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Association of Fertility, Fitness and Longevity with the Murine Ah Locus Among (C57BL/6N) (C3H/HeN) Recombinant Inbred Lines

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ABSTRACT

The *Ah* locus encodes a cytosolic receptor which controls the induction of enzymes that metabolize drugs, chemical carcinogens, and other environmental pollutants. B6NXC3N recombinant inbred lines have been developed from the progenitors C57BL/6N and C3H/HeN inbred mouse strains. *Ah* phenotyping at each generation has resulted in the establishment of some lines containing high levels of the high-affinity *Ah* receptor; other lines, very low levels. A genetic model involving two unlinked loci is offered to explain the distribution of *Ah* receptor levels among (C57BL/6N) (C3H/HeN)F_2 individuals.

Between generations 7 and 13, individual females and males from the B6NXC3N recombinant inbred lines were crossed with DBA/2N males and females. Presence of high levels of the high-affinity *Ah* receptor in both female and male B6NXC3N mice was found to be associated with greater fertility, fitness, and longer life span. The data suggest that these parameters are correlated with the *Ah* locus or a closely segregating gene.

INTRODUCTION

The *Ah* locus codes for a cytosolic protein receptor that binds avidly to relatively planar foreign chemicals such as 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[2] (reviewed in Eisen et al., 1983). Many of these ligands are naturally occurring combustion products which pollute our environment. If a normal physiologic ligand exists for the *Ah* receptor, it has not yet been discovered.

These foreign chemicals produce a wide range of biologic effects that appear to require, and be controlled by, the *Ah* receptor: the induction of drug-metabolizing enzymes such as aryl hydrocarbon hydroxylase, immunosuppression, and tumor promotion (Eisen et al., 1983). While performing experiments concerned with polycyclic hydrocarbon-induced carcinogenesis among recombinant inbred mouse lines (Nebert, 1980), we found interesting data with regard to a relationship between the *Ah* locus and fertility, general fitness, and longevity. These findings are summarized in this report.

The *Ah* locus has been characterized principally in the mouse. Two allelic forms of the *Ah* receptor have been identified among inbred mouse strains (Eisen et al., 1983). The C57BL/6N (B6; Ah^b/Ah^b) has a form with high affinity for inducers such as 3-methylcholanthrene and TCDD; the DBA/2N (D2; Ah^d/Ah^d) has a form with significantly lower affinity for these inducers. As a consequence, the D2 mouse is considerably less sensitive to many of the effects of these compounds. The B6D2F_1 heterozygote (Ah^b/Ah^d) exhibits additive inheritance of *Ah* receptor levels. Although the C3H/HeJ (C3) inbred mouse possesses a high-affinity form of the receptor, the receptor concentrations are considerably lower than those in the B6 mouse. Furthermore, in crosses between B6 and C3 mice, the receptor is not

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[2] Abbreviations used: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; [^{3}H]TCDD, [^{3}H-1,6]2,3,7,8-tetrachlorodibenzo-*p*-dioxin; B6, the inbred C57BL/6N mouse strain; D2, the inbred DBA/2N strain; C3, the inbred C3H/HeN strain; RI, recombinant inbred; cytochrome P_1-450, that form of polycyclic-hydrocarbon-induced enzyme most closely associated with induced aryl hydrocarbon hydroxylase (EC 1.14.14.1) activity.

inherited in a simple manner (Nebert et al., 1982b). We show in this report that, although B6C3F$_1$ mice have intermediate levels of receptor, B6C3F$_2$ mice demonstrate wide variation, including "D2-like" individuals[3] that appear to lack the high-affinity form of receptor. In order to investigate differences among B6, C3 and D2 inbred mice (Nebert et al., 1982b), B6NXC3N recombinant inbred RI lines from B6C3F$_2$ litters were developed (Nebert, 1980). We planned to perpetuate the "mix" of genetic elements that result in the D2-like character among B6C3F$_2$ individuals. Surprisingly, the D2-like character was found to be associated with decreased fertility. We also felt it was important to show in this report that very low levels of the high-affinity receptor occur among these D2-like individuals of the B6C3F$_2$ population.

MATERIALS AND METHODS

Chemicals

TCDD was a generous gift from Dow Chemical Co. (Midland, MI); [^{3}H]TCDD (52 Ci/mmol) was custom-synthesized by KOR Isotopes (Cambridge, MA). Zoxazolamine (2-amino-5-chlorobenzoxazole) was kindly donated from McNeil Labs., Inc. (Fort Washington, PA). β-Naphthoflavone (5,6-benzoflavone) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Zoxazolamine Paralysis Time

Ah phenotyping was performed by established procedures (Robinson and Nebert, 1974). The zoxazolamine paralysis test is normally performed between 4 and 6 weeks of age. β-Naphthoflavone (200 mg kg^{-1}) in corn oil (25 ml kg^{-1}) was given intraperitoneally about 40 h before zoxazolamine (250 mg kg^{-1}) in corn oil (50 ml kg^{-1}) was administered intraperitoneally. Within 3 min the mice were paralyzed; paralysis time was recorded as that period of time until the animal, placed on its back, was able to return repeatedly to its feet. This time ranged from several minutes to almost 2 h.

