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2.1 The Urogenital Ridge

The genital tract is derived initially from the intermediate mesoderm of the urogenital ridge. The urogenital ridge contains the developing kidney, which includes at first the pronephros, then the mesonephros and finally the metanephros (Hutson 2008). In addition, the developing gonad forms anterior to the mesonephros and its duct, the mesonephric (Wolffian) duct. The mesonephros develops at approximately 4 weeks of gestation after the pronephros begins to degenerate. It also undergoes regression at approximately 7–8 weeks, which is at the time that the gonad begins to develop into ovary or testis. The mesonephric duct persists from the pronephric duct, and was originally named by Wolff. The paramesonephric or Müllerian duct develops as an evagination of the peritoneum adjacent to the gonad and migrates to the cloaca following the Wolffian duct (Fig. 2.1a–c).

The germ cells develop in the extra-embryonic tissues surrounding the yolk sac. Between 3 and 5 weeks of development they migrate around the yolk sac, through the caudal umbilical stalk and into the urogenital ridge (Fig. 2.1d, e). Once they arrive in the developing gonad they stimulate the development of the ovary or testis. In the developing ovary, the granulosa cells surround the germ cells and trigger meiotic arrest. By contrast, in the testis the newly formed Sertoli cells,

which are derived from the surface epithelium of the gonad, enclose the primordial germ cells to form the testicular cords and trigger mitotic arrest of the germ cells (Fig. 2.2a–c).

2.1.1 Wolffian and Müllerian Duct Development

At the caudal end of the Wolffian duct, the metanephric mesenchyme of the urogenital ridge stimulates development of a ureteric bud from the Wolffian duct just before it reaches the cloaca. A ureteric bud will stimulate development of the definitive kidney or metanephros. When the cloaca separates into hindgut posteriorly and urogenital sinus anteriorly the distal end of the Wolffian duct beyond the ureteric bud becomes incorporated into the posterior surface of the urogenital sinus to form the trigone of the bladder. The caudal ends of the mesonephric ducts and ureters enlarge as they are incorporated into the urogenital sinus, and differential growth occurs so that ureters end up being cranial to the distal end of the Wolffian duct (Fig. 2.3).

The Müllerian ducts rotate antero-medially around the Wolffian ducts as they grow to the cloaca and fuse with each other. The distal end of the ducts contains no lumen and forms a solid utero-vaginal primordium, which contacts the endodermal urogenital sinus at the Müllerian tubercle. Proliferation of the endoderm of the tubercle produces the vaginal plate, which invades the utero-vaginal primordium to form the vaginal lumen by canalisation and also elongates caudally to bring the opening of the vagina to the exterior of the urogenital sinus (Figs. 2.4 and 2.5).

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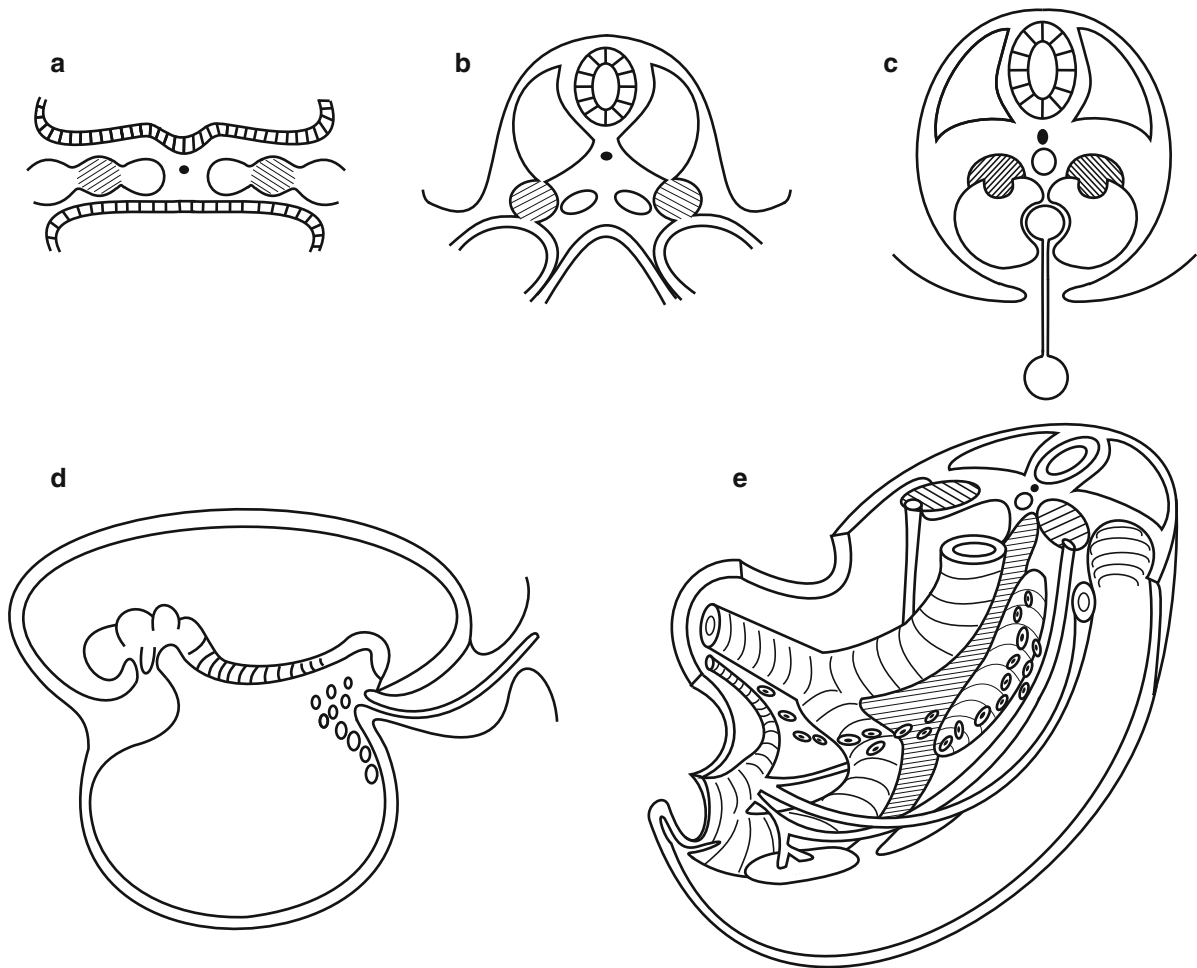


