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Strain C3H-A^{vy}fB Mice: Ninety Percent Incidence of Mammary Tumors Transmitted by Either Parent

Abstract. *Mammary tumorigenesis in C3H-A^{vy}fB female mice is not due to milk-borne mammary tumor virus but to factors transmitted at conception. Prominent among these is the* A^{vy} *gene which may increase the virulence or transmissibility of a variant of mammary tumor virus vertically transmitted by either parent, or may increase the tumorigenic response of the mammary tissue. These factors together with the influence of hormones of pregnancy resulted in the high incidence observed.*

In 1968 we (*1*) reported that mammary gland tumors occur at high incidence in our strain C3H-A^{vy}fB female mice in the absence of the mammary tumor virus (MTV) that is normally transmitted by the milk. Strain C3H-A^{vy}fB originated in 1964 from a C3H-A^{vy} litter delivered by cesarean section and nursed upon a strain C57BL female to prevent infection with MTV. Now, after 20 generations of inbreeding, mammary tumors continue to occur in approximately 90 percent of breeding females, appearing when the mice average 15 months of age. Tumors appear in 20 percent of the virgin females at an average age of 15 months.

The viable yellow mutation (*A^{vy}*), which characterizes strain C3H-A^{vy}fB, was discovered by Dickie (*2*) in a litter of mice born to C3H/HeJ parents. From a litter with the viable yellow mutation, sent to us by Dickie in 1961, we developed strain C3H-A^{vy} which is highly susceptible to mammary tumors and in which MTV is transmitted to the offspring by nursing mothers. The strain was introduced into our colony by a C3H-A^{vy} litter born by cesarean section and nursed upon a C3H/He female, which introduced MTV from our colony of C3H. Mammary tumors occur in 100 percent of breeding and virgin females of strain C3H-A^{vy} at approximately 6.5 months of age. In our strain C3H mice mammary tumors occur in 100 percent of breeding and virgin females, but at a later age (approximately 8 and 10 months, respectively). Strain C3HfB (*3*), derived from a litter of strain C3H born by cesarean section and nursed on strain C57BL, has an incidence of mammary tumors of 40 percent in breeding females; the tumors occur at an average age of 19 months. The incidence in virgin females is 2 percent at 22 months of age.

The higher susceptibility to mammary tumors in strain C3H-A^{vy}fB females, compared with that in C3HfB, appears to be determined by the *A^{vy}* gene, which also produces the yellow coat color and increases body weight. In addition to genetic susceptibility, the presence of type B particles [presumably nodule-inducing virus (NIV)] (*4*) and the hormonal stimulation of pregnancy represent the other factors which interact to produce the high incidence of mammary tumors in strain C3H-A^{vy}fB.

When we found that C3H-A^{vy}fB had such an unexpectedly high incidence of mammary tumors, it seemed worthwhile to examine further the transmission of this susceptibility. To determine whether it was transmitted in the milk as MTV is, through some other avenue of maternal transmission, or by the male parent as readily as by the female parent, we carried out foster nursing and hybridization experiments with strain BALB/c mice. This strain normally has a low incidence of mammary tumors, but it is genetically very susceptible to the tumorigenic influence of MTV and it has a high incidence of tumors after infection with MTV.

Reciprocal matings were made between the yellow strain C3H-A^{vy}fB (*A^{vy}A^{vy}BBCC*) and albino strain BALB/c (*AAbbcc*) to produce the F_1's that were genotypically *A^{vy}ABbCc*, and phenotypically large and yellow. Fifty-seven (C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 females and 54 (BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 females were set up as breeders, each being mated to an F_1 male of the same hybrid type. Most of these females were allowed to have only two litters, with a very few having either one or more than two

Table 1. Occurrence of mammary gland tumors, hepatomas, and cholangiomas in breeding strain C3H-A^{vy}fB females. MT, mammary tumors.

