Table 1. Female behavior of male rats castrated (1A), castrated and given estradiol dipropionate (2A), given estradiol diproprionate (3A), or not treated (4A) when newborn. Males in groups 1A and 2A were castrated 16 to 32 hours after birth.

Group	N	Mean latency to lordosis induced by males (hr)	Tests positive for crouching, ear-wiggling (%)	$\frac{\text{Lordoses}}{\text{Mounts}} \times 100$ (%)
1A	8	6.1*	70.8*	40.0*
2A	9	11.2	0	6.4
3A	9	9.9	0	14.0
4A	8	9.6	8.3	6.6

* Significantly different from all other groups by Mann-Whitney U-test ($p < .05$). Groups 2A, 3A, and 4A did not differ significantly from one another.

200 μg of estradiol benzoate 96 hours after birth; and group 4B, rats given 0.2 ml of peanut oil 96 hours after birth. At 180 days males in groups 3B and 4B were castrated. Starting at 200 days, three weekly tests were given. To induce estrous behavior, 10 μg of estradiol benzoate was given on each of two successive days, followed by 0.5 mg of progesterone the next day. Each subject was paired for 10 minutes with a stimulus male 3½ hours after the progesterone was injected. The frequency of mounting by a stimulus male and frequency of lordosis were recorded.

Results from experiments A and B show that the intact male rats treated with estrogen during the 1st week of life (groups 3A and 3B) did not display more female behavior than corresponding males of groups 4A and 4B (Tables 1 and 2). These findings indicate that estrogen injected into newborn intact males does not induce feminization.

On the other hand, the males in group 1A (castrated 16 to 32 hours after birth) and the males in group 1B (castrated 96 hours after birth) displayed significantly more feminine behavior than all the other groups in their respective experiments (Tables 1 and 2). Thus, it is confirmed that absence of androgen during neural differentiation induces feminization. It is evident that the injection of estrogen to the neonatally castrated males (groups 2A and 2B) not only failed to induce further feminization, but actually suppressed female behavior (Tables 1 and 2).

Table 2. Frequency of lordosis in male rats castrated (1B), castrated and given estradiol benzoate (2B), given estradiol benzoate (3B), or injected with oil (4B) when newborn. Rats in groups 1B and 2B were castrated 96 hours after birth.

Group	N	Total times mounted	$\frac{\text{Lordoses}}{\text{Mounts}} \times 100$ (%)
1B	6	174	19.5*
2B	5	145	0.0
3B	9	210	0.0
4B	8	168	6.5

* Significantly different from all other groups by the F-test ($p < .01$). Groups 2B, 3B, and 4B did not differ significantly in the number of lordoses shown.

Table 1 indicates that the males in group 1A displayed lordosis in response to mounting at a mean time of 6.1 hours after the injection of progesterone. The lordosis response was also elicited by fingering these males. The latency was 7.5 hours from the time of progesterone injection. Animals in groups 2A, 3A, and 4A never responded to fingering. Although not quantified, castrated males of group 1A displayed a deeper and more prolonged arching of the back when they responded to mounting or fingering. All other groups in experiment A exhibited weaker and shorter lordoses.

Animals in group 1A were mounted twice as frequently (33.4 times per test) as the next highest group 4A (16.9 times per test). This difference was significant [$p < .002$, Mann-Whitney U-test (*6*)], and indicates that stimulus males preferred the animals in group 1A to all other animals as sex partners. These results were not duplicated in experiment B. In experiment B, there were no significant differences between groups in the number of times the animals were mounted by stimulus males (Table 2). This discrepancy probably reflects differences in testing procedures in the two experiments.

We predicted that the males in group 1A (castrated 16 to 32 hours after birth) would show more female behavior than the males in group 1B (castrated 96 hours after birth), because of the already demonstrated rapidity with which neural differentiation occurs after birth (*7*). This prediction appears confirmed (Tables 1 and 2) but the differences in testing procedures between experiments A and B should be considered.

Group 1A males showed significantly more female sexual behavior (in all measures) than animals of groups 2A, 3A, and 4A. These last three groups did not differ significantly from one another in any of the measures shown in Table 1. In experiment B, males in group 1B exhibited the lordosis response on significantly more occasions than animals of the other three groups (Table 2).

Thus, estrogen injected into newborn male rats does not induce female behavior. In fact, such treatment suppresses its display. Inasmuch as early treatment with estrogen prevents receptivity in adult female rats, these results are not surprising (*8*). We conclude that it is the absence of androgen during the period of neural differentiation rather than the presence of estrogen which induces female sexual behavior.

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5. While this experiment was in progress, H. H. Feder learned that R. E. Whalen was attempting to answer this same question. A comparison of notes revealed that although procedural details differed, results were generally confirmatory. We therefore decided to present our work jointly. Experiment A (H.H.F.) was supported by grant NIH 08634 from the USPHS. Experiment B (R.E.W.) was supported by research grant HD-00893-02 from the National Institute of Child Health and Human Development of the USPHS.
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9. Estradiol diproprionate (Ovocylin) was supplied through the courtesy of Ciba Inc. Estradiol benzoate (Progynon) was generously supplied by Schering Corp., Bloomfield, New Jersey. We thank William C. Young for his many valuable criticisms of the manuscript.

