THE FOURTH LAWSON WILKINS MEMORIAL LECTURE

INTRODUCTION

Through generous gifts from the colleagues, the pupils and the friends of the late Dr. Lawson Wilkins, it has been possible to establish a distinguished lectureship designed to commemorate his great contribution to pediatric endocrinology. On behalf of Drs. Robert Blizzard, Avionam Kowarski, John Money and Robert Schultz, my colleagues at the Pediatric Endocrine Clinic, it is my privilege and pleasure to welcome you to The Fourth Lawson Wilkins Memorial Lecture.

In 1876, John Shaw Billings stated in his report on The Johns Hopkins Hospital: "This hospital shall advance our knowledge of the causes, symptoms and pathogenesis of disease and methods of treatment so that its good work shall not be confined to the city of Baltimore or to the State of Maryland, but shall in part consist in furnishing more knowledge of disease and more power to control it for the benefit of the sick and afflicted of all countries and of all future times." Dr. Wilkins fulfilled well this dream of the founders. He was a great clinician, who was never content with the present state of the art. He had a tremendous inner drive which compelled him to seek a better understanding of disease and hence, a better means to treat it. Also he had the need to share and discuss his ideas with his pupils who in this process became an extension of himself. His spiritual children are now carrying his tradition in many parts of the United States and the world. Many of them have returned to Baltimore today, to honor the memory of Dr. Wilkins.

Schools of medicine are presently reconsidering their overall role in public health. Although they are centers of teaching and learning, it is proper that they would consider the social problems of medicine and the way to solve them. It is our hope that, in the process of determining the reforms required by our changing times, medical institutions will take excellence instead of expediency as their goal. The example of the dedication to scholarly pursuits exhibited by Wilkins and many of our forefathers should be the basis for changes.

Excellence as an ideal brings us to our guest speaker. Professor Alfred Jost is Head of the Laboratory of Comparative Physiology, Faculty of Sciences, University of Paris, France. Professor Jost is a leader in the field of developmental endocrinology and its effect on fetal metabolism. He is a pioneer in the investigation of the embryological development of sex organs in mammals. He has contributed particularly to the delineation of the role of genetic and hormonal factors in sex differentiation. Now that most of us think that we understand the intricacies of fetal sex development, Professor Jost comes to remind us that the scientific truth of yesterday needs to be modified today and then again tomorrow, and that the labors of research have no end. In terminating this introduction I would like to express our deep appreciation to our distinguished lecturer for his willingness to give The Fourth Lawson Wilkins Lecture which is entitled: "A New Look at the Mechanisms Controlling Sex Differentiation in Mammals."

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A NEW LOOK AT THE MECHANISMS CONTROLLING SEX DIFFERENTIATION IN MAMMALS[1]

Alfred Jost

Today the memory of Lawson Wilkins will be celebrated by someone who did not have the privilege of being one of his pupils. I never worked under Dr. Wilkins at The Johns Hopkins Hospital, and I actually visited with him in Baltimore on only a very few occasions. On my last visit I saw him as a patient, a somewhat restless patient, in the hospital, after his car accident in 1960. My acquaintance with Dr. Lawson Wilkins and my admiration for him began in 1949, and since then it has become continually deeper and more affectionate. I have a vivid recollection of the time I met him that June. This first contact was very typical of him and had long-lasting consequences for both of us. I wish to recall it briefly.

MEETING LAWSON WILKINS

In 1947, I completed experiments on rabbit fetuses showing that early castration in utero resulted in feminine development of the whole body, whatever the genetic sex of the fetus (10-12). I concluded one of my first reports of these experiments, with a parallel between the cases of so-called *ovarian agenesis* in gonadless, feminine-looking patients and castrated rabbits, and I naively suggested that "if the results observed on rabbits are valid for humans, it seems that the soma of a genetically masculine, but agonadic human being, should present a feminine morphology" (11).

The next year I was invited to participate in the First Mexican Congress of Gynecology and Obstetrics to be held in Mexico City, in May 1949. It may be of interest for you to learn how I happened to receive an invitation to speak at that meeting. My brother, Dr. Marc Jost, lived in Mexico City, was familiar with my experiments and had shown reprints to some of his friends who became the organizers of the meeting. For such a gathering of many clinicians it seemed fit to discuss the clinical implications of the rabbit experiments, and for many weeks I reviewed the medical literature. Without any other clinical experience I constructed and presented an interpretative scheme for the feminine features in cases of gonadal agenesis, or Turner's syndrome, and in male pseudohermaphroditism (13).

On my return from Mexico, I visited several eminent American experts in the field of sex differentiation in animals. The first stop was in Baltimore, where I was to meet world-famous scientists at the Department of Biology of The Johns Hopkins University and at the Department of Embryology of the Carnegie Institution of Washington. Dr.

[1]The Lawson Wilkins Memorial Lecture was given in the Turner Auditorium, April 26, 1971 by Prof.

Robert K. Burns an Associate in Embryology was very friendly to me during this visit, and he suggested that I should meet at The Johns Hopkins Hospital a clinician by the name of Dr. Lawson Wilkins, who was interested in problems of human genital anomalies. Dr. Burns made the appointment for the early afternoon, and thus I was introduced to Lawson Wilkins. He was 55, I was 33; he was a well-known clinician, I was not even a doctor of medicine. He calmly and patiently followed my description of the rabbit experiments, looked at the rabbit pictures, asked many pertinent questions, and listened to the interpretations proposed for human anomalies. Then he submitted me to a keen clinical examination. I had to comment on the illustrations and reports concerning clinical cases. I thus had the privilege of being among the very first ones who saw the cases later to appear in the first edition of "The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence" (33). But not only did I have to look at pictures, I was asked for interpretations which were coldly evaluated and screened. Also I was introduced to extremely important clinical experiments, for instance those concerning the absence of sensitivity to androgens of "hairless women with testes."