Generation	Females (No.)	Incidence of MT (%)	Average age MT appeared (mo)	Average age died without MT (mo)	With hepatomas (No.)	With cholangiomas (No.)
F_1	1	100	15		1	
F_2	1	100	14		1	
F_3	2	100	17.5		2	
F_4	6	83.3	14.8	21.0	6	
F_5	17	76.5	16.5	18.3	16	1
F_6	15	93.3	15.3	19.0	15	
F_7	20	100	15.8		19	1
F_8	21	90.5	16.2	19.5	21	2
F_9	15	81.3	13.3	12.0	14	
F_{10}	13	84.6	15.6	18.0	13	2
F_{11}	24	83.3	15.4	17.8	23	1
F_{12}	17	94.1	14.4	13.0	16	1
F_{13}	5	100	14.0		5	1
F_{14}	13	92.3	14.0	20.0	12	1
F_{15}	21	90.5	15.7	15.5	21	
F_{16}	11	100	14.1		10	
F_{17}	8	100	12.6		6	
F_{18}	14	85.7	12.5	10.5	11	
F_{19}	19	94.7	13.9	18.0	19	1
F_{20}	15	73.0	14.2	16.3	13	
F_{1-20}	258	89.5	14.8	16.7	244	11

Table 2. Classification of mammary gland tumors occurring in strain C3H-A^{vy}fB females, F_1 to F_{20}, and in reciprocal F_1 hybrid females of strains C3H-A^{vy}fB and BALB/c.

Type	C3H-A^{vy}fB (No.)	F_1 hybrids (No.)
Adenocarcinoma A	77	56
Adenocarcinoma B	220	107
Adenocarcinoma C		15
Adenoacanthoma	97	105
Carcinosarcoma	6	2
Sarcoma	1	

litters, so as to make possible comparison with breeding females of strain C3H-A^{vy}fB, in which the average number of litters per breeder is approximately two. After giving birth to two litters, the animals were retired eight to a cage (*5*), segregated according to hybrid type, and allowed to live out their normal lives. They were observed regularly for the occurrence of mammary tumors. In addition, 48 (C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 females and 57 (BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 females were set aside as virgins, and also placed eight to a cage, segregated as to hybrid type, and observed throughout their lives for mammary tumors. The strain C3H-A^{vy}fB and BALB/c mothers used in the production of the two hybrid types were also retired in the same manner and observed for the occurrence of mammary tumors.

As a test of whether strain C3H-A^{vy}fB females contained MTV or some other agent transmitted in the milk, as MTV is transmitted, 26 BALB/c females were nursed upon C3H-A^{vy}fB females, kept as virgins, and observed throughout their normal lives for the occurrence of mammary tumors.

The animals were autopsied when they had mammary tumors, or when they were in poor condition. All mammary tumors, as well as all other tumors, were fixed in Fekete's modification of Tellyesniczky's fixative (70 percent ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part), mounted in paraffin, cut, and stained with hematoxylin and eosin for microscopic examination. All the mammary tumors were classified histologically according to Dunn's classification (*6*).

Table 1 lists the incidences of mammary tumors for strain C3H-A^{vy}fB in the F_1 through the F_{20} inbred generations. The average age at which the mammary tumors appeared and the average age of mice that died without mammary tumors are also shown. In all generations the occurrence of mammary tumors was high, with a range of 73 percent in the F_{20} generation to 100 percent in seven other generations. Of 258 breeding females there were 231 with mammary tumors, an incidence of 89.5 percent; the average age at which the tumors appeared was 14.8 months. The average age of the 27 that died without mammary tumors was 16.7 months.

The mammary tumors found in strain C3H-A^{vy}fB are listed according to type in Table 2. Although many tumors were either adenocarcinoma type A or B, there was a high number of adenoacanthomas, a type often seen in strains with mammary tumors but without the MTV. The cell arrangement of some tumors is similar to that classified as typical of type Y (*7*), but the tumors are listed here as type B because they appear to be of that cell type. The fact that there were also many small mammary tumors not included in this classification and many hyperplastic alveolar nodules observed in the mammary glands further emphasizes the high susceptibility of the strain.