Fig. 2.1 Origin of the intermediate mesoderm in the early embryo. **(a)** Day 19. Transverse section of the trilaminar embryo showing the notochord and developing neural groove in the midline. The mesoderm forms into the paraxial mesoderm (the future somites), the intermediate mesoderm (the future urogenital tracts) and the lateral plate. **(b)** Day 21. The neural tube and dorsal aortas have formed, and the lateral plate mesoderm has split into parietal (somatic) and visceral layers. **(c)** Day 21–22. The embryo has now grown into a three-dimensional form. One dorsal aorta remains and the intermediate mesoderm is now

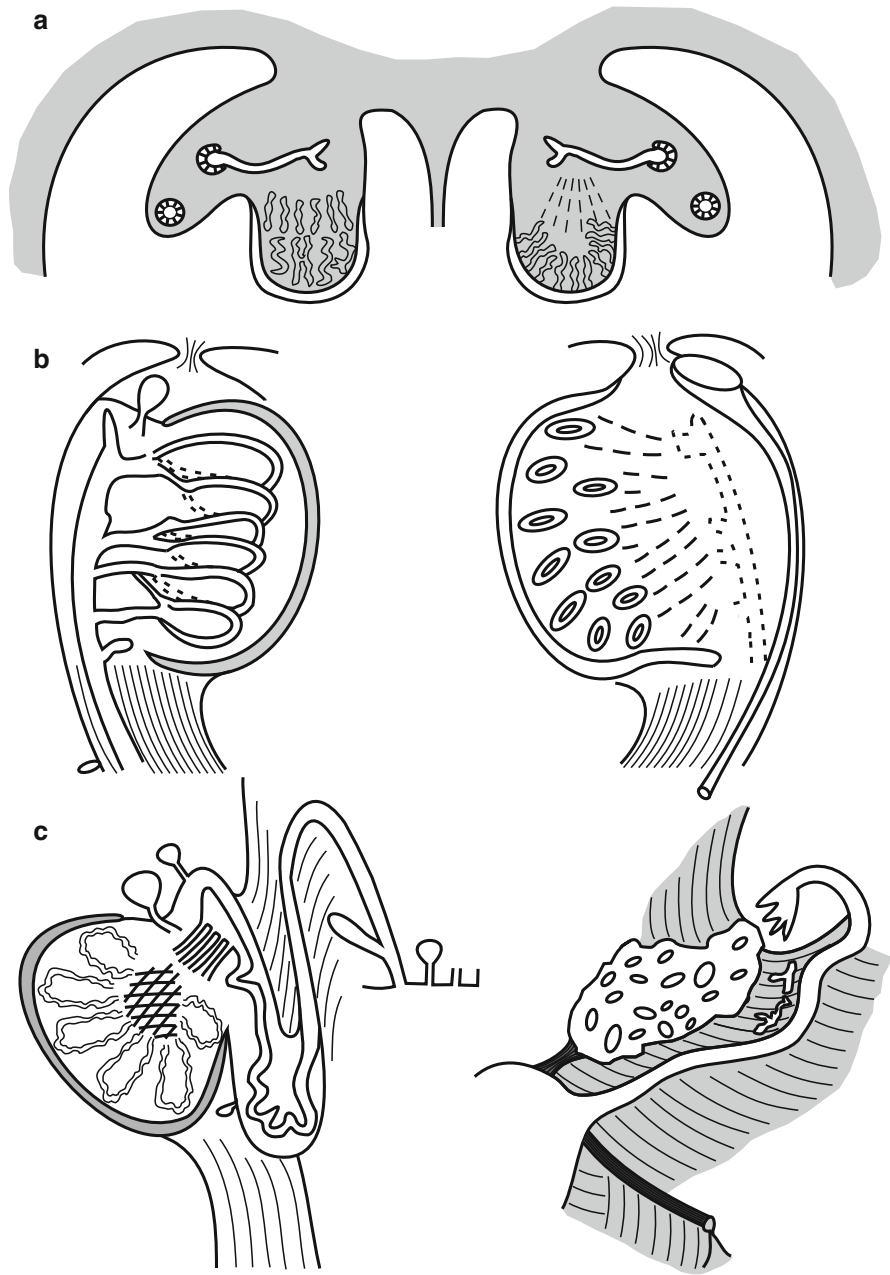
proliferating and bulging into the future intraembryonic cavity (future pleura and peritoneum). **(d)** At 3 weeks, the primordial germ cells proliferate in the caudal wall of the yolk sac near the allantois. **(e)** Migration of the germ cells by amoeboid movement around the hindgut and through its dorsal mesentery to reach the retroperitoneum, and then into the urogenital ridges to reach the primitive gonads, which is required for their further normal development. Migration is regulated by Steel, fibronectin and laminin, and the primordial germ cells are visible because of expression of c-Kit, Oct4 and alkaline phosphatase

Abnormalities of Müllerian duct development include fusion defects, such as a bicornuate uterus or uterus didelphys. Failed canalisation of the primordium leads to uterine or vaginal atresia. One relatively common anomaly is obstruction of one hemivagina associated with absence of the ipsilateral kidney. This is caused by failure of the Wolffian duct to reach the cloaca, which prevents caudal migration of the ipsilateral Müllerian duct and prevents development of a ureteric bud (Rokitansky sequence) (Fig. 2.6) (Stephens et al. 2002).

