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# The Influence of Sodium Phenobarbital on the Hepatic Microsomal Enzyme System which Reduces P-nitobenzoic Acid in Fetal and Newborn Rats

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# THE INFLUENCE OF SODIUM PHENOBARBITAL ON THE HEPATIC MICROSOMAL ENZYME SYSTEM WHICH REDUCES p-NITROBENZOIC ACID IN FETAL AND NEWBORN RATS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Pharmacology Department Date

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# INTRODUCTION

The duration and intensity of action of many drugs are largely determined by the speed at which they are metabolized in the body by enzymes in liver microsomes (1). Previous studies have shown that the activities of drug metabolizing enzymes in liver microsomes are markedly increased when animals are given various drugs, hormones, insecticides, and carcinogens. This increase in activity appears to represent an increased concentration of enzyme protein and is referred to as enzyme induction. Induction of liver microsomal enzymes leads to an accelerated biotransformation of drugs *in vivo*. This results in an altered duration and intensity of drug action. It has been reported that steroid hormones and other body constituents are also substrates of drug metabolizing enzymes in liver microsomes (1). This indicates the administration of a hepatic microsomal enzyme inducer may stimulate the metabolism of natural body constituents.

Richardson *et al.* (2) reported that 3-methylcholanthrene prevents 3'-methyl-4-dimethylaminoazobenzene, a potent carcinogen, from forming hepatomas in rats when both compounds are fed simultaneously. Brown, Miller, and Miller (3) provided an explanation for this observation when they showed that 3-methylcholanthrene increases the activity of the liver microsomal enzyme system that metabolizes aminoazo dyes to non-carcinogenic products. These investigations led to further studies of polycyclic hydrocarbons and other compounds that influence liver microsomal enzyme activity.

Conney and Burns (4) reported that pretreatment of rats with phenobarbital, barbital, aminopyrine, phenylbutazone, or orphenadrine enhances the activity of the azo dye demethylase system. It has been demonstrated that these drugs stimulate the activity of various other liver microsomal enzymes that metabolize zoxazolamine, phenylbutazone, hexobarbital, aminopyrine, and 3,4-benzpyrene (5). More than 200 drugs, insecticides, carcinogens, and other chemicals are known to stimulate the activity of drug metabolizing enzymes located in the hepatic microsomes (1).

There appears to be a broad range of activity both with respect to the type of molecule which is able to elicit stimulation of hepatic microsomal activity and with respect to the enzyme activities that are stimulated. Inducers are of at least two types, exemplified by phenobarbital and 3-methylcholanthrene. Compounds of the phenobarbital type stimulate varied pathways of metabolism by liver microsomal enzymes, including oxidation and reduction reactions, glucuronide formation, and de-esterification. The phenobarbital type also increases microsomal protein content and alters the morphology of the agranular endoplasmic reticulum. Liver microsomes from phenobarbital treated rats show greater amino acid incorporating activity when measured in vivo (6) and in cell free preparations (7), and exhibit a greater sensitivity to stimulation by polyuridylic acid (8). The polycyclic aromatic hydrocarbons, typified by 3-methylcholanthrene, stimulate a more limited group of reactions, do not affect microsomal protein content, nor do they affect gross morphologic changes in the agranular endoplasmic reticulum (1).

The two types of inducers differ in the course and intensity of induction. On daily administration of phenobarbital to rats the maximal increase of enzyme activity (3- to 10-fold) is not reached for at least 3 days (5). After injecting polycyclic hydrocarbons the enzyme activity is more than doubled within 3 to 6 hours and maximal increases (5- to 10-fold) are observed after 24 hours (9,10).

The age of the patient is one of the factors that is considered when a drug is used in therapy. It is known that the young of man and animals are more sensitive to certain drugs than adults. This greater sensitivity could result from differences in drug metabolizing enzymes present in liver microsomes. Studies have shown that adults have liver microsomal enzyme activity which is several times higher than the activity present in the embryo (11).

Fouts and Adamson (11) reported that a number of drugs that are metabolized by enzymes present in liver microsomes of adult rabbits are not metabolized by liver microsomes of newborn rabbits. There is some activity present two weeks after birth, and at the age of four weeks activity almost equals the adult level. The pathways studied include: oxidation of hexobarbital, N-dealkylation of aminopyrine, deamination of amphetamine, hyroxylation of acetanilide, oxidation of the ring sulfur of chlorpromazine, and the reduction of the nitro group of p-nitrobenzoic acid. When the supernatant fraction from newborn rabbit livers is added to the reaction vessels containing adult liver homogenates, the adult enzymes are inhibited in some cases. This indicates the possible presence of inhibitors of drug metabolism in the livers of newborn rabbits.

Other studies also indicate differences between hepatic microsomal drug metabolizing enzymes present in newborn and adult animals. Jondorf et al. (12) reported that liver microsomes of newborn mice and guinea pigs are deficient in certain drug metabolizing enzymes. The enzyme systems that N-demethylates aminopyrine, O-dealkylates phenacetin, oxidizes hexobarbital, and conjugates phenolphthalein as the glucuronide can not be demonstrated in newborn guinea pigs. The enzyme systems are absent 24 hours after birth but appear during the first week and increase in activity until the animals are about eight weeks old. Weatherall (13) reported that pentobarbital is more toxic to newborn than to adult rabbits and rats. It also produces a more sustained pharmacologic effect in newborn animals as measured by loss of the righting reflex.

