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The Bovine Freemartin: A New Look at an Old Problem [and Discussion]

R. V. Short, A. Jost, G. W. Harris and C. E. Ford

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The bovine freemartin: a new look at an old problem

By R. V. Short
Department of Veterinary Clinical Studies and A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge

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[Plates 17 and 18]

The first scientific study of the freemartin condition in cattle was published by John Hunter in 1779. One of the animals that he procured for his dissections came from a certain Mr Wright, and there was no history of this particular animal having been born co-twin to a bull. In addition to providing us with detailed anatomical notes of his dissections, Hunter commissioned William Bell to portray the dissected reproductive tract (figure 1, plate 17). The specimen itself, now almost 200 years old, is still in an excellent state of preservation and can be seen in the Hunterian Museum of the Royal College of Surgeons, Lincolns Inn Fields, London.

One glance at this reproductive tract is sufficient to convince anybody conversant with the condition that the animal cannot have been a freemartin. The gonads are clearly testicular in appearance, and in Hunter's own words 'were more than twenty times larger than the ovaria of the cow, and nearly as large as the testicles of the bull, particularly as those of the ridgill, the bull whose testicles never come down'. The gonads of true freemartins, on the other hand, are always small and atrophic, even though they may take on the morphological appearance of testes. This is clearly shown in William Bell's drawing of one of Hunter's authentic freemartins, which was obtained from a Mr Arbuthnot (figure 2, plate 18).

In spite of the large intra-abdominal testes of Mr Wright's animal, which were examined histologically by Berry Hart (1910) and more recently by J. S. S. Stewart (personal communication), there were almost no other signs of masculinization. 'There was nothing similar to the vasa deferentia . . . As the external parts had more of the cow than the bull, the clytoris, which may also be reckoned an external part, was also similar to that of the cow . . . Although I call these bodies testicles for the reason given, yet they were only imitations of such . . . From the seeming imperfection of the animal itself, it was not to be supposed that they should be testicles, for then the animal should have partook of the bull, which it certainly did not.'

Hunter was perfectly correct in stating that there were no obvious signs of testicular activity in this animal. But even though the testes had failed to stimulate the development of Wolffian duct derivatives, they had certainly succeeded in inhibiting the development of the Müllerian ducts, for 'the vagina terminated in a blind end, a little way beyond the opening of the urethra, beyond which the vagina and uterus were impervious'.

True freemartins, on the other hand, such as Mr Arbuthnot's, invariably show some signs of masculinization; if the gonads have become testicular in appearance, the internal genitalia may show extensive masculinization and the clitoris may also be hypertrophied (figure 2). There may even be some degree of masculinization of the external genitalia in very extreme cases (Lillie 1917; Short, Smith, Mann, Evans, Dickson, Fryer & Hamerton 1969).

Jost (1955) has postulated that the function of the testis in foetal life is twofold: stimulation of the Wolffian duct derivatives by an androgen, possibly testosterone, and inhibition of the Müllerian ducts by some other substance, as yet unidentified. Thus the testes in Mr Wright's animal seem to have produced the Müllerian-inhibiting hormone and not the Wolffian-stimulating one, whereas the gonads of Mr Arbuthnot's freemartin have apparently produced some of both substances.

Mr Wright's animal closely resembles the appearance of a cow with the testicular feminization syndrome (Nes 1966). In this syndrome, which is well recognized in man, individuals that are genetic males (XY) develop testes in the normal way, but grow into phenotypic females, with uterine aplasia, because the target organ tissues are apparently unresponsive to androgens. In man it has recently been suggested that this target organ defect may be related to the inability of the tissues to convert the testosterone secreted by the testis into its androgenically active metabolite, 5α-dihydrotestosterone. In normal individuals, testosterone is preferentially converted to dihydrotestosterone in some target organs, whereas this conversion does not take place in similar tissues from testicular feminization patients (Wilson & Walker 1969; Mauvais-Jarvis, Bercovici & Gauthier 1969; Northcutt, Island & Liddle 1969).

But to return to John Hunter for a moment, there can be little doubt that in his search for freemartins he had unwittingly stumbled across the first recorded case of testicular feminization (Short 1969). It is a tribute to his industry and meticulous attention to detail that we are able to give him full credit for this discovery, almost 200 years after the event.

