Disturbance of polyamine synthesis was reflected only a little in incorporation of the radioactive label into proteins of the regenerating liver and had practically no effect on RNA synthesis (Table 3). Meanwhile, a considerable (by more than 40%) inhibition of DNA synthesis was observed. This is evidence in support of the view that polyamines play an important role in DNA synthesis [14] and that they are probably essential not only for its initiation, but also for maintenance of the normal replicative function during the first 24 h of regeneration. Unlike the observations of Kallio et al. [6], who state that there is direct correlation between the spermidine concentration and DNA synthesis in the regenerating liver, we found that incorporation of $^{3}$H-thymidine into DNA correlates closely (coefficient of correlation $r = 0.93$) with the putrescine concentration and, at the same time, with incorporation of $^{14}$C-methionine into spermidine ($r = 0.71$). In this case spermidine synthesis probably undergoes parallel changes with DNA synthesis and evidently cannot determine it. The results suggest that maintenance of normal DNA synthesis in the course of cell proliferation is one of the functions of putrescine.

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# EFFECT OF MIXED AND SEPARATE ADMINISTRATION OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ON BENZPYRENE HYDROXYLASE IN THE LIVER OF SENSITIVE AND RESISTANT INBRED MICE


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KEY WORDS: induction; phenobarbital; 3-methylcholanthrene; benzpyrene hydroxylase; monooxygenases.


The existence of two classes of inducers of monooxygenase systems in the liver, differing in their mechanism of action, has now been demonstrated. Typical representatives of one of these classes are the barbiturates, and of the other, the polycyclic aromatic hydrocarbons (PAH) [2]. One member of the first group, namely phenobarbital (PB), induces a monooxygenase which possesses low substrate specificity. Administration of PAH, on the other hand, induces de novo synthesis of an aberrant enzyme (cytochrome P-448), which selectively catalyzes the hydroxylation of PAH in microsomes [2, 4].

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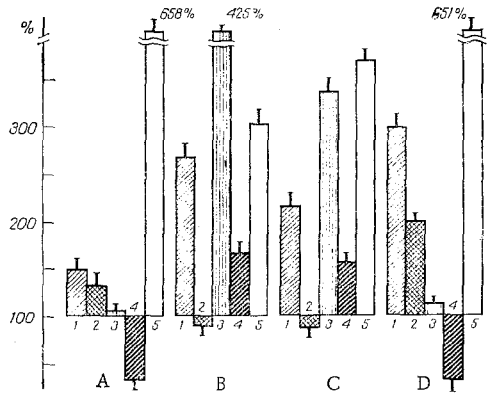


Fig. 1

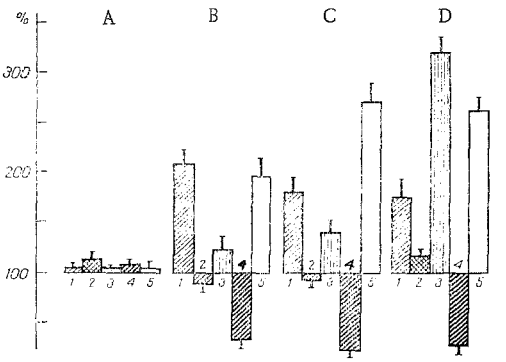


Fig. 2

Fig. 1. Spectral and kinetic parameters of activity of microsomal monooxygenases after injection of PB and 3MCh into C57BL/6 mice (in % of control). 1) Cytochrome P-450 (P-448): In the control group (100%) its content was 0.43 nmole/mg protein; 2) ratio of high-spin (type a) to low-spin (type b) forms of hemoprotein: In the control group (100%) it was 0.44; 3) spectral constant of benzpyrene binding: In the control group (100%) it was 4.25 $\mu$M; 4) Michaelis constant ($K_m$) for benzpyrene: In the control group (100%) it was 1 $\mu$M; 5) maximal rate of benzpyrene metabolism ($V_m$). Value in control group (100%) 365 pmole 3-hydroxybenzpyrene/min/mg protein. A-D) Groups (see Table 1).