2.2 Sexual Differentiation

At 7–8 weeks of development, regression of the mesonephros leaves the gonad suspended on a mesentery known as the mesogenitale. With sexual differentiation the gonad forms primary epithelial sex cords and mesenchymal medulla. Presence of XY chromosomes triggers activation of the SRY gene, which initiates development of a testis (as described in Chap. 1), where the primary sex cords develop into Sertoli cells (Fig. 2.2). The Sertoli cells produce Müllerian-inhibiting

Fig. 2.2 (a) Transverse sections through the testis (on right side of embryo) and ovary (*left*) at 7–8 weeks. The testis is developing a tunica albuginea, a rete testis (from regressing mesonephric tubules) and primitive cords containing primordial germ cells. In the ovary the primitive medullary cords degenerate and the germ cells are in cortical cords, with proliferating surface epithelium. (b) The testis (on right side of embryo) has developed seminiferous cords (horseshoe-shaped) connected via the rete testis to the ductuli efferentes and the mesonephric (Wolffian) duct. The ovary has degenerated medullary cords and mesonephric tubules. The cortex has now developed primitive follicles around each germ cell. (c) The definitive genital ducts after testicular descent and formation of the broad ligament. The seminiferous tubules are in a horseshoe shape, connecting to the rete testis, efferent ductules and the developing epididymis. Caudally the vas deferens has a lateral diverticulum to form the seminal vesicle. Note cranial and caudal remnants of the paramesonephric (Müllerian) ducts: appendix testis (Hydatid of Morgagni) and prostatic utricle, respectively

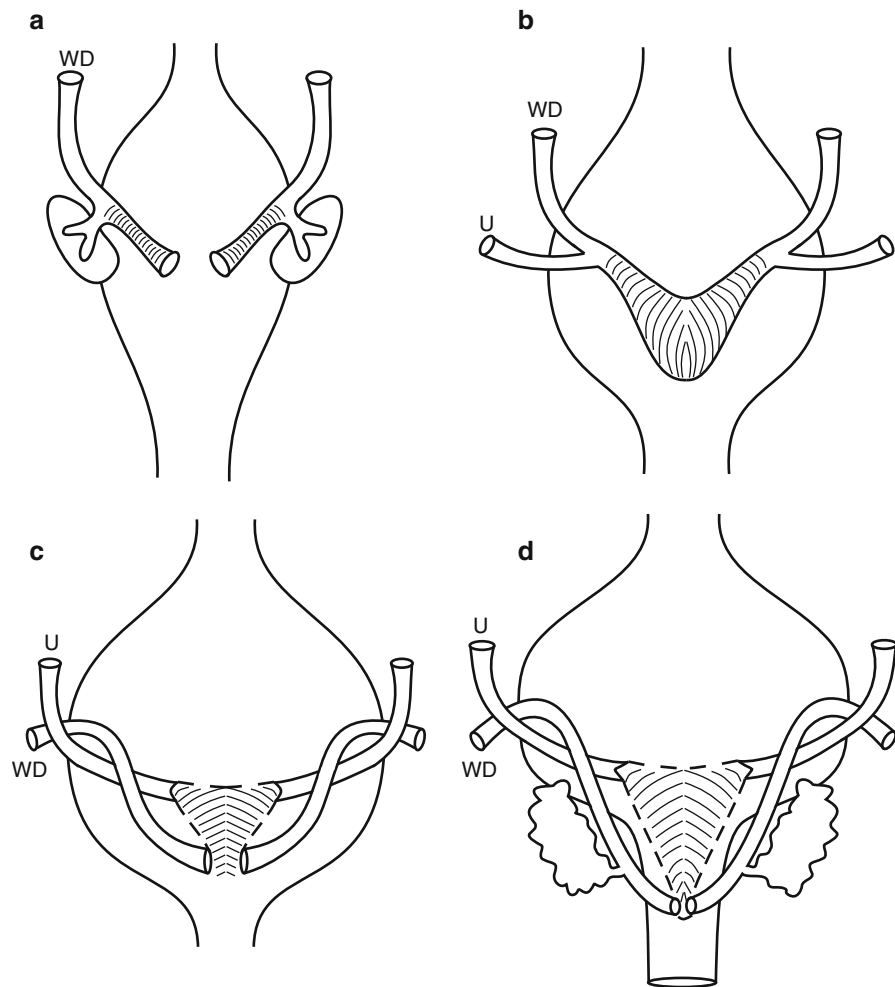


substance, also known as anti-Müllerian hormone (MIS/AMH), which leads to regression of the Müllerian duct (Seifer and MacLaughlin 2007). Leydig cells form outside the testicular tubules and produce testosterone, which stimulates the Wolffian duct to persist to form the epididymis, vas deferens and seminal vesicles (Tong et al. 1996). Insulin-like hormone 3 (INSL3) is also produced from the Leydig cells and is important for development of the gubernaculum (Nef and Parada 1999; Zimmermann et al. 1999).

2.2.1 Testosterone

Testosterone was first synthesised in the 1940s, and is a steroid formed from cholesterol and progesterone in the Leydig cells. It is secreted into the bloodstream as well as down the Wolffian duct in an exocrine manner, exposing the Wolffian duct to very high concentrations (Tong et al. 1996) (Fig. 2.7a). In addition, testosterone is secreted in an endocrine manner into the bloodstream where it will act on the

Fig. 2.3 The urogenital sinus, shown from behind, to demonstrate how the mesonephric duct below the ureteric bud is absorbed into the back wall of the urogenital sinus, to form the trigone of the bladder between the ureters, which migrate cranially around the lateral sides of the mesonephric ducts. **(a)** Common mesonephric ducts entering the urogenital sinus. **(b)** Common stem of mesonephric ducts being absorbed into back wall. **(c)** Origin of ureter rotating laterally around mesonephric duct to acquire independent opening into developing bladder. **(d)** Definitive anatomical arrangement with ureters opening into bladder at lateral corners of the trigone. Ejaculatory ducts enter into the prostatic urethra at the verumontanum. Seminal vesicles develop as lateral diverticula of caudal vasa deferentia



external genitalia to cause masculinisation. Sexual differentiation begins at approximately 8 weeks of gestation with production of MIS/AMH to trigger Müllerian duct regression, along with testosterone stimulating the Wolffian duct to persist to form the epididymis, vas deferens and seminal vesicles. The initial blood level of testosterone is probably too low to stimulate the external genital development without conversion to dihydrotestosterone (DHT) by the enzyme 5- α reductase type-2. Dihydrotestosterone binds about 5–10 times more tightly than testosterone itself to androgen receptors in the external genitalia, thereby increasing the effective concentration of testosterone ten-fold (Handelsman 2006). Some effects of testosterone in the brain may be mediated by conversion of testosterone to oestrogen via the enzyme aromatase, which is another mechanism to increase the effective concentration of androgen (Fig. 2.7b).

