# Genetic and Postgenetic Sex Determination

By E. WITSCHI*

Recent discoveries in the field of human sexuality are reviving the interest in the mechanisms which bring about male and female differentiation. As in the discussions which at the beginning of the century followed the discoveries of sex chromosomes and of genetic mechanisms of sex differentiation, there appears again a danger of misunderstanding the actual significance of the genetic factor. Even in organisms that have not been available for analysis by conventional heterozygoty methods, it must be assumed that sex morphology and physiology are based in an essential way on genetic constitution. Thus, in the hermaphrodite freshwater snail *Valvata* (Fig. 1a) the formation of ovarian cortex and testicular medulla certainly is hereditarily arranged for. However, when in the course of embryonic development cortical and medullary territories become established, the differentiation is not brought about by any sort of genic segregation. Primary germ cells become eggs or sperms according to their location in one or the other territory. Obviously, such a hermaphrodite may become a male by temporary or permanent inactivation of the cortex, or a female by deficiency of the medulla. Gonochorism of this type is widespread among invertebrates. For instance, in *Crepidula* testicular maturation occurs during the early phase of life; the small young snail is a male. Later testicles and male secondary sex characters regress, following which ovarian development changes the growing individual into a female. Predominance of testicular or ovarian induction seems to be determined mainly by internal milieu conditions correlated with aging processes. In other invertebrates and even in some marine fishes external factors, such as ectoparasitism on an adult female as against unattached larval development, decide over realization of male or female differentiation.

While in these primitive hermaphrodites and gonochorists activation and suppression of inherent potentialities of sexual differentiation depend on a variety of milieu conditions, in other instances the same decisions are under genetic control. In the typically monoecious (hermaphrodite) maize plant two recessive gene mutations were found which suppress, one the formation of female ears (ba ba), the other the differentiation

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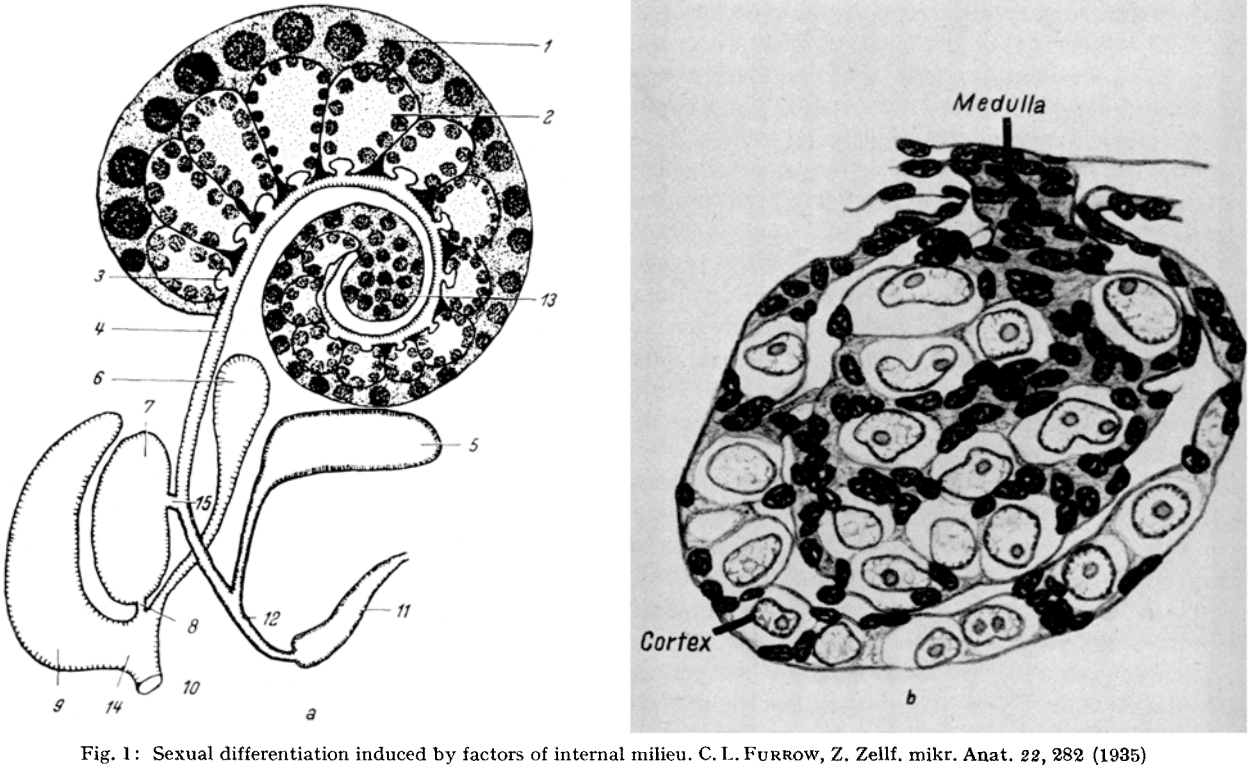