β-Naphthoflavone-induced cytochrome P$_1$-450 metabolizes zoxazolamine, thereby decreasing the intensity and duration of action of the paralytic effect (Robinson and Nebert, 1974). Mice having the high-affinity receptor thus are paralyzed for only several minutes; mice having the poor-affinity receptor are paralyzed for more than 1 h. For inducing P$_1$-450, we prefer β-naphthoflavone to 3-methylcholanthrene because of less gonadal toxicity and therefore better breeding (Mattison and Thorgeirsson, 1978). Thus, this test (Robinson and Nebert, 1974) has provided over the past decade a convenient, noninvasive means of *Ah* phenotyping.

Ah Receptor Assay by Sucrose Density Gradient Analysis

All procedures were similar to those described (Okey et al., 1979; Bigelow and Nebert, 1982). HEDG buffer is composed of 25 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid), 1.5 mM ethylenediamine-tetraacetic acid, 1 mM dithiothreitol, and 10% glycerol (v/v), pH 7.6. Dextran-charcoal solutions contained 5 mg of charcoal of 0.5 mg of dextran per ml of HEDG buffer. Liver cytosol (105,000 × *g* × 1 h supernatant) from individual mice was isolated. One ml of cytosol was incubated with 1 nM [^{3}H-1,6]2,3,7,8-tetrachlorodibenzo-*p*-dioxin ([^{3}H]TCDD) in the absence or presence of 100 nM nonlabeled TCDD for 1 h at 4°C. Dextran-coated charcoal was used to remove unbound [^{3}H]TCDD, following which centrifugation in a vertical rotor (Tsui and Okey, 1981) was performed at 235,000 × *g* for 2 h at 2°C in a linear 5%–20% sucrose density gradient. Twenty-five fractions from the gradient were each counted by scintillation spectrometry. Radioactivity in those three to six fractions exhibiting high-affinity and saturability properties was used to quantitate the receptor in femtomoles per mg of cytosolic protein. Each receptor determination performed is the average of duplicate centrifuge tubes, and never varied by more than 10%.

Animals

B6NXC3N RI lines were begun in this laboratory in 1974 (Nebert, 1980). From C57BL/6N and C3H/HeN inbred mice as the progenitors, F$_1$ hybrids were produced and allowed to breed. Strict brother-sister matings of B6C3F$_2$ mice constituted an RI line. Twelve such RI lines were developed (Nebert, 1980): seven having negligible hepatic P$_1$-450 inducible by 3-methylcholanthrene and therefore presumably the poor-affinity *Ah* receptor; five having hepatic P$_1$-450 highly inducible by 3-methylcholanthrene and therefore presumably the high-affinity receptor.

It should be emphasized that these 12 RI lines were not selected randomly (Nebert, 1980). From 609 B6C3F$_2$ mice obtained during the first 6 months of 1975 and phenotyped by the zoxazolamine paralysis test, 47 were considered D2-like and have been the basis for the seven RI lines having presumably the poor-affinity receptor. This frequency (47 of 609) is not statistically different from a ratio of 1 in 16. From about 200 B6C3F$_2$ individuals, the remaining five lines have been selected for the shortest zoxazolamine paralysis times and therefore are considered B6-like. At each generation between 1975 and 1982, the

[3] "D2-like" individuals are defined as mice having *Ah* receptor properties (as determined by our present-day methods) not experimentally different from those of D2 mice: *1)* negligible amounts (<1.5 fmol/mg cytosolic protein) of cytosolic *Ah* receptor measured by the sucrose density gradient assay (Okey et al., 1979); *2)* hepatic intranuclear inducer-receptor complex detectable in vivo following [^{3}H]TCDD injection intraperitoneally 4 to 24 h earlier (Tukey et al., 1982); and *3)* maximally inducible aryl hydrocarbon hydroxylase activity not significantly different from that in B6 by TCDD at doses sufficient to overcome the *Ah* receptor defect in D2-like mice (Poland et al., 1974).

zoxazolamine paralysis time was used for selecting every individual mouse for breeding in the ensuing generation. Although the genetic expression of P_1-450 inducibility appears to be more complicated than that determined by two alleles at a single locus (Robinson et al., 1974; Nebert, 1980), we have attempted for more than 20 generations to select for seven RI lines, and five RI lines, having increasingly longer and shorter, respectively, zoxazolamine paralysis times. Another measure of this complicatedness is the fact that it continues to be necessary to *Ah* phenotype each generation by the zoxazolamine paralysis test. A single-gene difference would have become fixed in these RI lines after several generations, and the zoxazolamine paralysis would no longer be required. The continuing emergence—even after 20 generations—of an occasional highly responsive individual in a nonresponsive line, and vice versa, therefore suggests a pattern of inheritance involving two or more genes.

