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Studies on the Early Development and Sexual Differentiation of the Mammalian Gonad*

Estudios sobre el desarrollo temprano y la diferenciación sexual de las gónadas de mamíferos

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According to the findings of Witschi (1951, 1957) and of Jost (1970, 1971; Jost *et al.*, 1973) the mammalian gonad has to pass through several developmental periods before it finally becomes a differentiated male or female gonad. Beyond this stepwise development of the gonad itself, the differentiation into a testis or an ovary seems to be influenced by genetic as well as other stimuli and the gonad itself stimulates the development and differentiation of the male and female organs of the genital system. Following this concept, the development of a male or of a female organism is primarily determined at fertilization and the "genetic sex" is required for a normal development of the sexual differentiation of the gonad. The "gonadal sex" on the other and, influences and regulates the differentiation of the "body sex" (somatic sex), whereby the testis influences the development of the male genital system and male characteristics.

Sex differentiation of the gonad is guided under the influence of a masculinizing mechanism and results in the formation of testicular structures. If the male organizer is absent, the gonad develops into an ovary. The male organizer has been discovered to be the HY-antigen. Evidently, sex determination of the male gonad would seem to depend on the presence of HY-antigen (Wachtel *et al.*, 1975; Ohno, 1971, 1976, 1977; Wolff, 1979). In turn, the sex differentiation of the male sex organs is guided under the influence of a masculinizing mechanism that emanates from the secretion of androgens. If these testicular hormones are absent, the Müllerian ducts and the urogenital sinus develop into female sex organs. If the testicular androgens are present, the Wolffian ducts and the urogenital sinus develop into male sex organs. The regression (involution) of the Müllerian ducts requires an anti-Müllerian hormone, which seems to be produced by the testes, too (Josso *et al.*, 1977).

These are the basic requirements and mechanism for the development and differentiation of the indifferent sexual anlage into a male or a female organism. This concept, however, does not do much to clarify the cellular development process: it does not explain the mechanism of determination of what kind of blastema interacts with the gonadal anlage in order to induce these tissues to differentiate into a male or female gonad.

The role of mesonephric cell on early gonadal differentiation

Before discussing the problem of differentiation of the indifferent gonad into a testis or an ovary, the early developmental

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events of gonadal differentiation should be reviewed. Textbooks normally do not pay much attention to the early differentiation of the gonad. Development of the indifferent gonad starts with the formation, by thickening, of the genital ridge, and terminates with the appearance of structures representing either testicular cords or the ovarian cortex. This period includes the immigration of the primordial germ cells and in human development requires 7 to 10 days. A thickening of the coelomic epithelium close to the mesonephric organ is the first sign of the formation of the genital ridge (Wartenberg, 1978).

In human embryo 12 mm long, the mesonephros is represented by nephric corpuscules and tubuli.

The thickening of the genital ridge does not show any primordial germ cells, but dark blastemal cells are intermingled with light cells, these latter being of epithelial origin. According to most authors, the dark cells are presumed to be mesenchymal cells, which should form a mesenchymal core of the gonadal primordium, whereas the light epithelial cells should form the superficial layer of the blastema (Gondos, 1974; Merchant-Larois, 1978). Since the first primordial germ cells appear in the genital ridge region after proliferation of the epithelium has started, there is much evidence that the germ cells do not induce the coelomic epithelium to differentiate into the gonadal blastema. There is, however, one point which seems very important: Witschi put forward the idea that the medullary structures do not derive from plain mesenchymal cells but are, instead, derivatives of the mesonephric blastema (Witschi, 1951). The concept of a cellular interaction between two blastemas that derive from the superficial epithelium and mesonephric structures might explain the developmental relationship which causes the gonadal differentiation (Wartenberg, 1978, 1979, 1980 a,b). The interaction of the two blastemal parts, and the origin of the medullary blastema of the gonad from the mesonephros, have been found in sections of many mammalian species (Upadhyay *et al.*, 1979; Zamboni *et al.*, 1979; Wartenberg, 1980 a).