19 October 1964

Albinism and Water Escape Performance in Mice

The interpretation given by Winston and Lindzey (*1*) of the interesting behavioral differences they found between certain albino and pigmented strains of mice does not do justice to differences reported elsewhere. We have shown, for example (*2*), that at least one albino strain (CF/1) demonstrates routinely short escape times from a water maze and that its overall performance is very much like that of two of the pigmented strains used

(C57BL/6 and DBA/2). Two other albino strains (AKR and BALB/c) performed much like the third pigmented strain (C3H/He), a close relative of one of the groups used in the Winston-Lindzey report. That the strains of animals could be divided into groups was clear, but albinism did not seem to be the decisive factor.

More recently, one of us has been able to show (*3*) that these relations between the albino and the pigmented strains become considerably more complex when the dimension of age is considered. The CF/1 animals were among the quickest to escape the maze at all ages studied (21 through 189 days of age), whereas the AKR were efficient at the earliest ages but quite sluggish thereafter. The BALB/c were slow at all ages, maximally so at 63 days. Again, however, the nature of the relations with the other non-albino strains used precluded the unique contribution of coloration.

Presumably, all four albino strains are homozygous for the recessive condition of the *c* allele at the *C*-locus in linkage group 1. Although the A and BALB/c strains have a common origin, the origins of the other strains are probably distinct (*4*). Possibly the explanation for the Winston-Lindzey results lies not in the presence of the *c* allele alone but in alleles, at other gene loci in group 1, which may be carried by some but not all of the common albino strains, and which are situated close to the *C*-locus, so that the probability of cross-over phenomena is minimized. It would seem that the performance of the CF/1, albeit a randomly bred strain, poses a major hurdle for their theoretical explanation.

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Inasmuch as the CF/1 is a randombred strain, it no more poses a hurdle than do the fast water-escape performances of our own albino subjects of the outbred genotypes (A × DBA/8) × (A × DBA/8) (F_2) and A × (A × DBA/8) (backcross), shown in Table 1 of our report. Mice homozygous for albinism but heterozygous at some (other) loci may exhibit rapid swimming in water because of hybrid vigor. Our cross between the A and BALB/c strains (as well as the two crosses mentioned above) led to offspring in which the effects of albinism were "discernibly expressed even in the presence of hybrid vigor resulting from heterozygosity at other loci." The research cited by Meier and Foshee (in which the appropriate crosses were not performed) provides no findings that contradict this.

The research by Meier and Foshee (their reference *2*) appears, if anything, to support our findings. The performance of their inbred albinos resembled the performance of our inbred albinos. The performance of their random-bred albinos resembled the performance of our hybrid albinos. That the C3H/He strain performed slowly in their test situation (while our C3H/Bi performed rapidly in ours) is not surprising, because of an important difference between the two test situations. We utilized a circular water container, and the speed of escape was highly correlated with the vigor animals displayed in swimming. Meier and Foshee used a three-choice water maze, a far more complicated learning task and one in which visual cues may have played an important role. Since the C3H is a blind strain, their slow performance in the Meier-Foshee apparatus seems hardly surprising. In any case, evidence of inferior C3H performance in a multiple-unit T-maze has been reported (*1*). We specified that "These data in no way suggest that albinism is the only factor that does or could make a 'major gene' contribution to phenotypic variance in water escape behavior; indeed, any one of a number of heritable factors could make such a contribution."

Meier and Foshee suggest that the relation between genotype and behavior becomes "more complex when the dimension of age is considered." Presumably there is a large number of variables, like age, that will interact, interestingly with specific genotypic influence on behavior. Sex, rearing, housing, and previous experience are well-known examples of the kind of variable Meier and Foshee are calling attention to. In our study, we held constant as many of these variables as possible, using as subjects standard adult laboratory mice. It may be that the findings we reported should not be generalized beyond certain age periods, although the age findings Meier and Foshee mention do not demonstrate this convincingly.

The hypothesis that these findings, theirs and ours, are due to other alleles, closely linked to the *C*-locus in linkage group 1, is certainly tenable. We do not assert in our report that albino genes are themselves the determining factor. In our abstract, as well as in paragraphs 1 and 8, we explicitly state that an *association* between albinism and slow water escape is what we are demonstrating. The available data do not permit one to decide whether slow water escape is a pleiotropic effect of albino genes or is due to alleles closely linked to the *C*-locus, and it is not an easy question to answer experimentally.

In summary, our findings make it clear that homozygosity for albinism is associated with slow water escape, though they do not prove that albino genes at the *C*-locus are the specific causal agents determining this association. No assertion was ever made to the effect that phenotypically pigmented animals were guaranteed to be fast water escapers. The data of Meier and Foshee are consistent with our own. The (apparent) inconsistencies they emphasize appear, in the case of their C3H/He strain, to be due to the nature of their test apparatus, and in the case of their CF/1 strain, to be due to the fact that it is a random bred strain in which hybrid vigor may be concealing the influence of homozygosity of the *c* allele at the *C*-locus in linkage group 1. Perhaps Meier and Foshee's letter is of value in amplifying an alternative explanation of our findings, but the data they cite are of little relevance to the issue.

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