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Hormonal Factors in the Sex Differentiation of the Mammalian Foetus [and Discussion]

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Hormonal factors in the sex differentiation of the mammalian foetus

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[Plates 14 and 15]

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1 Sex differentiates under genetic control during successive periods. Classical morphological and experimental data have shown the sexual bipotentiality of the developing structures. But, as a matter of fact, several observations indicate that both sexes are not equal or equipotential as to their developmental trends and mechanisms.

2 The developmental analysis of the body sex characteristics reveals a hormonal control. In animal experiments made by the author and by others it has been observed that many structures or systems develop along the feminine type in the absence of testes during several critical developmental stages. These structures include the genital tract, the hypothalamic centres controlling the pituitary function, the nervous structures mediating sex behaviour and possibly other tissues. The ovary is unnecessary for the feminine differentiation of these structures; in males, femaleness has to be repressed and maleness imposed by the testes.

3 The problem of gonadal sex differentiation is re-evaluated; developmental aspects occurring during normal development or in the gonads of freemartins in cattle are examined. During early sexual differentiation of the gonads, testes rapidly differentiate whereas ovaries are first characterized mainly by the fact that they do not become testes. These observations can be interpreted by assuming that in males a signal imposes masculinity on the gonadal primordia which otherwise would slowly become ovaries.

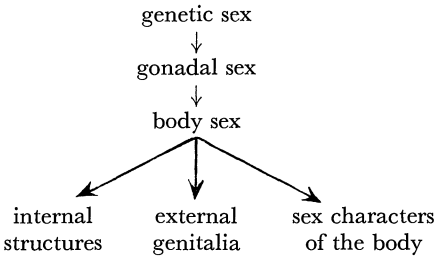
4 It is hypothesized that throughout sexual differentiation in mammals, maleness has to be actively imposed on a system which will become feminine if it escapes this control.

Introduction

The concept that the body is sexually *bipotential* during development has long been classical. It originated both from embryological and from physiological studies. The former established that the embryonic sex structures pass through a common indifferent phase before differentiating into male or female; the latter demonstrated that sexual characters can be postnatally masculinized or feminized by gonadal grafts or hormonal treatments.

During development sex differentiates by successive steps (table 1) which are controlled by the genetic constitution of the individual (genetic sex). Testes or ovaries arise from common undifferentiated primordia (gonadal sex). Male or female genital tracts and secondary sex characters (body sex) develop from anlagen present in both sexes. From the descriptive embryological data it might be deduced that both sexes are *equipotential* which could connote equal trends and possibilities to differentiate into the male or the female direction. In the frame of the hormonal theory of sex differentiation this would mean that genetically controlled hormonal or humoral mechanisms impose either maleness or femaleness on sexually neutral structures by discrete but similar means.

TABLE 1. CHAIN OF EVENTS IN SEXUAL DIFFERENTIATION



However it has long been known that human eunuchs display some feminine trends in their body features and that castrated hens usually acquire masculine plumage. From the beginning, the data supporting the hormonal theory of sex differentiation showed hormonal prevalence of one sex over the other: in cattle freemartins, Lillie (1916, 1917) and Keller & Tandler (1916) observed only an influence of the male co-twin on its female partner with no reciprocal influence; in parabiotic pairs of amphibian larvae of the same species and age, or after orthotopic unilateral gonad transplantation, the male is dominant (see Burns 1961). In birds, ovarian dominance over the testis is shown either in embryonic gonadal grafting experiments (Wolff 1947) or in the natural embryonic parabiosis established in eggs provided with two yolks (Lutz & Lutz-Ostertag 1959).

From the present evidence it seems justified to conclude that, during early sex differentiation, masculine influences are predominant in mammals and feminine influences in birds. From that viewpoint several aspects are opposite in the two groups and the present discussion will be limited to mammals. Emphasis will be laid on unsolved problems and for this reason data concerning body sex will be discussed before those pertaining to gonadal sex.

BODY SEX

The various sexual characteristics which can be studied under the heading of body sex share in common the fact that they develop at a stage which follows gonadal differentiation. Therefore the role of gonadal hormones in their development can be studied by removing the testes or the ovaries at an appropriate age. Other experimental methods have also been applied such as *in vivo* or *in vitro* grafts of gonads, hormonal administration or use of an antiandrogen. Some of the major results will be briefly recalled (appropriate references will be given for more complete information).

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Genital tract

The role of gonadal hormones in the differentiation of the genital tract has been elucidated in experiments done on rabbit foetuses *in vivo*. In rabbit foetuses castrated before the initiation of sexual differentiation of the genital tract, the whole internal tract and the external genitalia become feminine whatever the genetic sex of the foetus (figure 1) (Jost 1947, 1953). This result was confirmed *in vivo* on mice foetuses whose gonads had been destroyed by a beam of X-rays (Raynaud & Frilley 1947) and *in vitro* on isolated pieces of the genital tract of foetuses of the rat (Jost & Bergerard 1949; Jost 1950*b*; Picon 1969) or of the mouse (Brewer 1962). Both *in vivo* (Jost 1947, 1955) and *in vitro* (Brewer 1962; Picon 1969) grafts of testis achieved masculinization. A long series of observations on humans congenitally deprived of gonads shows that in the absence of gonads the genital tract becomes feminine whatever the chromosomal sex.