Fig. 2. Spectral and kinetic parameters of activity of microsomal monooxygenases after administration of PB and 3MCh to DBA/2 mice (in % of control). Control values taken as 100%; cytochrome P-450 = 0.48 nmole/mg protein; a/b = 0.44; $K_m$ = 0.67 $\mu$M; $V_m$ = 254 pmole 3-hydroxybenzpyrene/min/mg protein. Remainder of legend as in Fig. 1.

The results of experiments with preliminary administration of PB to rats showed that the intensity of the inducing effect of PAH depends on the level of their metabolic activation in the microsomes [1]. Unlike rats for which genetic regulation of cytochrome P-448 synthesis has not been demonstrated [7], various lines of mice have been found to differ in their sensitivity to the inducing action of PAH [6, 8]. Experiments involving crossing of inbred mice of different lines showed that the inducibility of the liver monooxygenases by PAH is inherited as a dominant character and is marked by a simple autosomal distribution. Under these circumstances C57BL/6 mice are carriers of the dominant inducibility allele, whereas DBA/2 mice carry the recessive allele [7, 13]. It has been suggested that in mice resistant to induction a mutant intracellular receptor for PAH, with weak ability to "recognize" the inducer molecule, is synthesized [9].

This paper describes an attempt to "overcome" the resistance of DBA/2 mice to the inducing effect of 3-methylcholanthrene (3MCh) by preliminary administration of PB to these mice. The inducing ability of 3MCh in C57BL/6 mice, sensitive to induction, also was studied after preliminary activation of the liver monooxygenase system by PB.

## EXPERIMENTAL METHOD

Male C57BL/6 and DBA/2 mice weighing 15 g were used. The animals were deprived of food for the night before each injection of inducer and sacrifice. 3MCh (40 mg/kg body weight), dissolved in the minimal volume of olive oil, and PB (70 mg/kg body weight), dissolved in 0.9% NaCl, were injected intraperitoneally (Table 1).

The concentration of cytochrome P-450 (P-448) and the content of microsomal protein were determined by the method in [1, 15]. Quantitative determination of high- and low-spin forms of cytochrome was carried out as in [5].

Benzpyrene hydroxylase activity in the liver microsomes of the control and experimental mice was determined by the method in [11] with the modifications in [3]. According to the results of the investigations cited, the use of low concentrations of microsomal protein (not more than 20 $\mu$g/ml) rules out nonspecific binding of benzpyrene and also possible errors connected with the activity of the microsomal enzyme epoxide hydratase. The fluorescence spectrum of 3-hydroxybenzpyrene (excitation at 446 nm, emission at 522 nm) was measured on a model MPF-4 Hitachi spectrofluorometer.

TABLE 1. Scheme of Administration of Inducers

| | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th |
|---|---|---|---|---|---|---|---|---|
| A | 3MCh | 3MCh | Sacrifice | – | – | – | – | – |
| B | PB | PB | PB | PB | PB | Sacrifice | – | – |
| C | PB | PB | PB | PB | PB | – | – | Sacrifice |
| D | PB | PB | PB | PB | PB | 3MCh | 3MCh | Sacrifice |
| Control | Control to groups A-D* | | | | | | | |

Legend. Scheme given for each line of mice. *) Corresponding volume of olive oil or 0.9% NaCl injected into control animals.

The spectral binding constant ($K_S$) for benzpyrene was determined by the method in [12], but "metabolic" protein concentrations were used in the determination. To measure the difference spectra of the cytochromes, p-octylamine, and binding of the substrates with microsomes, a dual-beam Hitachi 556 scanning spectrophotometer was used.

## EXPERIMENTAL RESULTS

It will be clear from Fig. 1D that injection of 3MCh into C57BL/6 mice after preliminary induction with PB led to a threefold increase in the level of CO-binding hemoprotein in the form of cytochrome P-448. By contrast with induction by PB alone, subsequent injection of 3MCh was followed by a predominant increase in the content of the high-spin form of the hemoprotein, a characteristic feature of induction by 3MCh. With respect to these parameters an additive effect of PB and 3MCh was observed, similar to that found previously in rats [1]. This confirms the view that the true inducers are products of microsomal metabolism of PAH. Otherwise in the animals of this group the changes in the parameters studied resembled those of induction by 3MCh alone (Fig. 1A, D).