Sections through the gonadal anlage of a 12-day old rabbit embryo explicitly demonstrate a very important aspect of the behaviour of the mesonephrogenic blastema in respect of the epithelial blastema: during the indifferent period, mesonephrogenic cells invade the superficial epithelium. Mesonephrogenic and epithelogenic cells intermingle and include the immigrating germ cells (Fraedrich, 1979). The final composition of the genital ridge consists of a common blastema, the so called primary gonadal blastema, which shows no separation into two distinct primordia. Contrary to Witschi's opinion no individual cortex and medulla are distinguishable throughout this early period.

These two characteristics of early gonadal development –the formation of a primary blastema consisting of two types of somatic cells and the participation of the mesonephros in supporting this common blastema by segregating the cells that induce the other type to proliferate– should be demonstrated in two other mammalian species. In the mouse, the mesonephros never attains a state of full activity (Fraedrich, 1979; Upadhyay *et al.*, 1979). When the genital ridge starts its proliferative process on day 11, the mesonephros has developed short tubuli which in the upper part of the organ connect with the Wolffian duct. Figure 1a-d demonstrates, cross-sections through four consecutive levels of the mesonephric organ. In the sections of the three lower levels the developmental process of the mesonephros is even less advanced: small groups of cells which form rudimentary vesicles represent the mesonephric structures. These rudimentary structures have one feature in common: mesonephrogenic cells are segregated from them forming a loose blastema below the proliferating superficial epithelium. This segregation of mesonephric cells is combined with three characteristics: cell-death of some cells within the area of segregation, interruption of the basal lamina and the appearance of lysosomes. Signs of the so-called "physiological cell death" is the commonest feature in areas of developmental interaction between two different blastemas.

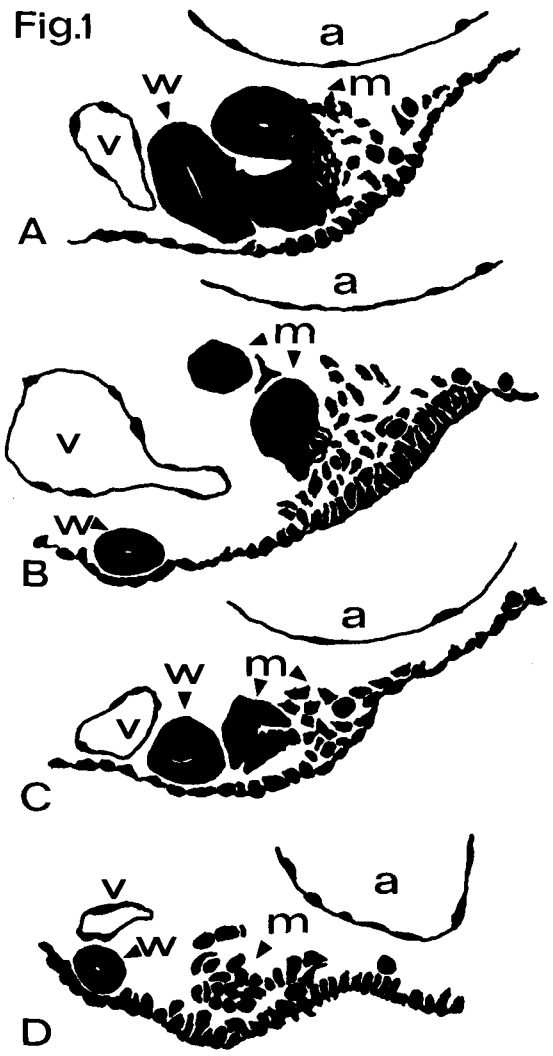

Fig. 1 A – D. Cross sections through 4 consecutive levels of the mesonephric organ and genital ridge of an 11 day old mouse embryo. *m* mesonephric structures, *w* Wolffian duct, *a* dorsal aorta, *v* Vena cardinalis caudalis (after Fraedrich, 1979).