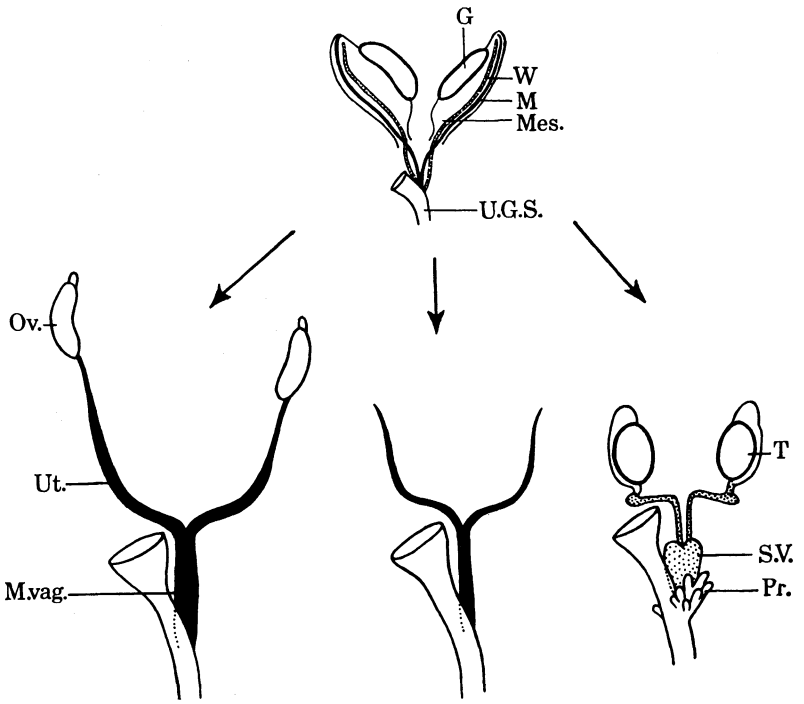

FIGURE 1. Schematic presentation of sexual differentiation of the sex ducts in the rabbit foetus. From the undifferentiated condition (top) may arise either the female structure (lower left), the male structure (lower right) or the feminine gonadless structure in castrated foetuses of either sex (lower 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). (From Jost 1960.)

It appears that the testes are the body sex differentiators; they impose masculinity on the whole genital sphere which would become feminine in their absence. The presence or absence of ovaries is of no significance.

The testicular influence forces masculine organogenesis upon the common anlagen (urogenital sinus, external genitalia), stabilizes the Wolffian ducts, and causes the retrogression of the Müllerian ducts. The mode of action of the testis upon the ducts remains unknown so far; it might be either direct or indirect via the agency of another structure such as the mesonephros.

Androgens, e.g. testosterone or similar steroids, masculinize the genital tract, but do not inhibit the Müllerian duct; the hypothesis has been expressed that separate testicular secretions

or distinct mechanisms could (1) inhibit the Müllerian ducts, and (2) masculinize the other parts of the genital tract (Jost 1953, 1965, 1969). The use of the antiandrogen cyproterone acetate which in rabbits opposed only the latter effects strengthened this hypothesis (Elger 1966; Jost 1966). However, in rats the same antiandrogen does not oppose the stabilization of the Wolffian ducts, a result which remains open to discussion (Jost 1966, 1967). In the human syndrome of testicular feminization which seems to result from lack of sensitivity of tissues to androgens, the whole body becomes feminine but the Müllerian ducts are inhibited. This suggests that the testicular inhibitory action took place, whereas no masculinization occurred. The condition of the genital tract is similar to that found in male rabbit foetuses given cyproterone acetate.

Hypothalamus and neural structures mediating sex behaviour

The early experiments of Pfeiffer (1936) on rats showed that the masculine pattern of gonadostimulating activity of the adult pituitary depends upon the presence of normal testes in males or grafted testes in females during the neonatal period, and that the absence of testes or the presence of ovaries results in a pituitary functioning in a cyclic feminine pattern. A long series of experiments later established that the pattern of pituitary functioning is controlled by the hypothalamus (Harris & Jacobsohn 1952) and that the testicular hormone modifies neural structures in the hypothalamus at a definite period of life (Barraclough & Gorski 1961). Administration of an antiandrogen to male rats in the neonatal period also causes a feminine cyclic type of pituitary function (Neumann & Kramer 1966). During normal development the male pattern is imposed upon neural structures by the testicular hormone.

Similarly, neural structures mediating sexual behaviour in adulthood can be modified by androgens in females, during a critical period which is prenatal in guinea-pigs (Phoenix, Goy, Gerall & Young 1959) or postnatal in rats (Harris & Levine 1962). The androgen permanently impairs the possibility of feminine behaviour. On the other hand, castration of newborn male rats permits them to develop definite female behavioural trends (Grady, Phoenix & Young 1965). It appears that the rat is born with a sexually undifferentiated central nervous system which is the female pattern and which will become fixed in type unless it is organized into the future male pattern by the testicular hormone (Harris & Levine 1965).

Other tissues

In the adult rat corticosteroid metabolism exhibits a distinct sexual difference due to differences in the level of activity of steroid metabolizing enzymes present in the liver. Denef & De Moor (1968) recently observed that in animals of either sex castrated at birth the feminine pattern of enzymes develops 4 or 5 months afterwards, while the male pattern is found in females or in neonatally castrated animals of either sex given testosterone or in males castrated after the 10th day. During adult life the effect of testosterone on these enzymes is only temporary.

Conclusions

From the study of the hormonal control of the genital tract it was concluded that the feminine pattern develops irrespective of the genetic sex of the individual, or of the presence or absence of the ovaries, in individuals which have no testes. In males the testis imposes masculinity during definite critical stages (Jost 1947, 1950*a*). The same conclusion has now been extended to the brain and to other structures. Full or partial femaleness appears if the body is deprived of testes or escapes testicular influence at definite developmental periods.

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Gonadal sex

The gonadal sex is an essential link between genetic sex, phenotypic sex and reproductive capacities. The testes and the ovaries arise from a common and small primordium developing on the inner side of the mesonephros and initially made of the coelomic epithelium, the underlying mesenchyme and the primordial germ cells. The action of the genes governing sex is first expressed when sexual differentiation of the gonads begins. It is generally accepted that this event results from local processes taking place inside the tiny gonadal primordium. Techniques such as surgical removal of one part of the primordium or of one kind of cell has so far proved impracticable and less direct techniques had to be used to explore the mechanisms involved.