Fig. 1: Sexual differentiation induced by factors of internal milieu. C. L. FURROW, Z. Zellf. mikr. Anat. *22*, 282 (1935)

*a* Hermaphrodite sex organs of the snail *Valvata tricarinata* (after Furrow); 1. cortex (female), 2. medulla (male), 3. atrium. 4. hermaphrodite duct, 5. Prostate gland, 6. albumen gland, 7. bursa copulatrix, 8. oviduct, 9. shell gland, 10. female genital aperture, 11. Penis, 12. sperm duct (vas deferens), 13. apical zone, 14. vestibule, 15. oviduct. *b* Cross section of amphibian gonad immediately before sexual differentiation

of male flowers (ts ts). By proper combination of stock, homozygous, and heterozygous for these two mutations, a self-perpetuating dioecious (gonochoristic) maize can be synthesized. Evidently this case is not a replica of the usual course of evolution of genetic sex determination; however, it serves to demonstrate that gene mutations bearing on the development of reproductive organs may also become factors of hereditray sex determination.

The comparative study of mechanisms of genetic sex determination in tetrapod vertebrates leads to the conclusion that their origin dates back to the Jurassic period, an estimated 150 million years ago (WITSCHI[1]). It is safe to assume that evolution started with a number of small gene mutations, leading to conditions similar to those now existent in fishes like Xiphophorus, Oryzias, and Lebistes. It is a fascinating problem how from such beginnings the heteromorphic sex chromosomes and the sex chromatin of mammals might have derived. The near future should provide possibilities for the experimental study of these phenomena.

Unfortunately most animal species favorable for genetic analysis do not equally lend themselves for embryologic and endocrine studies. This is particularly true for *Lymantria* and *Drosophila*, which served GOLDSCHMIDT and BRIDGES in the establishment of their concepts of *quantitative genic balance*. In Drosophila work on translocation of pieces of chromosomes has permitted to localize feminizing factors in the X chromosome (DOBZHANSKY and SCHULTZ[2]; PATTERSON, STONE, BEDICHEK[3]) and masculinizing factors in the third chromosome (PIPKIN[4]). BRIDGES' term of 'genic balance' seems to imply a direct interaction between female and male determining genes; but considering their localization in separate chromosomes this seems hardly acceptable. GOLDSCHMIDT in many of his writings suggested some interaction between gene-substances, graphically expressed as a competitive race toward gaining control of developmental processes.

The study of the tetrapod vertebrates proves that the competition is not one between genes but between the cortical and medullary inductors of sexual differentiation. Germ cells – whether of female or male genic constitution – differentiate into eggs under the influence of the cortex and into sperms under the influence of the medulla. Gonia lost outside the gonadal territory always remain sexually undifferentiated (WITSCHI[5–7]). The quantitative rate of early development of cortex and medulla is genetically controlled. However, decisive for the outcome of the competition between the two inductors becomes the fact that they act as a pair of antagonists, each tending to suppress the other (Witschi[8]). In summary, genic control of sex differentiation in higher vertebrates assures the prevalence of one or the other member of a pair of antagonistic inductors. The cross section Figure 1b, through the gonad of an amphibian larva just undergoing sexual differentiation, presents a condition which, temporarily, is identical with that of the hermaphrodite gland of *Valvata*. It shows medulla and cortex, each with a number of yet undifferentiated gonia. The relative strength of the medulla reveals that the specimen is a genetic male. Hence, germ cell development in the cortex already seems doomed.