In early 1977, at generations 7 to 9, B6NXC3N offspring at 6 weeks of age were set up in breeding cages with D2 mice; five females were placed with two males (Fig. 1). Ten cages of poor-affinity receptor-containing B6NXC3N mice were set up: five with B6NXC3N females and D2 males; five with D2 females and B6NXC3N males. Eleven cages of high-affinity receptor-containing B6NXC3N mice were established: five with B6NXC3N females and D2 males; six with D2 females and B6NXC3N males. Litter size (number of live births), life span, and general healthiness[4] of the B6NXC3N mice were recorded. The B6NXC3N mice were selected from all 12 RI lines.

In 1978, at generations 11 to 13, a repeat experiment was begun with ten cages each of poor-affinity and high-affinity receptor-containing B6NXC3N mice: five of each had B6NXC3N females and D2 males; five of each had D2 females and B6NXC3N males. Again litter size (number of live births), life span, and general vigor of the B6NXC3N mice were recorded, and the B6NXC3N mice were chosen from all 12 RI lines.

RESULTS

Zoxazolamine Paralysis Test and Aryl Hydrocarbon Hydroxylase Inducibility Both Correlate with Ah Receptor Concentrations

Robinson and Nebert (1974) demonstrated a strong association between a short zoxazolamine paralysis time and high aryl hydrocarbon hydroxylase inducibility by 3-methylcholanthrene. Poland and co-workers (1976) and Okey and co-workers (1979) showed an absolute correlation between aryl hydrocarbon hydroxylase inducibility by various aromatic compounds and the avidity with which these compounds bind to the *Ah* receptor. It is therefore presumed that the zoxazolamine paralysis test, aryl hydrocarbon hydroxylase (P_1-450) inducibility, and the quantity of high-affinity *Ah* receptor are all associated with one another.

Ah Receptor Concentrations Among B6C3F$_2$ Mice

Ah receptor levels were therefore quantitated among the B6C3F$_2$ population. Livers from five B6, five C3, or five B6C3F$_1$ mice were combined and the cytosolic receptor was found to be 31.2, 6.2, and 18.5 fmol/mg protein, respectively. These data clearly illustrate additive inheritance of the hepatic *Ah* receptor levels in the B6C3F$_1$.

The results among B6C3F$_2$ individuals, however, were more complex. The individuals shown in Fig. 2, *top* and *bottom*, represent the highest and lowest, respectively, among 35 B6C3F$_2$ examined. From the standpoint of this receptor assay, these two individuals appear "B6-like" and "D2-like," respectively. From various biological responses, such as hepatic aryl hydrocarbon hydroxylase inducibility as a function of increasing doses of intraperitoneal TCDD (Nebert, 1980), we believe that the D2-like B6C3F$_2$ has predominantly the poor-affinity *Ah* receptor, similar to that seen in D2 mice. Further physicochemical studies, when the *Ah* receptor is eventually purified, will be required to prove this hypothesis. The poor-affinity receptor in Ah^d/Ah^d mice is barely detected in the cytosol (Okey et al., 1979) but can be quantitated in nuclear extracts following in vivo treatment of [^{3}H]TCDD (Mason and Okey, 1982; Tukey et al., 1982).

With regard to levels of the cytosolic *Ah* receptor among individual B6C3F$_2$ mice (Fig. 3), at least three populations emerge: mice having large (30–38 fmol/mg protein), intermediate (8–24 fmol/mg protein) and small (<6 fmol/mg protein) amounts of the high-affinity moiety. These three distinct populations can be visualized clearly on normal probability paper (Fig. 4). If the two D2-like mice (having <1.5 fmol/mg protein) represent a fourth population, it is not evident in Figs. 3 and 4. A fourth population might become evident if 100 or 200 individual mice were evaluated, but the cost

[4] Each month throughout their lives, the mice were scored as "3+" (normal healthy appearance), "2+" (less vigorous than normal), and "1+" (obviously weakened and sickly). Susceptibility to infections must be included as a factor in generalized vigor. Unhealthy mice, for example, are more prone to die at a younger age from Sendai virus injection; Sendai virus has always been endemic in our mouse colony. No other virus, bacteria, mites, or parasites have been detected in our colony, however.