The very serious and positive discussion, free of any superfluous verbal ornament, went far into the afternoon and finally Dr. Wilkins concluded "I'm convinced." These few words from him were very important; they opened a long era of friendship and fruitful discussions. Lawson Wilkins adopted the new views in his book and he quoted my Mexico paper (33). From then on, he developed and strengthened the new interpretation in beautifully elaborated clinical investigations reported in 1955 with Melvin M. Grumback, Judson J. Van Wyk (8) and later with Howard W. Jones, Jr. and with many others whom you well know.

The aspects of sexual differentiation, normal or abnormal, which I discussed with Lawson Wilkins concerned the hormonal factors controlling the differentiation of the genital tract rather than the mechanisms governing sex differentiation of the gonad itself or its structure. Nevertheless, in April 1961, in a letter announcing his next visit to Paris, Dr. Wilkins asked for my "critical opinion" on an important paper, prepared with Drs. C. Bergada, W. W. Cleaveland and Howard W. Jones, Jr., concerning gonadal histology in 81 patients with male pseudohermaphroditism or testicular dysgenesis, which would appear in 1962 in Acta Endocrinologica (1). I shall refer to that paper later.

More recently I myself became interested in problems of gonadal differentiation in mammals (19-21). With Vigier and Prepin, we also studied again the old question of sex anomalies in freemartins in cattle. I should like to have a new look, or at least a glance, at the whole process of sexual differentiation of the mammalian fetus and to re-evaluate some old problems.

A GLANCE AT MECHANISMS CONTROLLING SEX DIFFERENTIATION

Please forgive me if I start the whole story at its beginning and if I recall very schematically questions with which you are familiar. I intend to have a free look at some classical notions.

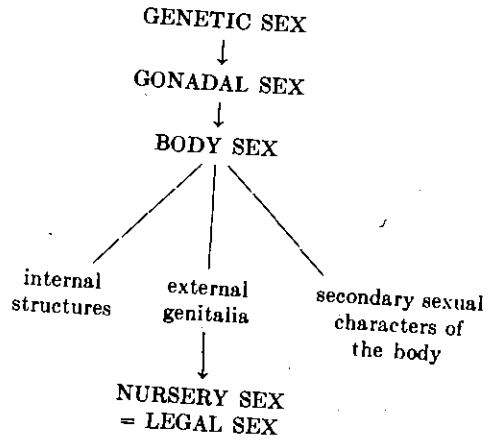


Fig 1. Chain of events in morphological sexual differentiation (15).

other body sex characters differentiate progressively. Under normal circumstances, *gonadal sex* and *body sex* develop in agreement with *genetic sex*. Since 1959 exceedingly interesting advances have been made in the study of sex chromosomes and of chromosomal anomalies in animals and in humans. This important field will not be discussed in the present paper.

It has long been recognized that during organogenesis, the gonads and each of the other sex structures first appear as undifferentiated primordia which appear identical in both sexes. Then divergent developmental patterns permit male and female organs to differentiate. In adulthood the testes and the ovaries play a symmetrical role in releasing germ cells and hormones. Moreover, in the late 1930's it was discovered experimentally that, in mammalian fetuses, androgens masculinize and estrogens feminize parts of the genital tract, such as the external genitalia or the urogenital sinus.

For all these reasons early theories explaining the mechanisms controlling sex differentiation suggested that discrete, but essentially similar or symmetrical mechanisms were responsible for male and for female differentiation. A dual system of competing embryonic components was thought to control gonadal sex differentiation, and a dual system of testicular and ovarian hormones was assumed to control the differentiation of the body sex (the bihormonal theory entertained in 1913 by Steinach (32) and in 1944 by Greene (7). Such interpretations are summarized in Figure 2. Later the experimental study of the hormonal control of body sex differentiation has invalidated the lower part of the scheme. The relevant data should be recalled rapidly before discussing the problem of the gonadal sex.

Differentiation of the *Body Sex:*

In the first part of this paper I mentioned experiments made a long time ago on rabbit fetuses (10-12, 14). When male or female rabbit fetuses were surgically castrated in utero before the onset of body sex differentiation, they uniformly acquired a feminine genital tract, whatever their genetic sex (Fig 3). This indicated that the fetal ovary is a dispensable organ for feminine organogenesis, and that the fetal testis prevents the genital tract from becoming feminine and imposes masculinity. The fetal testis plays this dual

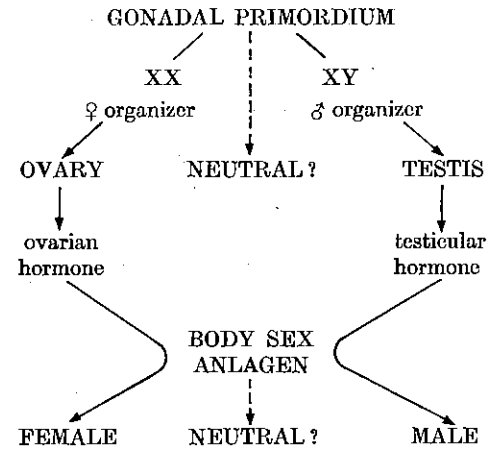


Fig 2. Theoretical scheme summarizing the concept of symmetrical mechanisms in the sex differentiation of the gonad and of the body sex. The hypothetical "neutral" condition remains undefined (21).

role in repressing the *presumptive* female ducts (Müllerian ducts) and in masculinizing the other parts of the genital tract (this involves the incorporation of the mesonephric or Wolffian system into the male genital sphere). These results were confirmed in vivo in 1947 by Raynaud and Frilley (30) working on mice and later in vitro on explanted parts of the rat fetal genital tract (22, 23, 29). The feminine features of patients suffering from gonadal agenesis or dysgenesis is further confirmative evidence. In that connection it is noteworthy that, in humans, feminine features develop whenever testes are absent and whatever the chromosomal constitution of the individual.

