Origin of Müllerian Duct and Its Later Development in Relation to Wolffian Duct and Anogenital Distance in the Rat

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ABSTRACT. On day 14 of fetal life in the rat, Müllerian (paramesonephric) duct in both sexes was first observed close to Wolffian (mesonephric) duct. Its caudal end joined Wolffian duct at about the level of the middle of gonadal anlage. Thereafter, Müllerian duct became independent of Wolffian duct through its whole length. Therefore, the main part of Müllerian duct may be formed by budding from Wolffian duct. On day 16, the anogenital distance in male started to increase with thickening of Wolffian duct and disappearance of Müllerian duct. Castration of male fetuses on day 19 stopped later masculinizing development. Therefore, it is clear that the fetal testis is an organ crucial for the masculinization of the urogenital tract as it activates the secretion of masculinizing hormone(s).—KEY WORDS: anogenital distance, masculinization, Müllerian duct, rat fetus, Wolffian duct. Jpn. J. Vet. Sci. 51(4): 693–701, 1989

Wolffian (mesonephric) and Müllerian (paramesonephric) ducts are present in both sexes in early fetal stages.

The descriptions of the growing process of Müllerian duct can be encountered with two divergent opinions. The first opinion is that Müllerian duct is formed from an active downward growth of the cells in the bottom of itself [1, 2, 10]. The second opinion is that Müllerian duct is originated from Wolffian duct [4, 5, 11, 12, 14].

In male fetus, Wolffian duct develops well along with the regression of Müllerian duct and the increase of the anogenital distance. In female fetus, Müllerian duct develops well together with the regression of Wolffian duct and with only a slight elongation of the anogenital distance. It has been reported that the fetal testis plays a very important role in controlling the masculinization of the undifferentiated fetal urogenital tract [8, 17]. The fetal testis stimulates the development of Wolffian duct as well as the external genitalia and induces the regression of Müllerian duct. However, it is unknown whether the fetal ovary participates in the development of the urogenital tract. The present study was designed to know how and from what origin Müllerian duct was made up and also to determine at what period the sex difference was noticed in development of Wolffian duct, Müllerian duct, and the external genitalia in rat fetuses. Furthermore, castration was performed in male fetuses to investigate the role of the fetal testis in the mode of development of the urogenital tract.

MATERIALS AND METHODS

Wistar rats were used in this study. They were given a commercial diet (Funabashi MM-1) and water, both ad libitum. Females in estrus were caged together with males overnight, and were examined for the presence of sperm in the vaginal smear in the following morning, which was counted as day 0 of gestation.

On days 13 to 21, the pregnant animals were sacrificed and two male and two female fetuses were obtained at random in each litter. The anogenital distance of each
fetus was measured, except for fetuses on days 13 and 14 which were too small to be measured.

After measurement, the cranial half of the body of each fetus was cut off at the diaphragmatic level and the caudal half of the body was fixed in Bouin's fluid. Then, the liver, intestine, limbs and tail were removed. The body was dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned serially at 7 μm. The sections were stained with hematoxylin-eosin or Masson's trichrome.

Diameters of Wolffian and Müllerian ducts were measured in each fetus on days 14 to 21 of gestation. Diameters of the cranial parts of Wolffian and Müllerian ducts were measured in the section in which the middle part of the gonad was cut. Also, the diameters of the caudal part of Wolffian duct at two different levels were measured in every fifth section from the bottom of the Wolffian duct nearly attaching the urogenital sinus. The diameters on both sides of Wolffian duct or Müllerian duct were averaged in each fetus. The caudal part of Müllerian duct was not measured because it was fused with Wolffian duct in both sexes on days 14 and 15 and the duct began to disappear in male on day 16.

In the next experiment, pregnant rats were subjected to fetal castration in utero on day 19 of gestation by the method of Eguchi and Morikawa [3]. In each litter, two male fetuses were castrated. Two days later, they were autopsied. At autopsy, each surviving operated fetus was matched at random with an intact littermate male fetus. In this experiment, the diameter of the cranial part of Wolffian duct was not measured because it was removed with the testis by castration. The sex of fetuses on and after day 18 was easily distinguishable by the anogenital distance; with the naked eyes it was longer in male than in female. The sex of fetuses prior to day 17 was determined by histological

inspection of their gonads.

Analyses of data were made with Student's t test. A P less than 0.05 was considered statistically significant.

RESULTS

Measurement observation (Table 1)

Anogenital distance: On day 15 of gestation, the anogenital distance was not different between male and female fetuses. On day 16, the distance was significantly longer in male than in female. Then, on and after day 17 this difference became greater.

Castration of male fetuses on day 19 stopped the increase of anogenital distance 2 days later. The distance of castrated male was significantly short as compared with that of littermate male but still significantly long as compared with that of littermate female.