Many observations accumulated during the last fifty years show that the differentiation of the gonad can be altered, and sometimes reversed, under the influence of humoral factors. Historically the hormonal interpretation of freemartins in cattle, in 1916, was supported by the experimental sex reversal obtained in parabiotic amphibian larvae in the late twenties and early thirties, and by the sex reversal produced after 1935 by sex hormones in submammalian species. These beautiful experiments and repeated successes—although sprinkled with unexpected or unexplained observations—strengthened the hormonal theory of gonadal sex differentiation. In this theory diffusible substances are produced by some cells and govern masculine or feminine organogenesis. The source, the nature and the mode of action of these substances should be ascertained.

The concept of cortico-medullary antagonism

Since 1914, Witschi has made thorough analyses of gonadal organogenesis in frogs and insisted on the dual constitution of the undifferentiated gonad: the cortex is composed of the superficial coelomic epithelium, some mesenchymal cells and the primordial germ cells, and is separated by a layer of mesenchyme from the internal medulla which is composed of solid cords of cells coming from the mesonephric blastema (see Witschi 1929*a*). In a gonad differentiating into an ovary, the cortex continues growing, whereas the rete cords stop proliferating and become progressively hollowed. In a gonad differentiating into a testis, the rete cords continue growing and they branch and attract the germ cells; the cortex thus is emptied and reduced to the thin coelomic epithelium. The germ cells become ovocytes if they remain located in the cortex, they become spermatogonia if embedded in the medulla; their fate apparently depends upon the somatic cells which enclose them.

Witschi developed the concept that cortex and medulla form a dual system of antagonistic inductors, which govern not only the differentiation of the germ cells but also the sex differentiation of the whole gonad. The cortexine(s) and the medullarine(s) are inductive substances respectively produced by the cortex or the medulla and which compete to impose either ovarian or testicular development under genetic control (details in Witschi 1931, 1937, 1967). One important consequence of this scheme is the compensatory development of the medulla in a female whose cortex is damaged, and vice versa in a male.

Witschi's fascinating analysis was pursued on a long series of experiments including: (1) female to male sex reversal in frogs developed from overripe eggs (Witschi 1924); however, since chromosomal anomalies occur in these animals (Beetschen 1957; Witschi & Laguens 1963) the cause of their sex anomalies is difficult to assess; (2) female to male sex reversal in frog larvae reared at a high temperature (Witschi 1929*b*); (3) heterosexual parabiosis: as a rule in these pairs the male is dominant, but in some instances fast growing females may influence the male partner (Witschi 1937).

Since in parabiotic salamanders the testicular substance is exchanged via the blood stream, the early concept of local cortico-medullary antagonism was later incorporated into the hormonal theory of gonadal sex differentiation by several authors, the more so as sex hormones may also reverse sex differentiation in lower vertebrates. Actually it has to be assessed whether the sex hormones directly control gonadal organogenesis or whether they interfere with the production of the cortico-medullary inductors (Witschi 1967).

The problem of freemartins

It is well known that freemartins in cattle are females (this has been proven on the basis of chromosomal studies) which are modified under the influence of their male co-twin, as a consequence of early fusion of their chorions and of vascular anastomosis. Freemartins are characterized mainly by: (1) ovarian reduction and sterilization, with sometimes some seminiferous tubules present; (2) more or less complete regression of the Müllerian ducts; (3) slight and inconstant signs of masculinization (seminal vesicles and sometimes clitoridal enlargement).

A programme was initiated several years ago in our laboratory to re-investigate the freemartin problem and to re-evaluate their bearing on theories of sex differentiation. Results have been reported only briefly so far (Jost, Chodkiewicz & Mauléon 1963; Jost 1965, 1966). Some data will be surveyed here, the work being done in collaboration with B. Vigier & J. Prépin, and will be published elsewhere.

Developmental history of the freemartin gonad

Multiple pregnancies were produced in cows given gonadotrophic treatment and inseminated under control; foetuses of known age could be obtained. Fusion of the chorionic blood vessels was verified; in several cases chromosome studies in liver tissue ascertained blood exchange.

Histologically discernible sex differentiation of the testes began in control foetuses on approximately day 40 (figure 2, plate 14). Presumptive freemartins were studied on various developmental stages between days 44 and 110. The size of the ovaries was the same in freemartins and in control females from days 44 to 48. Afterwards growth of the freemartin gonads was, practically nil; quantitative comparisons were made until day 62. In histological sections no clear difference could be seen between freemartin and control ovaries up to day 48 (figure 3, plate 15); from that stage on the 'germinative epithelium' of the freemartins lagged behind the controls and on day 60 it displayed signs of degeneration. In none of the 35 freemartins studied at ages ranging from 44 to 62 days was any structure of testicular appearance found in the core of the ovaries.

The first obvious conclusion of these observations is that the freemartins develop normal ovaries for a while; cortical inhibition starts secondarily when the males already have had well recognizable testes for a week. Therefore the situation of the freemartins gives no indication on the mechanisms which initiate gonadal sex differentiation. Interestingly enough, ovarian inhibition starts in the freemartin only a few days before the first signs of inhibition of the Müllerian ducts are recognizable both in the male co-twin and in the freemartin itself.

As mentioned before, in the material collected so far no seminiferous tubule was observed in the ovarian medulla. If our collection is representative for the general condition, this observation would suggest that such structures appear at stages later than day 60. It may be recalled that, in salamanders, ovarian inhibition under the influence of the developing testis begins after an early period of ovarian development and that true masculinization occurs still later (Humphrey 1929, 1931).

