racy in this task is difficult to measure. We must presume that the requester always asked for what he wanted and was therefore 100 percent correct. The recipient of the request generally replied in kind. "Errors" on the part of the recipient most often occurred when a highly preferred food was requested. The recipient appeared either to ignore the request or to act as though he hadn't understood but would be quite willing to offer a piece of chow instead of a piece of chocolate. Accuracy ranged across sessions from 70 to 100 percent, depending upon the willingness of the animals to comply with each other's requests. In general, the lower-ranking animal, Austin, always complied with Sherman's requests. Sherman also complied with Austin's requests but needed more frequent encouragement to do so.

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11. Photographs were made of all foods and drinks used in the study. The experimenter who baited the container handed three photographs, face down, to a blind experimenter who was to accompany the chimpanzee. One photograph was of the correct food, the other two were selected randomly.
12. Supported by grants from the National Institute of Child Health and Human Development (HD-06016) and Animal Resources Branch (RR-00165) of the National Institutes of Health. Large portions of this study were filmed. We thank J. Lawson, R. K. Davenport, N. Smith, T. Maple, and R. Nadler for helpful criticism. This research is part of an effort to determine the value of the chimpanzee as an animal model for language research that cannot be conducted with the human child. A major portion of our research program entails extending advancements made with chimpanzees as subjects to language-training research with mentally retarded children at the Georgia Retardation Center in Atlanta.

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Genetic Basis of XX Male Syndrome and XX True Hermaphroditism: Evidence in the Dog

Abstract. *Serological analysis of white blood cells from the members of a family of American cocker spaniels indicates that a form of abnormal sexual development, in which individuals with a female karyotype have testes or ovotestes, is caused by anomalous transmission of male-determining H-Y genes.*

In mammals the Y chromosome is male-determining. Under the influence of a Y-situated gene or group of genes the indifferent embryonic gonad becomes a testis. The testis then secretes hormones which actively impose maleness in a system that is inherently biased toward the female condition (*1*). Yet despite the male-determining role of the mammalian Y chromosome, testicular differentiation and subsequent male or hermaphroditic development have been observed in subjects with a female (XX) karyotype (*2*).

Generally, theories concerned with differentiation of testes in the absence of a detectable Y chromosome propose conservation of Y-chromosomal function in one form or another—for example, (i) Y-to-autosome translocation, (ii) Y-X interchange, (iii) mutational acquisition of Y-chromosomal function by autosomal or X-chromosomal genes, or (iv) presence of undetected Y-bearing cell lines. Cytological studies (*3*) have provided supporting evidence for (ii) and (iv) above, but in our experience testicular differentiation in the absence