Since now we realize that the decision on male or female differentiation actually depends on a balance of inductors rather than genes, the question arises whether a strict and necessary harmony always exists between the two systems, or whether under special circumstances, genetic and postgenetic determination may become contradictory. Simple evidence of 'sex reversal', that is of differentiation contrary to the genetically predetermined pattern, gives no sufficient answer unless supplemented by definite proof that the genic constitution has remained unchanged.

The most complete and extensive proof of the possibility and actual occurrence of sex reversal without genic readjustment is furnished by the case of continuous breeding of purely homozygous, male (ZZ) stock of *Xenopus laevis*. In 1950[7] the writer reported that treatment of larvae with small doses of estradiol results in complete feminization of the genetic males (193 ♀ + 0 ♂, as against 43 ♀ + 52 ♂ in control group). Breeding of sex reversed males (♀ ZZ) with fraternal males (♂ ZZ) gives entirely male offsprings (CHANG and WITSCHI[9,10]). However, by treating any chosen number of eggs (genetically all ZZ) with estradiol, one obtains the homozygous females for the perpetual propagation of this stock (Fig. 2). So far this experiment has been carried on through four generations. The chromosome number remains constant (36 in the diploid cells). The question of what may be the possible effects on sex chromosomes that are repeatedly carried through individuals of contrary sex expression gains interest through attempts by the author[11] and possibly also OHNO[12] to explain the heterochromia of BARR's sex chromatin on the basis of genetic inactivity.

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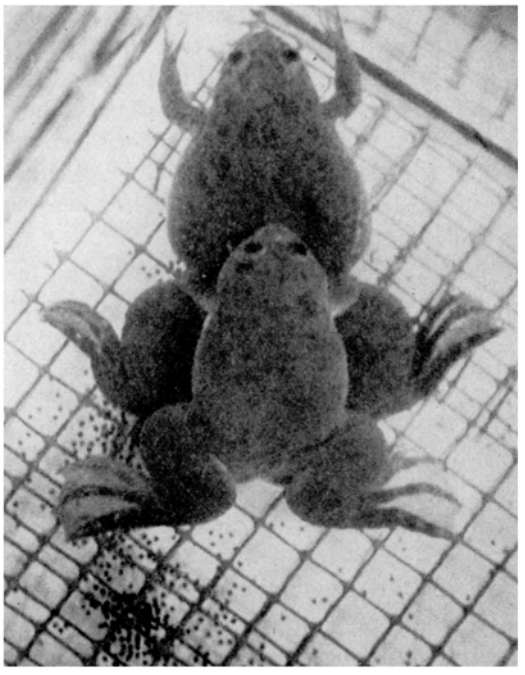

Fig. 2: Mating pair of *Xenopus*, consisting of a male (ZZ) and a sex-reversed ZZ individual now functioning as a female. The deposited and fertilized eggs are all of male (ZZ) constitution (phot. by K. MIKAMO)

Only a few additional cases of sex reversal are adequately supplied with proof of unaltered genic and chromosomal constitution. Two adult hermaphrodite frogs, bred with normal males and females, brought evidence of female genic constitution (XX), both in the ovarian and the testicular parts of their gonads (WITSCHI[13]). On the other hand, well known experiments by PONSE[14] establish the genetic maleness (ZZ) of both testicular and cortical parts of the composite sex glands of male toads. HUMPHREY[15] by testicular implants transforms female salamanders (*Ambystoma*) into males, and progeny tests prove persistence of the female genotype (ZW). GALLIEN[16] in the newt caused genetic males to differentiate as females by the administration of estrogenic hormones; their constitution remained unchanged (ZZ) as revealed in breeding tests. Finally MILLER[17] and WITSCHI[11] showed that in the hen, sex reversed by ovariotomy, spermatogenesis proceeds normally with unchanged female chromosomal arrangements (ZO, Fig. 3).