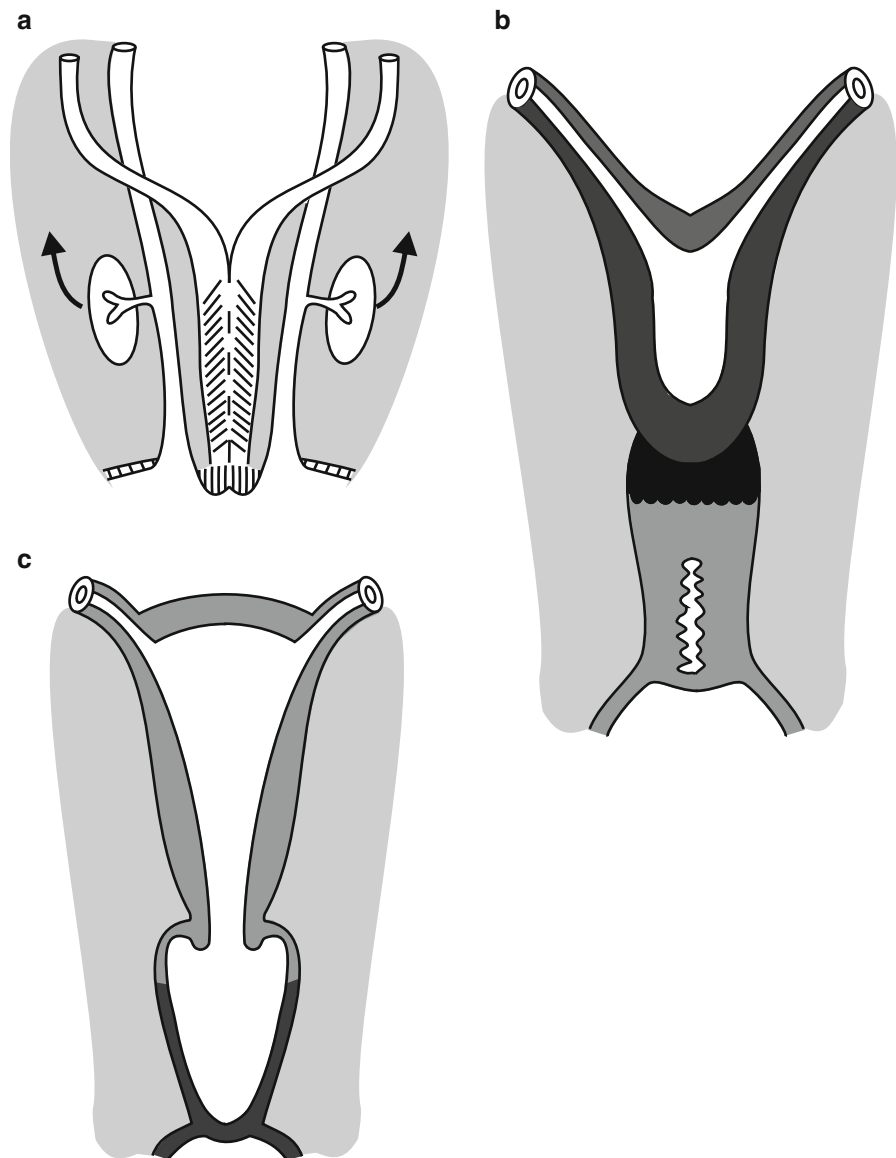
2.2.2 Müllerian-Inhibiting Substance/ Anti-Müllerian Hormone (MIS/AMH)

Müllerian-inhibiting substance (MIS/AMH) was first synthesised in the 1980s, and is a glycoprotein dimer (~MW 140,000) produced by Sertoli cells. It is also secreted into the Wolffian duct and then diffuses laterally into the adjacent Müllerian duct to trigger its regression in the male. It may also have some secondary role in development of the gubernaculum, and has post-natal functions in the ovarian cycle (Seifer and MacLaughlin 2007).

2.2.3 Insulin-Like Hormone (INSL3)

Insulin-like hormone 3 (INSL3) was discovered in the 1990s, and is a protein with homology to insulin that is produced by Leydig cells. It stimulates growth of the

Fig. 2.4 Development of uterus and vagina. **(a)** Paramesonephric (Müllerian) ducts migrate to urogenital sinus, fusing with contralateral duct. Distal ends are solid. Contact of solid tips of paramesonephric ducts triggers proliferation of endoderm to form sino-vaginal bulb (Müllerian tubercle). **(b)** Proliferation of the bulb cells forms the vaginal plate, which begins to breakdown centrally to form a lumen. Meanwhile, a lumen is developing in the fused Müllerian ducts to form a single uterine cavity. **(c)** Late gestation with lumen in uterus and cervix and vaginal vault now in continuity with lumen within vaginal plate to form the vagina. The hymen breaks down to become patent before term



genito-inguinal ligament, or gubernaculum, which is important for the first phase of testicular descent (Zimmermann et al. 1999).

2.3 External Genitalia

The external genitalia remain undifferentiated until 8 weeks of development (Fig. 2.8a). Initially they contain a central genital tubercle surrounded by inner and outer genital folds. Between the inner genital folds lies the endodermal urethral plate and the urogenital sinus opening. Masculine development is triggered by DHT,

causing growth of the genital tubercle into a penis, as well as growth of the perineal body to cover the urogenital sinus, canalisation of the urethral plate to form the urethra, and fusion of the outer genital folds to form the scrotum (Fig. 2.8b–d). In the female, the genital tubercle only enlarges a small amount to form the clitoris. In addition, there is apoptosis of the ventrally placed endoderm of the urethral plate, which leads to bending of the clitoral shaft. The inner genital folds remain separate to produce the labia minora, while the outer genital folds produce the labia majora. The urogenital sinus remains open to form the vestibule of the introitus (Fig. 2.9a–d).