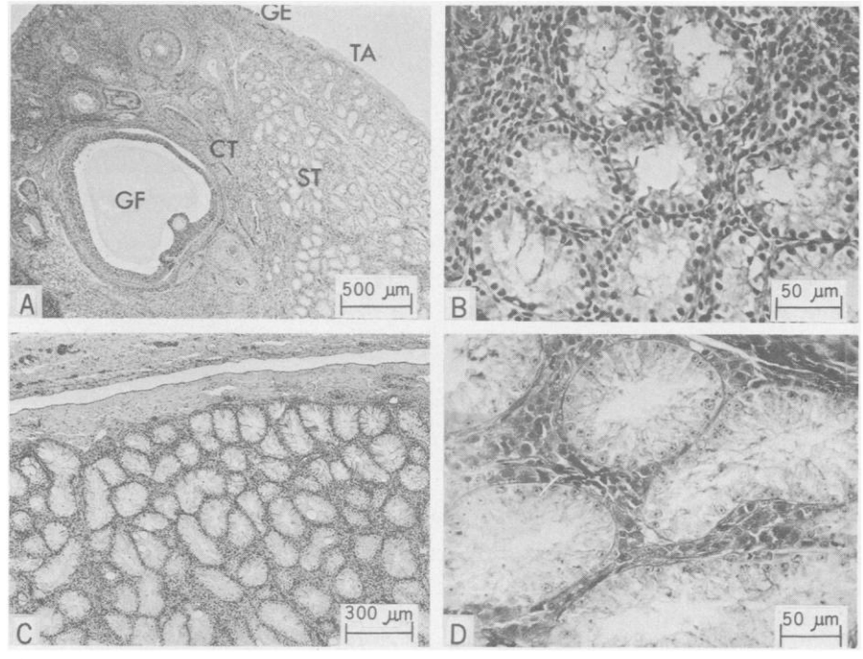

Fig. 1. Photomicrographs of gonadal tissue from XX male and XX true hermaphrodite. Ovotestis of the true hermaphrodite (A) (×36). This dog had bilateral ovotestes, both located in normal ovarian position (*7*). Under higher magnification (B) (×303), the seminiferous tubules are lined by vacuolated Sertoli cells; no spermatogenesis is evident. Leydig cells are present. Cryptorchid testicle of the XX male (C) (×53) contains lumenized seminiferous tubules varying in shape and size arranged in lobules by connective tissue trabeculae. Under higher magnification (D) (×283), the seminiferous tubules are lined by vacuolated Sertoli cells; no spermatogenesis is seen (Verhoeff's strain). Abbreviations: *CT*, connective tissue; *GE*, germinal epithelium; *GF*, Graafian follicle; *ST*, seminiferous tubules; and *TA*, tunica albuginea.

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of a detectable Y has invariably been correlated with serologically detectable H-Y (histocompatibility-Y) antigen, a phylogenetically conserved cell membrane component that is known to be determined under normal circumstances by Y chromosomal genes (*4*). For example, *Sxr*/XX males of the mouse are H-Y antigen positive (H-Y$^+$) (*5*) and so are XX males and XX true hermaphrodites of the human (*3*). On this basis we have proposed that the male-determining gene and the H-Y gene are one and the same (*6*). Here we describe a study of H-Y antigen expression in a family of dogs (*Canis familiaris*) which shows that XX male syndrome and XX true hermaphroditism may have a common genetic basis.

One of a litter of three pups whelped by an American cocker spaniel was a phenotypic and karyotypic female (78,XX), the second (sex unknown) failed to survive parturition, and the third was a 78,XX male with unilateral cryptorchidism (right inguinal testicle), hypoplastic penis, hypospadias, and a uterus. Histologic examination of the cryptorchid testicle revealed an abundant interstitium encompassing lobulated, small seminiferous tubules devoid of spermatogenic activity (Fig. 1). The mother was a karyotypic (78,XX) and phenotypic female. But surgical exploration revealed that she was in fact a true hermaphrodite with bilateral ovotestes containing prominent testicular tubules as well as mature ovarian follicles (*7*) (Fig. 1).

Assignment of the H-Y phenotype in dogs is based on the capacity of their white blood cells (WBC) to absorb H-Y antibodies from mouse H-Y antiserums which are then reacted with mouse sperm (*8*). Positive absorption (indicating that the absorbing cells were H-Y$^+$) is manifested as a decreased reactivity of H-Y antiserums with sperm cells in the sperm cytotoxicity test and in the mixed hemadsorption-hybrid antibody test. After the H-Y antiserums had been absorbed with WBC from the true hermaphrodite and from her XX male pup, we noted a decrease in reactivity in both our serological assays. On this basis, the XX male pup and hermaphrodite mother were typed H-Y$^+$ (Fig. 2).

These observations, implying vertical transmission of male-determining H-Y genes that are not on the Y chromosome, led us next to assay WBC from the parents of the true hermaphrodite (the grandparents of the XX male). The point is that increased amounts of H-Y antigen on the cell membrane, indicating supernumerary H-Y determinants within the nucleus, are detectable serologically (*9*). Since the mother of the true hermaphrodite was a normal female, the true hermaphrodite must have inherited non-Y-chromosomal H-Y genes from her father. If the Y chromosome of the phenotypically male, 78,XY father were intact, one might predict excess H-Y antigen on his WBC's. But this Y must be intact; one littermate of the true hermaphrodite is a 78,XY (H-Y$^+$) male who has sired numerous normal male offspring.