Conney et al. (5) reported that pretreatment of weanling rats with certain drugs causes a marked increase in the activity of various enzyme systems in hepatic microsomes. Inscoe and Axelrod (14) have shown that benzpyrene injected into newborn rats causes a significant increase in hepatic microsomal glucuronyl transferase as compared with untreated litter mates. They also reported that benzpyrene given to pregnant rats does not stimulate glucuronyl transferase in the fetus, though it does cause increased activity in the mother. Hart et al. (15) reported that administration of sodium phenobarbital to pregnant does or to newborn from untreated does, stimulates hepatic microsomal drug metabolizing activity present in the newborn. Concurrent administration of ethionine blocked this increase in activity.

Crigler and Gold (16) reported that the administration of 15 mg.

of phenobarbital removed the jaundice from two infants suffering from congenital nonhemolytic jaundice. Parallel studies on the metabolism of salicylate in these children showed a defective capacity to conjugate glucuronide. This defect did not exist when phenobarbital was being administered. This study suggests that enzyme inducers given to expectant mothers may have an effect on fetal liver microsomal enzyme activity. If enzymes can be induced in this manner it may be possible to treat congenital metabolic disorders resulting from the lack of certain enzyme systems by the administration of an enzyme inducer to the mother before delivery.

The study of Hart et al. (15) indicates that pretreatment of pregnant rabbits with sodium phenobarbital stimulates microsomal enzyme activity in the offspring of these animals. A search of the literature uncovered no further studies using phenobarbital to stimulate fetal enzyme activity by pretreating the mother. The fact that there are major differences in the hepatic microsomal enzyme activities of rats and rabbits along with the observation that benzpyrene causes no increase in activity in the offspring from pretreated pregnant rats (14) prompted our investigation of this phase of enzyme induction.

Fouts and Brodie (17) were the first to report on the enzyme system which reduces p-nitrobenzoic acid in liver microsomes. They found that this reductive system is located in both the microsomes and soluble fraction. It is now known that this system is located in the microsomes with only a trace of activity present in the soluble fraction (21,18). This system was used in these experiments to

determine the effect of sodium phenobarbital on the hepatic microsomal enzyme activity present in fetal and newborn from pretreated pregnant females.

## MATERIAL AND METHODS

Sprague-Dawley rats were used in this study. The animals were housed in an air-conditioned room (70-75°F) and provided with feed (Purina Laboratory Chow) and water ad libitum.

Sodium phenobarbital (30 mg./kg.) was dissolved in double distilled water and injected subcutaneously twice daily into pregnant rats. The injection schedule began approximately five days before delivery and continued throughout the experimental period. Some animals were given less than ten injections prior to delivery because the time of birth varied from 12 to 24 hours. A separate injection bottle containing the calculated dose in 0.2 ml. was prepared for each test animal. The control animals were given 0.2 ml. of double distilled water twice daily throughout the experimental period.

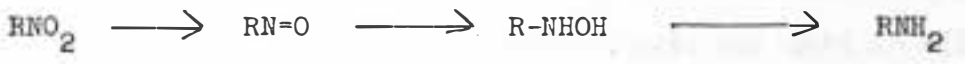
The activity of the enzyme system which reduces p-nitrobenzoic acid to p-aminobenzoic acid was determined using the method reported by Fouts and Brodie (17). During the four to five week experimental period the offspring were sacrificed by decapitation and their livers removed. All subsequent tissue manipulation was carried out at 0 to 4 degrees centigrade. Livers were immediately weighed and homogenized with a Potter-Elvehjem Teflon-glass homogenizer in 4 parts of 0.2 M phosphate buffer, pH 7.4, to provide a 20 per cent homogenate. Duplicate 1.0 ml. (200 mg. of tissue) aliquots of homogenate were placed in 10 ml. beakers. The reaction beakers also contained 5.7 micromoles of glucose-6-phosphate, 2.0 micromoles of adenosine triphosphate (ATP), 0.25 micromoles of triphosphopyridine nucleotide

(TPN), 0.3 micromoles of diphosphopyridine nucleotide (DPN), 100 micromoles of nicotinamide, 20.0 micromoles of potassium chloride, 10.0 micromoles of magnesium chloride, 3.0 micromoles of p-nitrobenzoic acid and sufficient 0.1 M phosphate buffer, pH 7.4, to give a final volume of 5 ml. This reaction system is similar to that used by Mueller and Miller (19).

The reaction beakers were incubated for 2 hours in a Dubnoff metabolic shaker at 37°C. in an atmosphere of nitrogen. At the end of the incubation period the reaction was stopped by the addition of 15 ml. of 6.67 per cent trichloroacetic acid. The precipitated protein was removed by centrifugation at 3700g for 10 minutes and 5 ml. aliquots of the supernate were analyzed for p-aminobenzoic acid.

The p-aminobenzoic acid present in the trichloroacetic acid supernates was diazotized and coupled as described by Bratton and Marshall (20). One ml. of freshly prepared sodium nitrite (0.1%) was added to the 5 ml. aliquot. After three minutes 1.0 ml. of 0.5 per cent ammonium sulfamate was added to destroy excess nitrite. The diazotized product was coupled by adding 1 ml. of 0.5 per cent N-(1-Naphthyl)-ethylenediamine dihydrochloride, allowing fifteen minutes to complete the reaction. The colored derivative was extracted with 10 ml. of isoamyl alcohol and assayed spectrophotometrically on a Spectronic 20 at 540 mu. The reduction of p-nitobenzoic acid to p-aminobenzoic acid probably takes place in three stages, involving the intermediate formation of nitroso and hydroxylamino compounds. There is no direct evidence indicating the presence of these intermediates, but Kamm (21) found that liver microsomes reduce