The testis thus appears as the sex differentiator and two aspects concerning this role deserve some further comment: 1) A detailed study of the effects of the fetal testis on the genital tract suggested that it probably produces two kinds of secretions, namely, a Müllerian inhibitor and a masculinizing hormone (14-16). The repressing effect of the former on the Müllerian ducts has never been duplicated by steroid androgens, whereas these androgens easily duplicate the masculinizing potency of the fetal testis on the fetal structures (Fig 4). Another interesting observation will be quoted, dealing with the effects on the rabbit fetus of the antiandrogen, cyproterone acetate. This drug opposes the effects of androgens at the level of the target organs. When it is given to pregnant rabbits, many male fetuses are profoundly abnormal (3, 17): the masculine sex characters are absent; their development is prevented by the antiandrogen; the Müllerian ducts are also absent, a fact which suggests that the testicular Müllerian inhibitor has not been opposed by the antiandrogen. The condition of the genital tract resembles that found in cases of so-called "testicular feminization," due to insensitivity to androgens, as Lawson Wilkins (33) had foreseen, "hairless women with testes". 2) The testis also imposes both prenatally or neonatally effects upon the nervous system which become manifest only in adulthood. A permanent "masculinization" of neural structures mediating sex behavior is produced by the testis of fetal guinea pig (28) or neonatal rat (9). Similarly the male pattern of hypothalamic control of the hypophyseal gonadostimulating activity depends upon the neonatal testes in the rat (9). In females or in neonatally-castrated rats the female pattern

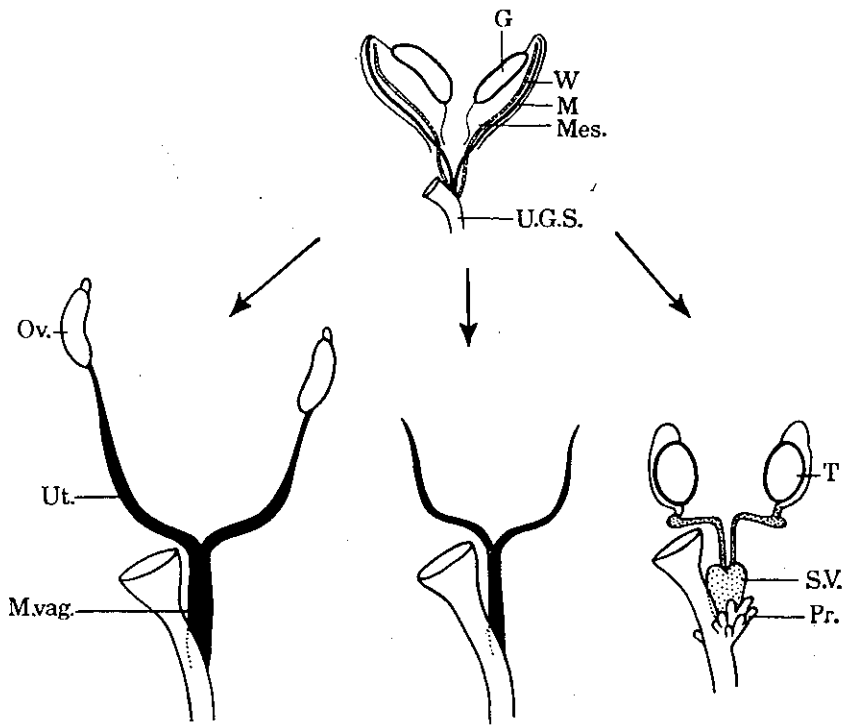


Fig 3. Schematic presentation of sexual differentiation of the sex ducts in the rabbit fetus. From the undifferentiated condition (top) may arise either the female structure (bottom left), or the male structure (bottom right) or the gonadless feminine structure in castrated fetuses of either sex (bottom middle). G., gonad; M., Müllerian duct; Mes., mesonephros; M. vag., Müllerian vagina; Ov., ovary; Pr., prostate; S.V., seminal vesicle; T., testis; U.G.S., urogenital sinus; Ut., uterine horn; W., Wolffian duct (stippled). After Jost: Mem. Soc. Endocrinol. No. 7, 49-61 (1960).

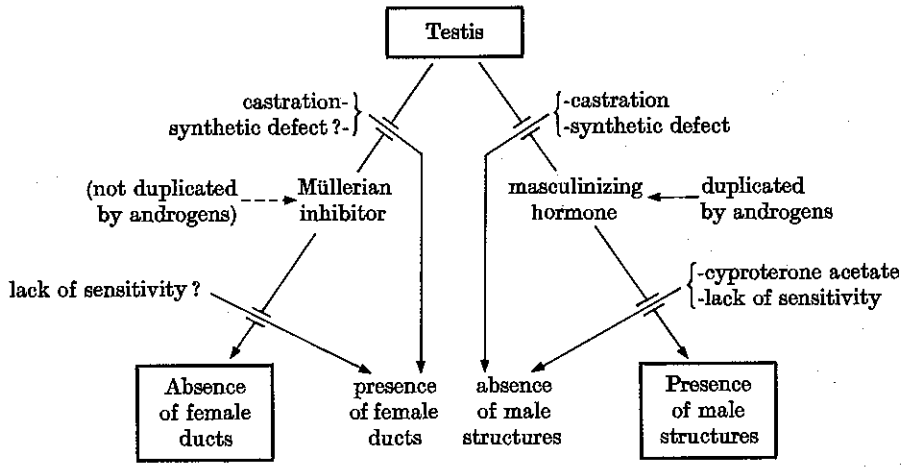


Fig 4. Scheme summarizing the testicular control of the differentiation of the sex ducts and sex

All the observations pertaining to body sex obey the same scheme. Female features develop in every individual in the absence of testes or in the presence of ovaries. In males the testes impose masculinity during an early and critical phase of development and forbid female features to appear. This is schematized in Figure 5 which modifies Figure 2 for *body sex*, but not for *gonadal sex*.