The scientific history of the freemartin has been an illustrious one, and it is humbling to realize that the true explanation of the condition continues to elude us. There can be no doubt that the precipitating event is the occurrence of a vascular anastomosis between the foetal circulations of dizygotic twins of unlike sex before gonadal differentiation. This anastomosis allows an interchange of cells and substances between the two individuals, and the subsequent differentiation of the reproductive tract of the female foetus is impaired as a result. We have recently suggested that the basic defect is in fact a partial sex reversal of the female gonad; as a result of the retention of some medullary tissue, the genetically female gonad starts to secrete male-type hormones. Thus the freemartin is probably masculinized by the secretions of its own gonads (Short *et al.* 1969).

Satisfactory though this explanation may seem at first sight, we still do not know what produces the gonadal sex-reversal in the first place. It is clear that testosterone itself is unable to interfere with normal ovarian development in the bovine foetus (Short *et al.* 1969), so we can no longer accept the time-honoured explanation of the freemartin condition proposed independently by Lillie (1916, 1917) and Keller & Tandler (1916). They believed that the female twin was masculinized by sex hormones derived from the testis of the conjoined male co-twin.

When it began to be realized that freemartins were chimaeras, containing in their bodies an admixture of their co-twin's red cells, white cells and possibly germ cells, it was postulated that the gonadal sex-reversal might have been a direct result of this cellular interchange (Fechheimer, Herschler & Gilmore 1963; Goodfellow, Strong & Stewart 1965). But which of these three cell types could influence the sexual differentiation of the foetal gonad? It is unlikely that red cells or white cells could be responsible. In the marmoset monkey, placental anastomoses commonly occur between unlike-sexed twins before gonadal differentiation, but in spite of the extensive erythrocyte and leucocyte chimaerism that follows as a result of this anastomosis, sexual development of the female marmoset foetus is unimpaired (Benirschke & Brownhill 1962).

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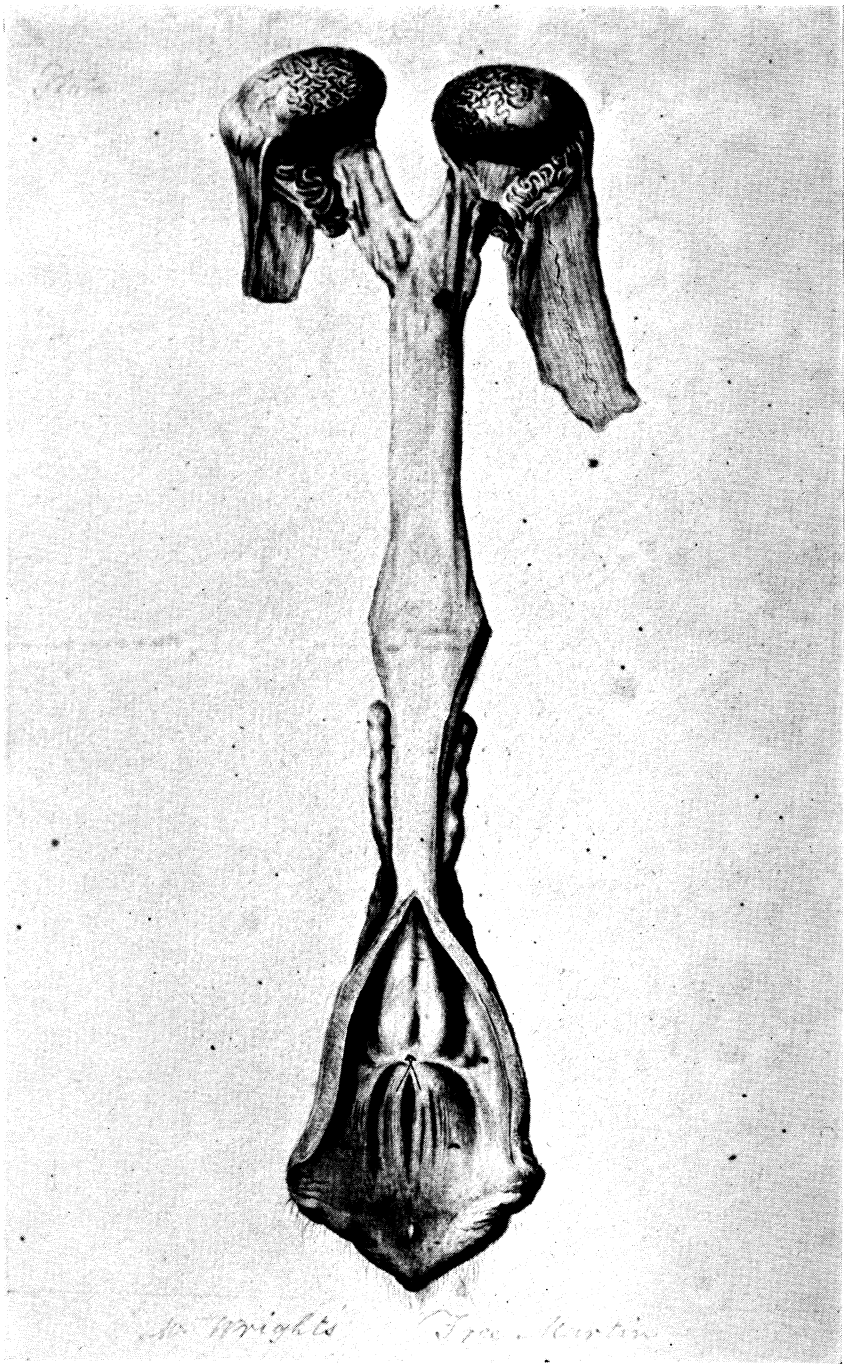