In human development, the mesonephros has reached full activity when differentiation of the gonadal blastema begins (Wilson, 1926; Wartenberg, 1978). In a 12 mm embryo the proliferating superficial epithelium has close contact to the Bowman's capsule of a mesonephric corpuscle. Dark cells are concentrated below the proliferating epithelium. This contribution of mesonephrogenic cells continues over a long period. In a cross-section of the testicular anlage of a 28 mm embryo, one still sees the mesonephric

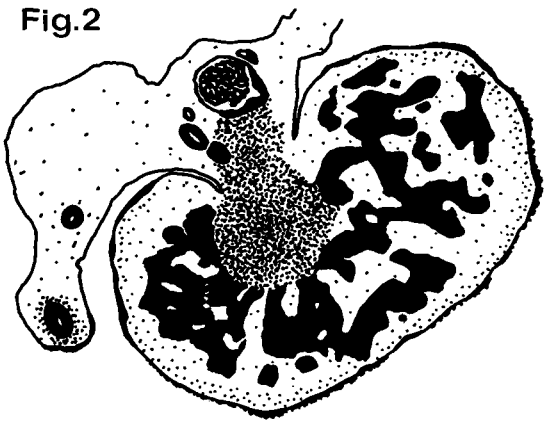

Fig. 2. Cross section through the upper level of the right testis and Wolffian body of a 28 mm long human embryo. The gonadal blastema of mesonephric origin (dotted) connects the regressing mesonephros with the new formed testicular cords.

structures near the gonad (Fig. 2). An undifferentiated blastema can be traced from the mesonephros through the broad mesogonadium into the center of the testis.

This kind of full gonadal-mesonephric relationship only shows up at this level. It is restricted to the upper pole of the gonadal anlage. This situation is visible quite well in longitudinal sections. In human gonadal development a so-called central gonadal blastema occupies the upper pole of the testicular as well as the ovarian anlage. From this central blastema testicular cords which finally differentiate into testicular tubules, are formed. The central blastema in turn is supplied by the mesonephros. The mesonephric glomerula of that upper region has almost completely regressed. Studies at cellular level demonstrate that the mesonephric nephroi do not degenerate; certainly they cease their activity, but continue releasing cells from their glomerular end. While the tubular part remains, the glomerula and their Bowman's capsules regress. The development of the Malpighian corpuscles seems to occur in reverse order. Single cells become detached from the Bowman's capsule, and partly disintegrated loops of the glomerulum become expelled through the vascular pole.

The podocytes "redifferentiate" by shedding their redundant processes; in their final appearance they are hard to distinguish from pure mesenchymal cells,