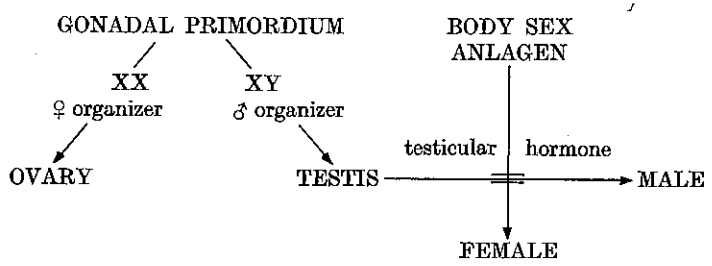


Fig 5. Theoretical scheme showing how the part of Figure 2 concerning the differentiation of the body sex characters should be modified after experimental studies (21).

Differentiation of the *Gonadal Sex:*

The differentiation of a testis or an ovary is classically described as resulting from the development of discrete components of the gonadal primordium. A century ago the concept was introduced that in the mammalian fetus the so-called *germinative epithelium* covering the early gonadal primordium proliferates *sex cords* into the underlaying mesenchyme (Fig 6). A first set of these cords is assumed to have testicular potentialities. The cords become the future seminiferous tubules in males while in females they are pushed in the hilar part of the ovary (medullary cords) where a new set of sex cords proliferates from the germinative epithelium. These cortical cords, or Pflüger's cords, differentiate into the ovarian cortex. It should be noted that the proliferation of sex cords from the germinative epithelium has never been demonstrated experimentally by studies of mitotic rates or so on. In some animals successive proliferations of sex cords have never been observed, for example, in the rat. In other cases the formation of the so-called cortical cords is controversial (4).

In his classical work on amphibians, begun in 1914, Witschi (36) introduced another concept of gonadal sex differentiation. In the undifferentiated gonad of these animals he recognized two components of opposing significance (Fig 7): 1) the internal medulla made of small cells and which has a potential testicular outlook, and 2) the superficial cortex which first contains the primordial germ cells beneath the coelomic epithelium and which has a potential ovarian outlook. Witschi (34-36) hypothesized that the two gonadal components are antagonistic and secrete opposing inductive substances, medullarine and cortexine. The final sex of the gonad results from the prevalence of one inductor over the other. The theory of cortico-medullary antagonism has been extended to the differentiation of the mammalian gonad (35) but in the literature the words cortex and medulla have frequently been given multifarious and somewhat confusing connotations.

It should be emphasized that, according to these views, there is no strict homol-

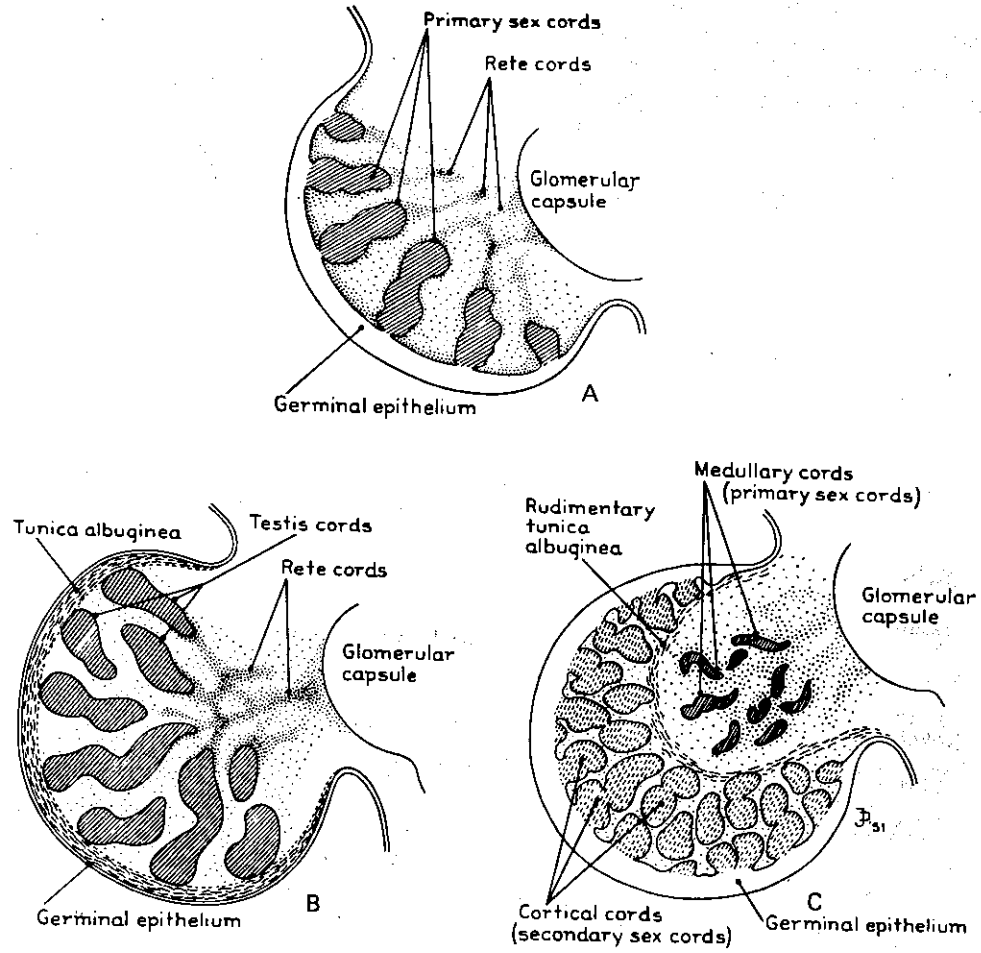


Fig 6. Classical views concerning gonadal differentiation in mammals and other amniotes, as outlined by R. K. Burns (1961). The diagram shows the origin of the medullary and of the cortical cords from the germinal epithelium. A. Gonad at the indifferent stage of sexual differentiation; the primary sex cords represent the male or medullary component, whereas the germinal epithelium represents, potentially, the cortical component. B. Differentiation of a testis consists in the further development of the primary sex cords, and the reduction of the germinal epithelium to a thin, serous membrane, accompanied by development of the tunica albuginea. C. Differentiation of an ovary consists in reduction of the primary sex cords to medullary cords of the ovary, whereas the cortex is formed by continued development of cortical cords from the germinal epithelium.