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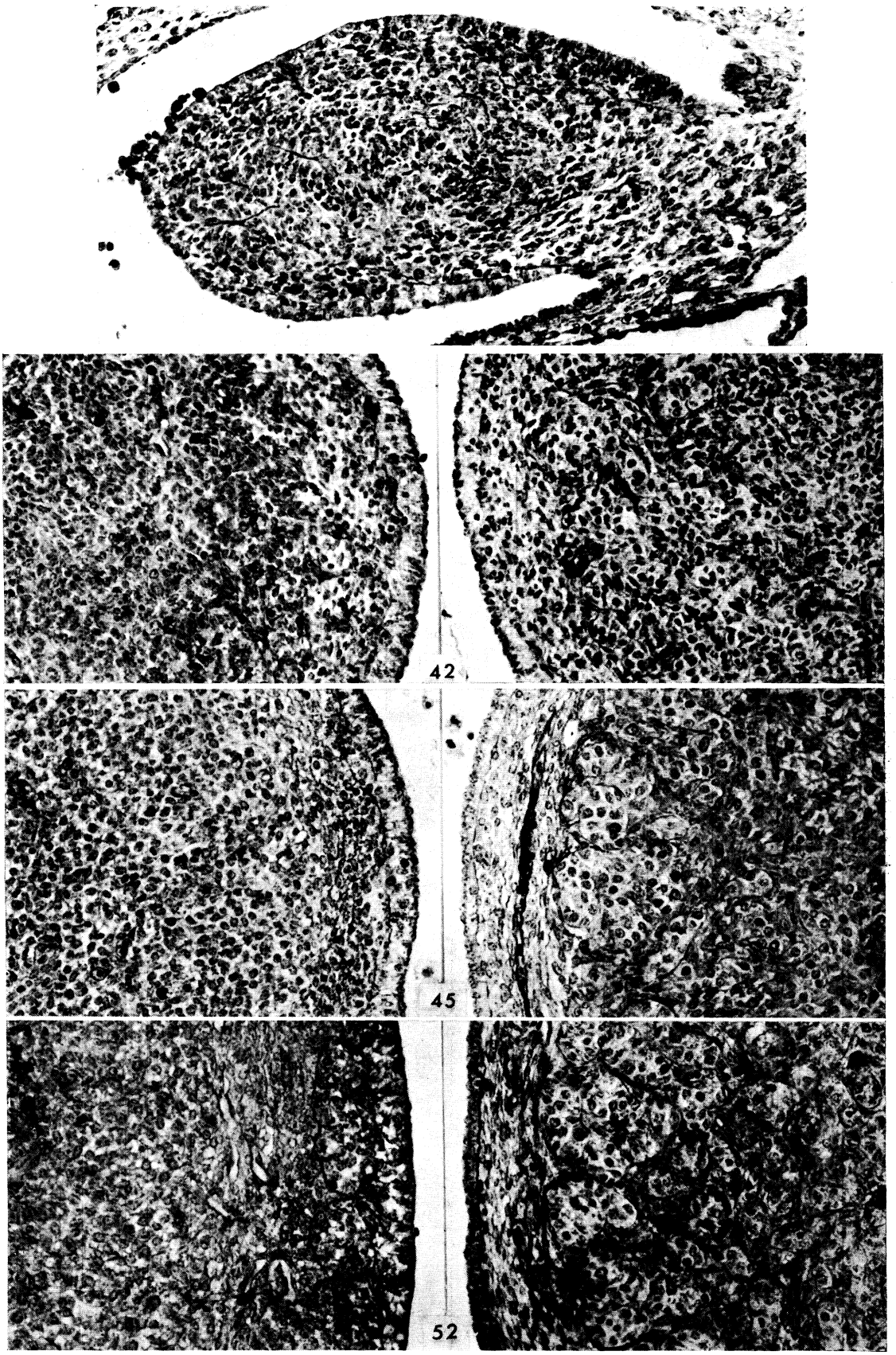

FIGURE 2. Gonadal development in the calf foetus.

On top: 36 days post-insemination. The foetus was a male as judged by the chromosomal complement in the liver. Histologically undifferentiated stage. The origin of the internal material is difficult to assess. Contact with mesonephros on the right side.

On the right side: three stages of testicular organogenesis at 42, 45 and 52 days post-insemination. Notice the progressing delineation of the sex cords, and the development of the albuginea. The coelomic epithelium persists throughout the period under study.

On the left side: three stages of ovarian organogenesis (42, 45 and 52 days). Notice that the medulla does not develop distinct cords. The persisting coelomic epithelium is thicker and more irregular on its lower aspect than in the male; on day 52 large cortical cords proliferate in it.

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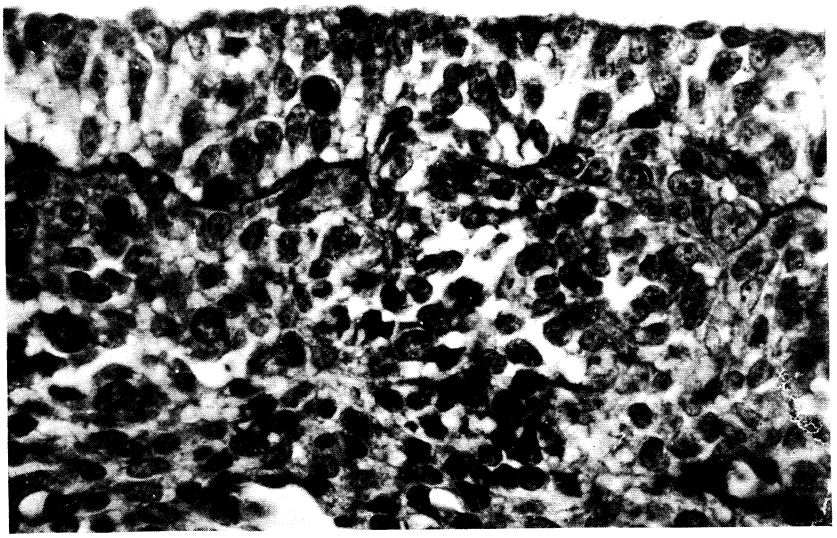

FIGURE 3. Section through the persisting 'germinative epithelium' of a 48-day-old foetal freemartin ovary.

