Benzpyrene Hydroxylase Activity and Its Induction by Methylicholanthrene in Morris Hepatomas, in Host Livers, in Adult Livers, and in Rat Liver during Development

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SUMMARY

Benzpyrene hydroxylase activity and its induction by a single injection of methylicholanthrene (2 mg/100 g body weight) were studied in liver from rats during developmental stages and in Morris Hepatomas 9618A, 9633, 7800, 7794A, and 8999 under controlled feeding conditions. The purpose of the experiment was to see whether naturally occurring levels of benzpyrene hydroxylase are lower in activity among the different hepatoma lines than in adult liver or newborn liver and whether induced levels of the activity in hepatoma by methylicholanthrene treatment could be referred to those induced in liver from rats of normal developmental stages.

Benzpyrene hydroxylase activity in Hepatoma 9633 is very low, but after administration of methylicholanthrene the activity was markedly increased, with some modification by variations in the time of injection. The benzpyrene hydroxylase activities in the host livers were strongly influenced by the size of the pooled hepatoma in rats bearing Hepatoma 8999 but not in rats bearing Hepatoma 9618A, while in the latter an influence of feeding regimen was shown.

Methylicholanthrene was able to induce benzpyrene hydroxylase activity in Morris hepatomas as well as in the corresponding host liver, although the induced levels in hepatomas were always lower than those in the host liver.

There was no consistency of the induced levels among hepatoma lines, although all the rats were sacrificed exactly 6 hr after onset of feeding under controlled dietary regimens and 24 hr after a single intraperitoneal treatment with methylicholanthrene.

Benzpyrene hydroxylase in fetal rat liver was very low in activity, and after birth a marked increase was observed. After administration of methylicholanthrene the fetal liver showed some increase in benzpyrene hydroxylase activities and in neonatal stages more profound responses were detected. The activity of benzpyrene hydroxylase and the magnitude of the induced levels in normal rat liver were completely dependent upon the age of the rat, and there were no differences in activity between male and female rats until after 21 days of age. The similarity of hepatoma lines to normal rat liver during developmental stages with respect to the activity of benzpyrene hydroxylase and its response to methylicholanthrene induction were discussed.

INTRODUCTION

Conney et al. (8) were first to describe the remarkable and now widely recognized induction of benzpyrene hydroxylase activity by the prior administration of BP itself, or methylicholanthrene, or of other polycyclic hydrocarbons (8, 15, 16).

Many hepatic tumors exhibited significantly lower activity of drug-metabolizing enzymes, compared with normal liver, but in some lines of Morris hepatoma the potent inducers, e.g., phenobarbital, caused the induction of drug-metabolizing enzymes (1, 7, 19, 21), and Rogers et al. (40) demonstrated...
stated that Morris Hepatoma 7800 had approximately the same levels of benzpyrene hydroxylase activity as did the host liver or normal liver. Similar Michaelis constants and estimated maximal enzyme reaction velocity for benzpyrene hydroxylation in the hepatoma and the host liver were observed.

In order to find a meaningful explanation for the diversity of biochemical patterns in Morris hepatoma lines in terms of a unifying theory of carcinogenesis, Potter and Watanabe (34) suggested that there are 2 different categories of the Morris hepatomas, i.e., the “minimal-deviation” hepatoma lines with normal diploid chromosome numbers and the “progressed hepatomas” on the basis of chromosomal and enzyme patterns. Potter (32) suggested recently that oncogeny may be interpreted as blocked or reverted ontogeny, indicating that as an embryonic hepatocyte undergoes maturation to an adult hepatocyte, random mutations could occur in any of a very large number of gene loci along the switching sequence and still result in a hepatoma if certain final stages of maturation were unattainable.

The metabolic significance of drug-metabolizing enzymes during developmental stages in mammals has been considered. Very low activity of some of the drug-metabolizing enzymes was observed in the liver from newborn and fetal rabbit, compared with that in adult liver (14, 19). Bresnick and Stevenson (3) demonstrated the failure of induction of \( N \)-demethylase activity in fetal rat liver by MC, in contrast to the increase of the activity in the liver from neonatal rats.