On the other hand, the concept of a cortico-medullary antagonism in gonadal sex differentiation implies the simultaneous activity of a double set of inductors in the early gonadal primordium. These points are far from evident when one studies histological sections through differentiating gonads of graded ages. It first appears that most of the fixative fluids which were used in the past are unsatisfactory for the watery fetal tissues and that the quality of the histological examination must be improved. I myself looked at

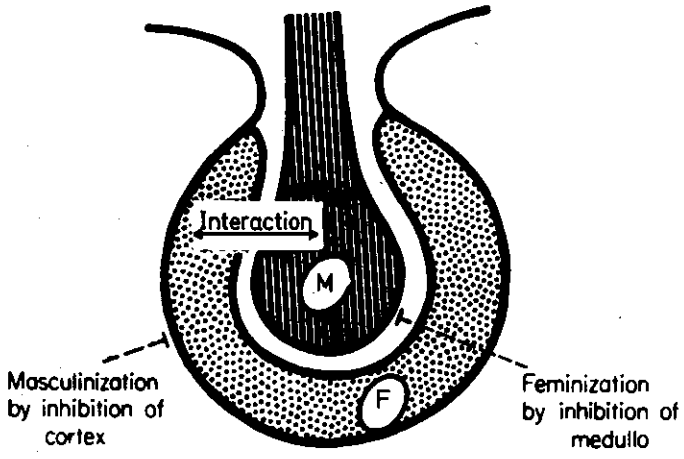


Fig 7. Witschi's interpretation of the sex differentiation of the gonads in amphibians showing the antagonistic cortex and medulla (36).

Some general and major conclusions emerge from such a study. At a definite stage, usually referred to as the stage of gonadal sex differentiation (day 15 in rabbits, stage of 15 to 17 mm in human fetuses), testicular organization rapidly becomes discernible in the male fetuses (early seminiferous tubules) whereas nothing happens in females. It has long been the common experience of many authors, that while the testes differentiate, the *presumptive* ovaries remain undifferentiated, that is, similar to the undifferentiated gonadal primordium. For instance, as Gillman (6) put it, in his study of the human ovary, "actually the gonad is an ovary, not because it has that structure, but rather because it is not a testis." Similar statements were made by many others.

The early and very fast differentiation of the testis must be contrasted with the very late and slow differentiation of the ovary. It is clear from the data concerning the human fetus (Fig 8) that, in males, sex differentiation progresses rapidly during three main developmental phases: 1) beginning differentiation of the seminiferous tubules (stage of 15-17 mm), 2) appearance of interstitial cells (28-30 mm), 2) masculine organogenesis of the genital tract (30-60 mm).

During this whole period of time the presumptive ovaries grow but remain undifferentiated. The cells, which in the testis rapidly became associated and which organized the future seminiferous tubules, continue to multiply in the presumptive ovary, but neither an ovarian nor a testicular organization is realized at this stage. Nothing like medullary cords may be observed at these early stages (Fig 11). Later, the germ cells become more or less tightly grouped in the so-called Pflüger's cords and they enter meiosis. Somewhat later they become surrounded by follicular cells, and finally (approximately 12 weeks after the testicular differentiation started in the human male fetus) an ovary provided with follicles and stroma slowly develops in the female fetuses.

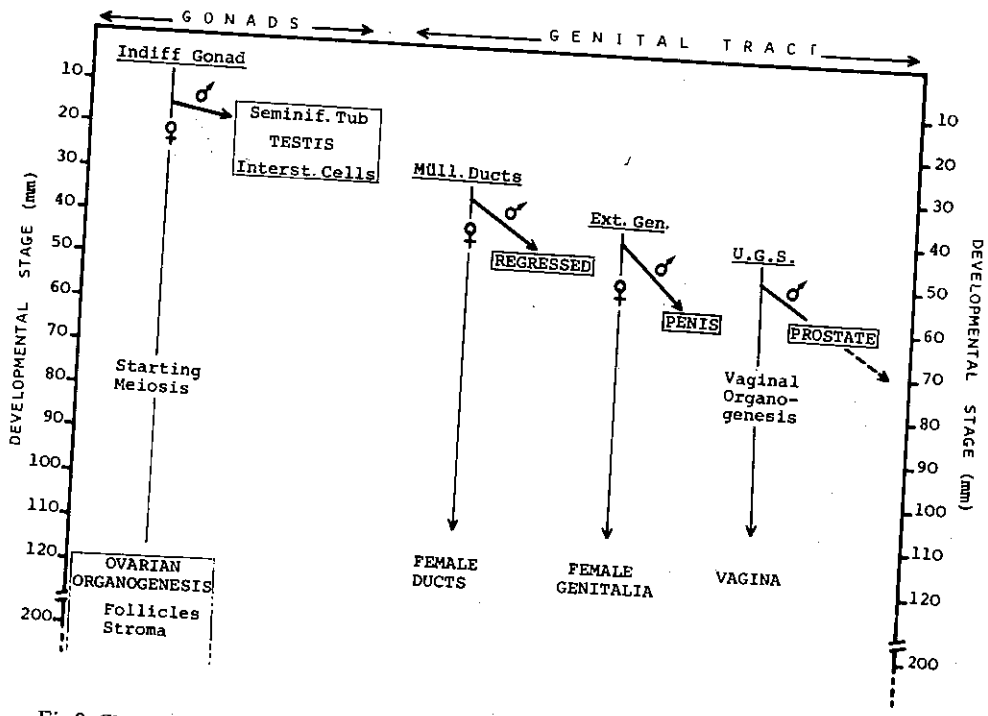


Fig 8. Chronology of sexual differentiation in the human fetus.

testicular triggering mechanisms were allowed to work at a later stage, on the still undifferentiated presumptive ovary, a delayed testicular organization still might be possible[2].