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Factors responsible for the freemartin effect

According to the hormonal theory the alterations observed in freemartins result from the action of the foetal testicular hormone transferred to the female (Lillie 1916, 1917). In order to verify whether adult-like androgen steroids could duplicate the freemartin effect, such steroids were injected in large amounts to pregnant cows, usually starting on day 35, i.e. before gonadal sex differentiation. The foetuses were studied either on days 104 and 112 (Jost *et al.* 1963; Jost 1965) or on days 49 and 56 (Jost, Vigier & Prépin, quoted in Jost 1966). In the androgenized female foetuses the ovaries were normal, and no freemartin effect was noted. Similarly in the older group, the Müllerian ducts were not inhibited, whereas the other internal or external genitalia were heavily masculinized. On day 49 the only somatic sex character recognizable in normal males was the beginning of urethral flexure and increased anogenital distance. Masculinization of this region was already noticeable in three female foetuses killed on day 49 after testosterone or methyltestosterone treatment, the latter being more effective. The androgen was available to the female foetus and effective on its genital tract before day 49 at a time when the first freemartin effects became recognizable. From these experiments it was concluded that the steroid androgens used and the factors responsible for the freemartin effect are not the same.

On the other hand, Herschler & Fechheimer (1967) developed the concept that transfer of cells from the male to the female partner and sex chromosome chimaerism (reflected in blood cells) might account for the freemartin condition rather than transfer of a testicular hormone. They found a positive correlation between the percentage of XY blood cells in adult freemartins and the degree of 'masculinization'. On a limited number of observations Goodfellow, Strong & Stewart (1965) favoured the same concept, whereas Ohno, Trujillo, Stenius, Christian & Teplitz (1962) and Kanagawa, Kawata, Ishikawa, Muramoto & Ono (1965) did not verify such a correlation.

In a recent analysis made on our material, B. Vigier (unpublished) studied the same problem on 12 presumptive freemartin foetuses, at the very moment when freemartinism becomes obvious. No correlation was observed between the percentage of cells containing XY chromosomes present in the foetal liver and the severity of the freemartin condition at the level of the developing ovary.

The causative factor of the freemartin condition in cattle remains an open problem. Early exchange of blood and of some cell types is obvious; in adult parabiotic animals protein hormones seem to be more readily transferred than steroids, largely because of liver inactivation of the latter, but in foetuses the ductus venosus permits large amounts of blood to by-pass the liver. Immunological processes involved in or resulting from early testicular differentiation might also be of importance in freemartinism.

A re-evaluation of the problem of gonadal sex

The exact mechanisms leading to and governing the sex differentiation of the gonad are still unknown and remain a matter of interpretation and theories. It will be rewarding to reopen the discussion, to re-evaluate the evidence and perhaps to consider simpler hypotheses. The role of genetic factors as evidenced by recent studies will not be discussed here (see Jost 1969).

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Some factors of gonadal organogenesis

The early gonads develop on the mesonephros; several types of tissue participate in its organogenesis, namely the coelomic epithelium, the underlying mesenchyme, probably cells originating from the mesonephric organ or blastema and finally the germ cells which have an extra-regional origin. The role of each of these types of cells in gonadal organogenesis and differentiation is difficult to assess.

The role of the mesonephric area blastema or of the mesonephros as an organ, in gonadal development and the significance of this participation still are insufficiently solved. Experimental results on amphibians (Houillon 1956) and on chick embryos (Bishop-Calame 1966) seem to clearly indicate that some cells arisen from the mesonephros necessarily participate in gonadal organogenesis. Sex differentiation of mouse gonadal primordia was obtained *in vitro*, but so far only in experiments in which the complex mesonephros + gonad was explanted (Wolff 1952; Asayama & Furusawa 1960).

The role of the germ cells in gonadal development has been explored under experimental conditions which permitted the differentiation of sterile gonads of either sex in frog larvae (Bounoure 1950; Padoa 1964) or in chick embryos (Simon 1960). Only very early gonadal stages have been obtained by these authors, but their results imply that early gonadal sex differentiation does not depend upon the germ cells in these animals. However, several lines of evidence suggest that germ cells are necessary for the maintenance of the ovarian structure. In XO human embryos, presumptive ovaries develop as long as germ cells are present; when the germ cells degenerate the ovaries themselves regress (Singh & Carr 1966).

Another important point has been raised by Van Limborgh (1966, 1968) who found that the number of germ cells present in the so-called undifferentiated gonadal primordium of bird embryos depends upon the genetic sex of the embryo. This observation would call in question the concept of an undifferentiated stage. It is noteworthy that in $14\frac{1}{2}$ day-old rat foetuses, immediately after sex differentiation, Beaumont & Mandl (1963) found more germ cells in testes than in ovaries.

The process of gonadal sex differentiation

At the onset of gonadal sex differentiation in the mammalian foetus, both genetic sexes behave in a very dissimilar way. Suddenly males differentiate testes, while females remain in the undifferentiated phase for a more or less prolonged period according to the animal species. There are variations from one animal species to the other in the histological structure of the undifferentiated gonad and in the details of early testicular organogenesis (see Brambell 1956). But the result is essentially the same: most of the germ cells are attracted towards the core of the gonadal anlage and are enclosed with somatic cells in primitive seminiferous tubules limited by connective tissue and a basal membrane. The coelomic epithelium soon becomes separated by a layer of connective tissue (albuginea) from the rapidly differentiating and growing testis.

During the first stages the ovary can be identified only because it does not become a testis (e.g. for humans: Gillman (1948); for the rat: Torrey (1945)). No structure similar to the testicular seminiferous tubules differentiates in the core of the early ovary. The material laid down in the inner gonad before sex differentiation remains more or less inert. It may contain some irregular cords of cells or be rather unorganized according to the animal species. In the

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calf foetus the covering epithelium is separated from an unorganized central cellular mass (figure 2); after day 50 it thickens and proliferates large outgrowths which will be used in the elaboration of the definitive cortex (figure 2). As a rule an adult-like ovarian cortex becomes organized only when the primary follicles and the cortical stroma develop, i.e. after the 14th week in the human ovary (Gillman 1948; Witschi 1962).