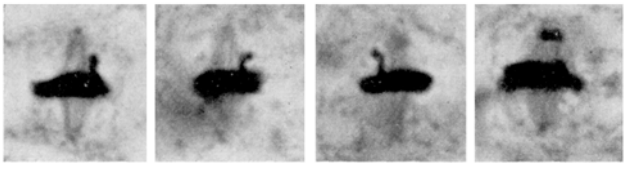

Fig. 3: Four spermatocytes from the testis of an ovariotomized hen showing precocious movement of single Z chromosome to one pole in anaphase of first meiotic division

It will be noticed that five of the six presented examples refer to amphibian species; but the case of the sex reversed hen is particularly valuable because it serves as unequivocal proof that sex reversal without chromosomal compensation is possible even in the class with the most highly specialized chromosome type.

This short review leads to a number of conclusions of fundamental value. (1) The sexual differentiation of primordial germ cells is directly controlled by induction. (2) Induction may arise from external or internal milieu conditions. In gonochorists it assumes also antagonistic qualities. (3) In tetrapod vertebrates genetic mechanisms gain control of the development of the inductors and thereby, indirectly, of the sexual differentiation of the gonads. (4) However, there remain possibilities of postgenetic interference with the course of gene-initiated chain reactions. (5) In all sufficiently analyzed cases, deviations from the genetically indicated sex have been brought about by inhibition or elimination of the epistatic inductor. This may result in near or fully complete sterility. (6) In some instances the hypostatic inductor, having been relieved of its antagonist, responds with a compensatory development that may lead to partial or complete sex reversal.

It seemed desirable to review and reaffirm the evidence for postgenetic sex determination before entering on the discussion of recent discoveries bearing on abnormal sex development in man. During the last three years a number of French[19] and British[20–30] investigators have published a series of reports on unusual chromosome numbers which tend to be associated

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with abnormalities of various degenerative types. Attempts to interpret them in the light of the classical findings in polyploid and heteroploid *Drosophila* flies soon became complicated by the realization that the very type of genetic sex differentiation in mammals may not be the same as in Drosophila (RUSSELL *et al.*[31]; WELSHONS and RUSSELL[32]). More serious is the inconstancy of relationship between chromosomal and morphologic aberrations. In the Table the known viable chromosome combinations in man are summarized, and listed together with the physical types of their bearers.

Mongolism is the best established aberrant type. Chromosomally it consists of triplo-22 males and females (Fig. 4). Over 30 cases have been adequately studied, cytologically; however little information is available about their sexual development and relative fertility. In other groups only 1 to 4 cases have been described (not taking into account the normal XX females and XY males). Manifestations of sexual abnormality are irregularly distributed and show no simple relationship to chromosomal patterns. An XX individual may be not only a normal female, but also a Klinefelter (pseudomale), an agonadal Turner, or a true hermaphrodite. A similarly wide range is found in the XY group; it includes also the case of a woman who apparently is a completely sex reversed genetic male (NILSSON *et al.*[30]). Or, viewed from the other side, we note that the Klinefelter syndrome appears under five different chromosomal groups (XXY, XX, XXY–XX, XY, XXY + 22).

Some remarkable results were obtained from studies on the inheritance of color blindness. In three chromatin-positive Klinefelter cases (XX or XXY?) NOWAKOWSKI *et al.*[33] find evidence that both X chromosomes are derived from the mother. It is not surprising that similar studies with 4 chromatin-negative Turner cases (XO or XY?) show that the single X is of maternal origin. Recently GRUMBACH *et al.*[25] reported on a chromatin-positive XO case. On the basis of OHNO's contention that it is particularly the paternal X that produces the sex-chromatin body, it would appear that in GRUMBACH's case the X was of paternal origin.