The mechanisms leading to ovarian differentiation in the presumptive ovary also have to be uncovered. It is noteworthy that ovarian structures normally develop after the number of primordial germ cells has reached its maximum, and that these structures fail to develop, or to be maintained when germ cells degenerate at an early stage (31). It is also possible that epithelial-mesenchymal interactions play a role in the differentiation of the ovary as in many other embryonic structures (5).

In the perspective of a cortico-medullary antagonism controlling gonadal sex differentiation, the role of the cortical inductor in females would be essentially to maintain the gonadal primordium undifferentiated for a long time. It could be hypothesized that no cortical inductor at all is at work at that time. In the testis some mechanism triggers the segregation and organization of cells in seminiferous cords at an early stage and leaves no possibility for later ovarian organogenesis. In the absence of this trigger the gonadal primordium slowly becomes an ovary. This working hypothesis is summarized in Figure 9. This scheme fits developmental chronology. If it were shown also to illustrate the basic mechanisms involved, a complete asymmetry in sexual development of both sexes would appear. Feminine organogenesis would result from the absence of the early gonadal masculinizing mechanism.

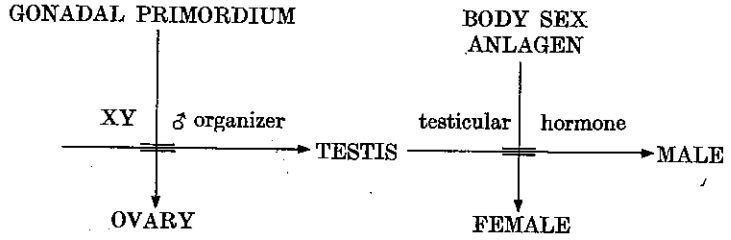


Fig 9. Scheme illustrating the concept of completely asymmetrical processes in sex differentiation: the gonads as well as the body sex would become feminine if not diverted from doing so by a masculinizing mechanism (compare with Figures 2 and 5) (21).

A NEW LOOK AT AN OLD PROBLEM: THE FREEMARTINS IN CATTLE

When two or more twin fetuses develop in a pregnant cow, they become sexually normal if they all belong to the same sex. If at least one male is present in a multiple heterosexual litter, most of the females become sexually abnormal and sterile and they are referred to as freemartins. In the freemartins the ovaries are usually stunted, they may contain sterile seminiferous tubules, the female ducts are inhibited and parts of the male ducts may develop (epididymes, seminal vesicles) but as a rule, the external genitalia remain feminine. Lillie (27) and Keller and Tandler (26) showed that in twin pregnancies in cattle, the fetal membranes and the superficial vessels fuse and the fetuses exchange blood. They proposed that the freemartins are genetic females which become abnormal because during sex differentiation their genital organs are influenced by the testicular hormone produced by their male twin fetus and transferred through the connecting blood channels. More recently, it has been shown that the fetuses exchange blood cells and perhaps other cells, since XY cells have been found in the freemartins. Some experts have expressed the opinion that exchange of cells rather than transfer of hormones is responsible for the freemartin effect. One way or another, fetal hormones still must be involved in the abnormalities of the genital tract of freemartins.

B. Vigier, J. Prepin and I (25) studied in detail the first steps of sex differentiation in freemartins in order to assess the mode of development of the sex anomalies. Cows were superovulated with gonadostimulating hormones and multiple pregnancies were obtained. As a rule, in these cases, the fetal chorions fuse (Fig 10). The fetuses were preserved a known number of days after insemination and we decided to explore the events taking place during the three weeks following the first differentiation of the testes in males, that is, between days 40 and 62. Most of the masculine features appear in normal male fetuses following a definite timetable:

Days 39-40: early testicular differentiation
From day 47 on: increasing ano-genital distance
From day 52 on: starting Müllerian regression
Days 56-58: scrotum, prostate and seminal vesicles appear

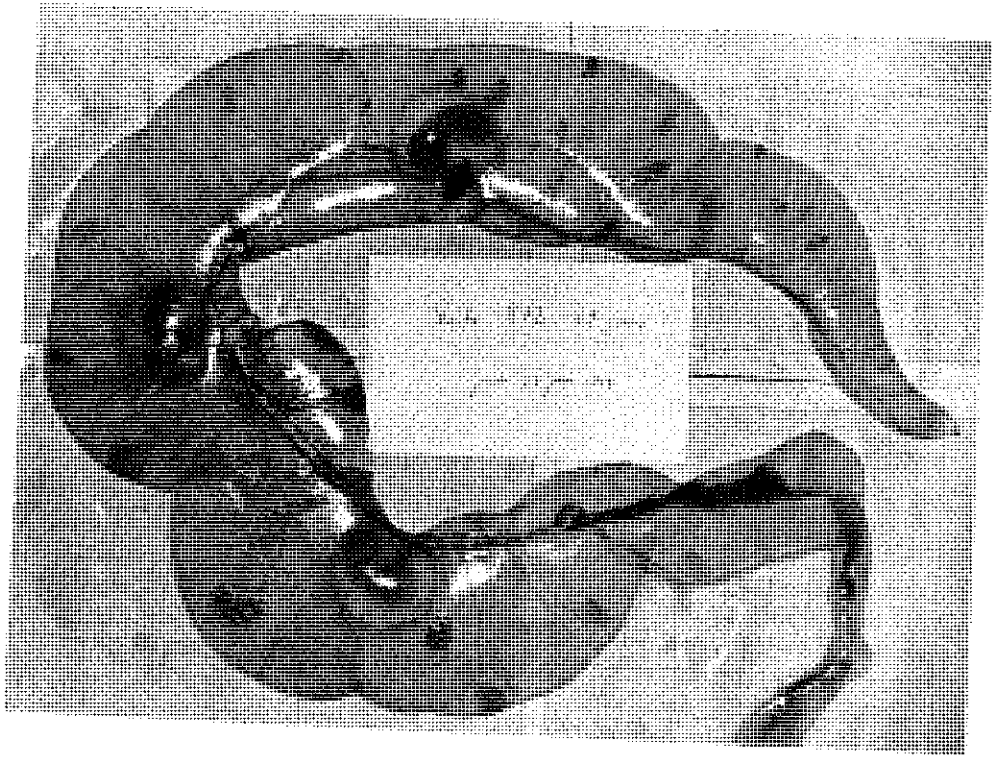


Fig 10. A group of triplet calf fetuses of a superovulated cow, 49 days after insemination. The large chorions and some large blood vessels are fused; each fetus is lodged in his smaller amniotic sac. (25).