In the experiment herein reported, the following hepatomas, i.e., Morris Hepatomas 9618A and 9633 with normal chromosome patterns; Morris Hepatomas 7800 and 7794A with normal chromosome numbers but different morphology; and Morris Hepatoma 8999 with significantly deviated patterns of chromosome morphology (27), were selected and studied. Cogeny may be interpreted as blocked or reverted ontogeny, indicating that as an embryonic hepatocyte undergoes maturation to an adult hepatocyte, random mutations could occur in any of a very large number of gene loci along the switching sequence and still result in a hepatoma if certain final stages of maturation were unattainable.

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**MATERIALS AND METHODS**

**Animals.** Morris Hepatomas 9618A (Generation 4), 9633 (Generation 7), 7800 (Generation 40), 7794A (Generations 24 and 25), and 8999 (Generation 7) were transplanted into male or female Buffalo strain rats at the National Cancer Institute, Bethesda, Md. The tumor-bearing animals were shipped to the McArdle Laboratory. Buffalo male rats used as controls were obtained from Simonsom Laboratories, Inc., Gilroy, Calif., and Charles River CD rats were used in the experiments which were performed under a variety of dietary conditions, and obtained from the Charles River Breeding Laboratories, Wilmington, Mass. Badger male or female rats, used as fetal, newborn, and weaning rats, were obtained from Badger Research Corporation, Madison, Wis. The hepatoma-bearing and normal adult rats were placed in a windowless room with an inverted and displaced lighting schedule in which lights were on from 8:30 p.m. to 8:30 a.m. (08:30) in a 24-hr cycle. Feeding dishes containing 12, 30, or 60% protein diets (79, 61, or 31% glucose respectively) (45) were placed in the cage just before the lights were switched off and removed after 8 hr. All rats except pregnant and weaning rats were adapted to 8 hr of feeding in every 24-hr cycle ("8 + 16" regimen) or in every 48-hr cycle ("8 + 40" regimen) (35, 45). Pregnant and weaning rats were fed ad libitum on 30% protein diets.

Methylcholanthrene was administered as a single injection intraperitoneally into newborn, weaning, and adult rats or directly into the amniotic sac at a dose level of 2 mg in 0.2 ml (for adult rat) or in 1 ml (for fetus, newborn, and weaning rats) of corn oil, U.S.P., per 100 g body weight. This dosage of MC is identical with that used by Bresnick and Stevenson (3) in their study of \( N \)-demethylase induction in adult, fetal, and newborn rat livers. Higher doses of MC were not tested in hepatoma-bearing animals, and the dosage used by Bresnick and Stevenson (3) in nontumorous animals was adhered to because all the hepatomas gave strong responses to this level of inducer although they contained near-zero levels of the enzyme before induction as shown in the final summary chart. Control rats received a comparable volume of corn-oil solution in every instance. The rats were sacrificed at the mentioned times in relation to the feeding schedule, as indicated in the charts.

**Preparation of Homogenate and Enzyme Assays.** At the clock times shown in each chart or legend, tumor-bearing and normal rats were killed rapidly by means of guillotine, with no prior stress to the animals, and the livers and tumors were quickly removed and cooled in chilled buffer. Connective tissue and necrotic tissue were carefully dissected away. Livers and tumors were weighed and homogenized in 4 volumes 0.2 M Tris-HCl buffer solution, pH 8.0, containing 10\( ^{-6} \) M dithiothreitol, by means of a Polytron homogenizer (Brinkman Instruments, Inc., Des Plaines, Ill.). The homogenate served as a source of the crude enzyme, and the medium was the same as used in other enzyme studies carried on at the same time.