Early phases of premeiosis usually appear in the ovarian germ cells even before the ovarian cortex is completely organized, i.e. the 12th week in the human foetus (Manotaya & Potter 1963) and the 75th day in the calf foetus (Erickson 1966). In amphibians premeiotic figures appear even earlier. Chronological descriptions of gonadal sex differentiation in amphibians are not numerous. From Witschi's (1929*a*) figures concerning *Rana sylvatica*, and from Asayama's (1959) data concerning *Bufo vulgaris*, for instance, it seems that in these forms testicular organogenesis also precedes ovarian development; for some time ovaries remain largely similar to undifferentiated gonads, whilst testes have become definitely different. The most prominent features of ovarian development is early appearance of premeiosis, arrested growth and hollowing of the rete cords. The transformation of the primitive ovary into the adult-like ovary is very late in *Rana sylvatica* (Witschi 1929*a*).

Interpretations and hypotheses

The process of sex differentiation of the mammalian gonads from the undifferentiated primordium begins when testes differentiate in genetic males. The morphogenetic mechanisms of testicular organogenesis have not been sufficiently elucidated. Several types of cells are involved: the germ cells are attracted and encapsulated with somatic cells in the seminiferous tubules, which are delineated by connective tissue; connexions occur with mesonephric elements; interstitial cells differentiate; and further thriving of the 'germinative epithelium' is inhibited.

During that period of time no clearcut morphogenetic event occurs in the future ovaries. A period of growth according to the initial pattern precedes the much later differentiation of the adult-type ovary.

In a previous paper (Jost 1965), it has been suggested that the simplest theoretical interpretation of this series of events would postulate that some mechanism working under the influence of the genes for maleness imposes testicular organogenesis at an early stage on the gonadal primordium. In the absence of this signal, the gonadal primordium slowly differentiates into an ovary.

The process of testicular organogenesis is probably controlled by one or more diffusible substance(s) produced by some cells and which influence other cells so as to impose male organogenesis. The identity of the leading cells and the nature of the inducing substance(s) remain unknown. It could be conceived either that these cells participate in the elaboration of the male duct system or that they remain somewhat apart from it (as do interstitial cells or mesonephric elements). A model for a separate gland governing sex differentiation of the reproductive organ is given by the crustacean androgenic gland (see Charniaux-Cotton 1965).

There is no indication that in those gonads which do not become testes (presumptive ovaries) there is any special mechanism or cortical inductor responsible for preserving the undifferentiated pattern of growth; the mere absence of the testicular inducing signal could explain their ovarian prospect. However, definitive ovarian differentiation is preceded or accompanied by the rather early onset of premeiosis in the ovogonia, whereas in the testis the germ cells remain

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in the gonial phase. In the absence of direct information it is difficult to assess whether premeiosis is blocked in the testes or stimulated in the ovaries by neighbouring cells. It has been observed that the mammalian ovogonia enter premeiosis before they are encircled by follicular cells and that these cells later prevent them from proceeding too far in the meiotic process, i.e. beyond the diplotene phase, and from degenerating (Ohno & Smith 1964). In the seminiferous tubules the germ cells are precociously surrounded by the sustentacular cells. Self-differentiating ovogenesis in the absence of the androgenic gland has been reported in crustaceans in which spermatogenesis is hormonally imposed (Charniaux-Cotton 1965). Although it is impossible to decide which mechanism prevails in vertebrates and especially in mammals, it seems conceivable that in the early ovary premeiosis should not necessitate incitement by a cortical inductor. Early premeiosis occurring unless it is hindered (as it is in the testes) might be another aspect of the female trends in mammalian development.

The foregoing interpretations and considerations remain as speculative as the hitherto accepted views and they should be submitted to new crucial experiments. One indirect and provisory way to test their validity is to verify to what extent they may account for older experiments, but it would be too long to undertake this discussion in the present paper.† For the same reason this presentation has been necessarily somewhat schematical and could not deal with several other interesting aspects.

Conclusions

The male infant develops as a male in the maternal womb despite the basic mammalian trend for femaleness. Under normal conditions the presence of the tiny Y chromosome in his cells permits the testes to impose masculinity on several parts of the body during successive critical stages. It is suggested that even during gonadal differentiation a genetically controlled masculinizing signal imposes testicular organogenesis on a gland which otherwise would become an ovary.

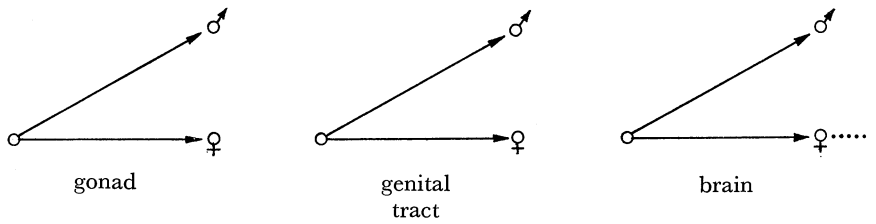

Figure 4

† The interpretative hypothesis proposed for mammals seems to permit also an interpretation of several observations made on amphibians in which male dominance is the rule under parabiotic conditions. (In those experiments in which rapidly growing female salamanders feminize young males, it should be ascertained if their ovaries already produce oestrogens which have a feminizing effect in males.) In 'undifferentiated races' of frogs the males pass through a phase during which their gonads first have the primitive ovarian aspect before being 'sex reversed': the so-called female phase (no adult-like ovary is formed) could result from the delayed onset of the masculinizing signal rather than from a temporary prevalence of a cortical inductor. Sex reversal in frogs reared at high temperature was interpreted by Witschi (1929*b*) as resulting from compensatory hypertrophy of the medulla after cortical involution. He noted that 'the cessation of inhibition of medullary growth coincides in time with cessation of ovocyte formation'. If activation of the masculinizing signal were the first effect of the treatment, it could be stated that the cessation of ovocyte formation would coincide in time with medullary growth, assuming that the former results from the latter or that both result from the same cause.

