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