How is it possible to interpret the remarkable diversity of chromosomal arrangements and morphologic manifestations presented by this relatively still very small number of cases? In two previous publications that were written without knowledge of chromosomal aberrations (WITSCHI[18,34]) it was shown that the peculiarities of the Turner and Klinefelter syndromes can be understood on common principles of developmental physiology. Experiments with amphibian eggs (WITSCHI[35,36]) show that overripeness (by delayed fertilization) or exposure to $CO_2$ result in stepwise deterioration of the developmental capacities of the eggs. As a consequence one observes among others the following ab-

*List of viable chromosomal types in man* with correlated types of sexual and somatic manifestations. Based on data by BAIKIE[20], FORD[21–24], GRUMBACH[25], HARNDEN[26,27], HUNGERFORD[28], JACOBS[29], NILSSON[30] *et al.*, unpublished material of ZELLWEGER, MIKAMO, and WITSCHI, and personal communications by Dr. C. E. FORD

| *Chromosomes* | | *Manifestations* (in parentheses: number of reported cases) * primary amenorrhea; ** secondary amenorrhea; + chromatin positive; – chromatin negative |
|---|---|---|
| number | pattern m mosaic | |
| 47 | XXX | + Super female (1)** |
| 47 | XXY | + Klinefelter (4) |
| 46 | XX | + Klinefelter (1); + Turner (1)*; + True hermaphrodite (2); + *Normal female* |
| 46–47 | XXY–XX$^m$ | + Klinefelter (1) |
| 46 | XY | – Klinefelter (4); – Turner (1)*; Female; – *Normal male* |
| 45 | XO | – Turner (4)*; + Turner (1)* |
| 45–46 | XX–XO$^m$ | + Turner (3 or 4)* |
| 47 | XX + 22$^{nd}$ | + Mongolism, female (5) |
| 47 | XY + 22$^{nd}$ | – Mongolism, male (6) |
| 48 | XXY + 22$^{nd}$ | + Klinefelter and mongolism (1) |
| 46–50 | irregular | Leukemia patients |

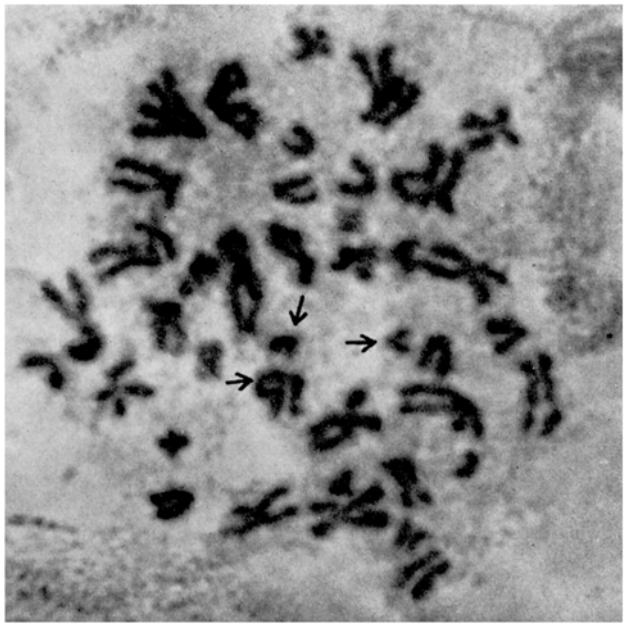

Fig. 4: Dividing bone-marrow cell of baby girl (possibly mongoloid) with 47 chromosomes; arrows point to the three chromosomes of next to smallest size; × 2600 (ZELLWEGER, MIKAMO, WITSCHI unpubl.) *Addition at time of proofreading:* Study of the parents indicates now that the father has 46 and the mother 48 (47-49) chromsomes. Both parents are mentally normal individuals; the development of the child is under observation

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normalities: Irregularity and incompleteness of gastrulation, defective development of rostral parts of the embryo, twinning, polymelia, and other defects of limb development, cellular pathology, reduction in number and quality of the germ cells, dysgenesis of the sex glands, and masculinization of genetic females. It is evident that the abnormalities produced in these experiments duplicate not only the peculiar germinal and somatic traits of the Turner and Klinefelter syndromes and of true hermaphrodites but also the characteristic features of human mongolism.