Table 1. Mixed hemadsorption-hybrid antibody tests showing reaction of mouse H-Y antiserum with mouse sperm after absorption with WBC from normal female (mother of true hermaphrodite), normal male (brother of true hermaphrodite), and father of true hermaphrodite. Suspensions of mouse sperm were exposed to H-Y antiserum portions A, B, C, and D, washed, and then reacted with a rabbit hybrid antibody of the specificity: antibody to mouse immunoglobulin/antibody to sheep red blood cells (SRBC). The sperm were again washed and next reacted with SRBC. Sperm which had already bound H-Y antibody (and therefore, also the hybrid antibody) now bound SRBC, thus forming rosettes. Any sperm cell to which three or more SRBC had adsorbed was scored as a rosette (*17*). In the tests shown in this table, H-Y antiserum portions absorbed with WBC from the father of the true hermaphrodite produced considerably fewer rosettes than H-Y antiserum portions absorbed with normal XY cells, indicating excess H-Y antigen on the cells of the father [compare with Fig. 2B, and also with table I in (*8*)]. The values shown are averages from two separate tests.

Unabsorbed	Absorbed		
A	B (Mother)	C (Brother)	D (Father)
46.4	46.0	36.4	16.5

As shown in Fig. 2 and Table 1, WBC from the father of the true hermaphrodite absorbed considerably more H-Y antibody than WBC from normal XY males, and this, together with the fact that he sired both a normal XY (H-Y$^+$) male and an XX (H-Y$^+$) true hermaphrodite, indicates the presence in his genome of both normal male-determining H-Y genes (on the Y) and supernumerary H-Y genes, either on an autosome, or on the X.

If, for the purpose of argument, one maintains that H-Y structural genes are normally situated on an autosome or on the X chromosome (*10*), then it is the activating or regulatory element (*4*) that is located on the Y. And if this is the case, the increased dosage of H-Y antigen in the grandfather and the differentiation of testicular tissue in his XX true hermaphrodite "daughter" and her XX male pup must represent two abnormal events: (i) duplication (and translocation?) of H-Y structural genes and (ii) activation of these genes in the apparent absence of the Y. The alternative explanation would seem less cumbersome: acquisition of Y-chromosomal function by an autosome (or the X) following either mutation of a gene in situ or translocation of material from the Y.

There is evidence that such a defect has existed in the cocker spaniel breed for at least four decades (*11*). Indeed, intersexuality has been reported more frequently in cockers than in any other breed of dog, the most common form involving presumptive or proven XX males and XX true hermaphrodites (*12*). Occurrence of XX male syndrome and XX true hermaphroditism within the

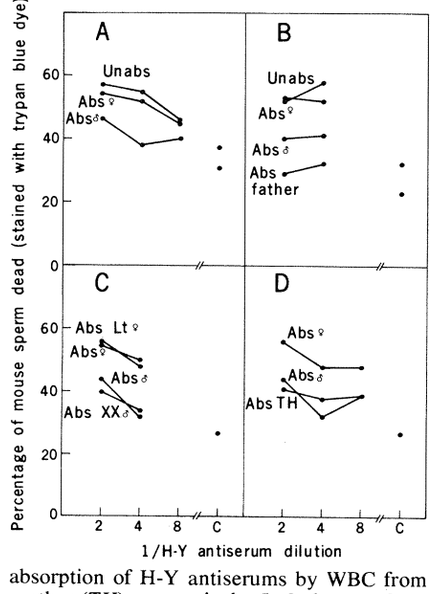

Fig. 2. Summary of cytotoxicity tests on mouse sperm showing absorption of mouse H-Y antiserum by white blood cells (WBC) of the dog. *Abs* denotes absorption with cells of the subject or subjects indicated; *C* denotes control: complement included, antiserum omitted. For each test selected H-Y antiserums were pooled and portions were treated as shown and then titrated for residual cytotoxic activity against mouse sperm. Sperm suspensions were scored as coded samples. For details of the procedures see (*3*, *9*). (A) Control data. The fall in cytotoxic titer after absorption with WBC pooled from normal XY males indicates that the absorbing WBC were H-Y$^+$. This graph is an average of five separate tests [compare with figure 1 in (*8*)]. (B) Excess H-Y antigen in the cells of the father of the true hermaphrodite. H-Y antiserums absorbed with cells from this male were invariably less cytotoxic than H-Y antiserums absorbed with cells from normal XY males. This is an average of readings from four separate tests [compare with table 1 and with figure 1 in (*9*)]. (C and D) Single tests showing absorption of H-Y antiserums by WBC from the XX male and from his true hermaphrodite mother (*TH*), respectively. Lt♀ denotes female littermate of XX♂.

same family is also documented in humans (*13*).