The observations made on freemartin fetuses may be summarized in the following way, according to the age at sacrifice:

1) *40 to 48 days.* During this period of time, testicular organogenesis proceeds rapidly in males. In females the presumptive ovaries show no differentiation and no so-called "medullary cords" may be distinguished. No clear-cut difference is seen between presumptive freemartins and normal females (Fig 11).

2) *49 to 52 days.* This period is important in males, because the penis begins to move along the belly wall toward its abdominal position characteristic of the adult bull and because the diameter of the upper part of the Müllerian ducts decreases, a sign heralding their retrogression. In females nothing much happens. In freemartins the upper Müllerian ducts become reduced as in males and the growth of the ovary stops more or less completely, according to the individual (Fig 12).

3) *53 to 60 days.* In males, most of the male sex characters become obvious or develop during days 56 to 58, namely abdominal position of the penis, presence of a scrotum, development of a prostate and of seminal vesicles, and rapid retrogression of the upper Müllerian ducts. In females there is not much change. In freemar-

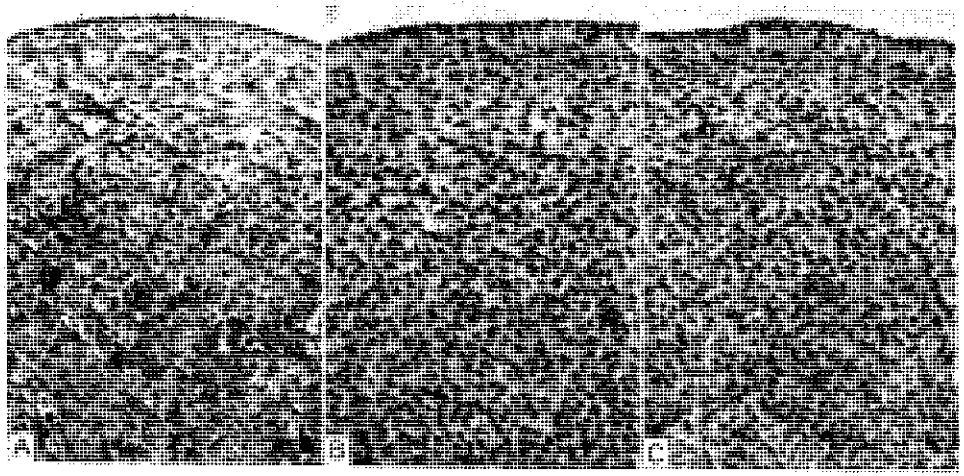
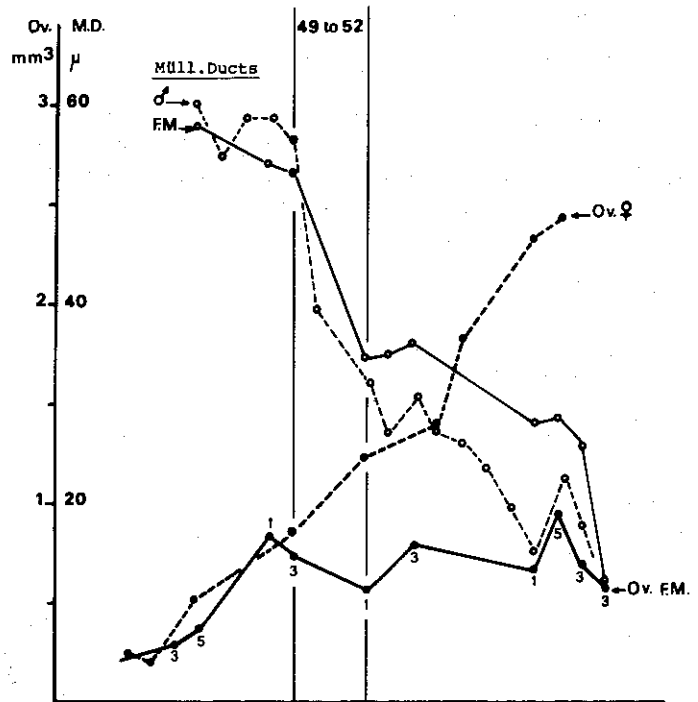


Fig 11. Histological sections through the gonads of three 45-days-old calf fetuses. A. Normal male showing the testicular structure; B. Normal female; C. Presumptive freemartin; note the absence of any medullary cord in both the presumptive ovaries (x185). (Unpublished picture from Jost, Vigier and Prepin).



