H-Y ANTIGEN and the BIOLOGY of SEX DETERMINATION

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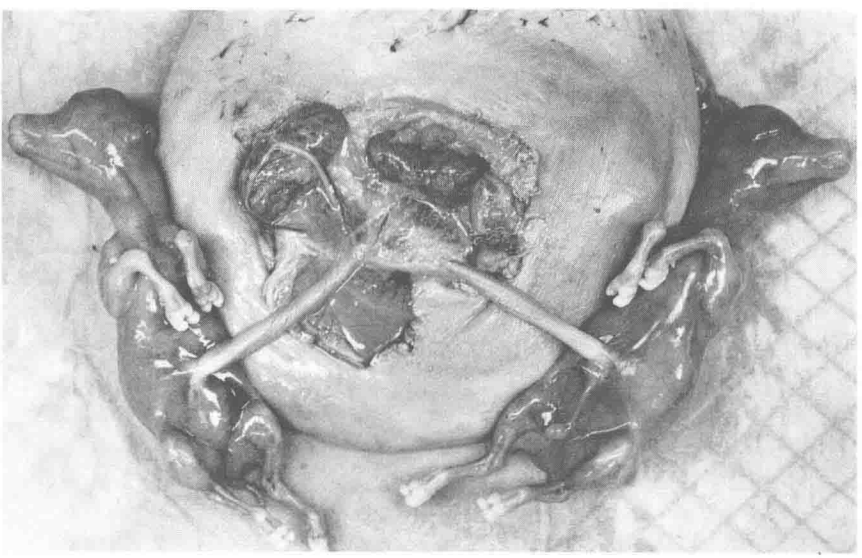

Synchorial male and female bovine twin fetuses of about 105 days of gestation; female at left. It is common knowledge among farmers that the female twin of a bull is sterile. In some cases, as in the twins that are pictured, the gonads of the female or freemartin are transformed to resemble those of the male. Freemartin gonads are H-Y$^+$ (see Section 6.6).

For Gwendolyn, Jonathan, and Todd

Wherever (the reader) finds that I have ventur'd at any small conjectures, at the causes of the things that I have observed, I beseech him to look upon them only as doubtful Problems, and uncertain ghesses, and not as unquestionable Conclusions, or matters of unconfutable Science.

Robert Hooke
Micrographia, 1665

I am grateful to Darcy Wilson for introducing me to the biology of transplantation, to R. E. Billingham and W. K. Silvers for initiating me in the study of the "male" antigen, and to Edward Boyse for providing the foundation of serology upon which much of this book stands. I thank my colleagues J. Bard, W. R. Breg, L. T. Cahill, G. C. Koo, O. J. Miller, U. Müller, M. I. New, I. R. Rosenthal, and P. Saenger for their several contributions to the work reviewed here; and I thank my fellows J. L. Hall and D. Nakamura for their critical reading. Elizabeth Simpson graciously agreed to review the section on cell-mediated cytotoxicity; Aster White, Merri Brenner, and Virginia Barsky helped in preparation of the manuscript; and Siri Mills and David Purnell provided the original artwork. Special thanks are due to my friend and colleague, Susumu Ohno, for sparking my interest in developmental biology and for his continuous and stimulating collaboration.

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PREFACE

In a sense, man begins his existence as a hermaphrodite. The primitive gonad retains the potential for male or female development, the external genitalia are ambiguous and ambisexual, and the primordial internal ducts of both sexes are present in the same individual. It is only after the second month of gestation that the distinct male and female morphologies become apparent. It is at that time that testosterone, a secretion of the newly differentiated testis, induces development of the male internal ducts, and it is at that time that the antimüllerian hormone, another secretion of the testis, suppresses development of the female internal ducts. It is then that dihydrotestosterone, a metabolite of testosterone, induces the development of the male external genitalia. In the absence of the testis and its secretions, the male ducts do not develop, but the female ducts do, and the external genitalia assume the female aspect. In certain abnormal cases, where neither male nor female dictate prevails, development is not clearcut: the ambisexual stage persists, and the result is mingling of the traits of male and female sexuality.

According to Hymie Gordon of the Mayo Clinic, the earliest written account of hermaphroditism is given in the cuneiform tablets unearthed at the remains of the Royal Library at Ninevah, which was built in the 7th century BCE. On one of the tablets there is a list of 62 birth defects with corresponding predictions of what each of the birth defects foretells, each being regarded as a significant portent

of one sort or another. Four of the birth defects deal with sexual development and two, apparently, with hermaphroditism:

> When a woman gives birth to an infant whose right ear is round, there will be an androgyne in the house of the newborn.
>
> When a woman gives birth to an infant that has no well-marked sex, calamity and affliction will seize upon the land; the master of the house shall have no happiness.*

Certainly hermaphroditism is as old as man himself. According to the dissertation of Aristophanes, as cited in the Symposium of Plato, the original human nature was different from what it is now—there being not two but three sexes: male, female, and a male–female combination called *androgynos.* Each was spherical in shape with two faces looking in opposite directions, two pairs of arms, two pairs of legs, and two sets of genitalia.