The problem of gonadal differentiation in birds cannot be discussed here (see Jost 1965, 1969).

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The feminine organogenesis follows a straight developmental line across several phases during which masculinizing agents or hormones must force several systems to deviate permanently towards the masculine type (figure 4). On repeated occasions masculine organization can fail to be imposed if the body escapes these controls.

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Discussion on paper by A. Jost, p. 119

D. Price: I have always had difficulty in applying to mammals the theory of cortico-medullary inductors in sex differentiation of gonads as it was developed from experimental work on amphibians. The labile sexuality of amphibians, the morphogenesis of the gonads, the ease with which experimental procedures can reverse sex and cause complete functional reversal of germ cells are all very different from the situation in mammals. For mammals, the results of grafting experiments using heterosexual pairs of foetal gonads seemed to support the existence of inductor substances that were postulated to be specific for foetal gonads, but the experiments of Turner and Asakawa (reviewed by Turner 1969 *Embryologia* **10**, 206–230) bring this into question. They produced sex reversal, formation of medullary tubules resembling sterile seminiferous tubules, in foetal rat ovaries by grafting them under the tunica albuginea of testes of adult hosts. The contralateral ovaries, put under the kidney capsule of the same hosts, formed normal vesicular follicles, not seminiferous tubules. In this case, high titres of endogenous testicular hormones of adult hosts were apparently effective in causing sex reversal of foetal ovaries.

A. Jost: Some of the amphibian experiments are difficult to interpret because animals were killed at too early stages. A very interesting case has been reported by L. Gallien (1969 *Embryologia* **10**, 206–230) who sex reversed male newts with oestrogens and bred them for 11 years: feminized males laid eggs for 2 or 3 years then became masculinized after 4 or 5 years and had testes at autopsy. Sex reversal was thus temporary, and a complete reversal of germ cells was produced twice.

R. G. Edwards: The evidence of X chromosome inactivation in the germ line of mammals is very dubious. Critical data on the germ cells appears to be lacking. The basic genetic situation

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in the ovary could be that germ cells with both X chromosomes active are present in a soma where the cells have only one active X chromosome. The basic XX/XY chromosome system determining the differentiation of sex would thus be active only in the germ cells, implying that these cells played a primary role in differentiation. This suggestion could be tested by examining female germ cells for sex chromatin or an inactive X: has anyone done this?

A. Jost: I have no personal evidence concerning X chromosome inactivation in germ cells. In early foetal freemartins there are practically no dividing germ cells: this made the study impracticable (B. Vigier, unpublished). Sex differentiation of sterile gonads has been reported in amphibians and in birds; this result would rather exclude the germ cells as important factors in gonadal sex differentiation. No similar effect has been described in mammals so far and confirmatory experiments would be welcome.

R. G. Edwards: Could the roles of germ cells versus steroids in determining the gonad be separated by killing or removing the male twin to the freemartin? The male twin could be removed between the period when germ cells entered the germinal ridge and the first secretion of hormones.

A. Jost: This would be the obvious experiment. We have been trying to collect such cases in superovulated cows, in which sometimes some foetuses die. The only case we found so far was unfortunately mishandled by accident.

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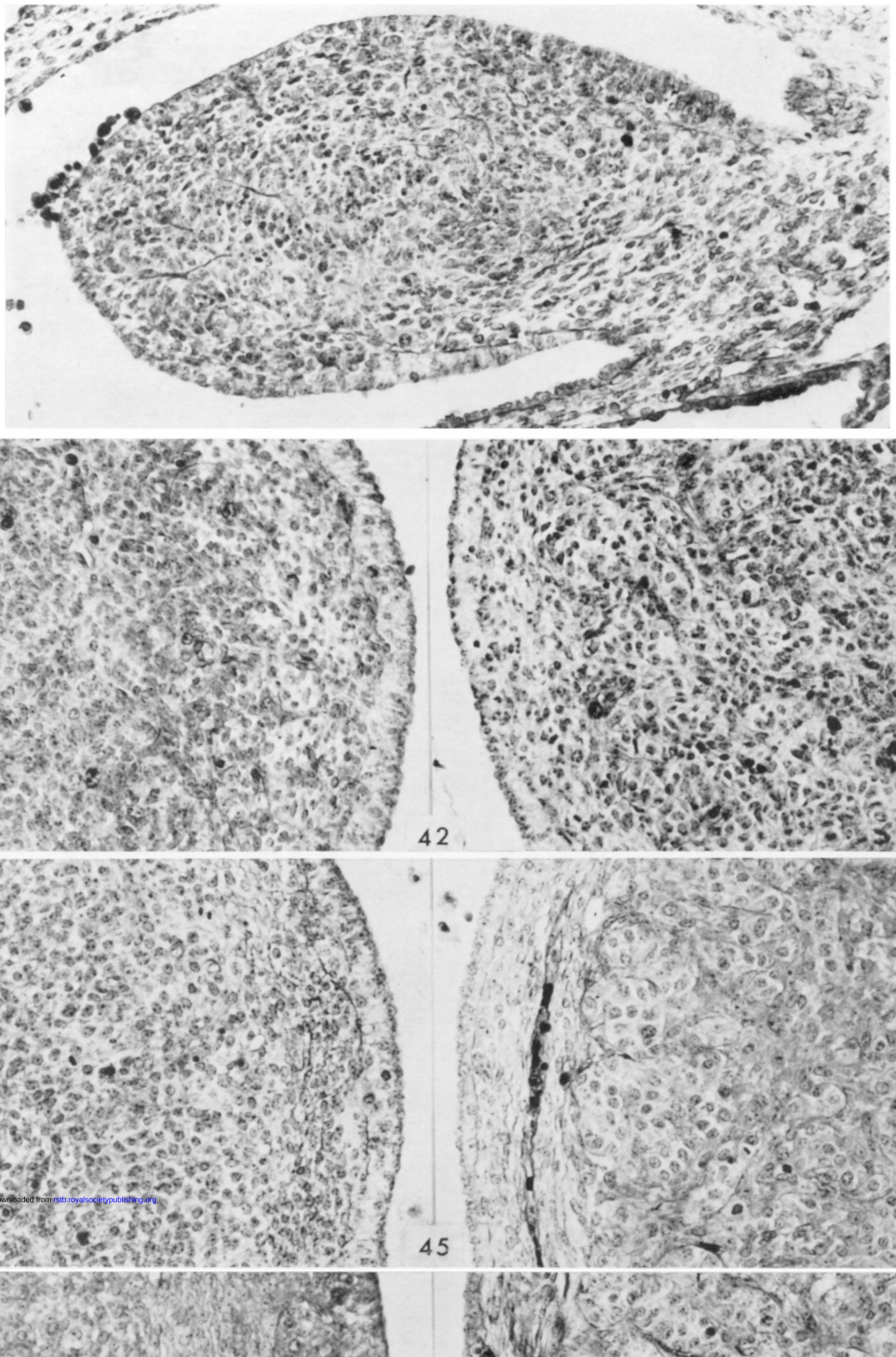