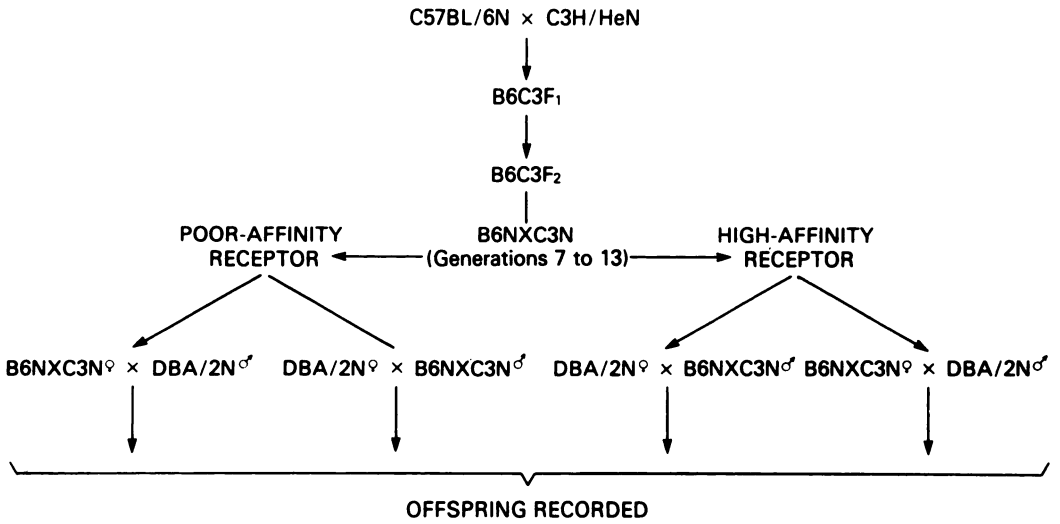
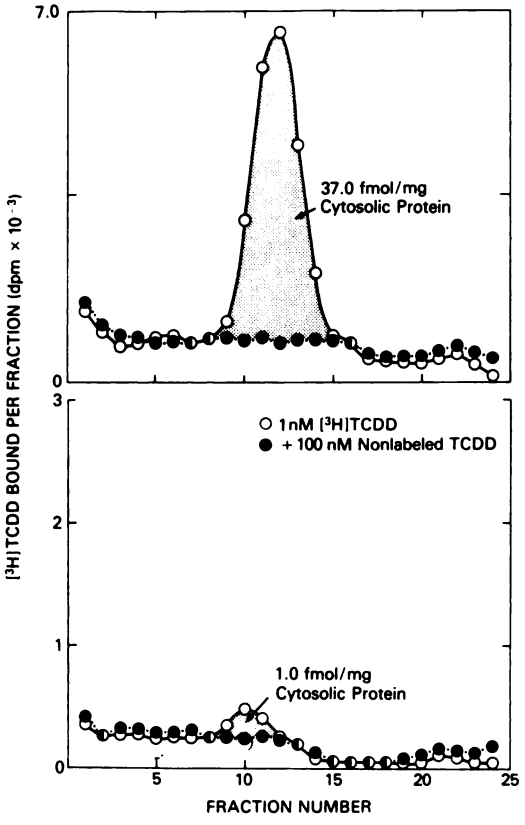


FIG. 1. Scheme by which B6NXC3N RI lines were generated from individual B6C3F$_2$ litters (followed by strict brother-sister mating). B6NXC3N were then crossed with D2 mice, and the size and number of litters were recorded.



and time involved for this number of *Ah* receptor assays would be prohibitive. It remains to be understood, therefore, why the receptor levels of the two D2-like mice are so similar to those of the other seven mice in the ≤6 fmol/mg protein group, yet the zoxazolamine paralysis time or aryl hydrocarbon hydroxylase inducibility by β-naphthoflavone (Nebert, 1980) clearly distinguishes these occasional D2-like mice (about 1 in 16) from the remaining B6C3F$_2$ individuals (Nebert et al., 1982b).

A genetic model consisting of two nonlinked loci (Fig. 5) is offered to explain the 1 in 16 incidence of D2-like mice among the B6C3F$_2$ population. Figure 5 depicts B6-like, C3-like, B6C3F$_1$, and D2-like mice, plus 9 of 16 B6C3F$_2$

FIG. 2. Velocity sedimentation analysis of the cytosolic *Ah* receptor in the liver of individual B6C3F$_2$ mice. Two extreme examples are shown. Cytosol (1 mg of protein per ml) from 5-week-old mice was incubated with 1 nM [^{3}H]TCDD in the absence (○—○) or presence (●—●) of 100 nM nonlabeled TCDD. Following dextran-charcoal treatment, gradients were centrifuged and fractionated as described in *Materials and Methods*. The amount of saturable receptor, i.e., that in which the radiolabel can be competed by a 100-fold excess of nonlabeled TCDD, is illustrated as a *stippled area* and can be converted to fmol of receptor per mg of cytosolic protein. Note differences in the values of the ordinates.