The present study, involving the only mammalian true hermaphrodite known to function as a fertile female (*14*), is informative for several reasons: (i) it implies that excess H-Y antigen on the cell membrane is associated with supernumerary male-determining H-Y genes in the nucleus; (ii) it shows that the H-Y$^+$ cellular phenotype does not rule out the presence of functional ovarian tissue in a fertile "female" of the dog and, by inference, of other mammalian species including the human; (iii) in view of the simultaneous gestation of XX male and XX female pups, it argues against the involvement of any environmental (or in utero) factor in the etiology of the XX male condition; and (iv) it indicates that the XX male syndrome and XX true hermaphroditism represent varying degrees of a common masculinizing event associated with abnormal transmission of Y-chromosomal (H-Y) genes.

Given a common genetic basis for XX male syndrome and XX true hermaphroditism, it remains to be determined how one gonad differentiates as a testis and another as an ovotestis. Earlier (*15*) we suggested that disseminated H-Y antigen is bound by plasma membrane receptors in the developing mammalian gonad. In this context it will be interesting to learn whether H-Y antigen expression differs in the two types of gonad and, if it does, whether this difference is related to the presence of other cell membrane components—for example, those determined by the major histocompatibility complex (*16*).

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17. Suspensions were scored as coded samples by an observer who recorded the counts of rosettes and free sperm. The values (percentage shown) were derived from the formula: (number of rosettes/number of rosettes plus free sperm). Positive absorption, indicating that the absorbing cells were H-Y$^+$, is indicated in the mixed hemadsorption-hybrid antibody test as a decrease in the number of rosettes when compared to the number of rosettes counted for unabsorbed H-Y antiserum [see (*9*) for details of procedure].
18. This work was supported in part by grants from NIH (CA 08748, AI 11982, GM 20138, HD 10065, HD 00171), ACS (FRA 167), and the Rockefeller Foundation. We thank K. Krupen-Brown, V. Kafantaris, C. L. Goldberg, and M. L. Oehlert for technical assistance; J. R. Church and R. F. Buchanan for referral of cases. R. S. Kenney assisted with the histology, and V. Scher with the manuscript.

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Morphological Alterations in Hippocampus After Long-Term Alcohol Consumption in Mice

Abstract. *Golgi methods were used to examine the hippocampus of laboratory mice that received alcohol-containing or control diets for 4 months followed by a 2-month alcohol-free period. Long-term alcohol consumption resulted in a significant loss of dendritic spines on hippocampal pyramidal cells and dentate granule cells. This study provides evidence that long-term alcohol consumption, in the absence of malnutrition, produces morphological damage to the central nervous system.*

The long-term consumption of ethyl alcohol is associated with a variety of neuropathological changes in central nervous system morphology (*1–3*). While many of these alterations are thought to be due to the secondary effects of malnutrition (*2, 3*), evidence of brain damage has been found in human alcoholic patients with no history of malnutrition (*4*). In addition, well-nourished animals receiving alcohol-containing diets show impairments in the performance of a variety of behavioral tasks, including shuttle-box avoidance (*5, 6*), timing behavior (differential reinforcement of low rate responding) (*7*), and maze learning (*8*). The ethanol-induced impairment in the acquisition of these behavioral tasks is present even after an ethanol-free period of 10 weeks (*5, 6*). Although a number of factors probably contribute to these ethanol-induced behavioral deficits, we report here that, in a nutritionally controlled animal model, long-term alcohol consumption results in the loss of dendritic spines on neurons in the hippocampal formation.

Three groups of mature (90 days old) female mice (C57B1/6J, Jackson Laboratories) were used. One group (group A) received an ethanol-containing liquid diet. A second group (group S) was pair-

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