> He could walk upright as men now do, backwards or forwards as he pleased, and he could also roll over and over at a great pace, turning on his four hands and four feet, eight in all, like tumblers going over and over with their legs in the air; this was when he wanted to run fast.

Now these primeval men were powerful, and they dared to plot against the gods, who thought to annihilate them with thunderbolts. Yet the gods had no wish to lose the dual advantages of worship and sacrifice, and so Zeus devised a scheme to humble Man without killing him. He cut them in two

> like a sorb-apple which is halved for pickling, or as you might divide an egg with a hair; and as he cut them one after another, he bade Apollo give the face and the half of the neck a turn in order that the man might contemplate the section of himself: he would thus learn a lesson of humility.

The divided humans were so distraught with their novel anatomy that they spent all their time in mutual embrace, in woeful contemplation of their separated halves: male–male, female–female, and androgynous male–female longing to be restored. They would have been destroyed by hunger and neglect, but the gods had pity on them and redesigned the human anatomy to promote more intimate

*Translation by J. W. Ballantyne: Teratologia 1:127, 1849, in Gordon H: Ancient ideas about sex determination, in Vallet H. L., Porter I. H. (eds): Genetic Mechanisms of Sexual Development. New York, Academic Press, 1979, pp. 1–32.

embrace and generation. This resulted in the current human form, and thus man survived, but as a mere half of his former self.

Accordingly, modern men and women are derived from the various severed halves of their rotund forbears. Men who are lovers of women are derived from the androgynos (adulterous men and women are included in this group); women who do not care for men are derived from the double female (female "companions" are among this group); and men who follow after men are derived from the original double male.

> they have the most manly nature . . . and these, when they grow up, become our statesmen . . .*

The androgynous nature of primeval man is treated rather less whimsically in the Babylonian Talmud (Berachoth 61a), representing an oral tradition committed to writing some 1500 years ago:

> According to Rabbi Jeremiah ben Eliazar . . . The Holy One Blessed Be He created two faces in the first man, as it is said, 'behind and before hast Thou formed me' (Psalms 139:5)

The implication is that primeval man was hermaphroditic with two aspects, and that Eve was created from one of them, an interpretation upheld in this Midrashic commentary (Bereishith Rabbah 8.1):

> Rabbi Jeremiah ben Eliazar said: in the moment that He created the first man, The Holy One Blessed Be He made him an androgynos, as it is said, "male and female created He *them*" ("and blessed *them* and called *their* name Adam") (Genesis 5:2)†

It follows that Eve was created by physical separation from the androgynous male, as expounded in the same argument:

> Rabbi Shimeon ben Nachman said . . . He gave him two faces (and one form); but He cut him and made for him two backs (and separate male and female forms)—one here and one there.

Indeed, the Hebrew word *tsela,* translated as "rib" in the English rendering of the creation of the sexes (Genesis 2:22), also means side, as in the *side* of a tabernacle (Exodus 26:20). Thus the cut was made at the side of Adam; and thus, another commentator (Hanoch Zundel) indicated that "they were joined back-to-back so that the (individual) backs were not discernible but became discernible when He cut them."

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*From the Symposium of Plato, The Jowett translation.
†Author's italics; and see Genesis 1:27.

Though it may seem that we have come quite a way from the ancients, in our treatment of sexual differentiation, we are perhaps not so far removed from their attitudes. The English word *sex* is after all derived from the Latin *sexus,* akin to *secus,* which comes from *secare,* which means *to cut.*

As for the modern credo, male development is viewed as an effect of the Y chromosome and female development as due to absence of the Y chromosome. In mammals XY embryos become males and XX embryos become females—under normal circumstances. But male, female, or hermaphroditic development may occur regardless of karyotype—under abnormal circumstances. So it is not the Y chromosome *per se* that causes maleness, but another factor that is usually under control of the Y.

In this book we shall review the history of Y chromosome-determined H-Y antigen, and we shall develop the notion that this cell surface molecule is the inducer of the mammalian testis. We shall consider evidence that the genes that code for H-Y antigen are phylogenetically conservative, being held in common among members of the heterogametic sex of every vertebrate species so far studied, and we shall describe experiments in which soluble H-Y of one species can induce development of the heterogametic gonad of another.

It is remarkable that the inducer of the heterogametic gonad should be found among the armamentarium of transplantation antigens, but evidently that is the case. H-Y was alluded to as a female-specific transplantation antigen in chickens in 1932; and it was discovered as a male-specific transplantation antigen in mice in 1955. Thus the book begins with a review of the transplantation biology of H-Y antigen (Chapters 1–3), continues with a discussion of the nature of H-Y and its function in sex determination (Chapters 4–8), and concludes with a section on medical genetics and their clinical implications (Chapters 9–12).