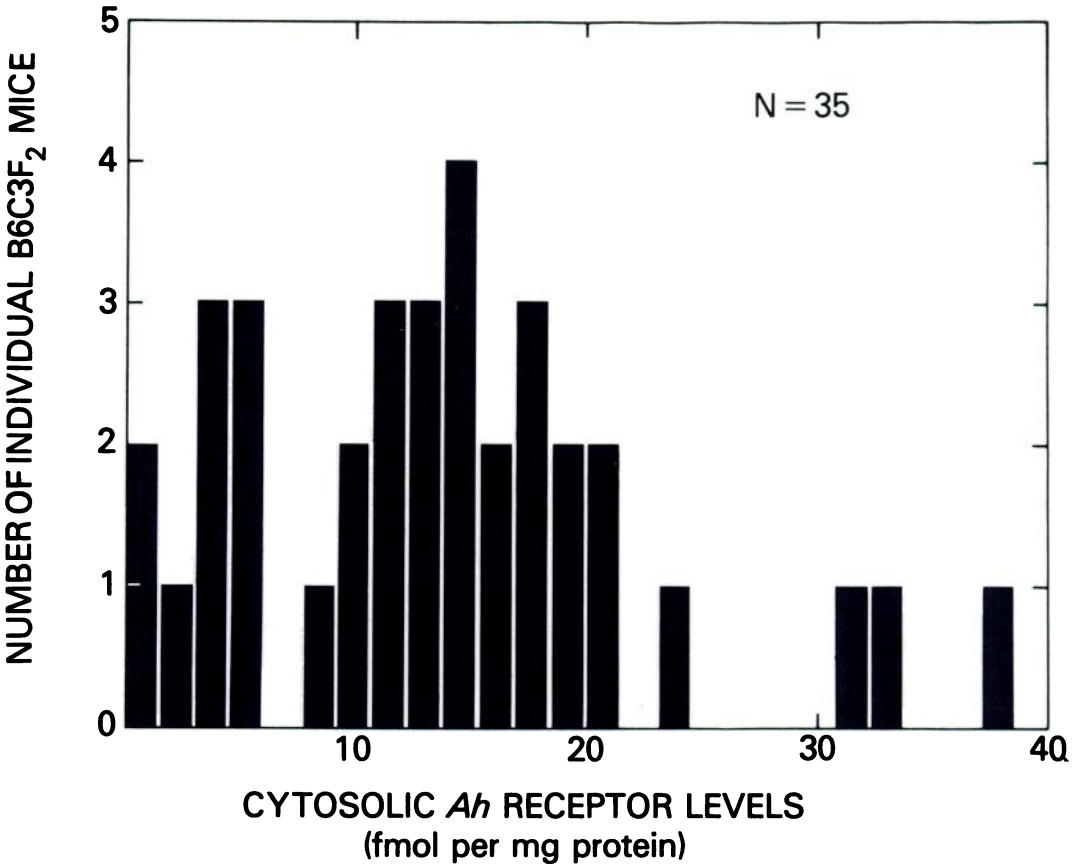


FIG. 3. Distribution of high-affinity cytosolic *Ah* receptor levels among the livers of 35 individual $B6C3F_2$ mice. The two individuals having ≤1.5 fmol/mg of cytosolic protein, shown at far *left*, are considered D2-like.

described only as "intermediate." The $\frac{h^-/h^-}{b^+/b^-}$ individual (incidence of 2 in 16), for example, could represent the seven mice in the low receptor group (≤6 fmol/mg protein) other than the two D2-like mice. This type of genetic model will be difficult to prove or rule out with the present-day *Ah* receptor assay, which is expensive, time-consuming, and requires killing each mouse. The genetic expression might be more complicated than that suggested in Fig. 5. For example, B6 and C3 may have the same two alleles at the *Ah* locus, encoding the receptor protein, but different alleles at a (non-linked) locus regulating the level of receptor expression.

The receptor data in Fig. 3 are consistent with the previously described zoxazolamine paralysis times and aryl hydrocarbon hydroxylase induction in β-naphthoflavone-treated $B6C3F_2$ mice (Robinson et al., 1974; Nebert et al., 1982b). A small group (in this study, 2 out of 35) of individual $B6C3F_2$ are D2-like, with low levels (≤1.5 fmol) of the high-affinity receptor and exhibit a negligible P_1-450 induction response to β-naphthoflavone. It was on this basis of *Ah* phenotyping by the zoxazolamine paralysis test (Robinson and Nebert, 1974) at each generation that the high- and low-inducible P_1-450 B6NXC3N RI lines were generated (Nebert, 1980).

Fertility

As described in *Materials and Methods* and illustrated in the Fig. 1 scheme, 21 breeding cages were set up in early 1977 for other experiments. B6NXC3N females and males were crossed with D2 males and females, respectively. Due to unexpectedly low birth rates in certain cages, a second experiment of 20 breeding cages was begun in August, 1978, 18 months after the first. Because we have

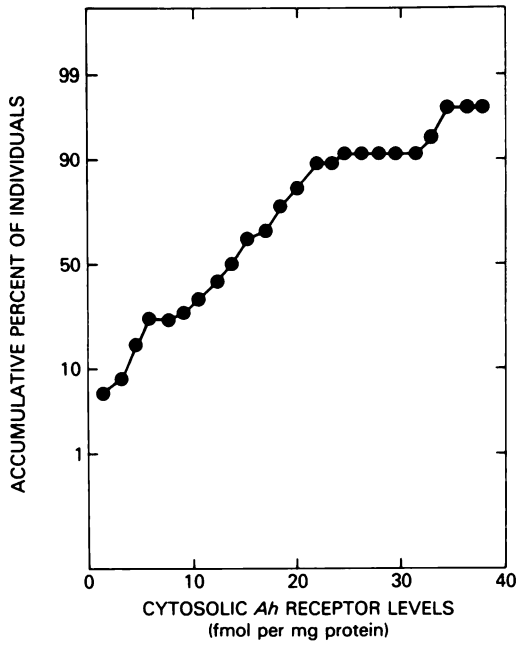


FIG. 4. Normal-probability graph showing the accumulated percent of the 35 B6C3F_2 individuals (shown in Fig. 3) as a function of hepatic receptor content. Any cumulative-normal-distribution curve would be represented in this plot as a straight line.

observed seasonal variations in breeding and litter size, setting up the second experiment 6 months out of phase with the first experiment was designed to cancel any possible circannual effect.