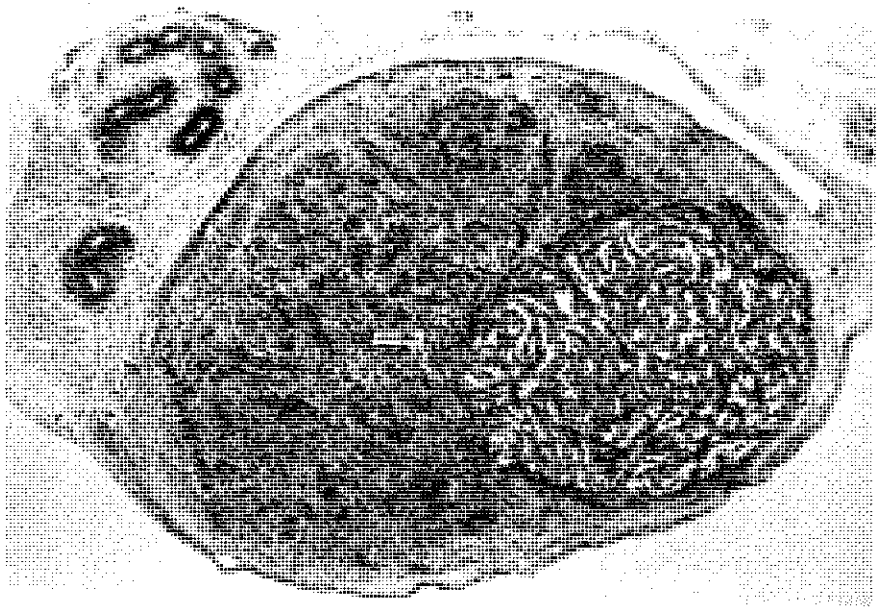
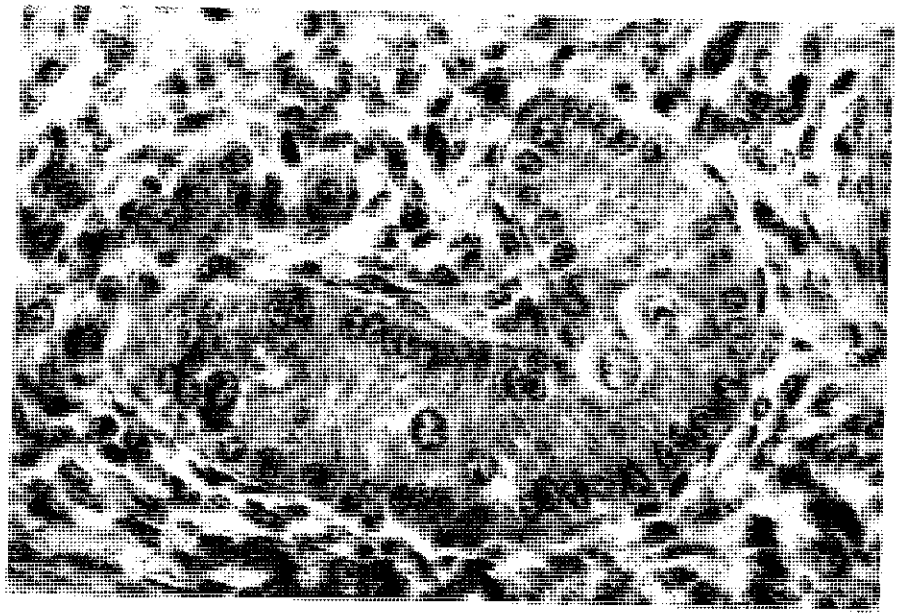


Fig 13. Histological section through one gonad of a 290 mm long freemartin fetus (approximately 125 days). The whole structure is very small in comparison with a normal ovary of the same age; it is surrounded by an albuginea-like layer and the rete system is well developed (the rete is well developed in normal ovaries also). Epidydimal tubules are seen on the left side. The arrow indicates the tubule illustrated in Figure 14. (Unpublished Figure from Jost, Perchellet, Vigier and Prepin) (x 37).



4) *60 to 62 days.* In a few freemartins, 3 of 17, some signs of masculinization, such as small seminal vesicles, make their appearance, but the freemartin effect still is conspicuous mostly at the level of the ovaries and of the upper Müllerian ducts.

It is obvious that until day 60 the freemartin effect is essentially an inhibition of the ovaries and of the upper Müllerian ducts. There is no sign of masculinization of the genital tract. On the contrary, in fetuses of a pregnant cow given large doses of methyltestosterone or other androgens, the effect is the reverse. All male characters develop penis, scrotum, prostate, whereas the Müllerian ducts and the ovaries are not inhibited (16, 17, 24). It would appear that in the freemartin some factor or factors is/are present which inhibits/inhibit the growth of the outer part of the gonads and induces/induce the retrogression of the upper Müllerian ducts. The concomitancy of these two effects might suggest that they result from the same cause, but other tentative interpretations have been considered (25). One possibility would be that the fetal testis produces a hormone, which at the same time favors the differentiation of the albuginea and inhibits the Müllerian ducts. If such a hormone plays a role in normal masculine organogenesis, its absence during the development of abnormal subjects might result in the simultaneous persistence of the uterus and abnormal cellularity of the albuginea. This condition has been described by Bergada, Cleveland, Jones and Wilkins (1), in the paper that Lawson Wilkins sent me in 1961. The recent observations on freemartins thus permit a late and still hypothetical comment on some observations mentioned in that paper.

Another point is of great interest. As has already been mentioned, postnatal gonads of freemartins frequently contain structures resembling sterile seminiferous tubules. Such structures were absent in the 48 freemartins studied to a fetal age of 62 days (19, 25). A search in the literature indicates that these structures never were observed by previous authors before the fetal stage of 20 cm, that is, at approximately 100 days. We recently decided to study older freemartin fetuses collected at the slaughter house. I was assisted in this undertaking by my associates, J. P. Perchellet, B. Vigier and J. Prepin, but so far we have not published the results of our investigations. So far we have studied the gonads of 10 freemartins ranging from 175 to 390 mm in length, that is, at approximately 95 to 125 days of age. They were covered by a thick layer of connective tissue resembling an albuginea testis and contained well-developed rete tubules (Fig 13). In five animals we found small formations, unequally developed and which looked similar to the seminiferous tubules described in postnatal freemartins (Fig 14). As was expected (25), these formations seemed to appear at a stage which roughly coincided in time with the formation of ovarian follicles in normal females. It still is too early to make further comments, but it is worth while to remember that organogenetic processes still are possible in the freemartin gonad at late developmental stages.

CONCLUDING REMARK

The extent of our ignorance concerning some of the major mechanisms controlling sex differentiation in mammals still seems a challenge. An open mind and a critical appraisal of theories and facts should incite new research with newer techniques and per-

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