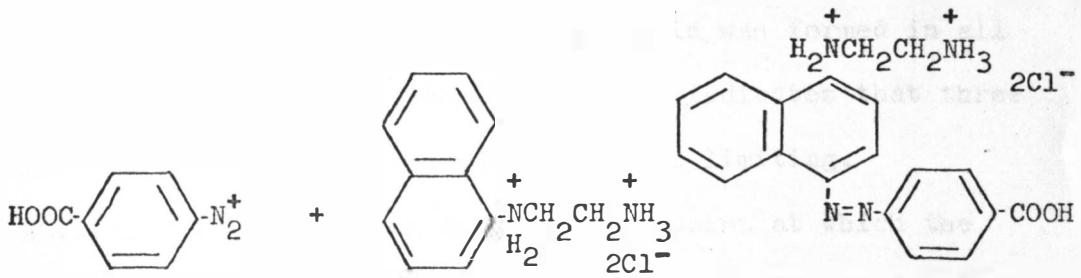
nitrosobenzene and phenylhydroxylamine to aniline more rapidly than they do nitrobenzene. The probable pathway for the reduction of nitro groups by microsomal enzymes is the following (17):




Nitro Nitroso Hydroxylamino Amino

The reactions involved in forming the colored derivative are the following:


p-Aminobenzoic acid Sodium nitrite Diazonium intermediate




Diazonium intermediate N-(1-Naphthyl)-ethylene-diamine dihydrochloride Colored product

The following experiments were performed previous to studies using pregnant rats.

An experiment was conducted to determine the relationship between tissue levels and the amount of p-aminobenzoic acid formed. The amount of p-aminobenzoic acid formed by 100, 150, 200, and 300 milligrams of tissue was determined. Three reaction vessels for each tissue level were prepared using a 20 per cent adult female liver homogenate.

A control containing liver homogenate and all other ingredients except p-nitrobenzoic acid was also prepared. The results of this experiment are shown in Fig. 1. The results indicate that a direct relationship exists between the amount of tissue used and the amount of p-aminobenzoic acid formed. The 200 mg. tissue level was used in all other parts of this study to measure the effect of sodium phenobarbital on the enzyme system which reduces p-nitrobenzoic acid.

A test was performed to determine if the substrate was rate limiting. Seven reaction vessels were prepared using 1 ml. of a 20 per cent adult female liver homogenate and the previously listed ingredients. Three vessels contained six micromoles of p-nitrobenzoic acid, three contained three micromoles, and one served as a tissue blank. The same amount of p-aminobenzoic acid was formed in all reaction vessels containing substrate. This indicates that three micromoles of p-nitrobenzoic acid is not rate limiting.

A trial was conducted to determine the point at which the reaction is complete. Nine reaction vessels were prepared using 200 mg. of tissue. Two reaction vessels were removed after 30, 60, 90, 120, 150, and 180 minutes and assayed for p-aminobenzoic acid. The results of this trial are shown in Fig. 2. The results indicate that the reaction is essentially complete in 120 minutes.

An experiment to determine the minimal dose of sodium phenobarbital capable of stimulating a significant increase in the activity of the enzyme system which reduces p-nitrobenzoic acid was conducted. Five groups, each containing three adult female rats,