FIGURE 1. Mr Wright's 'freemartin'. Drawing by William Bell from John Hunter's dissection; viewed from the dorsal aspect.

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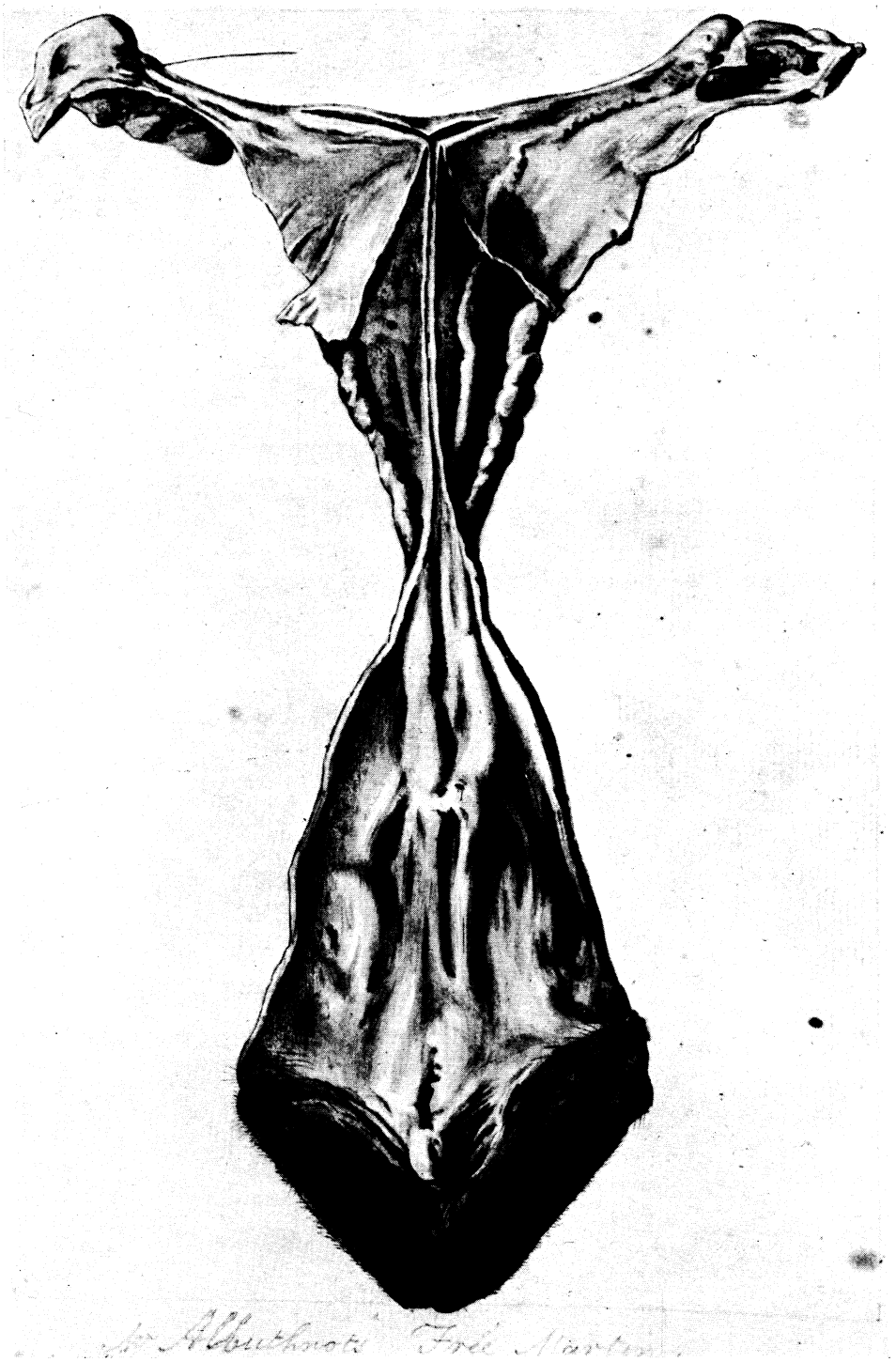