The metabolism of 3, 4-benzpyrene was followed in an assay system which was essentially that of Conney et al. (8) and Wattenberg et al. (49), with the modification by Nebert and Gelboin (26) outlined below. The composition of the assay system in a total volume of 1.0 ml included 50 \( \mu \)mol of Tris buffer, pH 7.5; 0.36 \( \mu \)mol reduced triphosphopyridine nucleotide; 3 \( \mu \)mol MgCl\(_2\); 0.10 ml 2.5 or 5% (w/v) cell homogenate; and 80 \( \mu \)mol BP (in 50 \( \mu \)l methanol). The substrate BP was added just prior to incubation, and the mixture was shaken in a Dubnoff metabolic shaking incubator (Precision Scientific Co., Chicago, Ill.) at 37° in 25-ml Erlenmeyer flasks with air in the gas phase. Under these conditions the enzyme reaction was linear with respect to time (for at least 15 min) and to the amount of enzyme solution. Routinely, a 15-min period was chosen for this incubation time.

The reaction was stopped with 1.0 ml cold acetone, 3.0 ml n-hexane were added, and the mixture was incubated with shaking at 37° for 15 min. After storage in the dark at 3° for 24 to 36 hr a 1.0-ml aliquot of the organic phase was shaken with 3.0 ml of 1 N sodium hydroxide for 1 min to extract the hydroxylated BP formed during the incubation. The extended dark storage time gave better results than 1, 6,
or 12 hr of storage (cf. also Ref. 8). The fluorescence of the extracted hydroxylated metabolite of BP in alkaline solution was determined in a Turner fluorometer (activation, 396 mμ; fluorescence, 525 mμ) 10 min after adding NaOH solution to the organic solvent. The hydroxylation of BP is expressed as μmole equivalents of 8-hydroxy-3,4-benzpyrene (sample kindly supplied by Dr. H. V. Gelboin) formed, although the fluorescence measured represents a mixture of hydroxylated metabolites (6). Because of the limited supply of 8-hydroxy-3,4-benzpyrene, quinine sulfate solution (3 μg/ml) in 0.1 N sulfuric acid was used to adjust the spectrophotometer for measurement of metabolite formation. The molecular weight of the product is 244 and we calculate that 50 μg product/min/mg tissue as expressed by Gelboin and Blackburn (16) would be equivalent to 12.3 nmole/mg/hr in our charts. Thus our values appear to be higher than the previous data cited, but the assay system is the more recent one used by Nebert and Gelboin (26), whose data are expressed as a function of protein in a cell fraction.

Chemicals. BP and Acti-dione (cycloheximide) were obtained from California Corporation for Biochemical Research, Los Angeles, Calif.; MC and puromycin dihydrochloride were from Nutritional Biochemical Corp., Cleveland, Ohio; actinomycin D was from Mann Research Laboratories, New York, N. Y.; corn oil, U.S.P., was from Matheson Coleman & Bell, Norwood, Ohio; and n-hexane suitable for use in spectrophotometry was from Fisher Scientific Co., Fair Lawn, N. J.

RESULTS

Increase of Benzpyrene Hydroxylase Activity in Normal Rat Liver after Administration of Methylcholanthrene. Conney et al. (8) reported that intraperitoneal injection of 0.1 to 1 mg BP or MC into weaning male rats caused a rapid increase in the hepatic benzpyrene hydroxylase activity to about 5 to 10 times the control value by 24 hr. Chart 1 shows increased activity of benzpyrene hydroxylase in liver from male and female rats after administration of MC. All the rats were sacrificed at 2:30 p.m. (14:30) under controlled dietary conditions. The activity in liver from control male rats injected with corn oil was apparently higher than that from control female rats. No differences in the induced activities of benzpyrene hydroxylase in liver from male and female rats were observed, and a linearly increased activity was observed for 24 hr after the injection of MC in male rats, whereas activity increased for 48 hr in female rats.

Conney and Burns (6) noted that the activity of a variety of drug-metabolizing enzymes can be influenced markedly by nutritional status of the animals. Radzialowski and Bousquet (37, 38) reported daily rhythmic variation in hepatic drug metabolism in the rat and mouse fed ad libitum under controlled lighting conditions, and the maximal levels were at 2:00 a.m. (02:00), i.e., 6 hr after onset of darkness.

It was of interest to see whether the activity of benzpyrene hydroxylase in rat liver showed daily variation under our conditions (45) but no significant variations in normal rat liver were observed (not shown). As shown in Chart 2, the activities of naturally occurring benzpyrene hydroxylase in livers from the rats adapted to the "8 + 16" dietary regimen were to some extent higher than those from the rats adapted to the "8 + 40" regimen. No significant differences of the enzyme activities were observed among the 3 different protein levels in the diet, i.e., 12, 30, and 60%.