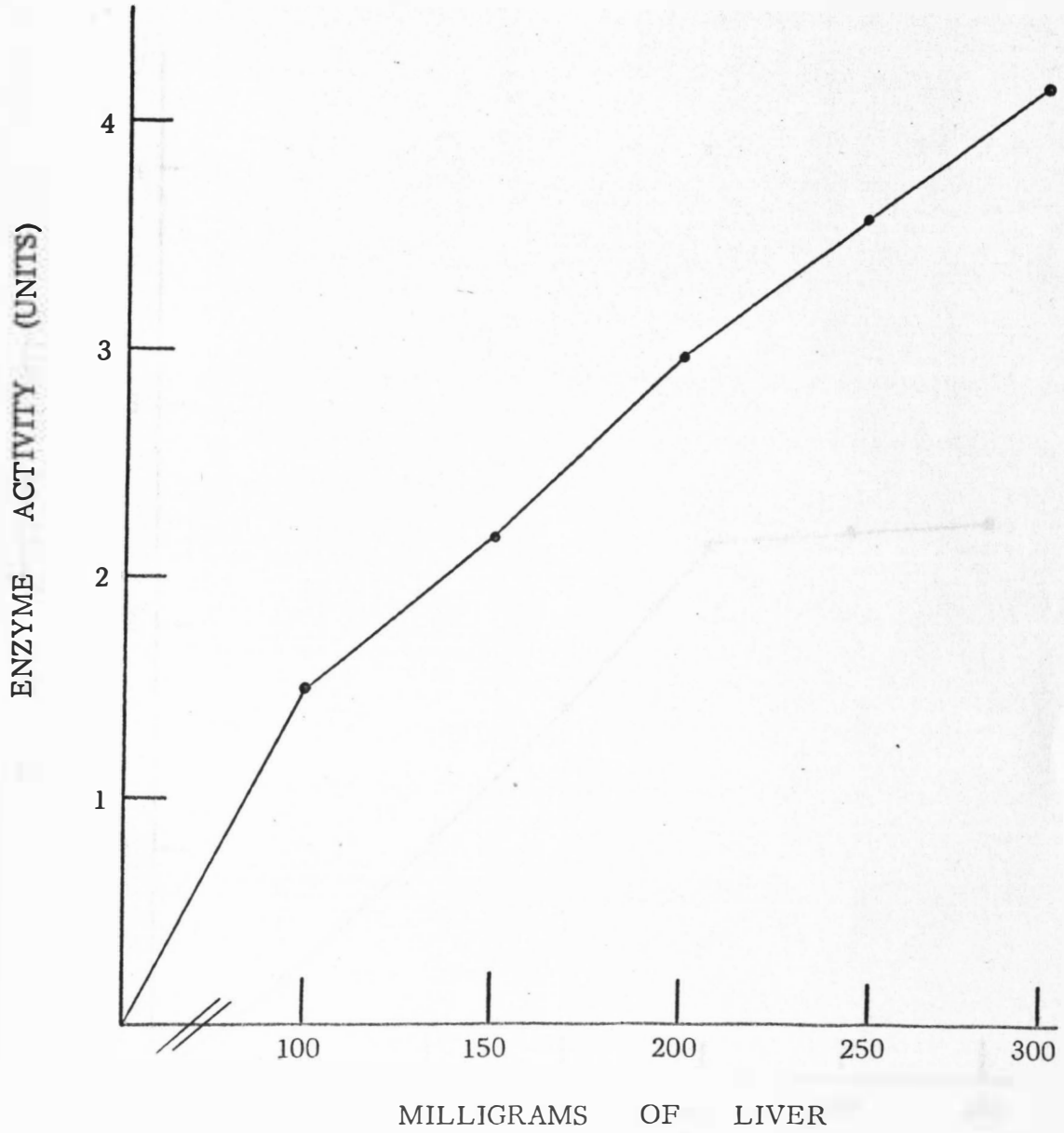



Fig. 1. The activity of the enzyme system which reduces p-nitrobenzoic acid in adult female rats. One unit represents 0.1 micromole of p-aminobenzoic acid formed by 200 mg. of tissue in 2 hours.

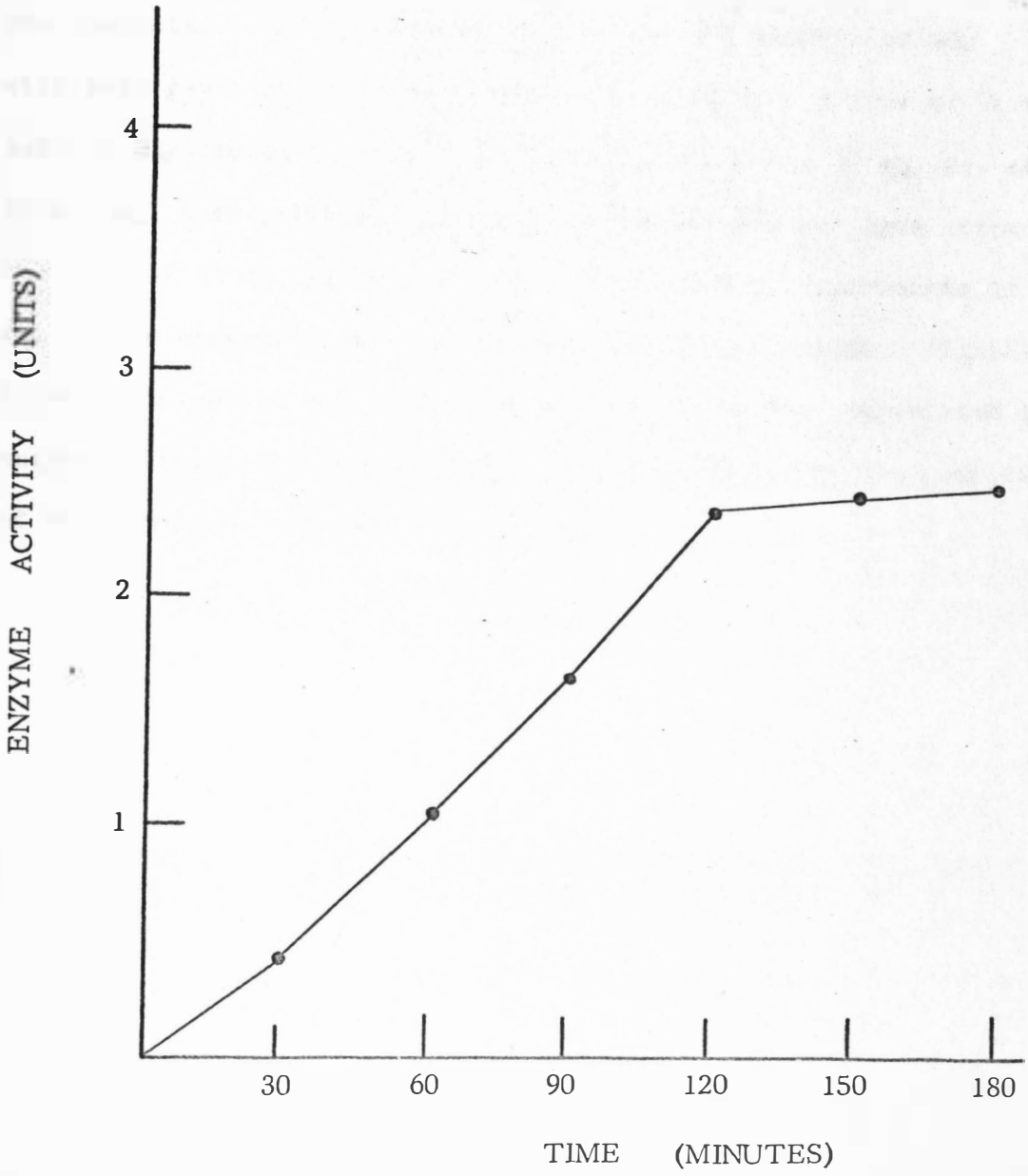



Fig. 2. The activity of the enzyme system which reduces p-nitrobenzoic acid in adult female rats. One unit represents 0.1 micromole of p-aminobenzoic acid formed by 200 mg. of tissue in 2 hours.

were given sodium phenobarbital, dissolved in double distilled water, twice a day at different dosage levels. The results of this experiment are presented in Table 1. The administration of sodium phenobarbital 5 mg./kg. and 10 mg./kg. did not significantaly stimulate p-nitrobenzoic acid reductase activity. A dose of 20 mg./kg. twice a day caused a 29 per cent increase while the 30 mg./kg. and 40 mg./kg. dose increased activity by 176 and 243 per cent respectively. The dose of 30 mg./kg. was used in all subsequent experiments in this study because it was the minimal dose which caused a significant increase in enzyme activity. The dose of 40 mg./kg. stimulated the enzyme activity to a higher level, but also increased the possibility of causing fetal and newborn sedation.