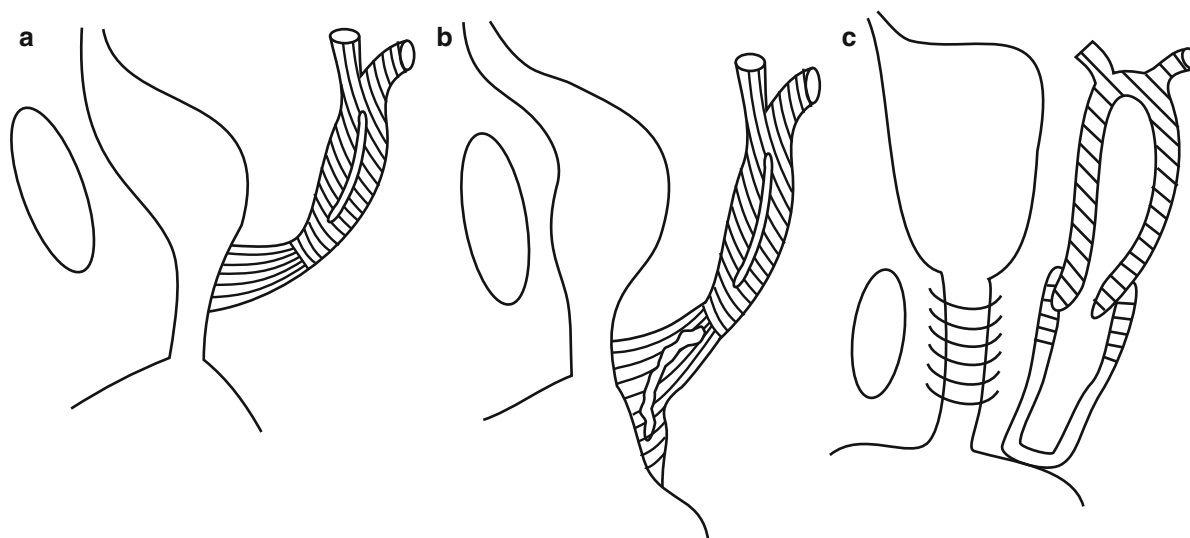


Fig. 2.5 Sagittal views of Müllerian tubercle (a) developing into vaginal plate (b), which by relative growth extends caudally to open eventually via a separate opening in the urogenital sinus to form the introitus (c)

Fig. 2.6 Rokitansky sequence. Ipsilateral failure of caudal growth of the Wolffian duct leads to absence of ureteric bud and agenesis of the ipsilateral kidney. In addition, the Müllerian duct cannot migrate caudally in the absence of the Wolffian duct as a guide, which leads to an obstructed hemi-uterus and hemi-vagina with haematometra or haematometocolpos

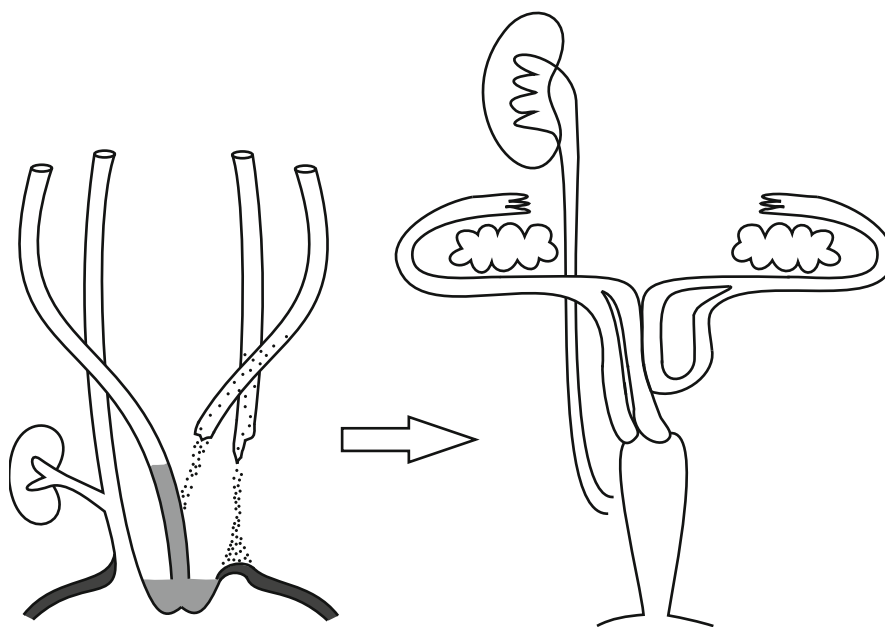


Fig. 2.7 (a) Schema of urogenital ridge at 8–10 weeks showing developing testis and ducts. Testosterone and Müllerian inhibiting substance (MIS/AMH) produced in the testis by Leydig cells and Sertoli cells diffuse into the duct system and pass via an exocrine manner down the mesonephric (Wolffian) duct. The testosterone is in a very high concentration which causes preservation of the Wolffian duct and its differentiation into epididymis, vas deferens and seminal vesicle caudally. Where there is gonadal dysgenesis or a block in androgen synthesis, only the cranial segment of the Wolffian duct is preserved. MIS/AMH also passes down the Wolffian duct and diffuses into the adjacent Müllerian duct to trigger regression. As with testosterone, the degree of regression is proportional to the amount of exocrine hormone reaching the duct system. (b) Testosterone acts by both exocrine and endocrine pathways in early development. Endocrine levels of hormone are low, presumably because the developing testis is initially very tiny, so adaptations have arisen to augment endocrine function at this stage. In some parts of the brain (in some animals) testosterone is converted to oestrogen, which then acts via the oestrogen receptor. Circulating oestrogens in females are bound to α -fetoprotein, which prevents crossing the blood–brain barrier, while testosterone can enter the brain freely. In the external genitalia and prostate the testosterone is converted by 5- α -reductase type-2 to dihydrotestosterone (DHT), which binds 5–10 times more tightly to androgen receptors than testosterone itself. Without this effective concentrating factor, the external genitalia cannot be masculinised by the testis, as its testosterone production is insufficient. However, at puberty, the testis is now so much larger that testosterone is capable of virilising remote tissues as the blood levels are more than 10–100 times higher than in the early foetus