FIGURE 2. Mr Arbuthnot's freemartin. Drawing by William Bell from John Hunter's dissection; viewed from the dorsal aspect.

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The possible role of germ cells in gonadal development is less clear. There is reasonable evidence that germ cells from the female foetus may migrate into the testes of the conjoined male twin in marmosets and in cattle (Benirschke & Brownhill 1963; Ohno 1969; Short *et al.* 1969). Although the breeding records of one bull born twin to a freemartin apparently showed an increase in the percentage of female offspring (McSparrin & Patrick 1967), this is not usually the case (Dunn, Kenney, Stone & Bendel 1969), and it seems unlikely that XX germ cells can complete spermatogenesis (Short *et al.* 1969). This is in accord with Tarkowski's findings in experimentally induced chimaeric mice (Mystkowska & Tarkowski 1968), and with our own observations on intersex goats in which XX germ cells migrate successfully into an XX testis (the correct genotype but the wrong phenotype), but degenerate before birth (Hamerton, Dickson, Pollard, Grieves & Short 1969).

Although it has been customary to assume that bulls born twins to freemartins are normal in every respect, there is a growing body of evidence to suggest that they, too, may be abnormal. Steroid synthesis by the testis, both *in vivo* (Short *et al.* 1969) and *in vitro* (Pierpoint, Stewart & Rack 1969) is impaired, and some animals may eventually develop testicular degeneration leading to complete sterility (Dunn *et al.* 1969). Although this could be an effect of the XX germ cells, it seems unlikely.

An even more interesting problem is what happens to the XY germ cells that migrate into the developing female gonad. Unfortunately, there is no direct information available on this point. In the marmoset, reciprocal germ cell exchange probably occurs between male and female chimaeric twins, and yet sexual development of the female is normal (Benirschke & Brownhill 1963).

Embryologically, there is no reason to suppose that germ cells are capable of influencing gonadal development; Burns (1961) has gone so far as to state that 'The role of the germ cells in the formation of the gonad appears to be a purely passive one, since the non-germinal tissues are capable of producing the typical structure of a testis or an ovary in the absence of all germinal elements'. So until some direct experimental evidence is forthcoming to show that XY germ cells in an XX genital ridge cause it to develop as a testis, the cellular theory of the origin of the freemartin condition must be regarded as unproven. If XY germ cells do migrate into the gonad of the female foetus and cause the development of seminiferous tubules, it is curious that these same germ cells are then unable to survive in the environment that they have created for themselves, for none can be seen in the seminiferous tubules after birth (Ohno 1969; Short *et al.* 1969).

With the hormonal theory untenable, and the cellular theory unproven, what other alternatives are there to explain the freemartin condition? It seems worth recalling an alternative theory first advanced by Witschi in 1934. He thought that in the freemartin, male inductor substance, 'medullarin', might pass into the female and induce testicular development.

In figure 3, an attempt has been made to outline the principal steps in foetal sex determination and differentiation. In some way that is not understood, the genotype of the cells in the genital ridge of the male foetus leads to the persistence of the medullary sex cords, possibly by the release of some inductor substance like Witschi's 'medullarin'. Once medullary cords have developed, they become populated by XY germ cells which migrate there from the yolk sac, and the development of cortical cords is inhibited. The resultant product is a testis, capable of secreting hormones that will stimulate the Wolffian duct and inhibit the Mullerian duct.

Although Witschi has put forward the theory of cortico-medullary antagonism, one can envisage female gonadal development as a much simpler process. It may be that in the absence

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of male inductor substance, the development of the cortical cords is no longer suppressed, so that they come to form the substance of the gonad. They are then populated by XX germ cells from the yolk sac in the normal way. Any hormones produced by the foetal ovary are of no consequence in the development of the female reproductive tract before birth.