TABLE I

EFFECT OF SODIUM PHENOBARBITAL ON THE ACTIVITY OF THE ENZYME SYSTEM WHICH REDUCES p-NITROBENZOIC ACID IN THE ADULT FEMALE RAT.

| Days of Injection | Dose (mg./kg.) | Enzyme Activity (micromoles of p-aminobenzoic acid formed per 200 mg. tissue per 120 minutes). | Per Cent of Control | Per Cent Change In Activity |
|---|---|---|---|---|
| Control | -- | .25 | -- | -- |
| 3 | 5 | .25 | 100 | 0 |
| 3 | 10 | .27 | 104 | 4 |
| 3 | 20 | .35 | 129 | 29 |
| 3 | 30 | .69 | 276 | 176 |
| 3 | 40 | .95 | 343 | 243 |

# RESULTS

Standard p-aminobenzoic acid curve. An experiment to determine the relationship between the concentration of p-aminobenzoic acid present in a 5 ml. aliquot of the trichloroacetic acid supernate and the optical density at 540 mu. was conducted. Reaction vessels containing the previously listed ingredients and concentrations of p-aminobenzoic acid ranging from 0 to 1.2 micromoles were prepared. The vessels were incubated for two hours in an atmosphere of nitrogen. The reaction was then stopped by the addition of trichloroacetic acid and 5 ml. aliquot samples were analyzed for p-aminobenzoic acid content by the previously described method. The results of this experiment are shown in Fig. 3. Each point on the curve represents the average value obtained from triplicate determinations from three trials. The results of this experiment indicate there is a direct relationship between the concentration of p-aminobenzoic acid and optical density in the range of 0 to 1.2 micromoles. Enzyme activity is directly related to the amount of p-aminobenzoic acid formed, therefore, this curve was used to determine the results of this study.

The influence of sodium phenobarbital on the hepatic microsomal enzyme system which reduces p-nitrobenzoic acid in newborn and fetal rats from pretreated pregnant females. To determine the effect of sodium phenobarbital pretreatment on newborn and fetal rats three pregnant animals were given subcutaneous injections of 30 mg./kg. twice daily beginning approximately five days before delivery and