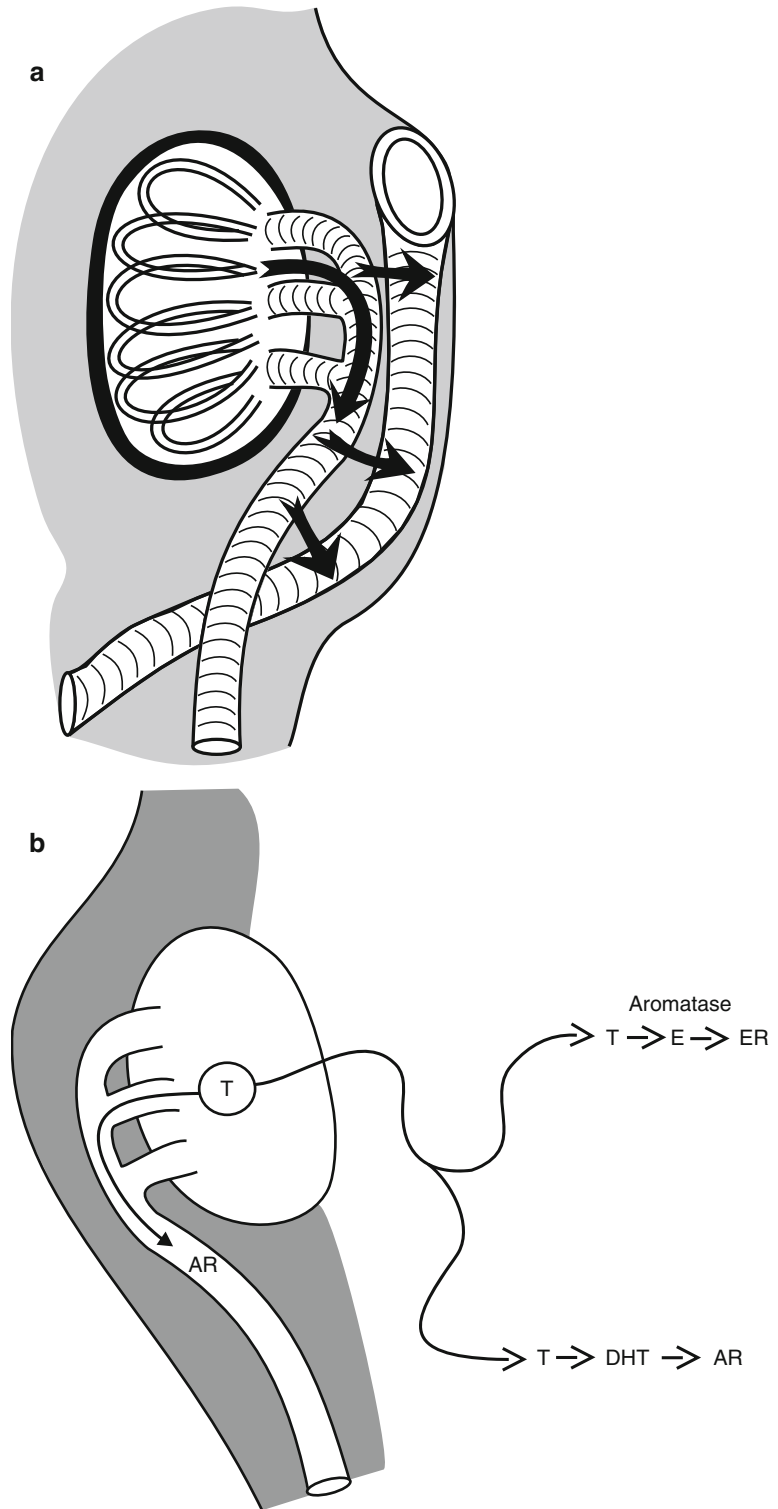
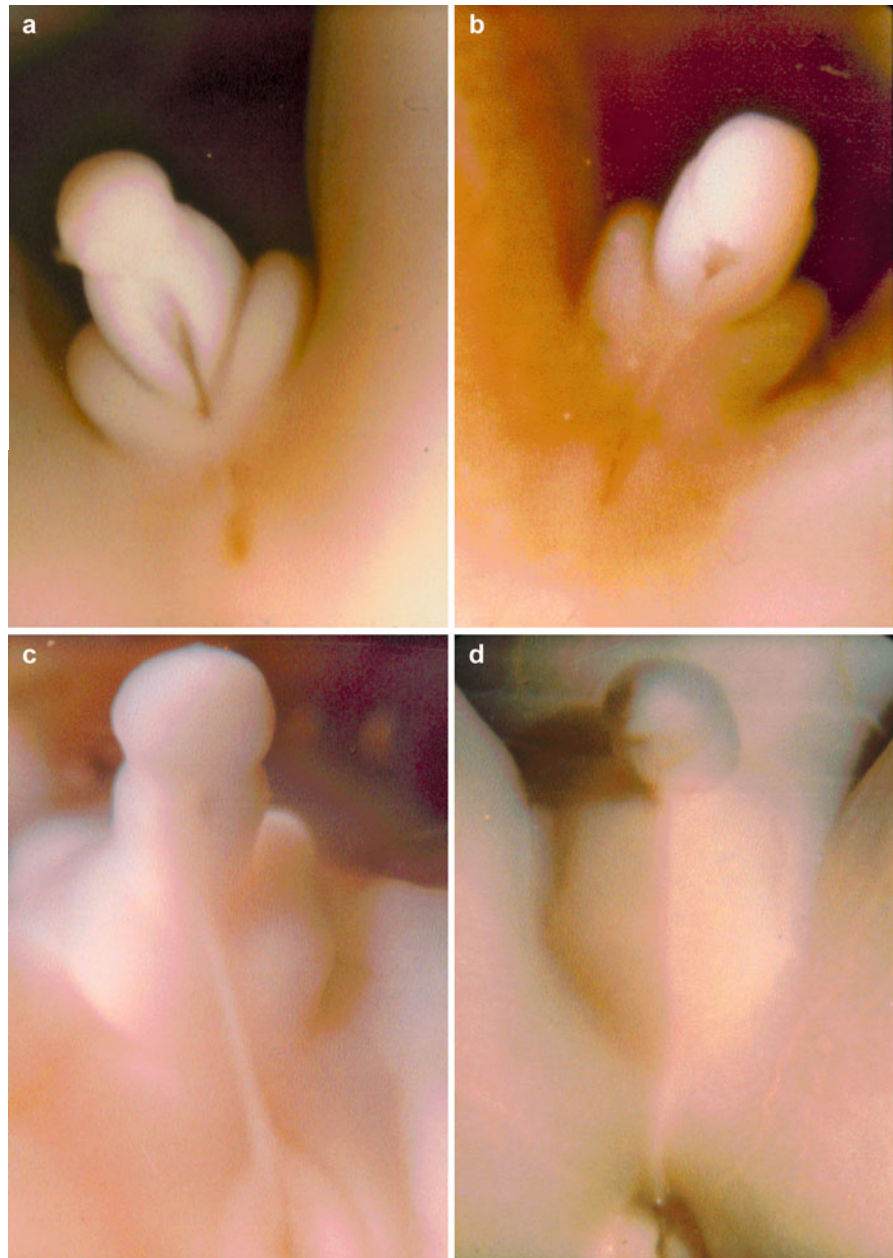