Furthermore the increased activities of hepatic benzpyrene hydroxylase following administration of MC were compared among the different dietary conditions, as shown in Chart 2. Except for the groups of rats fed on 30% protein diets, the levels of the enzyme activities in the rats adapted to the "8 + 16" dietary regimen were somewhat increased compared with the enzyme levels in the rats adapted to the "8 + 40" regimen. However, if we calculate the mean values and the standard deviation of the means from the results in all the experimental samples, most of the values under the different dietary conditions fall within the range occupied by the standard deviations.

Benzpyrene Hydroxylase Activity and Its Induction by Methylcholanthrene in Rat Liver during Developmental Stages. The liver microsomes of newborn animals have little or no ability to metabolize drugs (5, 6, 14). Hart et al. (19) and Bresnick and Stevenson (3) demonstrated that the activity of drug-metabolizing enzyme could not be induced in fetal liver after administration of the drug, but in neonatal liver significantly increased activities were observed. Furthermore it was reported that MC could be transported to a small extent across the placenta into the fetuses when the labeled isotope was injected intraperitoneally into the pregnant rat (3).

Chart 3 shows the activities of native and induced benzpyrene hydroxylase in rat liver during the developmental stage.
In fetal, neonatal, and preweanling stages of rats one half of the litter mates were treated by injection with MC and the other half were treated with corn oil as controls. No differences in the enzyme activities in livers among nontreated rats, rats treated with 0.9% NaCl solution, and corn oil were observed (not shown). The lower activities of benzpyrene hydroxylase were observed in fetal rat liver and also within 15 hr after the birth, and in 1-day neonatal rats the activities increased significantly. During preweanling stages of male and female rats higher activities were observed in comparison with those in weanling and adult female rats. There were no differences of the enzyme activity between fetal and neonatal rat liver.

After MC administration the activities of benzpyrene hydroxylase were increased even in fetal rat liver, but the induced levels were lower, compared with the induced levels in neonatal rat liver. In liver from preweanling rats, i.e., 15 to 17 days old, the induced levels of the enzyme were higher than those in neonatal rat liver and were similar to the values in adult rats in which no differences in the induced levels were observed between male and female rat liver. A tremendous difference in the activities of benzpyrene hydroxylase as well as the induced levels by MC treatment was observed between fetal and neonatal rat liver.

Naturally Occurring Benzpyrene Hydroxylase Activity in Hepatoma and the Host Liver from Rats Bearing Morris Hepatomas 9618A and 8999 under Controlled Feeding Conditions. In comparison with the responses of normal liver from adult rats to drug treatment, hepatic tumors including Morris hepatoma lines failed to metabolize the foreign drugs (1, 20, 21). However, Hart et al. (20), Conney and Burns (7), and Rogers et al. (40) demonstrated that after administration of phenobarbital or MC into the host the hepatic tissue showed increased activity of drug-metabolizing enzyme to some extent, but the induced levels in the hepatomas were lower than those in normal liver from adult rats.

The rats bearing Hepatoma 9618A were adapted to an "8 + 40" dietary regimen on 30 and 60% protein diets for 400 days and half of them were shifted to an "8 + 16" regimen 54 days before sacrifice. As shown in Chart 4, lower activities of benzpyrene hydroxylase in the hepatoma were observed without any relation to dietary conditions, in comparison with the responses of normal liver from adult rats to drug treatment, hepatic tumors including Morris hepatoma lines failed to metabolize the foreign drugs (1, 20, 21). However, Hart et al. (20), Conney and Burns (7), and Rogers et al. (40) demonstrated that after administration of phenobarbital or MC into the host the hepatic tissue showed increased activity of drug-metabolizing enzyme to some extent, but the induced levels in the hepatomas were lower than those in normal liver from adult rats.