In the author's earlier work not much attention had been given to chromosomal conditions in embryos from overripe cells. Only the occurrence of gross abnormalities in highly damaged eggs had been noted. In the meanwhile BEETSCHEN[37] has reported on extensive irregularities in chromosome distribution in blastulae derived from overripe salamander eggs. A study of overripe rat eggs is now in progress in our laboratory. Overripeness is obtained by delayed ovulation induced in constant estrus females. One of the first eggs examined showed non-disjunction of one diad in the second meiotic division (Fig. 5). If permitted to develop further, this egg should have retained either two elements or none of this chromosome type. This observation in itself is sufficient to explain the origin of the triplo-22 condition in mongoloids, the presence of two maternal X chromosomes in colorblind, chromatin-positive Klinefelter cases and of the chromatin-positive XO Turner case that apparently received no maternal sex chromosome. Moreover, the observations by BEETSCHEN parallel the reports by FORD *et al.* on XXY–XX and of XX–XO mosaics. Both indicate irregularities of chromosome distribution during cleavage. That pathology of cell development may lead to chromosomal abnormalities is well known to students of cancer cells, and is reaffirmed again by chromosome studies on human leukemia (BAIKIE[20] *et al.*), which reveal increases in chromosome numbers up to 50, particularly in cells of bone marrow.

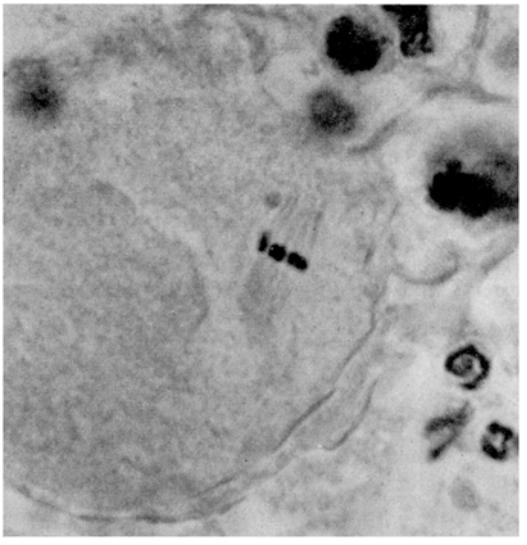

Fig. 5: Second polar spindle of a damaged unfertilized Rat egg. One diad (slightly out of focus) moves undivided to the upper pole; × 2000

Summing up old and new evidence and thought about the etiology of human sex deviations that are characterized by primary germinal dysgenesis and by frequent association with degenerative somatic malformations, the following generalizations may now be arrived at:

(1) The primary cause is a degenerative modification of the egg or the early germ which in itself is sufficient to explain gonad dysgenesis and teratologic development in general.

(2) The same original damage may also become the cause of chromosomal aberrations most of which seem to be lethal. The non-lethal ones probably exert some modifying influences on the course of sexual and somatic development in accordance with their genic value.

This interpretation which applies also to several other types of abnormal development, including monozygotic twinning, is compatible with the established fact that the frequency of most non-hereditary types of malformations shows a small increase among children of very young mothers and a more definite one in those from mothers older than 40. This may well be a consequence of difficulties in the ovulation process, resulting in delayed release of the egg from its follicle. At any rate, age dependency points to physiologic rather than pure chance determination of these abnormalities.

While it is concluded that degenerative types of sex deviation and somatic malformation have a postgenetic origin, evidently all cases which suffer chromosomal aberrations of any kind thereby acquire changes in their genetic constitution. Hereditary transfer of extra chromosomes may possibly become available for study in offsprings from mongoloid parents. However, sterility of the involved types of sexual abnormality precludes the further genetic analysis of this class.

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*Zusammenfassung*

Die relative Bedeutung genetischer und postgenetischer Faktoren der Geschlechtsbestimmung wird diskutiert. Nach einer Übersicht über Fälle von experimenteller Geschlechtsumkehr bei Wirbeltieren, die alle ohne genetische Regulation bleiben, werden die degenerativen Typen von Geschlechtsabnormalitäten beim Menschen kritisch bewertet. Es wird angenommen, dass in den sogenannten Turner- und Klinefelter-Syndromen die somatischen, genitalen, und chromosomalen Aberrationen wahrscheinlich gemeisam durch Keimschädigung erzeugt werden.

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