Wolffian duct: On days 14 and 15 of gestation, diameters of both the cranial and caudal parts of Wolffian duct were not different between male and female fetuses. On day 16, both the cranial and caudal parts of the duct became significantly thick in male. On day 17 in male, the diameter of the cranial part of the duct was the same as that on day 16. Then, the cranial part of the duct was gradually reduced in diameter toward the end of gestation. To the contrary, the caudal part was continuously increased in diameter. In female, the cranial part of Wolffian duct was definitely decreased in diameter, and it disappeared on day 18. The caudal part of the duct still persisted on day 18 with a markedly reduced diameter. The duct of female completely disappeared on day 19.

Castration of male fetuses on day 19 resulted, 2 days later, in a significant decrease in diameter of Wolffian duct as compared with that of intact littermate males.

Müllerian duct: On days 14 and 15 of gestation, the diameter of Müllerian duct
was not different between male and female fetuses. The duct of male almost disappeared on day 16. However, in the female, the size of the duct was increased continuously up to the end of gestation.

**Histological observation**

Wolffian duct: On day 13 of gestation, Wolffian duct in fetuses of both sexes was situated along the ventrolateral side of the mesonephros. The duct already reached the urogenital sinus. The duct was composed of single columnar epithelial cells and was provided with a wide lumen. Up to day 15, there was little change in histological feature of Wolffian duct in both sexes. Leydig cells were observable in fetal testis on day 15.

On day 16, male Wolffian duct was increased in cell number and in cell size, but the female duct remained unchanged.

On day 17, male Wolffian duct developed more in caudal part than in cranial part. In female at this stage, the cells of Wolffian duct were more irregular in shape, the cytoplasm contained many small eosinophi-
lic granules, and the duct itself disappeared from place to place. These degenerating characteristics were yet not observed in the caudal part of the duct.

On day 18, the number and size of cells in the cranial part of male Wolffian duct was decreased, together with the normal regression of the mesonephros. However, the caudal part of the duct continuously developed with an increase in cell number toward the end of gestation. A primordium of the seminal vesicle budded on the lateral side of the duct near the urogenital sinus. In female, the cranial part of Wolffian duct completely disappeared but its caudal part persisted. The caudal part became thin, apparently undergoing degenerative changes, as did the cranial part on day 17.

On day 19, female Wolffian duct completely disappeared from the urogenital tract. In male at this stage, the prostatic buds were observable at the region near the urogenital sinus.

Castration of male fetuses on day 19 caused, 2 days later, involution of Wolffian duct. Wolffian duct was provided with a slight lumen and was composed of small cells as compared with that of littermate males (cf. Figs. 1a and 1b). The duct cells apparently changed from columnar to cuboidal in shape by castration. Cytoplasm of the duct cells contained many small eosinophilic granules, as observed in female Wolffian duct. The seminal vesicle and the

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Fig. 1. a: Section, at the level where Wolffian and Müllerian ducts reached the urogenital sinus (US), showing well-developed Wolffian duct (W), and Müllerian remnant (M) in a male fetus on day 21 of gestation. Hematoxylin-eosin ×315. b: Section, at the level where Wolffian and Müllerian ducts reached the urogenital sinus (US), showing degenerative Wolffian duct (W), arrows showing eosinophilic granules) and Müllerian remnant (M) in a castrated male fetus on day 21 of gestation. Hematoxylin-eosin ×315.
prostatic buds were either degenerated or undifferentiated (cf. Figs. 2a and 2b).

**Müllerian duct:** On day 13 of gestation, Müllerian duct was not yet observed in the urogenital tract.

On day 14, the duct was first observed in fetuses of both sexes. At this stage, Müllerian groove and Müllerian duct were situated on the ventrolateral surface of the cranial part of the mesonephros, just caudal to the region where the mesonephro-diaphragmatic ligament attached. Müllerian groove was lined with a single columnar epithelium. Somewhat caudal to this groove, Müllerian duct ran parallel with Wolffian duct and showed a cord-like structure only partially provided with a lumen. Further caudally, Müllerian duct came to be in close contact with Wolffian duct. At this level, Müllerian duct came to be in close contact with Wolffian duct. At this level, Müllerian duct was enveloped with its own basal membrane, independent of Wolffian duct. However, the caudal solid end of Müllerian duct was enveloped with a basal membrane common to Wolffian duct. At the region where Müllerian duct was fused with Wolffian duct, cells of the caudal tip of Müllerian duct were common to Wolffian duct. Mitotic figures were very often observed at this region.

On day 15, Müllerian duct in both sexes was extended close to the urogenital sinus. Fig. 3 shows the relationship of Müllerian duct and Wolffian duct, particularly at the caudal part, in a female fetus on this day.

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Fig. 2. a: Section, at the level near the urogenital sinus showing well-developed Wolffian duct (W), the seminal vesicle (S) and prostatic buds (P) in a male fetus on day 21 of gestation. Hematoxylin-cosin ×170. b: Section at the level near the urogenital sinus showing degenerative Wolffian duct (W), seminal vesicle (see arrows) and prostatic buds (P) in a castrated male fetus on day 21 of gestation. Hematoxylin-cosin ×170.
Fig. 3. Serial sections showing the caudal tip of Müllerian duct attaching Wolffian duct in a female fetus on day 15 of gestation. Photomicrographs are arranged toward caudal direction in the alphabetical order. Müllerian duct is fused with Wolffian duct and both Müllerian and Wolffian ducts are enveloped with a common basal membrane. Mitotic figures (arrows) are found. Masson's trichrome ×600.
The duct was provided with the lumen and was lined with single columnar epithelial cells (Figs. 3a and 3b). The duct became devoid of the lumen toward the caudal end (Figs. 3c and 3d). Histological feature of the caudal end of Müllerian duct was the same as described on day 14 (Figs. 3e—3h).