Fig. 2.8 (a) The external genitalia of an 8–9-week human embryo showing the ambisexual stage, just before the onset of sexual differentiation. (b) Transformation of the ambisexual genitalia into that of a male over 8–12 weeks. Week 9: partially fused urogenital folds. (c) Week 10: urogenital fusion progressing. (d) Week 12: fused urogenital folds. (Miller et al. 2004; Clarnette et al. 1997) (Reproduced with permission from England 1983, Clarnette et al. 1997)



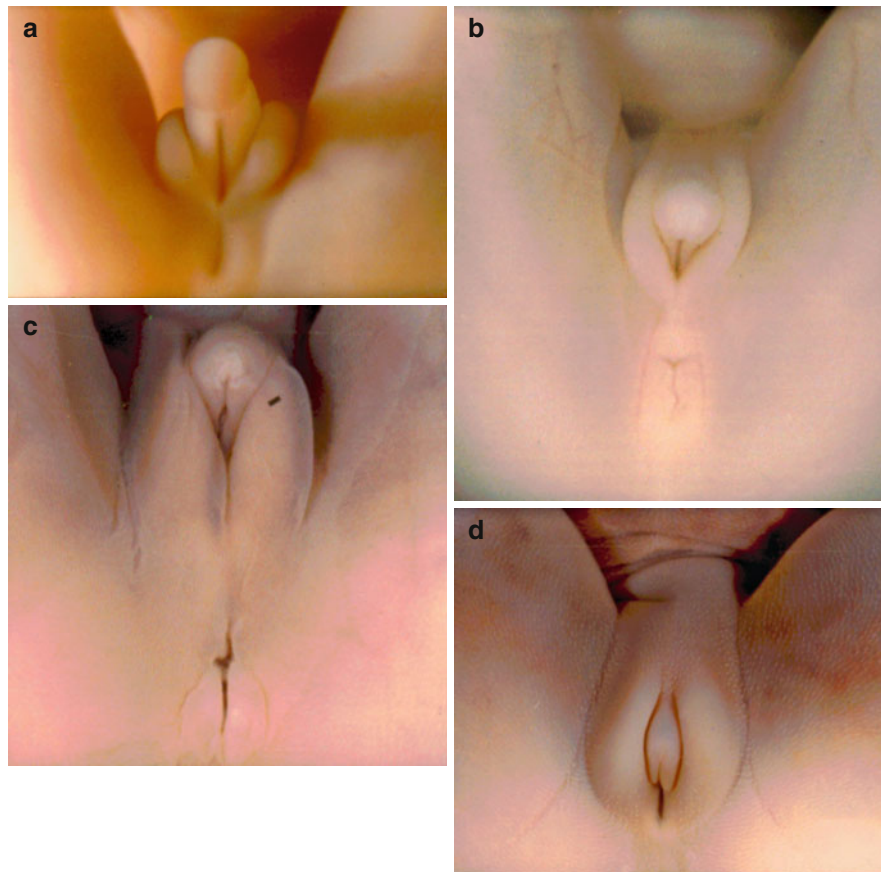


Fig. 2.9 Development of the external genitalia in female foetuses between 8 and 12 weeks. (a) Week 9: The clitoris is growing, but at a much slower rate than the rest of the foetus, making it look progressively smaller. (b) Week 13: The folding of the

clitoris caused by apoptosis of the endoderm forming the urethral plate is visible. (c) Week 17. (d) Week 20. (Reproduced with permission from England 1983)

2.4 Gonadal Descent

The ovary descends relatively in the abdomen as the Müllerian ducts and their mesentery, the broad ligament, do not grow as quickly as the lower part of the abdominal cavity (Fig. 2.10). By contrast, the position of the testis is changed radically by exposure to male hormones. Between 8 and 15 weeks, INSL3 stimulates the gubernaculum to enlarge, known as the swelling reaction, which holds the testis near the groin as the abdomen enlarges. This enlarged gubernaculum in the male anchors the testis passively to the site of the future inguinal canal as the abdominal cavity grows: the passive nature of this process is probably the reason that this mechanism is rarely abnormal, and hence undescended testes inside the abdomen are rare. Around the same time, testosterone causes regression of the cranial

suspensory ligament, which is the residual cranial mesentery of the urogenital ridge. In many mammals, the gonadal position is the vector sum of traction of the cranial and caudal ligaments (Fig. 2.11).

Between 25 and 35 weeks, testosterone stimulates the gubernaculum to grow out of the abdominal wall guided by the genitofemoral nerve, which releases a neurotransmitter (calcitonin gene-related peptide, CGRP) from its sensory nerve endings that provide a chemotactic gradient for the gubernaculum to follow (Hutson and Hasthorpe 2005). The processus vaginalis grows inside the elongating gubernaculum, as does the cremaster muscle. This second phase of testicular descent, requiring migration of the gubernaculum, is very complex mechanically (Hutson et al. 2010) and hence failure of this process, leading to undescended testis, is common (Fig. 2.12).

