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H-Y antigen and the growth of the dominant gonad

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SUMMARY The evolutionary conservation of H-Y antigen and its expression in the heterogametic sex regardless of whether this is male or female must mean that the antigen is associated with a common denominator underlying the development of mammalian testes and avian ovaries. It is suggested that one such common denominator is an enhanced growth rate of the dominant heterogametic gonad at a critical stage of its development.

The H-Y antigen, which was originally discovered in strains of inbred mice in which females rejected skin grafts from males (reviewed by Gasser and Silvers, 1972), has recently attracted intense interest as a result of the suggestion made by Wachtel *et al.* (1975b) that this antigen is the product of the testis determining gene on the Y chromosome and is an essential starting point for testicular differentiation in mammals. Though it is possible to accommodate most of the known facts of normal and abnormal sex development on the basis of this hypothesis there appear to be two major difficulties. The expression of the antigen is reduced in mice which were castrated soon after birth (Gasser and Silvers, 1972), which suggests that the antigen could be autosomally modified and its expression modified by androgens (Erickson, 1977) at an early stage of development. Secondly, though the antigen is not found in the females of mammals and amphibians which are homogametic (XX), it is present in the females of birds and of *Xenopus*, which are heterogametic (XY) (Wachtel *et al.*, 1975a). Thus, the antigen can no longer be regarded as confined to males, and it was suggested by Wachtel *et al.* (1975b) that its function may be to channel the embryonic gonad into whichever is the mature gonad of the heterogametic sex, that is the testis in mammals and the ovary in birds. This must mean that the antigen is associated with a common denominator underlying the development of mammalian testes and avian ovaries. I should like to suggest that one such common denominator is an enhanced growth rate of the embryonic gonad at a critical stage of its development.

Dominant embryonic gonad

It is recognised that the gonad of the heterogametic sex is the dominant embryonic gonad whose secretions play a major role in the development of the sexual phenotype (Jost, 1965; Price *et al.*, 1975). In mammalian embryos androgens secreted by the fetal testis are needed to maintain the Wolffian ducts and a further testicular secretion is thought to be necessary to cause the Müllerian ducts to regress; however, the corresponding processes in female embryos, the regression of Wolffian ducts, and the maintenance of Müllerian ducts occur spontaneously, and there is no evidence of any ovarian hormones at this stage. In avian embryos, on the other hand, the ovary secretes oestrogens which are necessary for the regression of the right Müllerian duct. Furthermore, the secretion of the dominant gonad can modify the sexual development of embryos of the opposite sex. Thus, in mammals androgens have a masculinising effect on the reproductive tract of chromosomally female embryos by stabilising the development of the Wolffian ducts, whereas the administration of oestrogens has not led to clear-cut results (Burns, 1971). In birds, the opposite situation holds. When chick embryos were treated with diethyl-stilboesterol, the left gonads of genetic males at one day after hatching were macroscopically and histologically indistinguishable from normal females, whereas treatment with testosterone propionate had no effect (van Tienhoven, 1957). In turkeys a variety of oestrogens administered to male embryos was found to induce ovotestes or ovaries on the left side; again, testosterone propionate, even in massive doses, had no macroscopical effect on females (Jaap *et al.*, 1951).

This contrast between embryonic birds and mammals is also illustrated by the freemartin condition. In the bovine freemartin, which occurs in cattle twins of unlike sex, the ovary of the female is modified into a sterile gonad by the male twin, whose own gonads normally become functional testes. The

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freemartin gonad may develop seminiferous tubules and in extreme cases resemble a small sterile testis which secretes testosterone (Short *et al.*, 1972), and it is noteworthy that H-Y antigen was detected in three highly masculinised freemartins (Ohno *et al.*, 1976). The freemartin condition can also occur in birds which develop from double-yolked eggs and it has been shown in duck embryos that the gonads of the male twin contained islands of cortical tissue, while the ovaries of the female were unchanged (Wolff and Lutz-Ostertag, 1961).

The freemartin condition emphasises the fact that the testis in mammalian embryos and the ovary in avian embryos will dominate the development of the homogametic gonad if per chance it comes into contact with it. This dominance appears to be associated with superior growth of the heterogametic gonad. In fetuses of rats and mice, testes are larger than the ovaries of litter mates (Mittwoch *et al.*, 1969; Jean, 1971; Mittwoch and Buehr, 1973) and in human fetuses, testes are larger than ovaries in fetuses of the same crown-rump length (Mittwoch, 1976). In chick embryos, male gonads are larger than female gonads until about the eighth day of incubation; however, at the time when the gonads become histologically differentiated, the left female gonad, which alone develops into an ovary, outgrows the male gonads (Mittwoch *et al.*, 1971). In the ovaries of chicks between the ninth and eleventh day of incubation there is a rapid proliferation of the germinal epithelium accompanied by a rapid increase of the primordial germ cells (Lillie, 1952). It seems, therefore, that ovarian differentiation in chick embryos is accompanied by a growth rate which is greater than that of the gonads of male embryos, that is the situation is the opposite to that in mammals (Mittwoch, 1971).