It is interesting to consider in more detail the way in which genetic sex is translated into the phenotypic sex. In insects, the sex chromosome complement of each individual cell may influence its behaviour; for example, the sex of brain cells determines the type of sexual behaviour in some species (Caspari 1965). In mammals, on the other hand, the generalized effects of sex are mediated by a special class of compounds, the steroidal sex hormones. These are manufactured by the somatic cells of the genital ridge, and it is the sex chromosome complement of

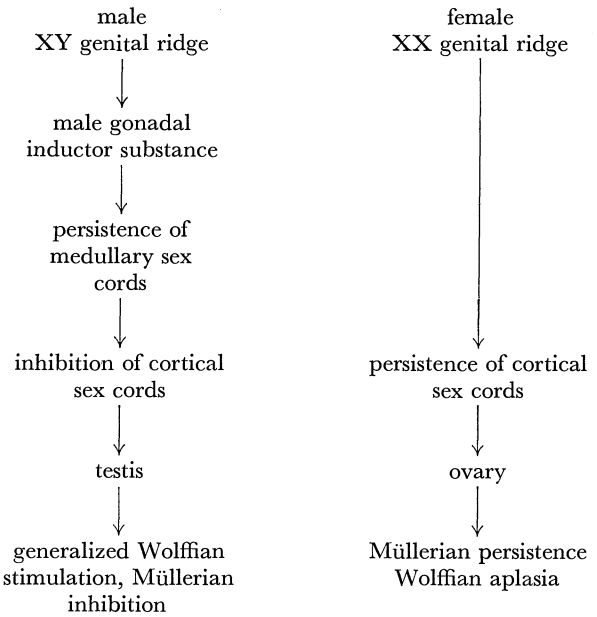

FIGURE 3. Normal sex determination and differentiation in the foetus.

these cells that is all important in determining the phenotype of the individual. The only other group of cells whose activity is apparently fixed by their sex chromosome complement is the germ cells themselves. The genetic sex of all other somatic tissues is apparently irrelevant to their subsequent sexual differentiation and development. This is well illustrated by the paradoxical case of a pair of monozygotic human twins of dissimilar sex (Jacobs 1969). Both twins showed extensive XY/XO mosaicism, and it is assumed that the female twin had XO tissue in the gonads, leading to the formation of the 'streak' ovaries of Turner's syndrome, whilst the male twin had mainly XY tissue in the gonads, resulting in normal testicular development. The proportion of XY and XO cells in the peripheral tissues bore no relation to the sex phenotypes of either twin.

Endocrinologically speaking, one might think that the essential difference between a male and a female is whether the gonad makes androgens or oestrogens (see figure 4). For androgen formation, the inactive precursor, androstenedione, has to be converted to testosterone by the 17β-hydroxysteroid dehydrogenase enzyme system. In the female, on the other hand, most of the androstenedione is hydroxylated at position 19 and then undergoes aromatization of the A ring. It would be attractive to suppose that genes on the X and Y chromosomes might control these various enzyme systems, which in turn determine the type of hormone secreted by the gonads.

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However, even this basic sex difference in hormone production is not in fact under the direct control of the sex chromosomes. In goats with the autosomal gene defect that brings about testicular development in genetic females, genetically XX testes can secrete testosterone (Hamerton *et al.* 1969); likewise the freemartin gonad which is still genetically XX, nevertheless secretes testosterone (Short *et al.* 1969). The only conclusion we can draw is that if medullary tissue persists in the gonad, it will secrete androgens, irrespective of its genotype; sex chromosomes therefore normally determine the presence or absence of medullary tissue.

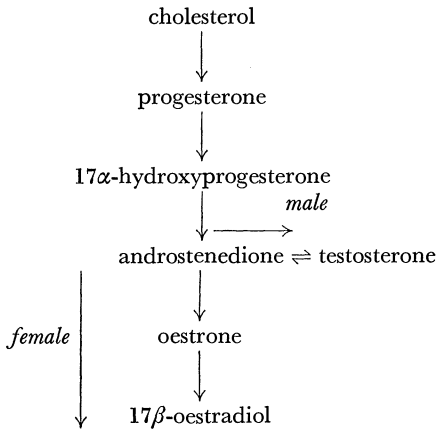

FIGURE 4. Pathways of steroid synthesis in the adult male and female gonad.