In the "resistant" mice of line DBA/2 (Fig. 2) administration of 3MCh caused virtually no change in the quantity and functional activity of the microsomal monooxygenases (Fig. 2A).

Induction of these mice with PB (Fig. 2B, C) was characterized by a twofold increase in the level of CO-binding hemoprotein in the form of cytochrome P-450 and a maximal velocity of benzpyrene metabolism. However, unlike in the C57BL/6 mice, the affinity of benzpyrene hydroxylase for the substrate increased in the mice of this group and the Michaelis constant fell to 40-30% of the control. Meanwhile, the maximal velocity of benzpyrene hydroxylation was induced to the same degree as in the sensitive mice. In addition, after administration of PB the quantity of PAH-binding h-protein in the liver cells increased [10]. After 5-day induction by PB the concentration of the possible intracellular receptors for 3MCh or its products thus increased.

However, administration of 3MCh after PB (Fig. 2D) caused no significant changes in monooxygenase activity compared with induction by PB alone. It is an interesting fact that in response to injection of 3MCh after preliminary induction by PB the value of $K_S$ for benzpyrene increased. This requires further study. It is important to note that in neither case was the spectrum of cytochrome P-448, characteristic of induction by PAH, discovered.

The results show that an increase in the number of intracellular receptors for PAH (cytochrome P-448 and h-protein) evidently does not play an essential role, for under these conditions no signs of induction of monooxygenases by 3MCh appeared in the DBA/2 mice.

It may be that the "breakdown" in these mice takes place not at the level of the receptor for PAH, but either at the stage of genome activation (the transcription stage) or in the catalytic properties of the active centers of the newly synthesized hemoprotein. Data supporting this last hypothesis have been obtained in the writers' laboratory [14].

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# CHARACTERISTICS OF NAD-DEPENDENT α-GLYCEROPHOSPHATE DEHYDROGENASE FROM MUSCLES OF SOME VERTEBRATES AND MAN DETERMINED BY ELECTROPHORESIS AND ISOELECTRIC FOCUSING IN POLYACRYLAMIDE GEL

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KEY WORDS: electrophoresis; isoelectric focusing; red and white skeletal muscles; isozyme spectrum of NAD-dependent α-glycerophosphate dehydrogenase.

Stable electron transport in the respiratory chain and oxidative phosphorylation in the mitochondria are maintained by preservation of the reduced state of the mitochondrial pyridine-nucleotide pool at a sufficiently high level. In muscles one of the most important systems responsible for the uninterrupted supply of hydrogen to the mitochondria [7] is the α-glycerophosphate dehydrogenase (α-GPD) system.

Two forms of α-GPD are found in mammalian cells [8]. The first is located in the cytosol and requires NAD as coenzyme (L-glycerol-3-phosphate, NAD-oxidoreductase). The mitochondrial form of the enzyme is considered [12] not to require NAD: As its coenzyme it uses flavin-adenine dinucleotide (FAD) (L-glycerol-3-phosphate acceptor-oxidoreductase). Despite many investigations devoted to the study of the molecular forms of α-GPD, the number of its isozymes and their distribution in different tissues have not yet been finally explained.

The aim of the present investigation was accordingly to study the distribution of isozymes of NAD-dependent α-GPD in the muscles of some vertebrates and man.

## EXPERIMENTAL METHOD

Supramitochondrial supernatants from red (m. soleus) and white (m. quadratus lumborum) muscles of intact animals (hens, rats, rabbits) were used for analysis. Investigations of α-GPD from human muscles were carried out on autopsy material obtained from 10 persons dying from accidents at the age of between 20 and 28 years. The time between death and taking samples for analysis did not exceed 12 h. A commercial preparation of α-GPD from rabbit muscles was first dialyzed against isotonic NaCl solution for 48 h at 4°C. Mito-

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