CONTENTS

1

MALE-SPECIFIC TRANSPLANTATION ANTIGEN OF THE MOUSE

I wonder whether the Y antigen may not be something different in principle from the "ordinary" histocompatibility factors, since it seems to be identical in different strains. Could it be an obligatory, as opposed to variable character, related for example, to the differentiation of maleness?

Klein, in discussion
Zaalberg, 1959

1.1 FAILURE OF INTRASTRAIN MALE SKIN GRAFTS

According to the "laws of transplantation" as formulated by George Snell (1953), tissues should be accepted when grafted from one member of a highly inbred strain to another member of the same strain or from a member of a *parental* strain (designated "A" or "B") to a member of the F_1 hybrid generation (A × B) (Fig. 1-1). In 1955 Eichwald and Silmser, working at the Montana Deaconess Hospital in Great Falls, presented the results of a study showing that Snell's laws were not inflexible. In certain highly inbred lines of the laboratory mouse, intrastrain skin grafts and parental strain to F_1 skin grafts were rejected when the donor was a male and the recipient a female (Table 1-1).

Commenting on the data presented by Eichwald and Silmser in the same journal, Hauschka (1955) suggested the alternative explanations that (1) male skin grafts require an *androgenic* milieu for their sustenance and (2) male cells possess an antigen determined by genes on the "non-pairing short segment" of the Y chromosome. According to the latter scheme, successful male-to-female grafts (as in many A strain mice; see Table 1-1) could result from crossing-over between X and Y chromosomes. Hauschka pointed out that the alternatives could be tested readily, and indeed the discovery of male-to-female incompatibility and a possible Y-linked histocompatibility gene stimulated considerable research in the budding new field of transplantation immunology that had previously been devoted largely to the study of histocompatibility loci such as H-2.

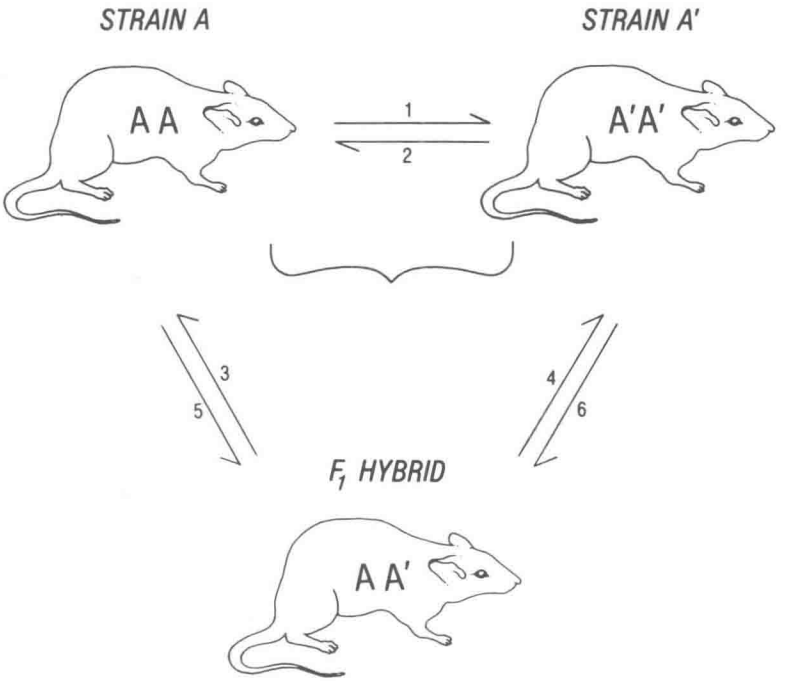

Figure 1-1. *The laws of transplantation. Consider two inbred strains of mice homozygous for alternative histocompatibility alleles, A and A'. Because histocompatibility genes are codominant, the alternative alleles each should be expressed in the F_1 hybrid generation, which is produced by mating members of the two strains. Grafts exchanged between members of the parental strains (arrows 1 and 2) are rejected because the alleles determine the formation of cell surface transplantation antigens that are foreign in the host. Grafts from the F_1 to members of either parental strain (arrows 3 and 4) are rejected for the same reason. Grafts from members of either parental strain are not rejected in F_1 hybrid recipients (arrows 5 and 6).*

In early experiments designed to test the effect of hormones on rejection of male skin *isografts,* female mice of the rejector strain C57BL were castrated and exposed to subcutaneous injections of testosterone proprionate before, during, and after transplantation. The protocol of hormone administration did not seem to significantly influence survival of the male skin grafts, but the results were not clear-cut, and male graft survival may have been extended in some of the treated females (Eichwald et al., 1957; 1958; and see the following discussion).

In other experiments designed to test the immunologic nature