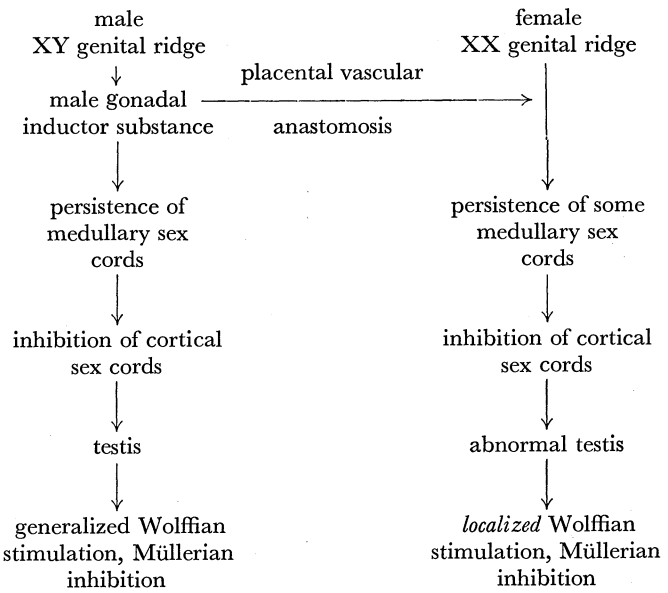

FIGURE 5. Sex determination and differentiation in the freemartin foetus.

The basic defect in the freemartin must be looked upon as the retention of medullary tissue n an XX gonad. The most likely reason for this would seem to be that male gonadal inductor ubstance has leaked across into the female's circulation via the placental anastomosis, and inter-ered with ovarian development (figure 5). Perhaps there is also a female gonadal inductor ubstance which could interfere with gonadal development in the male twin. There are a

number of facts that argue against such an inductor substance hypothesis; the normal development of the chimaeric female marmoset is one of them. If gonadal inductor substances enter the systemic circulation, it also becomes increasingly difficult to account for those rare cases of true hermaphroditism in which there is a testis on one side of the body and an ovary on the other.

The freemartin condition is of widespread occurrence in Nature, and is known to exist in sheep, goats and pigs (Short *et al.* 1969). In the past, freemartins have attracted the attention of some of the leading anatomists, embryologists, immunologists, haematologists and cytogeneticists of the day, and yet the cause of the condition still remains unexplained. Therefore it may be helpful to re-define the problem. It is essentially a question of the transfer of genetic information from a male to a female individual. More specifically, we must seek to explain how information, coded in the Y chromosome of a few cells in the genital ridge of a male foetus, is transferred to cells in the genital ridge of a female foetus. The problem becomes simpler if we accept Hamerton's thesis (Hamerton 1968) that all the male-determining genes are in fact located on the X chromosome, and so are present, but latent, in normal female individuals. The Y chromosome of the male is postulated to act merely as a controlling centre, 'switching on' the male-determining genes of the X chromosome. Something, cell or substance, passes across the placental anastomosis from the male to the female twin and causes genetically female somatic tissue in the gonad to function like that of the male, possibly by switching on its male-determining genes. However, the behaviour of the female germ cells appears to be immutably controlled by their genotype, and they cannot survive if the gonad is transformed into a testis.

While anatomists, histologists, cytologists and endocrinologists have helped to define the problem posed by the freemartin, and bring it into sharper focus, the ultimate solution seems to await the attention of those concerned with information transfer from cell to cell.

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Discussion on papers by D. Price, p. 133 *and R. V. Short, p.* 141

A. Jost: It is probably an oversimplification to consider that the syndrome of testicular feminization is entirely explained by lack of sensitivity of the tissues to androgens. The Müllerian ducts are usually suppressed and must have been sensitive to Müllerian-inhibiting testicular secretion.

R. V. Short: There are probably Müllerian-inhibiting substances as well as Wolffian-stimulating substances. In Hunter's case of testicular feminization, well-developed seminal vesicles were present, and this observation obviously poses some problems.

G. W. Harris: Could the evidence about dihydrotestosterone and testicular feminization be amplified?

R. V. Short: This is the work of several people, especially J. D. Wilson. In target organs, particularly the seminal vesicles, testosterone is metabolized to dihydrotestosterone. We and others have shown that dihydrotestosterone is quite as potent as an androgen as is testosterone. It therefore appears that target organs convert testosterone to a biologically active androgen and other tissues convert it into biologically inactive metabolites. Further evidence comes from recent studies on testicular feminization in man where the normal conversion of testosterone to dihydrotestosterone in target tissues such as the clitoris, does not take place. It is well known that these patients do not respond to testosterone.