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comparison with the host liver, although significant differences in the activities of benzpyrene hydroxylase between the rats adapted to an ‘8 + 16’ feeding regimen and ‘8 + 40’ dietary regimens were observed. The host liver from the rats bearing Morris Hepatoma 9618A showed significant responses of the enzyme activity to the dietary conditions, in contrast to the naturally occurring activities in the hepatomas and the host livers from the rats bearing Hepatoma 9633 presented in relation to the killing time in rats under controlled feeding conditions. All the rats had been adapted to an ‘8 + 16’ dietary regimen on 60% protein diets and were treated by injection with MC exactly 24 hr before sacrifice, as shown in the chart. There was an increase in the naturally occurring activity of benzpyrene hydroxylase in the host liver in the rats sacrificed at 14:00, i.e., 5.5 hr after onset of feeding, but there was no increase in the negligible natural level of activity in Hepatoma 9633. However, there was increased activity in the hepatomas from the rats administered with MC and these increases were apparently dependent upon the time of day, showing higher activity in hepatomas from rats killed at 14:00. MC was injected at the corresponding times 24 hr earlier. The induced levels in the host livers were much higher than those in hepatomas, as shown in the chart.

The time relations for the induced activities in Hepatoma 9633 shown in Chart 6 suggest a possible difference in decay rate for the enzyme in the hepatomas, on the assumption that the values at 14:00 would decline to the values shown at 6:30, in contrast to the situation shown in the host livers. If the decay rate were more rapid in hepatomas than in liver it could conceivably account for the negligible activities in the hepatomas in the noninduced state. A further experiment on the induction and decay of benzpyrene hydroxylase in Hepatoma 9633 is shown in Chart 7. In this experiment the decay rate in the hepatomas implied from Chart 6 is more directly confirmed if we compare the levels at 24 and 48 hr after MC. Unfortunately, there was wide variation in the host liver values at 48 hr after induction, but the curve was drawn through the average value because of the clear-cut data on normal livers shown earlier (Chart 1). On the basis of the combined results from Charts 6 and 7 it seems unlikely that the decay rate in the hepatomas is fast enough to account for their low naturally occurring levels of the enzyme.

Effect of Actinomycin D, Puromycin, and Cycloheximide on the MC Induction of Benzpyrene Hydroxylase. Gelboin and Blackburn (15, 16) reported that actinomycin D and puromycin inhibited the induction of benzpyrene hydroxylase by MC in several tissues of normal rats. It is of interest to see whether the induction mechanism of the enzyme in hepatoma is similar to that in normal rat liver. Chart 8 shows the results in which actinomycin D, puromycin, and cycloheximide inhibit the induction of the activity of benzpyrene hydroxylase by MC treatment in hepatoma as well as in the host liver from both male and female rats bearing Morris Hepatoma 7800. In this experiment all the rats were treated with MC injections at 10:00 (10 a.m.), and treated with either actinomycin D, puromycin, or cycloheximide at different times, as shown in the legend of Chart 8, before sacrifice at 17:00 (5 p.m.), i.e., 7 hr after the MC injection, in order to observe initial rates of synthesis (see Chart 1). A different induced level of the enzyme activity by MC
treatment was observed in the host liver of males compared to females but not in the corresponding hepatomas. Similar sex differences were observed in the basal enzyme activity in the host livers. Actinomycin D inhibited enzyme induction to the same extent in both male and female rats, showing approximately 57 and 33% inhibition of the activity in the host liver and hepatoma respectively compared with the corresponding induced level of the enzyme after the MC treatments. The data on male host liver suggest that the naturally occurring activity responds to actinomycin the same way the induced activity does, but direct experiments were not done. Puromycin in female rats and cycloheximide in male rats also resulted in the inhibition of the induced activity in the host liver and the hepatomas. Cycloheximide completely inhibited the induced increment of activity in both host liver and hepatoma. These results indicate that at least part of the stimulatory effect of MC in hepatoma tested was prevented by actinomycin D, an inhibitor of DNA-dependent RNA synthesis, and by puromycin and cycloheximide, inhibitors of protein synthesis. Further experiments with all the possible combinations of inhibitors and dosages were not carried out since the results did not seem to indicate any notable differences from the earlier work on normal liver by Gelboin and Blackburn (15, 16).