Hermaphroditism in man

The question whether the enhanced growth of the heterogametic gonad is the cause or the result of its sexual differentiation is clearly crucial. Recent data on human hermaphrodites suggest that superior growth is a necessary prerequisite for testicular differentiation in human fetuses. Wachtel *et al.* (1976a) have shown the presence of H-Y antigen in three patients with hermaphroditism whose chromosomes appeared to be those of normal females, 46,XX, though the presence of a rare cell line containing a Y chromosome remains a possibility. In spite of the antigen, however, such patients have ovarian as well as testicular tissue. Ferguson-Smith (1966) has postulated that the condition is the result of a cytologically undetectable interchange of part of the Y chromosome, including the male determining region, onto the X chromosome, so that the sex chromosomes of patients could be designated as XX^{y}. It is further assumed that during embryogenesis one or other of the X chromosomes will be randomly inactivated in different cells (Lyon, 1972) and thus give rise to either ovarian or testicular tissue. However, this theory does not fit the observed distribution of gonads in patients with hermaphroditism. Of 211 patients who had two different types of gonads, the testis (or ovotestis if the other gonad was an ovary) was on the right side in 62% of cases, while the ovary (or ovotestis if the other gonad was a testis) was on the right side in only 38% of cases (van Niekirk, 1974). This asymmetry cannot be explained in genetic terms, but must have an epigenetic origin. It is of interest, therefore, that the gonads of normal human fetuses show a consistent bias in favour of the right side, right gonads having more cells than those on the left side (Mittwoch, 1976). The concurrence of greater cell number in right versus left gonads, in testes versus ovaries, and the preferential development of testes on the right side in patients with hermaphroditism provides a strong argument in favour of the hypothesis that cell proliferation is a prerequisite for testicular differentiation in mammals (Mittwoch, 1973).

It may thus be possible to trace back the production of H-Y antigen to the presence of a fast growing dominant gonad in the heterogametic embryo.

XY females

Once it is accepted that there is a causal relation between gonadal growth and differentiation, several genetic abnormalities of sex development in man and other mammals can be simply explained on the basis of known mechanisms. In woodlemmings, *Myopus schisticolor*, a locus situated on the X chromosome renders XY animals into functional females (Fredga *et al.*, 1976). Such females lack H-Y antigen, and it has been proposed (Wachtel *et al.*, 1976b) that the mutant gene on the X chromosome suppresses the gene on the Y which should be coding for H-Y antigen; in the absence of the antigen, the gonads of XY embryos develop into ovaries. An alternative, and possibly simpler, hypothesis is that the mutant locus inhibits the growth of the developing gonads, which fail to undergo the mitoses necessary to reach a critical size by a given stage in development and consequently develop into ovaries (Mittwoch *et al.*, 1969).

The human condition known as pure gonadal dysgenesis may have a similar aetiology. Patients are typically female with streak or other abnormal gonads, and some have XY chromosomes; familial occurrence consistent with X chromosomal inheritance has been repeatedly shown (Espiner *et al.*, 1970) and the locus

responsible could be the same as that involved in the XY female woodlemmings. The fact that humans have streak gonads while the lemmings have functional ovaries is not surprising, since mammalian ovaries containing only one X chromosome lose their germ cells at an increased rate, and ovaries which have lost their germ cells at an early stage of development lose their characteristic architecture and degenerate into streaks of connective tissue. A similar difference is seen between patients with Turner's syndrome and 45,X chromosomes, who have streak gonads and are sterile, and XO (39,X) mice, which are fertile (Morris, 1968). The difference is undoubtedly connected with the difference in times of development between mice and women. In woodlemmings, the fertility of XY females is further ensured by a process of non-disjunction taking place in the ovary, so that XY cells give rise to XX oocytes (Fredga *et al.*, 1976).

There is an obvious parallel between a mutant locus postulated to reduce the growth of the fetal gonad and the *Bar* locus in *Drosophila melanogaster*. This is a mutation situated on the X chromosome which decreases the number of facets of the compound eye, so that in hemizygous XY males the eye is reduced to a narrow band (Lindsley and Grell, 1968). Embryological studies have shown that the phenotype results from a reduction in the number of cells in the optic disc and a reduced rate of cell division in the anterior part of the eye. Studies of the giant chromosomes in the salivary glands have revealed that *Bar* is the result of a tandem duplication of a small chromosomal region. A change of similar magnitude in a mammalian chromosome would probably not be detectable by present techniques.

Sex-reversed mice

In the mutant *bithorax*, flies appear to have two thoraces; the dorsal metathoracic disc of mature larvae was found to be significantly larger than normal (Chen, 1929). It is conceivable that cases of sex reversal in mammals in which XX individuals develop testes might have a similar origin. Mice carrying an autosomally transmitted mutation for sex reversal, *Sxr*, and XX sex chromosomes are phenotypic males with testes, and they express H-Y antigen (Bennett *et al.*, 1977). The authors regard this finding as evidence for a cytologically undetectable translocation between the Y chromosome and an autosome. The data, however, are equally compatible with an autosomal location of the gene responsible for antigen formation if we assume that its expression requires the presence of the dominant embryonic gonad, in this case the testes. In mouse embryos there is a very pronounced difference in growth between testes and ovaries. At 16 days' gestational age the mean volumes of fixed ovaries were found to be 0·033 mm^3 compared with 1·33 mm^3 for testes in embryos with XY chromosomes and 0·115 mm^3 for testes in *Sxr*, XX embryos (Mittwoch and Buehr, 1973). If we assume that the *Sxr* mutation increases the growth of the gonadal rudiment at a critical stage of development so that it passes the threshhold necessary for testicular differentiation, the interpretation of an undetectable Y-autosome translocation becomes superfluous.

The hypothesis that the expression of H-Y antigen is related to the growth of the dominant embryonic gonad in the heterogametic sex links the process of sex determination in higher vertebrates with that in some fish (Harrington, 1975) and amphibia (Witschi, 1942), in which primary sex determination can be affected by temperature. We may assume that in the course of evolution the Y chromosome has become increasingly efficient in making the sex of an individual independent of its environment by enhancing the growth of the embryonic gonad, thus ensuring its dominance. It seems that the early secretion of sex hormone as well as the expression of H-Y antigen are closely associated with this process. The question whether growth precedes antigen production or vice versa must at present be left open.

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