C. E. Ford: Evans and I have analysed spermatocytes and spermatogonia in ten bulls that were twins to freemartins. All cells were XY. Bull twins examined by Ohno were younger than ours and his differing observations may therefore be reconcilable. In a recent paper in *Cytogenetics*, analysis of tissue cultures from testicular explants of the bull twin was done and the authors claimed that some XX cells were present. If true, these workers must have grown *in vitro* either female germ cells that had migrated from the freemartin, and if so this would be the first time germ cells had ever been grown *in vitro*, or they have grown somatic cells, but how could cells from the female twin enter the testis of the male twin? They might possibly have come from the lymphomyeloid complex. Barnes and Kruschov have recently found evidence in mouse radiation chimaeras of donor cells of the fibroblastic type. If transport can happen between twins in one direction it can also presumably happen in the other. No one appears to have cultured the ovarian remnants in freemartins to examine their cellular constitution.

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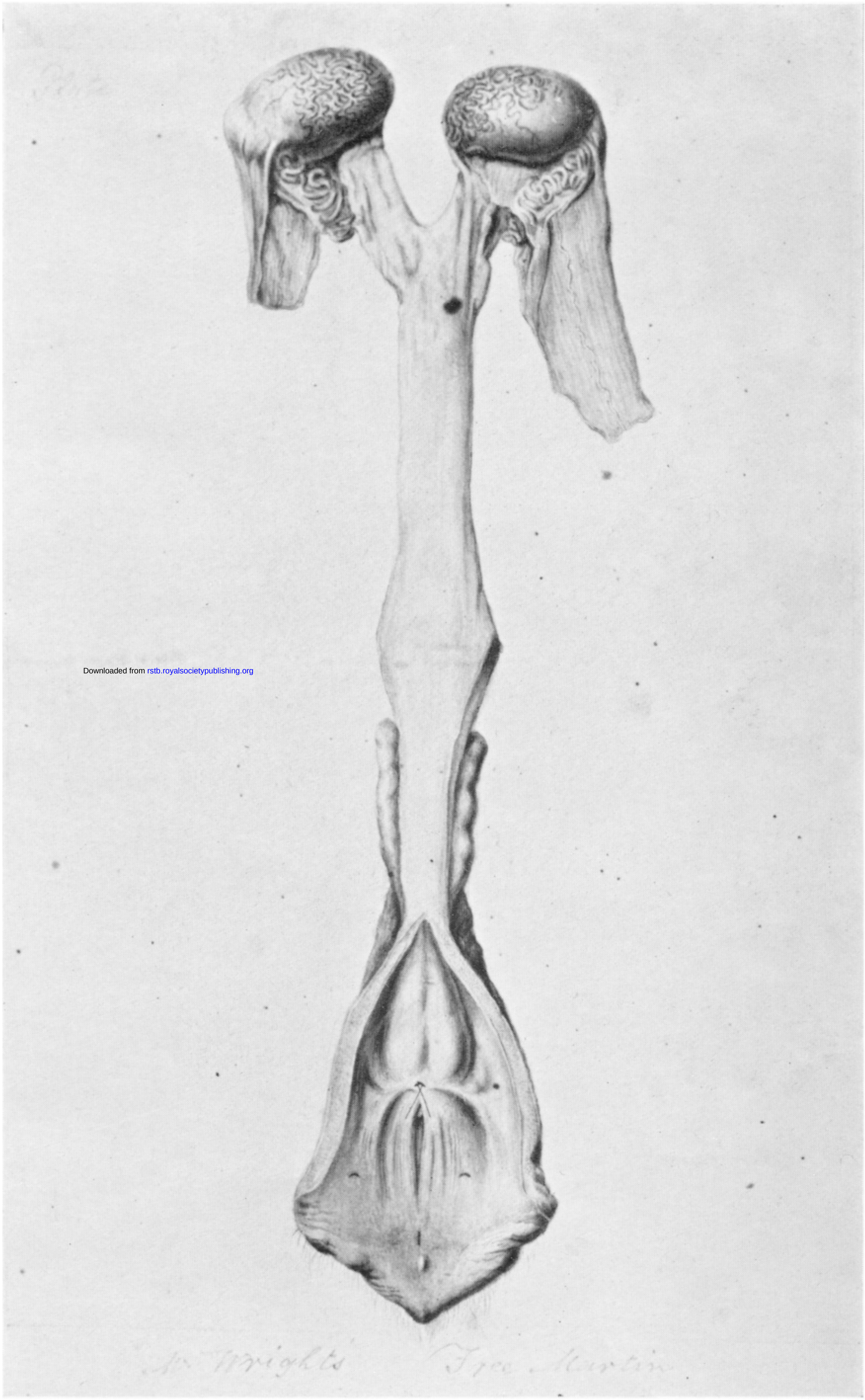

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FIGURE 1. Mr Wright's 'freemartin'. Drawing by William Bell from John Hunter's dissection; viewed from the dorsal aspect.