Survey on the Induction of Benzpyrene Hydroxylase by MC Treatment in Morris Hepatoma Lines and in the Host Livers. As shown in Chart 9 the activities of benzpyrene hydroxylase in hepatomas herein reported were much lower than those in the corresponding host liver, even after the administration of MC into the hepatoma-bearing rats. Different levels of naturally occurring enzyme activity were observed in the host liver from male and female rats bearing Morris Hepatoma 9633 and 7800, but no striking differences of the induced levels of the enzyme activities were seen in hepatomas or in the host liver from male or female rats. Significantly different responses of the activity of benzpyrene hydroxylase to the MC treatment were observed in the different lines of Morris hepatomas. Among the diploid lines of Morris hepatomas tested in this experiment, Hepatoma 9618A showed lower inducibility of the enzyme by MC treatments, when compared with the other lines, and significantly different induced levels of the enzyme were observed between Hepatoma 9618A and Hepatoma 9633, although their chromosome number and morphology were considered to be the same and not distinguishable from the number and morphology observed in normal diploid hepatocytes (27).

Thus the previous observations (34, 36) that these 2 lines are biochemically distinguishable are reinforced by the present experiments.

DISCUSSION

The profound diversity of metabolic patterns and their modes of regulation by hormones among various hepatoma lines have been demonstrated even in hepatoma-bearing rats under controlled feeding conditions and even when Morris hepatoma lines with the same chromosome number as in rat hepatocytes were surveyed (30, 31, 34, 36, 44, 46, 47). However, such a widely deviated and scattered distribution in enzyme activities in Morris hepatoma lines might be covered within the ranges occupied by fetal and neonatal rat
A variety of drug-metabolizing enzymes is located in the hepatic microsome fraction (4), and the induction of these enzymes is evoked by administration of carcinogens, barbiturates, steroids, insecticides, and other drugs (5). These inductions are associated with increases in the relative quantity of the smooth endoplasmic reticulum (12, 28, 39). Physiological factors influencing drug metabolism have been discussed by several authors (6, 13, 17). Sex differences in drug-metabolizing enzyme activity of rat liver have been shown to be related to a difference in substrate affinity for the mixed function oxidase reaction and not to a difference in the content of cytochrome P-450 (41).

Benzpyrene hydroxylase activity and its induction by polycyclic hydrocarbons were observed in liver and the other tissue in rats in vivo (8, 49), in human liver (24) and in hamster embryonic cells or pulmonary tissue grown in vitro (2, 26, 48). A good correlation between the capacity of rat-liver microsomes to metabolize BP and the amount of the carbon-monoxide-binding pigment, P-450, was observed in rats treated with either BP or phenobarbital (42). Treatment of rats with MC resulted in elevated levels of P-450 (18) or the formation of a new hemoprotein (P-450) in liver (43) as well as stimulation of incorporation of orotic acid-14C into nuclear RNA of liver from intact and adrenalectomized rats (25).

Potter et al. (33, 36) and Watanabe et al. (45) demonstrated that a number of metabolic activities oscillated in daily cycles in normal liver and Morris hepatomas from rats adapted to controlled lighting and feeding schedules, and that some enzyme activities were markedly modified by the change of dietary protein levels. Kato (22, 23) reported that the activities of some drug-metabolizing enzymes in rat liver and their inductions by treatment with phenobarbital were also markedly regulated by the dietary situations, and that such a dietary regulation of the drug-metabolizing enzyme may involve some relation to the changes in the content of P-450 in liver microsomes. In contrast to the results which showed daily rhythmic variations in some hepatic drug enzymes, e.g., the metabolism of aminopyrine, p-nitroanisole, hexobarbital, and 4-dimethylaminoazobenzene in rat and mouse (37, 38), the normal rat liver (Chart 2) did not show any remarkable daily variation of benzpyrene hydroxylase activity and its induced level by MC treatment. However, apparently different activities of benzpyrene hydroxylase at different times of day were observed in the host liver from rats bearing Morris Hepatoma 9633, and in Hepatoma 9633 the higher induced levels of benzpyrene hydroxylase were observed at 14:00 (2:00 p.m.) than at 6:30 a.m. (Chart 6). On the other hand there were different responses of the activity of benzpyrene hydroxylase to some extent in normal rat liver to the controlled feeding regimens, i.e., the "8 + 16" and "8 + 40" regimens, and these different responses were markedly amplified in the case of the host liver from