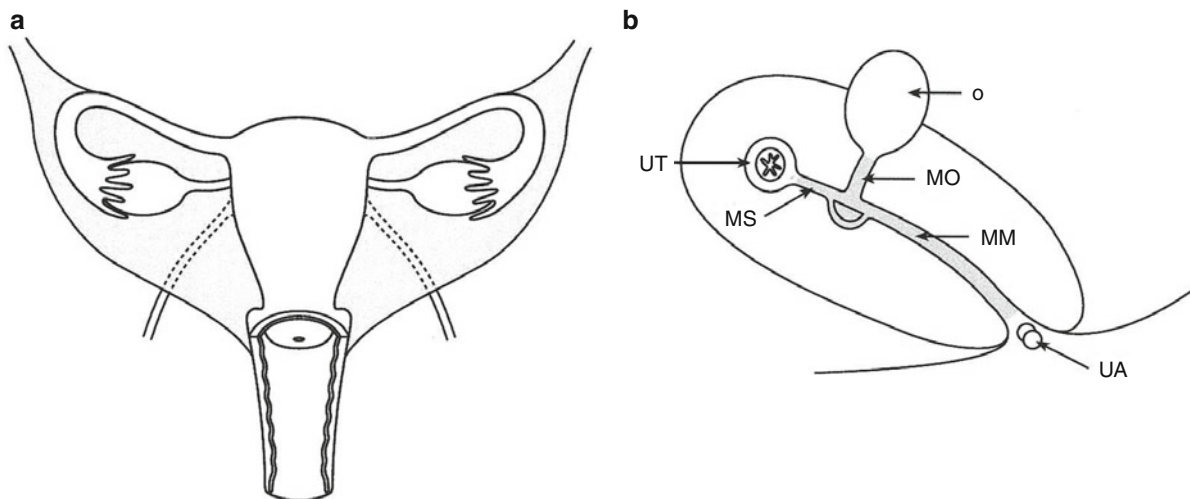
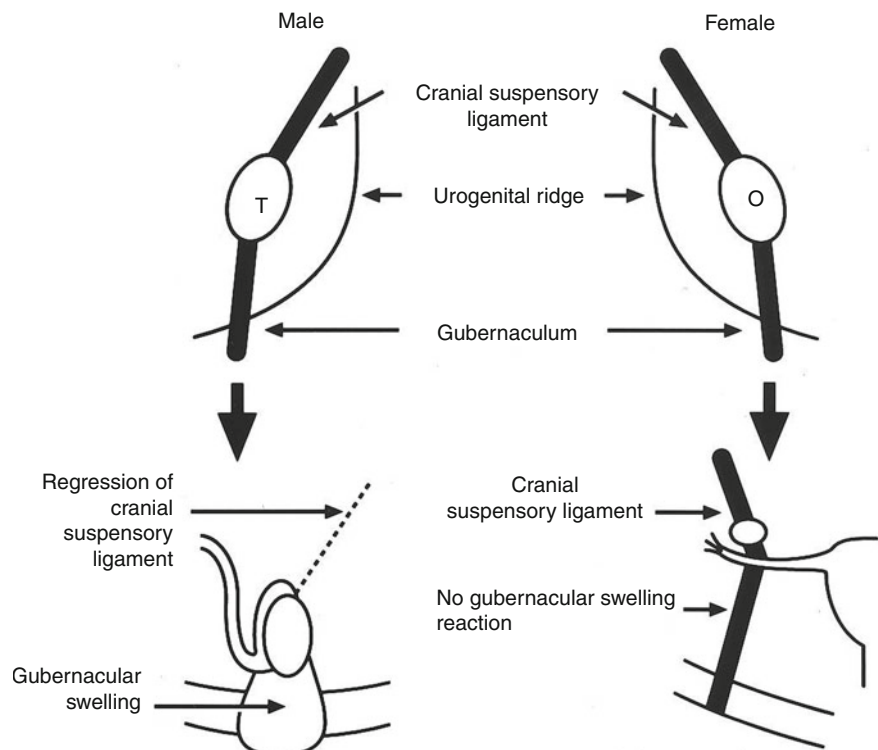


Fig. 2.10 Drawing of broad ligament (*shaded*) in adult female human shown from the back (**a**), and from the side (**b**). *UT* uterine tube, *O* ovary, *MO* mesovarium, *MS* mesosalpinx, *MM*

mesometrium, *UA* uterine artery (Redrawn from Gray's Anatomy (Williams et al. 1995), and reproduced with permission from Miller et al. 2004)

Fig. 2.11 Schematic diagram illustrating the role of the gubernaculum and cranial suspensory ligaments in the final position of the gonad. In the male a combination of gubernacular swelling and regression of the cranial suspensory ligament allows the testis to remain close to the internal inguinal ring. In females the gubernaculum remains long and thin, allowing the ovary to move away from the inguinal region with growth of the embryo. The cranial suspensory ligament helps to maintain the position of the ovary on the posterior abdominal wall (Reproduced with permission from Clarnette et al. 1997)



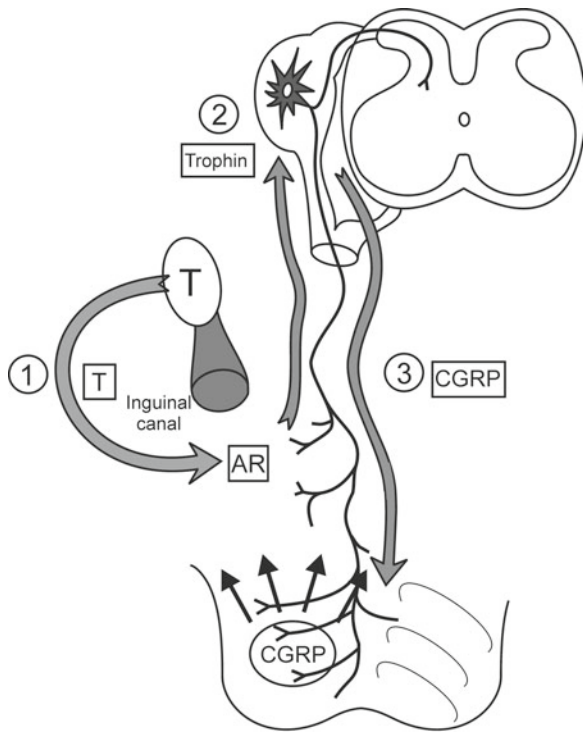


Fig. 2.12 The inguino-scrotal phase of testicular descent occurs between 25 and 35 weeks of gestation, under the control of androgen. However, the mechanism is very complex and indirect, with androgen acting mostly indirectly via androgen receptors in the inguinal region to trigger masculinisation (via an unknown trophin) of the genitofemoral nerve, which releases a neurotransmitter via its sensory nerve endings in the groin and scrotum (calcitonin gene-related peptide; *CGRP*). *CGRP* provides a chemotactic gradient for the migrating gubernaculum to the scrotum

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