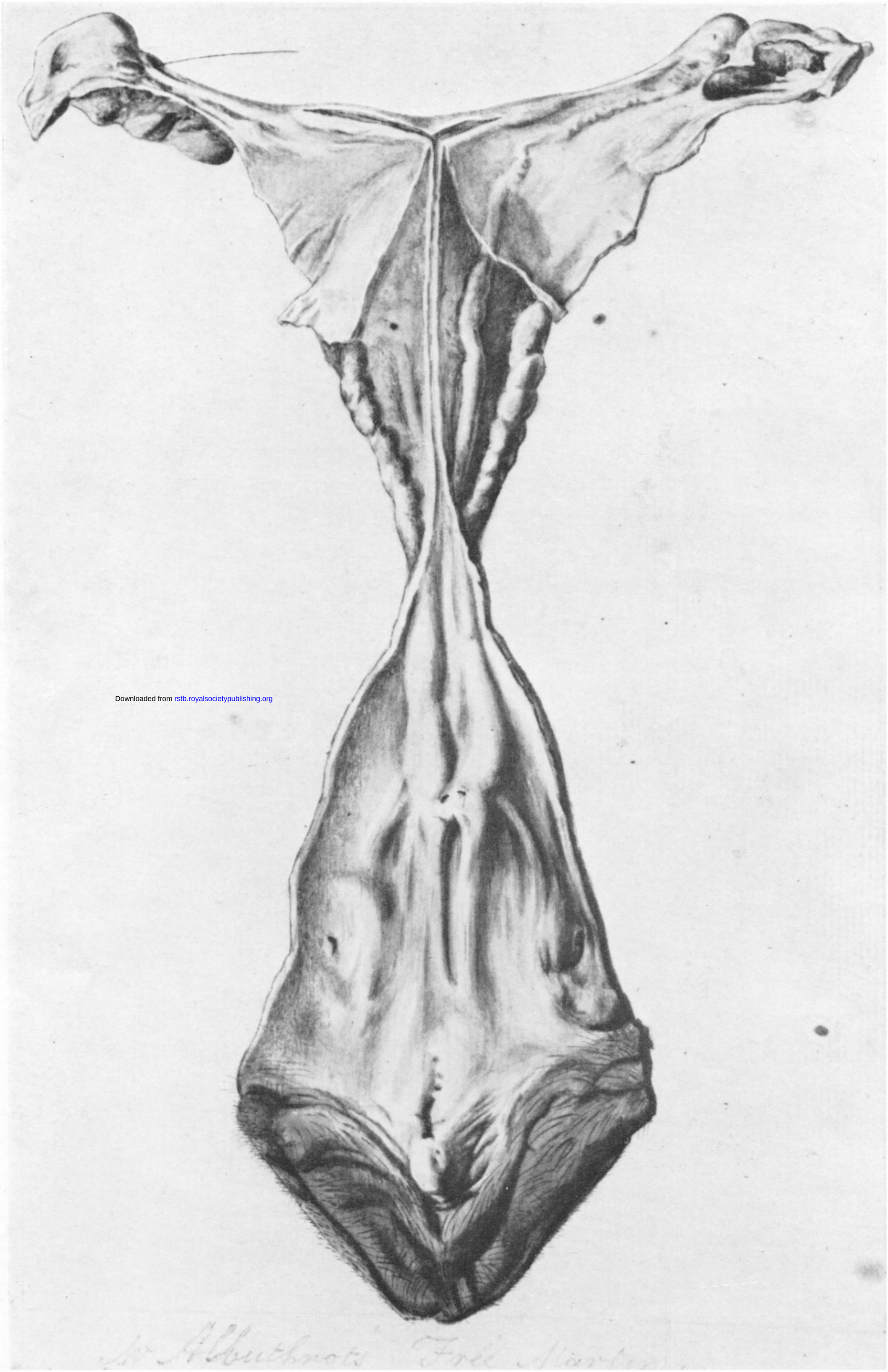

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FIGURE 2. Mr Arbuthnot's freemartin. Drawing by William Bell from John Hunter's dissection; viewed from the dorsal aspect.