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**Chart 6. Benzpyrene hydroxylase activity and its induction by methylcholanthrene in hepatoma and the host liver from male rats bearing Morris Hepatoma 9633, Generation 7, at different times in a day under controlled feeding schedules; methylcholanthrene was injected at the same time of day but 24 hr earlier in each case. All the rats were adapted to an "8 + 16" feeding regimen on 60% protein diets and sacrificed exactly at the time indicated. **

Open columns and cross-hatched columns, mean value of the activity in the liver from the rats given the injection of corn oil or methylcholanthrene 24 hr before sacrifice respectively. The vertical line on the top of the column represents the standard errors of the mean for 4 rats.
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Chart 8. The effect of actinomycin D, puromycin, and cycloheximide on the methylcholanthrene-induction of benzpyrene hydroxylase activity in hepatoma and the host liver from the rats bearing Morris Hepatoma 7800, Generation 40.

In each experiment methylcholanthrene (2 mg/100 g body weight) was injected intraperitoneally at 10:00 a.m., and the rats were killed at 17:00 (5:00 p.m.). Actinomycin D (50 µg/100 g body weight) or cycloheximide (2 mg/100 g body weight) were injected intraperitoneally at 8:00 a.m., 10:00 a.m., and 2:00 p.m., and puromycin (5 mg/100 g body weight) was injected at 8:00 a.m., 10:00 a.m., 11:30 a.m., 1:00 p.m., 2:00 p.m., and 3:30 p.m.

The vertical line on the top of the column represents the standard error of the mean for 4 rats, and the number in the bottom of the column indicates the relative activity in each group of rats in the experiment, compared to the methylcholanthrene-induced level.

During developmental stages in rat liver a characteristic modulation of many metabolic parameters occurs (32). Some drug-metabolizing enzymes showed negligible or little activity in liver microsomes from fetal and newborn animals (5, 6, 14) and, contrasted to the highly induced level of the enzyme activity by administration of drugs in adult and neonatal liver, no induction was observed in fetal liver (3, 19). In the experiments herein reported benzpyrene hydroxylase in fetal rat liver was also very low in activity, compared with that in neonatal liver, and was able to be induced to a slight extent by administration of methylcholanthrene, so that the induced level in fetal rat liver is approximately the same as the noninduced level in the liver from neonatal and preweanling rats (Chart 3). Thus our data on benzpyrene hydroxylase induction by MC are in general agreement with data involving other enzymes with the same or other inducers (3, 20). Drastic changes of the activity of benzpyrene hydroxylase occurred within 24 hr after birth and also, in rats 14 days old, hepatic activity is higher than in either rats 20 days old or in adult female rats. The livers in fetal and newborn rats contain hematopoietic cells up to about 5 to 7 days after birth but the enzyme inductions are too rapid to be ascribed to changes in the cell population.