The numbers and sizes of litters are recorded in Table 1. In the first experiment, B6NXC3N mice having the poor-affinity receptor gave birth to a total of 45 pups while the B6NXC3N mice having the high-affinity receptor gave birth to a total of 209. Most of the breeding occurred between 2 and 7 months of age, with occasional litters still appearing between 7 and 13 months of age. This more than 4-fold discrepancy between low- and high-receptor B6NXC3N was present when either females or males were crossed with D2 mice. The results were similar when these breeding experiments were repeated 18 months later (Table 1).

Each inbred strain has characteristic levels of reproductive success (Green, 1966), and F_1 hybrids or any other mice derived from two or more inbred strains often exhibit enhanced breeding efficiency due to heterosis or hybrid vigor (Clarke and Maynard-Smith, 1955; Manwell and Baker, 1970). As a comparison in our mouse colony, the high breeding rates of B6D2F_1 × D2 backcrosses, set up at the same time as Experiments 1 and 2, respectively, are included in Table 1; the reproductive performance of this backcross was at least three times greater than that of crosses involving B6NXC3N and D2 mice. The breeding rate for the B6D2F_1 × D2 cross was highest between 2 and 8 months of age, with occasional litters still appearing between 8 and 18 months of age.

Longevity

The life span likewise varies among inbred mouse strains, and increased longevity generally occurs in F_1 hybrids or any other mice derived from two or more inbred strains (Green, 1966; Manwell and Baker, 1970). B6NXC3N mice, both females and males, lived significantly longer if they possessed high levels of the high-affinity *Ah* receptor (Table 2). Their general appearance, vigor, and life span were thus associated with their reproductive capability.

DISCUSSION

B6NXC3N mice having very low levels of the high-affinity *Ah* receptor display less reproductive capacity, are generally less healthy, and have a shorter life span than corresponding B6NXC3N mice having high levels of the high-affinity *Ah* receptor. This observation is the same with both females and males. Measurement of reproductive capacity is the same with both females and males. Measurement of reproductive capacity is complicated because there are large differences in litter number and size among inbred mouse strains and because there are seasonal variations. We believe we have circumvented these difficulties by crossing both female and male B6NXC3N mice into a third (neutral) background, i.e., the D2 inbred strain. Also, the second experiment performed 6 months out of phase with the first experiment, was designed to avoid circannual variations. It remains to be determined whether the same association between the *Ah* locus and fertility or longevity could be found if an F_1 heterozygote, composed of strains other than B6 and C3, were crossed with D2 or another inbred strain.

One might consider the possibility that the β-naphthoflavone and zoxazolamine treatment at 4 weeks of age, or some dietary or environmental exposure, has a permanent effect on the reproduction and health of both females and

(~6 fmol)		(~30 fmol)
C3H		B6
$\frac{h^+/h^+}{b^-/b^-}$	×	$\frac{h^-/h^-}{b^+/b^+}$
F_1	$\frac{h^+/h^-}{b^+/b^-}$	(~18 fmol)

	$h^+ b^+$	$h^+ b^-$	$h^- b^+$	$h^- b^-$
$h^+ b^+$	$\frac{h^+/h^+}{b^+/b^+}$	$\frac{h^+/h^+}{b^+/b^-}$	$\frac{h^+/h^-}{b^+/b^+}$	$\frac{h^+/h^-}{b^+/b^-}$
$h^+ b^-$	$\frac{h^+/h^+}{b^+/b^-}$	$\frac{h^+/h^+}{b^-/b^-}$	$\frac{h^+/h^-}{b^+/b^-}$	$\frac{h^+/h^-}{b^-/b^-}$
$h^- b^+$	$\frac{h^+/h^-}{b^+/b^+}$	$\frac{h^+/h^-}{b^+/b^-}$	$\frac{h^-/h^-}{b^+/b^+}$	$\frac{h^-/h^-}{b^+/b^-}$
$h^- b^-$	$\frac{h^+/h^-}{b^+/b^-}$	$\frac{h^+/h^-}{b^-/b^-}$	$\frac{h^-/h^-}{b^+/b^-}$	$\frac{h^-/h^-}{b^-/b^-}$

(1) $\frac{h^+/h^+}{b^+/b^+}$ = Intermediate

(2) $\frac{h^+/h^+}{b^+/b^-}$ = Intermediate

(2) $\frac{h^+/h^-}{b^+/b^+}$ = Intermediate

(1) $\frac{h^+/h^+}{b^-/b^-}$ = C3H-like (~6 fmol)

(4) $\frac{h^+/h^-}{b^+/b^-}$ = B6C3F$_1$ (~18 fmol)

(2) $\frac{h^+/h^-}{b^-/b^-}$ = Intermediate

(1) $\frac{h^-/h^-}{b^+/b^+}$ = B6-like (~30 fmol)

(2) $\frac{h^-/h^-}{b^+/b^-}$ = Intermediate

(1) $\frac{h^-/h^-}{b^-/b^-}$ = D2-like (<1 fmol)

FIG. 5. Genetic scheme that might explain the observed data of Figs. 3 and 4. The 16 possible combinations of four alleles within the F_2 generation are shown in the boxes. Their frequency of occurrence and postulated *Ab* receptor levels are shown below and discussed in the text.

