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Strain Differences in Aryl Hydrocarbon Hydroxylase Induction by 3-Methylcholanthrene in Rabbits (38842)

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Aryl hydrocarbon hydroxylase belongs to a class of NADPH-dependent oxidative microsomal enzyme systems, is present in tissues of many species, and is "inducible" both *in vivo* (1–3) and *in vitro* (4–7). For example, in man it is present both in adult (8, 9) and fetal liver (10), in the placenta (11–14), and the skin (15, 16). However, there are differences in both basal and induced level of hydroxylase activity among the various tissues of the same individual (17, 18), tissues of different strains of the same species (19–21), and between different species (17, 22).

In the present investigation we determined basal levels of enzyme activity as well as the hydroxylase activity induced by 3-methylcholanthrene (3-MCA) in livers of several partially inbred strains of rabbits that we use in studies on chemical carcinogenesis. We hoped to identify strains of rabbits that exhibit differences in enzyme activity that we might correlate with the carcinogenic effects of hydrocarbons.

Materials and Methods. Treatment of animals. About equal numbers of male and female rabbits of partially inbred strains AC, ACEP, OS, X, III, and WH and their hybrids, (III × WH)F_1 and (WH × III)F_1, were used. These strains have previously been studied for a number of biochemical (23, 24) endocrinological (25, 26), and physiological (27, 28) parameters in addition to their genetic predisposition to tumorigenesis (29). All rabbits were obtained from the rabbit colony of the Jackson Laboratory. The mean age for the treated and control rabbits was 9.7 ± 0.2 and 9.3 ± 0.3 wk, respectively.

Treated rabbits were given intraperitoneal injections of 3-MCA (10 mg/ml in trioctanoin) at a dosage of 100 mg/kg body wt.

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All rabbits were killed 24 hr after injection and their livers removed. All experiments were performed at approximately the same hour of day.

Preparation of tissues for assay. The livers from each rabbit were placed on ice, washed in cold 0.9% saline and then homogenized in a glass–Teflon homogenizer using 20 vol (w/v) of buffer (pH 7.2 at 25°) containing 0.25 *M* sucrose and 0.05 *M* Tris. The homogenates were centrifuged at 9000*g* for 15 min in a Beckman refrigerated centrifuge and the supernatant fractions were used for determination of aryl hydrocarbon hydroxylase (AHH) activity.

Enzyme assays. The hepatic levels of AHH were determined by the procedure of Nebert and Gelboin (17) as modified by Thomas *et al.* (19). AHH activity was calculated in terms of U/g wet weight liver. A unit of AHH activity is defined as the amount of enzyme causing the fluorescence equivalent of 1 nmole of 3-hydroxybenzo(a)pyrene (3 OH-BP, kindly given to us by Dr. R. Kouri, Microbiological Associates, Inc., Bethesda, Maryland) per min at 37°. The 3 OH-BP was determined in an Aminco Bowman spectrophotofluorometer with the activation at 398 nm and emission at 522 nm.

Results. In in the present study, inducibility is defined as the ratio of the average AHH activity/g wet weight of liver of rabbits given MCA divided by the average enzyme activity determined in rabbits not given MCA. Rabbits of strain III showed the highest induced activity, i.e., 18.66 ± 0.59 U/g wet weight liver compared with 5.21 ± 0.16 U/g wet weight liver of basal activity (Fig. 1). In contrast, no significant AHH induction was observed in the WH, AC, ACEP, X, or OS rabbits although the average activity for each strain was somewhat higher than that in untreated rabbits. No significant sex difference was observed in the mean

TABLE I. HEPATIC ARYL HYDROCARBON HYDROXYLASE INDUCIBILITY AMONG DIFFERENT STRAINS AND HYBRIDS OF RABBITS.

Strains	No. of animals E/C[a]	AHH inducibility	Remark
III	11/11	3.7	Positive
OS	10/8	1.8	Negative
WH	11/10	1.1	Negative
X	11/9	2.0	Negative
ACEP	10/7	1.8	Negative
AC	9/7	1.5	Negative
(III × WH)F_1	6/6	3.4	Positive
(WH × III)F_1	20/9	2.3	Negative

[a] E/C, no. of experimental/no. of control rabbits.

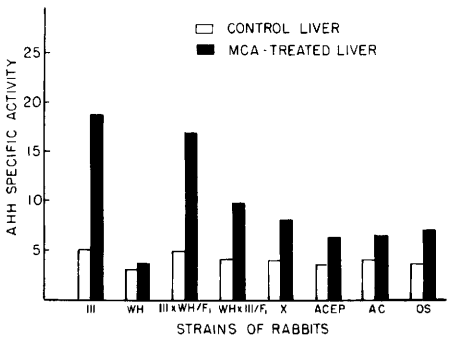

FIG. 1. Aryl hydrocarbon hydroxylase (AHH) induction of 3-MCA in different strains and hybrids of rabbits.

activity of either MCA treated or control rabbits.