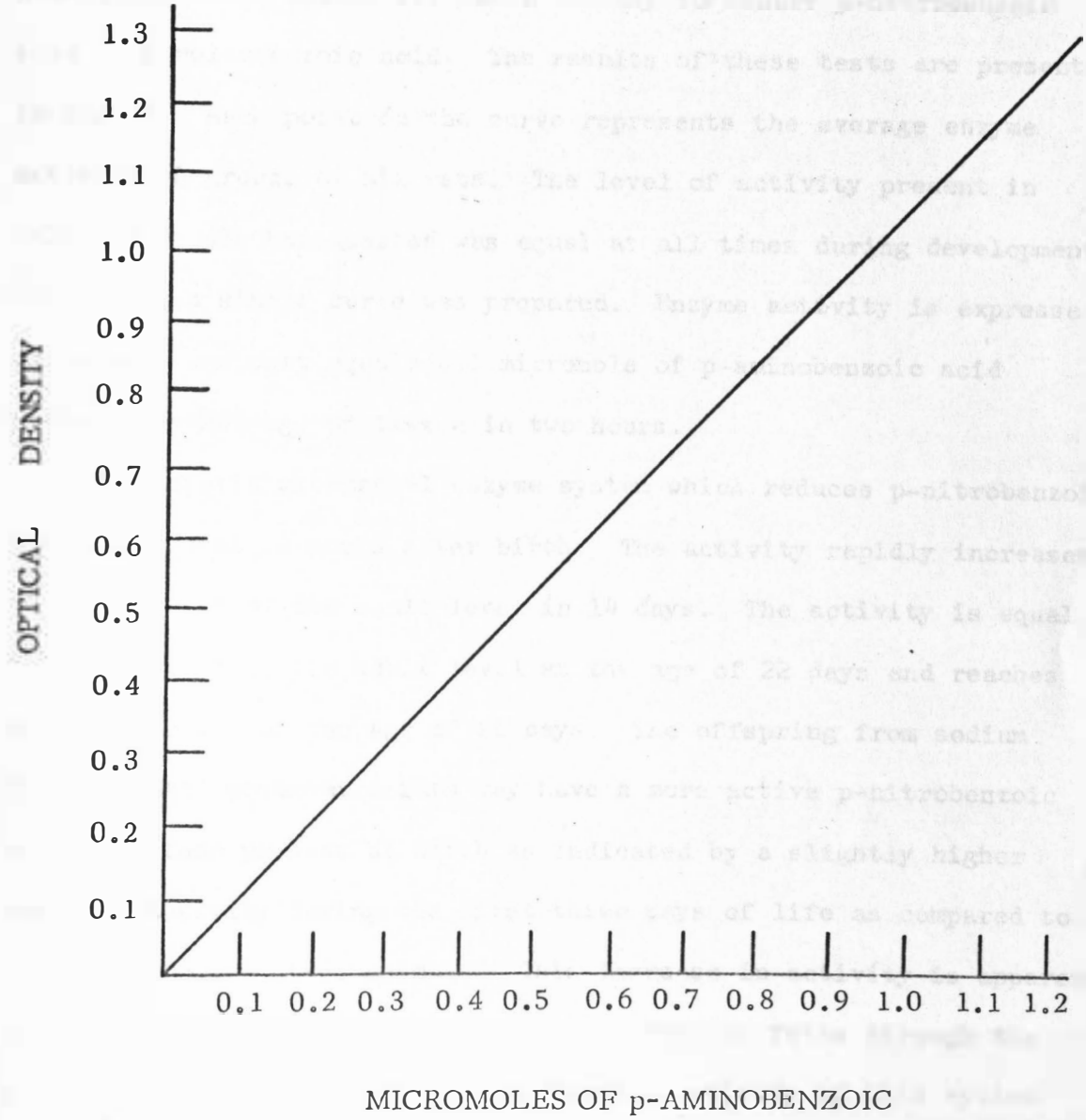



Fig. 3. Standard p-aminobenzoic acid curve. Optical density versus micromoles of p-aminobenzoic acid present in a 5 ml. aliquot.

continuing throughout the experimental period. The offspring were sacrificed at various intervals and their livers removed. Separate homogenates were prepared from male and female livers. The liver homogenates were tested for their ability to reduce p-nitrobenzoic acid to p-aminobenzoic acid. The results of these tests are presented in Fig. 4. Each point on the curve represents the average enzyme activity of groups of six rats. The level of activity present in male and female homogenates was equal at all times during development, therefore, a single curve was prepared. Enzyme activity is expressed as units. One unit equals 0.1 micromole of p-aminobenzoic acid produced by 200 mg. of tissue in two hours.

The hepatic microsomal enzyme system which reduces p-nitrobenzoic acid is present 24 hours after birth. The activity rapidly increases to 33 per cent of the adult level in 14 days. The activity is equal to 75 per cent of the adult level at the age of 22 days and reaches the adult level at the age of 26 days. The offspring from sodium phenobarbital pretreated rats may have a more active p-nitrobenzoic acid reductase present at birth as indicated by a slightly higher level of activity during the first three days of life as compared to offspring from control animals. This increase in activity is apparently caused by sodium phenobarbital which reaches the fetus through the placenta. There is a definite increase in activity of this system in offspring from pretreated females as indicated by the increased rate of development and the higher level of activity reached. Sodium phenobarbital in the mammillary secretion may be the cause of this increased activity.