The results of the reciprocal crosses between strains C3H-A^{vy}fB and BALB/c (Table 3) gave no evidence of a maternally transmitted agent that could be implicated as the cause of a high incidence of mammary tumors in the C3H-A^{vy}fB strain. There was a high incidence in both reciprocal groups. In this respect strain C3H-A^{vy}fB is like strain GR described by Bentvelzen (*8*) in which transmission is also by the egg and by the male.

The incidence of tumors (96.3 percent) in the breeding (BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 females (with BALB/c mothers) was slightly higher than the incidence (82.5 percent) in the (C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 breeding females. Had there been a maternally transmitted MTV involved, the latter group would have been expected to have a much higher incidence than the former. If this slight difference is real, there are two possible explanations for it. Those with the BALB/c mothers lived a little longer than those with C3H-A^{vy}fB mothers, and although they were not weighed they could have been expected to have weighed more because BALB/c females give more milk than C3H-A^{vy}fB females do. The groups kept as virgins had approximately the same life span and approximately the same incidence of mammary tumors as the breeders; the incidences for the virgins were relatively high in the absence of the hormonal stimulation of pregnancy and lactation, but they were significantly lower than those in the breeders.

There was no evidence of an MTV transmitted to BALB/c test females by the milk of C3H-A^{vy}fB females (Table 3). The incidence of mammary tumors in the BALB/c females was low (7.7 percent), which is characteristic for strain BALB/c without MTV.

Mammary tumors from four females from each hybrid type and from both breeders and virgins were examined for virus particles. A few type B particles were observed in three adenocarcinomas of type B from reciprocal hybrid breeders, but none were found in the tumors of the virgins. These particles undoubtedly represent the NIV variant of MTV which is passed to the (C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 females by

Table 3. Occurrence of mammary gland tumors, hepatomas, and cholangiomas in breeding females of strains C3H-A^{vy}fB and BALB/c, in their reciprocal F_1 hybrid females, and in BALB/c test virgin females. MT, mammary tumors.

Females	Females (No.)	Incidence of MT (%)	Average age MT appeared (mo)	Average age died without MT (mo)	With hepatomas (No.)	With cholangiomas (No.)
C3H-A^{vy}fB (breeders)	17	88.2	13.5	13.5	10	
BALB/c (breeders)	23	8.7	20.5	19.4		
(C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 (breeders)	57	82.5	16.4	16.1	34	2
(BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 (breeders)	54	96.3	17.8	21.0	34	1
(C3H-A^{vy}fB ♀ × BALB/c ♂) F_1 (virgins)	48	60.4	18.6	19.0	45	15
(BALB/c ♀ × C3H-A^{vy}fB ♂) F_1 (virgins)	57	57.9	17.5	19.9	54	22
BALB/c nursed upon C3H-A^{vy}fB (virgins)	26	7.7	17.5	20.4		

egg-borne transmission or by intrauterine infection, but NIV probably is transmitted to the reciprocal hybrid with BALB/c mothers by C3H-A^{vy}fB sperm or seminal fluid, since BALB/c is not infected with NIV.

The occurrence of mammary tumors in the parent females used to produce the reciprocal hybrids can be seen in Table 3. The incidence of mammary tumors (82 percent) for C3H-A^{vy}fB females is typical for the strain, and the incidence (8.7 percent) for the BALB/c female parents is not significantly different from the incidence (20 percent) for strain BALB/c.

The classification of mammary tumors occurring in the reciprocal hybrids is shown in Table 2. Most of the tumors were adenocarcinoma type A or B and adenoacanthoma; this finding compares well with the results obtained for strain C3H-A^{vy}fB. However, there were 15 type C mammary tumors. Tumors of this type have been seen before in hybrids where one of the parents was of strain BALB/c and in old females of strains without the MTV.