The liver enzyme activity was about five times higher in strain III than in strain WH rabbits (3.58 ± 0.15 U/g wet weight liver), which have the lowest enzyme activity of all strains examined. AHH was induced in the (III × WH)F_1 hybrids (Table I, Fig. 1). In contrast, injection of MCA did not appear to have an appreciable effect on the induction of AHH levels in (WH × III)F_1 hybrids. Thus, the induction of AHH activity in these two types of F_1 hybrids was dependent on the genotype of the mother. This relationship between the AHH induction and maternal genotype is clearly seen in Fig. 1 (16.89 ± 0.83 vs 9.58 ± 0.51).

Discussion. AHH-enzyme activity induced by MCA differs markedly in six strains of rabbits tested. Strain III rabbits revealed the highest induced enzyme activity, whereas strain WH had the lowest liver enzyme activity.

Strain X rabbits have slightly higher enzyme (8.21 ± 0.91 U/g wet weight liver) activity than rabbits of strain AC, ACEP, or OS (Fig. 1). Finally, the induction of AHH measured by the absolute increase in activity was higher in (III × WH)F_1 hybrids than in (WH × III)F_1 offspring indicating a maternal effect. Our preliminary experiments on (AC × WH)F_1, (WH × ACEP)F_1, and (ACEP × III)F_1 rabbits indicated that these F_1 hybrids were noninducible. Thus, crosses either between noninducible parental strains or when the noninducible parent was the mother appeared to be noninducible.

The enzyme AHH is a class of mixed-function oxidases which is responsible for the detoxification (22, 32) or activation (33–37) of a variety of compounds including polycyclic hydrocarbons (30–32). Induction or increase in AHH activity by aromatic hydrocarbons was observed only in certain inbred strains of mice (19), and inducibility segregated as a single autosomal dominant gene in crosses of strains C57BL/6J and DBA/2J (19, 38). However, in crosses between strains C3H/HeJ (inducible) and DBA/2J (noninducible) inducibility segregates as a single gene and in an additive manner, with the inducibility of hybrid animals falling between that of inducible and that of noninducible parent (39). This suggests that the genes responsible for inducibility of AHH in strains C3H/HeJ and C57BL/6J are not identical. In contrast to the one-gene theory proposed above (19, 38), Robinson *et al.* (40) now postulate that the enzyme induction, even among various inbred strains of mice, appears to involve at least two, and probably more than two, nonlinked genetic loci.

Cram *et al.* (21) have observed a wide range of variability in various genera of lagomorphs in the response of hepatic drug metabolizing enzyme systems to *in vivo* pretreatment with phenobarbital. The results of the present investigation clearly corroborate their observations. Our findings, however, indicate that, in contrast to mice, F_1 hybrids between the inducible strain III and nonin-

ducible strain WH rabbits differed in their response to AHH induction depending on the genotype of the mother. Thus, whereas (III × WH)F_1 rabbits are AHH inducible, (WH × III)F_1 hybrids show intermediate response, i.e., their inducibility was higher than that of strain WH but considerably lower than that of the strain III parents. It is difficult to interpret these results at present, however, work in progress on the backcross and F_2 generations using these two strains and the F_1 hybrids between strain III and other noninducible strains of rabbits will hopefully help clarify the situation as to the role of genetic factors determining drug-metabolizing enzymes.

Kouri *et al.* (41) have recently found a direct correlation between susceptibility to 3-MCA-induced subcutaneous tumors and the hepatic AHH inducibility. In crosses of strains in which AHH inducibility segregated as a single autosomal dominant gene, mice carrying the *Ah*^{b} (or inducible) allele were significantly more susceptible to MCA tumorigenesis than were their noninducible littermates (42). It would be interesting to know if such a relationship between enzyme activity and susceptibility to carcinogenesis is seen in other species. We are continuing parallel biological studies on different rabbit strains in attempts to correlate enzyme induction with sensitivity to polycyclic aromatic hydrocarbon carcinogenesis.

Summary. We determined both basal and induced AHH activity in livers of six partially inbred strains of rabbits. Strain III rabbits had the highest enzyme activity upon induction by 3-MCA, i.e., four to five times that in strain WH (noninducible), which has the lowest enzyme activity. AHH induction was also "low" in strains X, OS, ACEP, and AC. F_1 hybrids between strains III and WH revealed a differential response to the induction of liver AHH activity by MCA: the levels of induced hydroxylase activity were consistently higher in (III × WH)F_1 rabbits than in the reciprocal (WH × III)F_1 hybrids. All possible crosses between these two "extreme" strains are now being analyzed to estimate the number of genes involved in their response difference to MCA.

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