Adamson and Fouts (1) pointed out in 1961 that, whereas ability to metabolize certain drugs by enzyme from Novikoff and Morris 5123 hepatomas was negligible, no decrease was observed in the hepatic enzymes metabolizing codeine, hexobarbital, or p-nitrobenzoic acid during the "precancerous"
stage in the rats fed dimethylaminoazobenzene. In preliminary experiments involving 6 rats on 0.05% 3'-methyl-2-methylaminoazobenzene for 7 weeks we found average BP hydroxylase activities only 17% of 6 control values, which suggested that direct comparisons between benzpyrene hydroxylase and some other drug-metabolizing enzymes in precancerous livers are needed. Conney and Burns (7) demonstrated decreased activity of azo dye N-demethylase in Morris Hepatoma 5123 as well as in the tumors induced by feeding 3'-methyl-2,4-dimethylaminoazobenzene to male Holtzman rats, in comparison with the activity in the respective host liver. However, MC was able to induce the enzyme activity in Morris Hepatoma 5123 to the same levels as shown in the host liver, in contrast to the results in the azo-dye-induced hepatoma in which there was no induction of the enzyme. Hart et al. (20) studied extensively the activities of drug-metabolizing enzymes, e.g., metabolism of aminopyrine, aniline, p-nitrobenzoic acid, Neoprontosil, and hexobarbital and the inducibility of the enzyme by phenobarbital treatment in Morris Hepatomas 5123B and D, 7316B, 7800, and Reuber Hepatoma H-35. They concluded that the above-mentioned hepatomas had detectable enzyme activity for nearly all the drug substrates, although these activities were considerably less than those in normal liver, and that phenobarbital was able to stimulate increases in several enzyme activities in these tumors, but the induced levels were still lower than the noninduced levels in normal rat liver. Relatively high activity in oxidation of hexobarbital in Morris Hepatoma 7800 was seen after phenobarbital treatment, compared to that in the other Morris hepatoma lines, thus showing different responses to such a treatment.

In the survey of benzpyrene hydroxylase activity and its induction by MC treatment in Morris hepatoma lines herein reported, all hepatomas have very low or even negligible naturally occurring activities and also lower induced levels compared to those in the host liver, and there was also tremendous variety in the induced levels among the Morris hepatoma lines (Chart 9). There were approximately the same induced levels of benzpyrene hydroxylase in Morris Hepatomas 9633 and 7800 as well as the corresponding host liver in male and female rats, although much higher levels of naturally occurring activities were observed in the host liver from male rats compared with those in the female rats.

Dallner et al. (9—11) reported that during the fetal period of rapid differentiation of rat hepatocytes the endoplasmic reticulum increases in volume, remaining predominantly rough-surfaced, and that after birth the increases continue but affect mainly the smooth-surfaced part of the system, corresponding to the synthesis of new enzyme, rather than the activation of preexisting, potentially enzymatic proteins. Furthermore there was heterogeneity of rough-surfaced liver microsomal membranes in adult, phenobarbital-treated, and newborn rats with respect to the distribution patterns of some enzyme activities. Piot (29) has postulated that interaction of polysome messenger RNA with the membranes of the endoplasmic reticulum occurs and that a change in the mosaic structures of the endoplasmic reticulum can lead to an alteration of this complex resulting in hepatoma formation.

Considering the significant, characteristic enzyme changes in fetal and neonatal rat liver, Potter and Watanabe (34) and Potter (32) strongly suggested that the tremendous diversity among hepatoma lines could be referred to the changes of the enzyme levels during developmental stages. In studies of benzpyrene hydroxylase activity herein reported, Morris Hepatoma 9618A showed relatively lower induced levels by MC treatment, compared to those in the other hepatoma lines, and the lower sensitivity of enzyme to MC treatment and the lower naturally occurring levels in the hepatomas may be compared to the responses in fetal rat liver. Furthermore the induced levels of the activity in the other hepatoma lines were also able to be covered within the range occupied by the values for liver from the neonatal rats within 1 day of age. It is possible that the development of the benzpyrene hydroxylase system in the Morris hepatoma lines used here might be blocked or reverted to some point in the maturation process at which the enzyme is formed in smooth endoplasmic reticulum or at the point where the rough endoplasmic reticulum is transformed to new smooth endoplasmic reticulum (10). Further studies on the relation between the Morris hepatomas and fetal rat liver with other biochemical parameters involving the endoplasmic reticulum and the plasma membrane might provide data on the time sequence of gene modulations in the developing hepatocyte and might provide more rigorous tests of the recent proposals regarding the Morris hepatomas (29, 32, 34). Since our data on naturally occurring levels of benzpyrene hydroxylase are so uniform for all hepatomas studied (Chart 9), it would probably be desirable to survey all available hepatomas to see if there are any exceptions to the findings that have been documented up to this time.

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REFERENCES


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Benzpyrene Hydroxylase in Morris Hepatomas


Benzpyrene Hydroxylase Activity and Its Induction by Methylcholanthrene in Morris Hepatomas, in Host Livers, in Adult Livers, and in Rat Liver during Development

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