On day 16, Müllerian duct in both sexes reached the urogenital sinus. In male, the cranial part of the duct partially or completely disappeared, except for Müllerian groove which disappeared at about the end of gestation. The medial part of the duct began to degenerate, becoming smaller than that of females. The cytoplasm of cells contained small basophilic granules. However, the caudal part of the duct did not yet undergo such involution. Involution of Müllerian duct advanced in such a way that the duct cells diminished in size, the duct itself decreased in diameter and the lumen disappeared. The duct then was squeezed by mesenchyme and completely disappeared on day 17. The caudal end of Müllerian duct persisted as a remnant, so called prostatic utriculus. In female, Müllerian duct developed well continuously until the end of gestation.

DISCUSSION

Foregoing observations offer some lines of information as to the development and degeneration of Müllerian duct, Wolffian duct and other sexual structures. First, Müllerian duct is originated from Wolffian duct. Second, sexual differentiation of the external genitalia (anogenital distance) and that of Wolffian and Müllerian ducts start simultaneously on day 16 of fetal life. Third, castration of a male fetus stops the later masculinizing development of the urogenital tract. Fourth, regression of Müllerian duct begins at the time when the duct reaches the urogenital sinus.

As regards the first line of information, it has been well known that Müllerian groove is formed from the thickening of the coelomic epithelium of anterolateral surface of mesonephros.

Regarding the origin of Müllerian duct, Gruenwald [4, 5] favored Wolffian origin by his experimental result in that, by injury of Wolffian duct, Müllerian duct stopped growing the caudal direction beyond the injured region. In the present study, Müllerian duct in both sexes was first observed on day 14 of gestation, running parallel with Wolffian duct. On days 14 and 15, the caudal end of Müllerian duct was always fused with Wolffian duct. At this region, mitotic figures were very often observed, and it was very difficult to distinguish cells of Müllerian duct from those of Wolffian duct. Therefore, it is suggested that Müllerian duct is established by separating from Wolffian duct.

Concerning the second and third lines of information, it is thought that masculinization of the urogenital tract in undifferentiated fetuses is attributable to testicular androgen [8, 13, 16, 17, 21]. In the present study, Leydig cells were first observed on day 15. On day 16, sexual differentiation occurred in the urogenital tract. In male, the anogenital distance was increased in accordance with the thickening of Wolffian duct and disappearance of Müllerian duct. These changes were absent in female. Furthermore, fetal castration prevented the urogenital tract of male fetus from being masculinized in later development, indicating that the fetal testis is an organ principal for masculinization of male fetuses. The present result is in favor of the view that the fetal rat testis can secrete masculinizing hormone(s) already before day 16.

Regarding the fourth line of information, it has been shown that regression of Müllerian duct in male fetuses is induced by a testicular factor differing from testosterone [8]. The factor is called Müllerian inhibiting
substance, synthesized by fetal Sertoli cells located in seminiferous tubules [6, 7, 20]. Jost [9] and Price et al. [18] in vivo and Price et al. [19] in vitro noted in rats that the cranial part of Müllerian duct began to degenerate in male on day 15. In vitro, Picon [15] showed that male and female Müllerian ducts did not generate if explanted without the gonad on day 14 but degenerated if explanted with the fetal testis. After day 15, Müllerian duct was regressed in male and maintained in female with or without the fetal testis. In the present work, on day 15, the diameter of Müllerian duct did not reach the urogenital sinus. However, in one male fetus, which was not described in the result, Müllerian duct already reached the urogenital sinus and began to degenerate. On day 16, Müllerian duct of all fetuses reached the urogenital sinus, and the male duct began to disappear. Thus, it seems that until male Müllerian duct reaches the urogenital sinus, it does not begin to degenerate.

REFERENCES

要約

ラット胎仔における中腎管および中腎傍管の発生と消長について：猪設智夫・江口保暢1・中村経紀（麻布大学獣医学部実験動物学講座，1剖解学第二講座）—妊娠14日目に雌雄ラット胎仔に初めて中腎傍管が観察された。中腎傍管は中腎管のすぐ側に位置しており、発生の途中においてその尾側端は常に中腎管と融合していた。その後中腎傍管は中腎管から完全に分離独立していた。このことから、中腎傍管の主体は中腎管から分化するもののと考えられた。妊娠16日目になると、雄胎仔では雄のそれとは異なり、中腎傍管の消失、中腎管の発達、肛門生殖結節間距離の増加が認められた。妊娠19日目に胎仔精巣を除去すると、その後の胎仔の雄性化は妨げられた。これらのことから、胎仔精巣は生殖道の雄性化に必要不可欠な器官であることがわたった。