TABLE 1. Reproductive capacity of B6NXC3N RI lines to breed with D2 mice.

Experiment	*Ab* phenotype[a] of B6NXC3N	Cross ♀ (N)	Cross ♂ (N)	Total number of pups	Number of pups per mother	Number of litters	Number of pups per litter	Range of number of pups per litter
1	d	B6NXC3N (25)	D2 (10)	28	1.1	9	3.1	2– 6
	d	D2 (25)	B6NXC3N (10)	17	0.7	6	2.8	1– 6
	b	B6NXC3N (25)	D2 (10)	108	4.3	20	5.4	3– 9
	b	D2 (30)	B6NXC3N (12)	101	3.7	21	4.8	3– 8
		B6D2F$_1$ (25)	D2 (10)	384	15.4	48	8.0	5–14
2	d	B6NXC3N (25)	D2 (10)	33	1.3	10	3.3	2– 6
	d	D2 (25)	B6NXC3N (10)	29	1.2	9	3.2	2– 6
	b	B6NXC3N (25)	D2 (10)	114	4.6	21	5.4	3– 9
	b	D2 (25)	B6NXC3N (10)	122	4.9	22	5.5	3–10
		B6D2F$_1$ (25)	D2 (10)	351	14.0	47	7.5	4–14

[a]The "d" denotes poor-affinity, the "b" high-affinity, receptor, as determined by the zoxazolamine paralysis time *Ab*-phenotyping (Robinson and Nebert, 1974). In terms of cytochrome P_1-450 inducibility controlled by the *Ab* receptor, the former are D2-like and the latter are B6-like or C3-like.

males. The P_1-450 induction process is known to exist among inbred strains in both the ovary (Mattison and Thorgeirsson, 1978, 1979) and testis (Mattison and Thorgeirsson, 1978); however, polycyclic hydrocarbon-induced ovarian toxicity occurs to a greater degree in high-affinity receptor mice than in poor-affinity receptor mice (Mattison and Thorgeirsson, 1978, 1979). This result is just the reverse of the data in Table 1, and therefore we suspect that β-naphthoflavone and zoxazolamine treatment is not an important determinant in the reproductive failure and poorer health observed in both female and male low-receptor B6NXC3N mice.

Alternatively, it is conceivable that β-naphthoflavone or zoxazolamine in D2-like individuals, because of decreased metabolism, is more toxic and contributes to the infertility, decreased physical fitness, and shorter life span. The breeding results with other RI lines are evidence against this possibility, however. In our mouse colony, since 1973 we have developed B6NXAKN RI lines, whose progenitors are C57BL/6N and AKR/N inbred strains (Nebert, 1980). These lines have also been selected at each generation with β-naphthoflavone treatment followed by the zoxazolamine paralysis test. Among these lines we have never seen breeding or health differences between lines having high levels, and lines having low levels, of the high-affinity receptor. During the past 8 years of development of the B6NXC3N RI lines, however, we have noticed the tendency for greater reproductive capacity and vigor among these lines having short zoxazolamine paralysis times, compared with lines having prolonged paralysis times. It therefore appears that—somewhere in these B6NXC3N RI lines—there exists one or more genes which contribute to hybrid vigor. This interesting trait in the B6NXC3N lines does not seem to be expressed in the B6NXAKN lines. All of these RI lines in this laboratory are available to anyone interested.

The reasons for an association between the *Ab* locus and fertility, general fitness, or longevity are presently not known. It is interesting to note, however, that, among the 24 low-receptor and 44 high-receptor inbred mouse strains that have been phenotyped to date (Nebert et al., 1982a), many of the low receptor-containing strains are among those exhibiting decreased size and number of litters, greater incidences of viral expression and

TABLE 2. Longevity and general vigor of B6NXC3N RI mice.

Experiment	*Ah* phenotype[a] of B6NXC3N	Sex	Number	Life span[b] (days)	P values	General vigor[d]
1	d	♀	25	218 ± 16[c]	<0.001	1.5
	d	♂	10	349 ± 40	<0.1	1.7
	b	♀	25	338 ± 22[c]	<0.001	2.3
	b	♂	12	450 ± 53	<0.1	2.7
2	d	♀	25	235 ± 19[c]	<0.001	1.4
	d	♂	10	341 ± 42	<0.05	1.9
	b	♀	25	354 ± 26[c]	<0.001	2.6
	b	♂	10	464 ± 52	<0.05	2.6

[a]The "d" denotes poor-affinity, the "b" high-affinity, receptor, as determined by the zoxazolamine paralysis time *Ah* phenotyping (Robinson and Nebert, 1974). In terms of cytochrome P_1-450 inducibility controlled by the *Ah* receptor, the former are D2-like and the latter are B6-like or C3-like.

[b]Mean ± SEM. The *p* values were determined by the two-tailed Student's *t* test.

[c]The time of appearance of spontaneous mammary tumors was an important determinant in making the female life span shorter than the male life span. The earliest appearance of mammary tumors was during the eighth month and had no significant effect on fertility, nor was there any correlation with the *Ah* phenotype. Incidence of tumors at time of death was 42%.