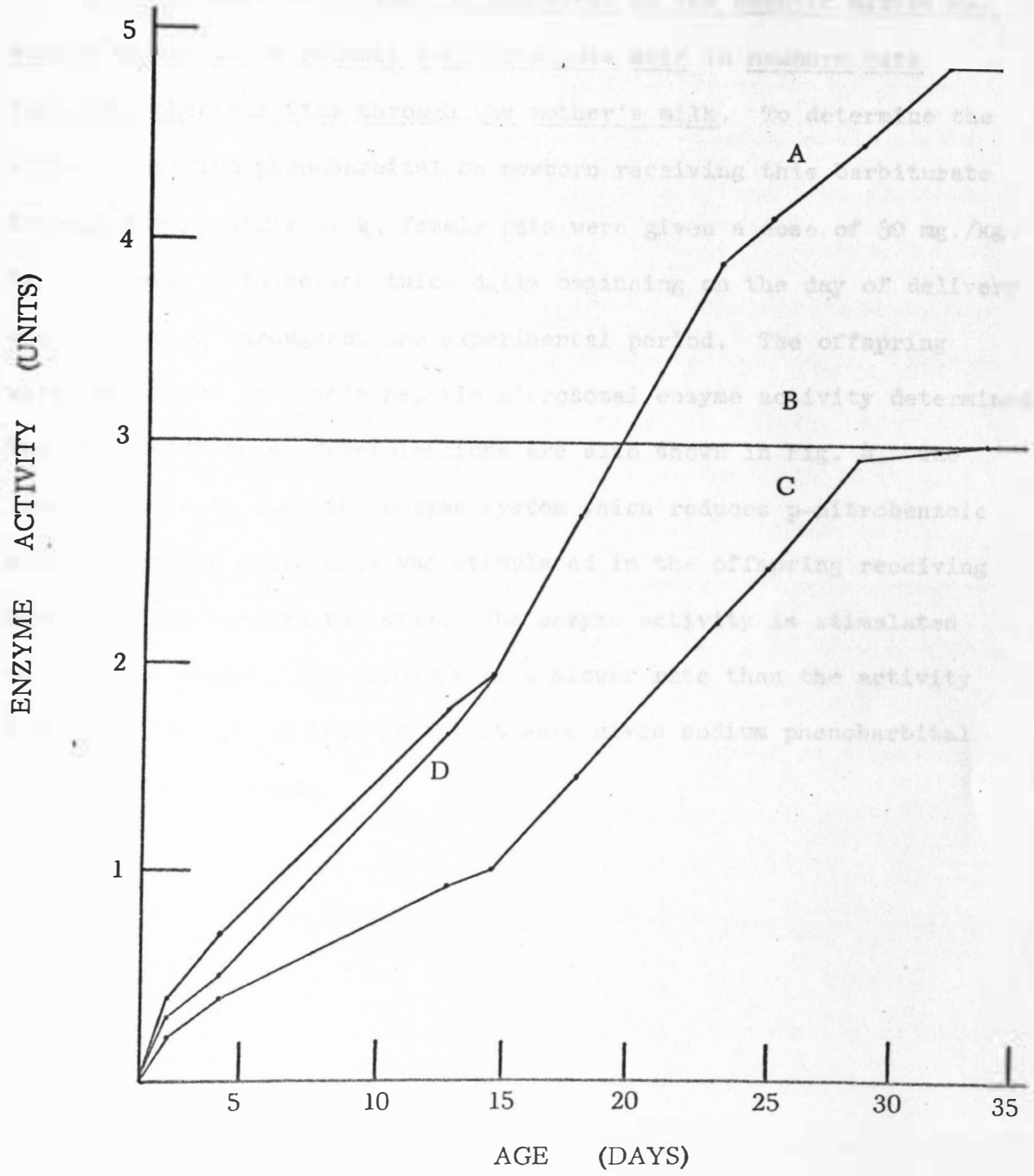



Fig. 4. The effect of sodium phenobarbital on the activity of the hepatic microsomal enzyme system which reduces p-nitrobenzoic acid in fetal and newborn rats. One unit represents 0.1 micromole of p-aminobenzoic acid formed per 200 mg. of tissue in 2 hours. A-activity of offspring from pretreated females, B-normal adult activity, C-activity of control offspring, and D-activity of offspring receiving phenobarbital through milk.

The influence of sodium phenobarbital on the hepatic microsomal enzyme system which reduces p-nitrobenzoic acid in newborn rats receiving phenobarbital through the mother's milk. To determine the effect of sodium phenobarbital on newborn receiving this barbiturate through the mother's milk, female rats were given a dose of 30 mg./kg. by subcutaneous injection twice daily beginning on the day of delivery and continuing throughout the experimental period. The offspring were sacrificed and their hepatic microsomal enzyme activity determined. The results of these determinations are also shown in Fig. 4. The results indicate that the enzyme system which reduces p-nitrobenzoic acid in hepatic microsomes was stimulated in the offspring receiving phenobarbital through the milk. The enzyme activity is stimulated to the same extent, but develops at a slower rate than the activity present in offspring from rats that were given sodium phenobarbital during their pregnancy.

## DISCUSSION

This study was undertaken to obtain information on the influence of sodium phenobarbital on the hepatic microsomal enzyme system which reduces p-nitrobenzoic acid in fetal and newborn rats from pretreated females. The enzyme system in liver microsomes that catalyze the reduction of p-nitrobenzoic acid exhibited low activity in newborn rats and developed rapidly to the adult level in four weeks. Results of this study indicate that the administration of sodium phenobarbital (30 mg./kg.) to pregnant rats twice daily by subcutaneous injection beginning five days before delivery and continuing throughout the experimental period stimulates the rate of development and increases the level of p-nitrobenzoic acid reductase twofold. Offspring from pretreated pregnant females seem to have a slightly more active reductase system present at birth. Activity developed at a more rapid rate in offspring from pretreated females as compared with controls and offspring receiving phenobarbital through the mother's milk.

The mechanism by which structually unrelated drugs, carcinogenic hydrocarbons, insecticides, and other compounds increase hepatic microsomal enzyme activity has been the subject of intensive research in recent years. It has been demonstrated that phenobarbital, chlordane, 3-methylcholanthrene, or 3,4-benzpyrene do not increase the activity of drug metabolizing enzymes when added to <u>in vitro</u> enzyme systems (5,9,10). Phenobarbital administration increases the maximal velocity of ethylmorphine N-demethylation, chlorpromazine sulfoxidation, and hexobarbital oxidation, but does not influence the

Michaelis constants or the susceptibility of the enzyme system to various inhibitors (22). This indicates the increased activity of hepatic microsomal enzymes from phenobarbital treated rats can not be explained by an altered affinity of enzyme for substrate.

Several attempts to demonstrate that polycyclic hydrocarbons or phenobarbital enhance drug metabolizing activity by increasing the level of an inhibitor have been unsuccessful (5,9,23). The discovery that certain compounds that inhibit the synthesis of protein also prevent drug induced increases in microsomal enzyme activity indicates increases in activity are related to an increased synthesis of enzyme protein. The stimulation of enzyme activity present in hepatic microsomes by 3-methylcholanthrene or phenobarbital is blocked by ethionine (5,9,10,24), puromycin (25,26), or actinomycin D (26,27). It has been reported that these inhibitors block protein synthesis by different mechanisms. Ethionine blocks protein synthesis by lowering the level of ATP in the liver (28), puromycin blocks the transfer of soluble RNA-bound amino acid into microsomal protein (29), and actinomycin D binds with DNA to block the DNA-directed synthesis of nuclear RNA which is required for protein synthesis (30). The inhibitory effect of ethionine on liver protein synthesis and the induction of increased drug metabolizing activity in hepatic microsomes can be prevented <u>in vivo</u> by the administration of either ATP (28) or methionine (5,9,10). This suggests that adequate levels of S-adenosylmethionine may be important for the synthesis of microsomal enzymes. The blocking effect of actinomycin D suggests that phenobarbital and 3-methylcholanthrene

may accelerate the DNA-directed synthesis of drug metabolizing enzymes on ribosomes. When puromycin, ethionine, or actinomycin D is administered several hours after 3-methylcholanthrene further increases in activity of aminoazo dye N-demethylase is prevented. This may indicate the formation of a short lived messenger-RNA required for the increased synthesis of aminoazo dye N-demethylase (24,9). Loeb and Gelboin (31) reported that treatment of rats with 3-methylcholanthrene increased the content of messenger-RNA in isolated liver nuclei and that nuclear RNA from treated rats was more active than equivalent amounts of RNA from normal rats in directing the incorporation of L-phenylalanine-$C^{14}$ into protein in a cell free Escherichia coli system. Jervell et al. (32) confirmed the blocking action of ethionine, puromycin and actinomycin D on the induction of aminoazo dye N-demethylase by 3-methylcholanthrene, but reported the induction of this enzyme by starvation is blocked by ethionine or puromycin but not by actinomycin D. This suggests the involvement of two different mechanisms. These authors also reported that induction of the N-demethylase system was blocked by the administration of glucose or fructose.