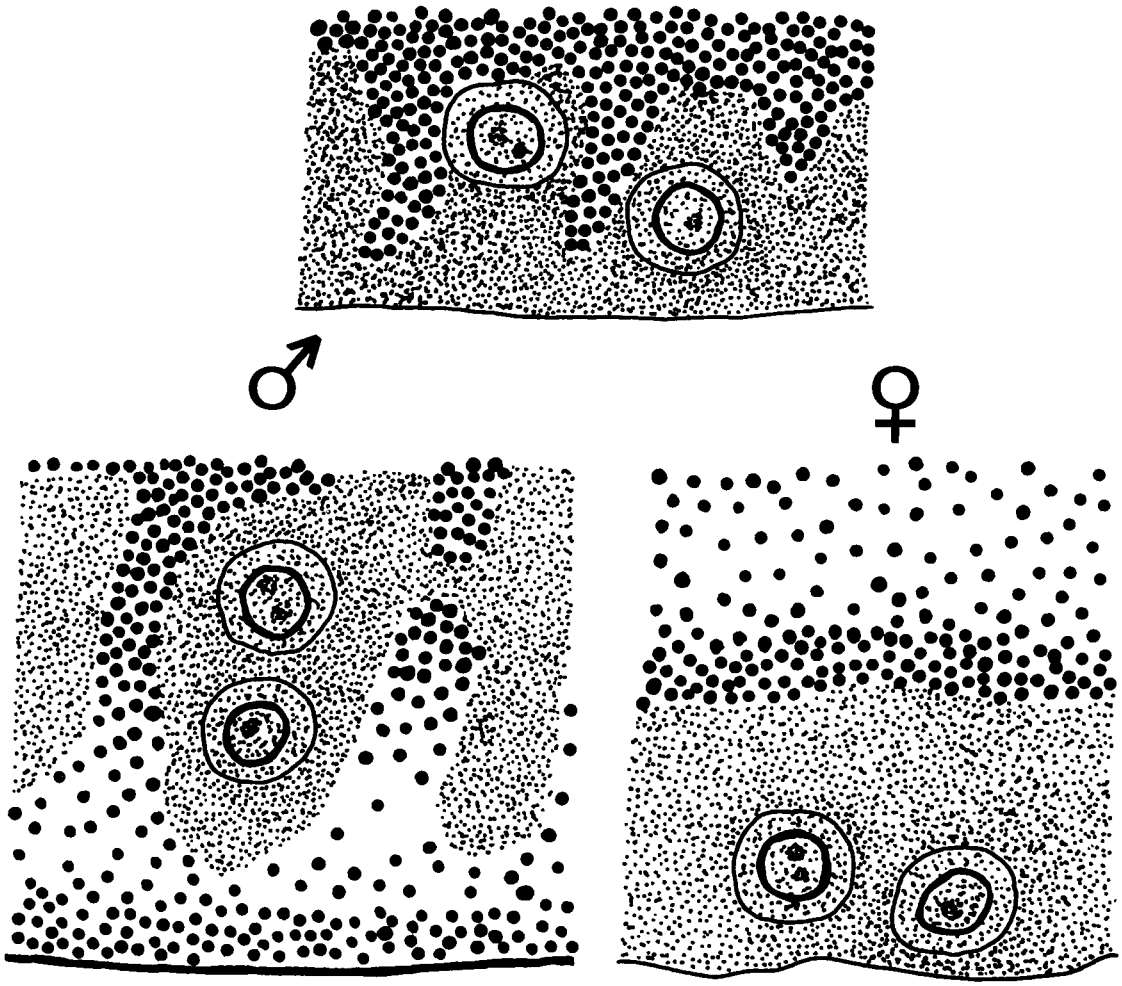

Fig. 3. Schematic drawing of the blastemal relationship during sexual differentiation of the gonadal primordium. In the indifferent gonad blastemal cells of mesonephric origin (coarse dotted) are intermingled with cells of the superficial epithelium (fine dotted). In the male anlage mesonephrogenic cells penetrate the superficial epithelium forming the Tunica albuginea. In the female anlage the mesonephrogenic blastema and the proliferating epithelium remains separated during the period of sexual differentiation (from Wartenberg, 1979).

although their cytoplasm and chromatin is denser (Wartenberg, 1978; 1979).

Developmental events during the sexual differentiation of the gonad into testis and ovary

Students of sexual differentiation maintain that the realization of the maleness of the gonad expresses itself by the sudden formation of testicular cords, while the onset of femaleness does not exhibit any concomitant remodelling of the basic structural situation (Jost, 1965; 1970). Careful histological studies on the period of sexual differentiation reveal a somewhat different aspect of the problem. Rabbit indifferent gonads display an alternative behaviour in male-determined anlage, in comparison with the future female gonad (Fig. 3). While the male blastemal cells continue to follow the tendency of becoming intermingled, in the female anlage an increasing segregation of the two blastemal parts becomes apparent (Wartenberg, 1980a). In the rabbit gonadal anlage of day 15, this is evident. In the future ovary the segregation of the two blastemal parts is apparent. One or two days later, on days 16 and 17, testis and ovary are clearly distinguishable. In the testis, mesonephrogenic blastemal cells have penetrated the core of the primary

blastema and now separate a small layer of the superficial epithelium from the blastema, forming a primitive tunica albuginea. The core of the primary blastema, which still consists of cell of epithelial and mesonephric origin, has lost its connection to the surface of the genital ridge. It forms the medulla of the testis. Primordial germ cells have participated in this centripetal movement and have become enclosed in the seminiferous tubules. The latter begin forming by separation of the blastema into a network of plate-like strands and interstitial tissue. The female gonad exhibits what amounts to a reversion to the original situation. Although both blastemal parts have proliferated, the penetration of the epithelial part by the mesonephrogenic cells has been unsuccessful and two blastemas have become largely separated. This process results in formation of the female "cortex" which, for the time being, consists of epithelial cells. Most of the primordial germ cells are enclosed within the cortical layer.

Once the male or female arrangement of the two blastemas has been achieved, the process of sexual differentiation is complete.

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