Hepatoma, multiple in many cases, was the other principal neoplasm occurring in strain C3H-A^{vy}fB and in the reciprocal hybrid females (Tables 1 and 3). Cholangiomas (*9*), an interesting lesion, also occurred in these animals with the A^{vy} gene. Thus far cholangiomas have been found only in the livers of animals with multiple hepatomas.

The results from reciprocal hybridizations and experiments with foster nurses demonstrate that the factor responsible for the very high incidence of mammary tumors in C3H-A^{vy}fB mice is not maternally transmitted but is passed equally well by either parent. Since the hybrids were allowed to have a set number of litters or were maintained as virgins, the hormonal stimulation of the gland was reasonably equivalent in the reciprocal groups; however, the higher incidence in the breeders than in the virgins was a significant hormonal effect. Two other factors, however, must be considered. The A^{vy} gene and the NIV are transmitted equally to the F_1 offspring by the C3H-A^{vy}fB parent, and both apparently play an important role in the development of mammary tumors in these mice. It is, therefore, of interest to ascertain whether the C3H-A^{vy}fB strain harbors a variant of NIV with a high tumorigenic capacity, or whether the effect of the A^{vy} gene is to enhance the usually poor oncogenic ability of NIV and thus to promote a high incidence of mammary tumors, or whether A^{vy} acting in some other way causes the high incidence.

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Sex Ratio of Newborns: Preponderance of Males in Toxemia of Pregnancy

Abstract. *The ratio of males to females in 1061 babies born to mothers with toxemia of pregnancy is 1.24. The ratio increases as the severity of the disease increases, being 1.71 in cases in which the urinary output of protein is equal to or greater than 3 grams per 24 hours. Histoincompatibility of the fetus and mother, including incompatibility due to an antigen (or antigens) dependent on the Y chromosome, is suggested to function in the pathogenesis of pregnancy toxemia.*

Incompatibility of fetus and mother as a cause of toxemia of pregnancy was first suggested by Dienst in 1905 (*1*). His findings, based on ABO groups in the mother and fetus, were supported by some others (*2*). Later studies, however, suggest that the distributions of different combinations of ABO and Rh groups in the fetus and mother are similar in toxemic and normal pregnancies (*3*). Nevertheless, the possible role of other antigens, at that time unknown, was considered by some geneticists as early as 1946 (*4*). We now report that the ratio of males to females in babies born to toxemic mothers is significantly increased; we suggest that this indicates the importance of feto-maternal histoincompatibility in the pathogenesis of toxemia of pregnancy.

Table 1. Ratio of males to females in 1061 newborn babies (including 18 sets of twins and one set of triplets) of mothers with toxemia of pregnancy and of 8257 control babies. The controls were healthy babies born to healthy mothers during the same period and in the same hospital as babies of toxemic mothers. The difference between the sex ratio of babies born to toxemic mothers and that of controls is significant ($\chi^2 = 7.97$; $P < .01$).

Group	Newborns (No.)		Ratio of males to females
	Males	Females	
Toxemia	588	473	1.24
Control	4196	4061	1.03

Data were collected from records taken in the Department of Obstetrics and Gynecology, University of Turku, from 1961 to 1969. All babies, both premature and full-term, born to toxemic patients, including those with the mild preeclamptic form, were considered. The criteria of Dieckmann (*5*) were used to establish the diagnosis of toxemia.

Mothers with toxemia of pregnancy have boys significantly more often than

Table 2. Ratio of males to females in 1061 babies born to mothers with toxemia of pregnancy. The degree of the maternal disease was determined from the urinary protein output (UPO) or the diastolic blood pressure (DBP).

Sex	Newborns (No.) to mothers with toxemias of					
	UPO (g/24 hour)			DBP (mm-Hg)		
	< 0.1	0.1–2.9	$\geqq 3.0$	< 90	90–109	$\geqq 110$
Males	332	203	53	41	277	273
Females	296	146	31	39	242	192
Ratio of males to females	1.12	1.39	1.71	1.05	1.14	1.42