[d]For each individual mouse, the consensus of "general vigor" was determined at least once a month by at least one animal caretaker, one chemist, and one senior investigator, "3+" being healthiest, and "1+" being least healthy (*cf.* footnote 3). The score for each month during a lifetime was divided by the number of months lived. Twenty-five B6NXC3N females, all receiving "3+" for 3 months, thus would receive maximal score of "3.0," all "2+," a score of "2.0," all "1+," a score of "1.0," etc. Whereas no statistics are attempted for these subjective evaluations, the trend seems clear: those with the B6-like *Ah* phenotype appear less sickly.

autoimmune diseases, and shorter life spans (Green, 1966).

When specific foreign chemicals bind avidly to the *Ah* receptor, a multitude of biochemical responses result. This pleiotypic response includes the induction of several forms of the drug-metabolizing enzyme cytochrome P-450, UDP glucuronosyltransferase activity, a cytosolic DT diaphorase, and ornithine decarboxylase activity. The gene for one of these forms of P-450, called P_1-450, has been cloned and characterized (Nakamura et al., 1983). The above-mentioned responses are known to be strictly correlated with the *Ah* receptor by means of studies involving Ah^b/Ah^d and Ah^d/Ah^d progeny from the B6D2F_1 × D2 backcross (Eisen et al., 1983). Other enzyme activities or proteins that are induced by polycyclic aromatic compounds that bind with high affinity to the *Ah* receptor include: cytosolic aldehyde dehydrogenase (Deitrich et al., 1977), mitochondrial δ-aminolevulinic acid synthetase (Poland and Glover, 1973), prostaglandin biosynthesis and cellular lipid deacylation (Levine and Ohuchi, 1978), α-fetoprotein (Becker and Sell, 1979), γ-glutamyltranspeptidase (Gupta et al., 1981), choline kinase and ethanolamine kinase (Ishidate et al., 1980), phospholipase A_2 (Bresnick et al., 1981), nucleolar and nucleoplasmic protein kinase (Kleeberg et al., 1982), and RNA polymerase A and B (Kleeberg et al., 1982). A strict relationship between the murine *Ah* receptor and these latter dozen inducible activities, however, has not yet been proven with the use of progeny from the B6D2F_1 × D2 backcross.

The potent eicosanoids (prostaglandins, thromboxanes, prostacyclin and leukotrienes) are known to play important roles in fertility, smooth muscle contractility, blood platelet aggregation, leukocyte migration, and gastric secretions. Inhibitors of prostaglandin or leukotriene biosynthesis have been recently shown to block the dietary fat enhancement of 7,12-dimethylbenzo[a]anthracene-induced mammary tumorigenesis in the rat (Carter et al., 1983) and polychlorinated biphenyl-induced toxicity in the fetal chick (Rifkind and Muschick, 1983). The *Ah* receptor is required for the manifestation of both the tumorigenesis

and the toxicity. It is thus conceivable that the *Ah* locus, under certain conditions in the mouse, could play a role in fertility, fitness and life span. Perhaps some endogenous eicosanoid is the normal-body ligand for the *Ah* receptor.

Other complex responses are associated with the *Ah* locus. These polycyclic aromatic inducers cause epidermal keratinization (Knutson and Poland, 1980) and are potent immunosuppressors (Blumer et al., 1982; reviewed in Nebert, 1979), teratogens (Shum et al., 1979; Poland and Glover, 1980), and tumor promoters (Pitot et al., 1980; Poland et al., 1982). The *Ah* induction response is demonstrable even in the preimplantation embryo (Galloway et al., 1980; Filler and Lew, 1981). These data thus suggest that the *Ah* receptor plays a vital role in certain growth processes such as differentiation and tumor progression. It is tempting to speculate that somewhere herein lies the link between the *Ah* receptor and fertility or longevity.

Even in the absence of polycyclic aromatic hydrocarbon pretreatment, mice having the high-affinity form of receptor have been shown to differ from mice having the poor-affinity form. Hence, mice that are genetically identical except for one allele at the *Ah* locus, or perhaps at closely linked loci, have differences in: microsomal P-450 reductase kinetics (Blumer and Mieyal, 1978), inhibition of the spread of epileptic audiogenic seizures of the juvenile type (Seyfried and Glaser, 1981), NADPH-regenerating capacity (Conway et al., 1983), and response to acute intraperitoneal doses of ethanol (Sanford W. Bigelow, Allan C. Collins and Daniel W. Nebert, manuscript in preparation).

Among individual offspring from the B6D2F_1 x D2 backcross, strict correlations have been shown between allelic differences at the *Ah* locus and enhanced risk of chemically induced tumors, mutagenesis, immunosuppression, ovarian toxicity, and several types of drug toxicities (reviewed in Nebert, 1981). Genetic differences in certain types of malignancy and drug toxicity within the human population also may be associated with the *Ah* locus (reviewed in Nebert, 1981). Further studies are needed to determine if reproductive capacity, general fitness, and longevity in the mouse are related somehow to the *Ah* locus or to closely segregating gene(s).

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