FIGURE 2. Gonadal development in the calf foetus.

On top: 36 days post-insemination. The foetus was a male as judged by the chromosomal complement in the liver. Histologically undifferentiated stage. The origin of the internal material is difficult to assess. Contact with mesonephros on the right side.

On the right side: three stages of testicular organogenesis at 42, 45 and 52 days post-insemination. Notice the progressing delineation of the sex cords, and the development of the albuginea. The coelomic epithelium persists throughout the period under study.

On the left side: three stages of ovarian organogenesis (42, 45 and 52 days). Notice that the medulla does not develop distinct cords. The persisting coelomic epithelium is thicker and more irregular on its lower aspect than in the male; on day 52 large cortical cords proliferate in it.

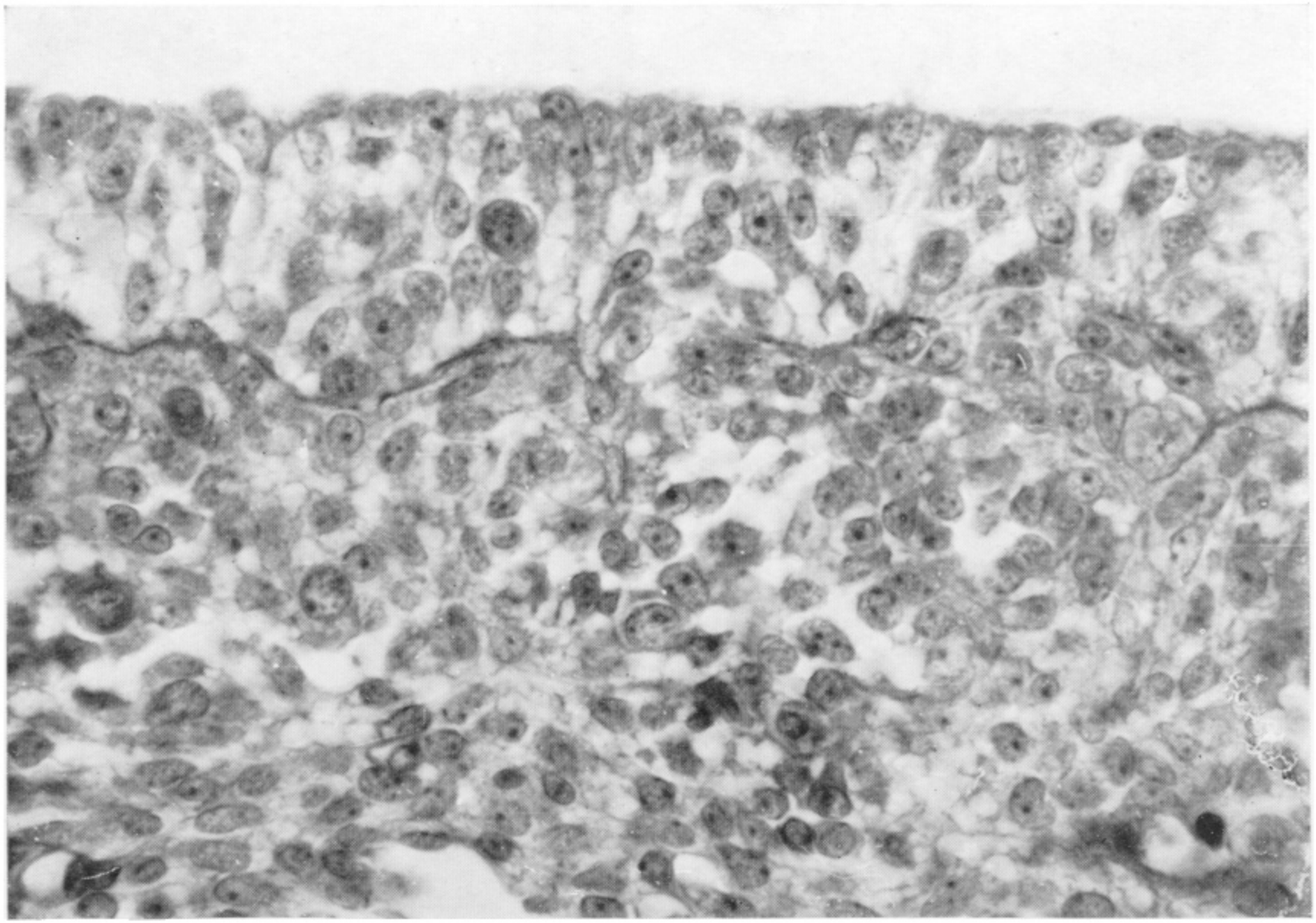

FIGURE 3. Section through the persisting 'germinative epithelium' of a 48-day-old foetal freemartin ovary.