The possibility that inducers of hepatic microsomal enzymes may increase activity by stabilizing enzymes, as well as by accelerating their synthesis, is now being studied in several laboratories. Jick and Shuster (33,34) found that treatment of mice with phenobarbital increases liver microsomal TPNH-cytochrome c reductase levels by increasing its rate of synthesis and decreasing its rate

of breakdown. These two workers also reported that phenobarbital increases total liver microsomal protein in rats by the same mechanism. Holtzman and Gillette (35) reported that phenobarbital inhibits the degradation of hepatic microsomal phospholipid in fasted rats.

Gelboin and Sokoloff (7) reported that the stimulatory effect of phenobarbital and 3-methylcholanthrene on the synthesis of microsomal enzymes and protein *in vivo* is paralled by increased incorporation of amino acids into microsomal protein *in vitro*. Phenobarbital produces a greater stimulation of amino acid incorporation into microsomal protein than does 3-methylcholanthrene. This agrees with the observation that phenobarbital stimulates more synthesis of microsomal protein *in vivo* than 3-methylcholanthrene (25). Gelboin and Sokoloff (7) also reported that 3-methylcholanthrene stimulates the incorporation of soluble RNA-bound amino acids into liver protein *in vitro*. This indicates that it does not act on reactions leading to activation of amino acids but on steps between transfer-RNA and the formation of protein on ribosomes. These investigators suggest that the increase in amino acid incorporation induced by 3-methylcholanthrene is due, at least in part, to an increase in the number of active microsomal incorporation sites and an apparent increase in the messenger-RNA content of microsomes. Kato *et al.* (36) suggest that a portion of the greater amino acid incorporating activity of microsomes from phenobarbital treated rats may be due to a phenobarbital induced shift to a greater proportion of membrane bound ribosomes. Kato *et al.* (37) showed

that treatment of rats with phenobarbital stimulates the incorporation of $C^{14}$-leucine into microsomal protein *in vivo* but has no effect on the incorporation of this amino acid into protein in other subcellular fractions.

Treatment of rats with phenobarbital increased by 108 to 266 per cent the incorporation of arginine, leucine, lysine, phenylalanine, and valine into liver microsomal protein *in vitro*. This effect could not be attributed to changes in cofactor level, amino acid concentration, or factors present in the cell sap. After the removal of endogenous messenger-RNA by incubation of the microsomes, the microsomes from phenobarbital treated rats displayed greater ability to incorporate L-phenylalanine-$C^{14}$ in the presence of either saturating or subsaturating amounts of polyuridylic acid. The ribosomes from phenobarbital treated and control rats have equal ability to incorporate this amino acid. This indicates that phenobarbital stimulated increases in microsomal enzyme activity are not directly related to alterations in the ribosomes, but suggests that it alters the translation of polyuridylic acid on the ribosomes (36).

The ability of several stimulators of liver microsomal enzyme activity to increase the concentrations of TPNH oxidase, TPNH-cytochrome c reductase, and cytochrome P-450 in liver microsomes suggests that the induction of these electron transport systems may play a role in the stimulatory effect of these compounds. This scheme is incomplete and cannot explain the selective effect of enzyme inducers that stimulate some liver microsomal hydroxylations without influencing others (1).

The results of this study indicate that a significant increase in activity of the hepatic microsomal enzyme system which reduces p-nitrobenzoic acid does not take place in offspring from sodium phenobarbital pretreated pregnant rats until the fifth day after delivery. This may be due to the limited capability of enzyme forming systems at this stage of development. It has been reported that the transformation of a normal liver cell into a cancer cell is accompanied by a decrease of drug metabolizing activity (38,39). It is of interest that hepatomas that resemble normal liver histologically and biochemically possess little or no microsomal drug metabolizing activity (38,39,40). Low levels of activity are also present in regenerating liver (41,42,43). The limited capability of fetal and newborn (less than 5 days old) liver microsomal enzymes to be stimulated may result from a short supply of cofactors or the incomplete development of the endoplasmic reticulum or may involve other factors. Little work has been done in this area of study and as a result no comprehensive theory can be presented at this time.

## SUMMARY

1. The method reported by Fouts and Brodie (17) was used to determine the effect of sodium phenobarbital on the activity of the liver microsomal enzyme system which reduces p-nitrobenzoic acid in fetal and newborn rats.

2. Sodium phenobarbital (30 mg./kg.) was given twice daily by subcutaneous injection to pregnant rats beginning approximately five days before delivery and continuing throughout the experimental period.

3. The liver microsomal enzyme system which reduces p-nitrobenzoic acid developed at a more rapid rate and to a higher level of activity in offspring from pretreated females than in offspring from controls.

4. The slightly more active reductase present at birth and the observation that offspring from pretreated pregnant rats develop activity faster than offspring given phenobarbital through the milk may indicate that some activity was induced in the fetus.

5. The liver microsomal enzyme system which reduces p-nitrobenzoic acid is present in male and female rats at the same level of activity before and after reaching maturity.

6. The inductive effect of sodium phenobarbital on the enzyme system which reduce p-nitrobenzoic acid is the same in male and female rats.

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