

[COMMUNICATION]

Origin of Somatic Cells in Bidder's Organ and the Gonad Proper in the Toad, *Bufo japonicus formosus*

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ABSTRACT—The development of Bidder's organ was observed ultrastructurally and made a comparison with the gonad proper in *Bufo japonicus formosus*. The primordia of Bidder's organ and the gonad proper consisted of coelomic epithelial cells and primordial germ cells. The gonad proper showed a typical cortico-medullary structure. After the gonadal sex differentiation, the medulla in the primordial testis developed together with migration of germ cells from the cortex, and the medulla in the primordial ovary developed as a somatic cell mass. Bidder's organ showed no cortico-medullary structure and no sexual difference. Numerous gonial cells enlarged without meiotic nuclear change, and were surrounded by a layer of epithelial cells. The origin of somatic cells in Bidder's organ was identical with that in the gonad proper, so that the peculiar development of Bidder's organ is not attributed to the cellular origin. We discuss the possible cause of the development of Bidder's organ.

INTRODUCTION

Bidder's organ, which is peculiar to Bufonidae, develops into an ovary-like organ irrespective of the genetic sex [1, 2]. In this organ, follicles appear at earlier stages than in the ovary [2, 3–8], but remain immature [9, 10]. The ovary-like development of Bidder's organ has been attributed to the agenesis of the medulla [2, 8], which derived from mesonephric or interrenal blastemal cells [11, 12]. In the last decade, however, ultrastructural studies on anuran gonads have shown that the gonadal medulla is formed with coelomic epithelial

cells [13–18]. These findings have cast doubt on the theory presented by Witschi [19] that the gonadal sex differentiation is attributed to the antagonistic development of the cortex and medulla.

In the present study, the embryological origin of somatic cells in Bidder's organ and the gonad proper in the toad *Bufo japonicus formosus* was examined first by electron microscopy.

MATERIALS AND METHODS

Freshly spawned egg strigs of *Bufo japonicus formosus* were collected in Niigata City, in the beginning of April. Hatched larvae were kept in dechlorinated tap water at $18 \pm 1^\circ\text{C}$ and were fed commercial fish meal. Under these conditions, larvae metamorphosed 45–50 days after fertilization. To determine the developmental stages of the larval specimens, the normal table of development of this species [20] was used.

Gonads and surrounding tissue obtained from 10–15 animals were fixed every 3–6 days in Karnovsky fixative (1.3% glutaraldehyde and 1.3% paraformaldehyde in 0.08 M cacodylate buffer) for 1.5–2 hr at room temperature, then postfixed in 1% OsO_4 in the same buffer for 2 hr at 4°C . After dehydration through a graded series of ethanol, specimens were embedded in Epok 812. Thin and semithin serial sections were cut using a Porter-Blum MT-1 ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate, and examined using a Hitachi H-300 electron

microscope. Semithin sections were stained with a mixture of 1% toluidine blue and 1% borax for light microscopic observations.

RESULTS

The genital ridge, the primordium of the gonad proper and Bidder's organ, first appeared at stage 30 (external gill stage) at the ventral medial region of the mesonephros, and consisted of coelomic epithelial cells and primordial germ cells (PGCs) (Fig. 1). The primordium projected into the coelom in stages 32–33, and then the cortico-medullary structure was observed clearly at the middle to posterior region of the gonad proper at

stage 40 (toe development stage) (Fig. 2a). Mesenchymal tissue invaded into the gonad proper simultaneously with the appearance of the cortico-medullary structure. The cortex and medulla were connected directly to one another and the two regions were covered with a continuous basal lamina (Fig. 2b), which suggests that both the cortical and medullary somatic cells are derived from coelomic epithelial cells. Sexual differentiation occurred at stage 41 (hindlimb completion) in the gonad proper. In the primordial testis, the germ cells and somatic cells migrated to the medulla through the connections between the cortex and medulla in stages 42–45 (climactic metamorphosis) (Fig. 4). In the primordial ovary during these stages, germ cells were situated in the cortex, and the medulla developed as a somatic cell mass (Fig. 5).

At stage 40, Bidder's organ consisted of epithelial cells, filled with lipid droplets, and germ cells (Fig. 3). At stage 42, lipid droplets within the epithelial cells decreased rapidly and numerous gonial cells which had a lobular nucleus enlarged without meiotic nuclear change. At stage 45, the enlarged germ cells were surrounded by follicular cells which originally consisted of the epithelial tissue of Bidder's organ (Fig. 7a). The follicular cells were covered with the basal lamina, and were connected with an enlarged germ cell with microvilli (Fig. 7b). Mesenchymal cells and blood vessels invaded to the periphery at stage 42 (Fig. 6), and then were distributed around the follicles (Fig. 7). These extragonadal cells were separated from the epithelial cells by the basal lamina (Fig. 6b and 7b). Neither the cortico-medullary structure nor sexual difference was observed in Bidder's organ.

DISCUSSION

The present study is the first to conclude with electron microscopic findings, that Bidder's organ consists of the coelomic epithelium-derived cells and germ cells, and that the medullary structure is not developed. Light microscopic studies have reported the absence of a medulla in Bidder's organ [2, 8], while the presence of slight medullary elements have also been claimed [5, 6]. The

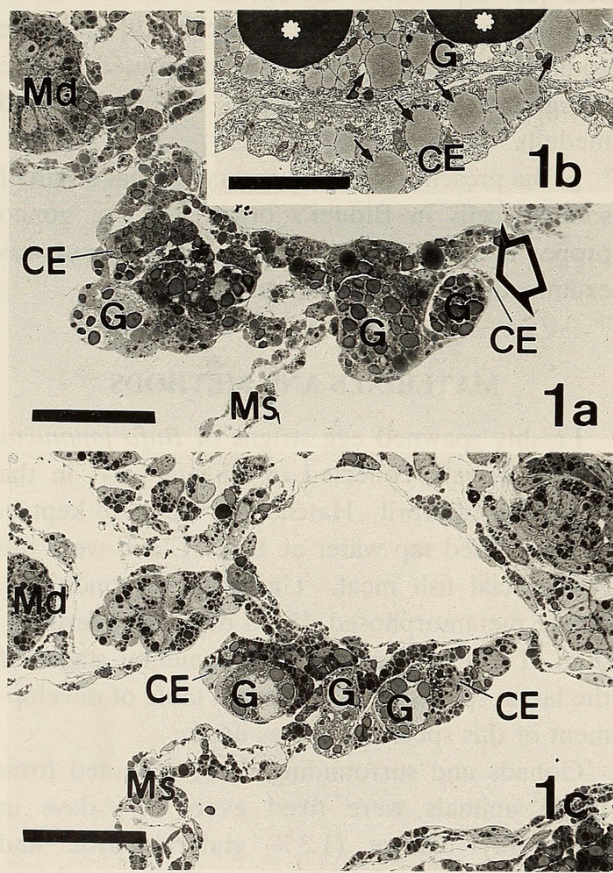


FIG. 1. Cross sections of the urogenital region at stage 30. a: The anterior region of the genital ridge, the primordium of Bidder's organ. Scale bar: 50 μ m. b: High magnification at the open arrow in Fig. 1a. Scale bar: 5 μ m. c: Middle region of the genital ridge, the primordium of the gonad proper. Scale bar: 50 μ m. CE: coelomic epithelial cells, G: primordial germ cell, Md: mesonephric duct, Ms: mesentery, arrow: lipid droplet, asterisk: yolk granule.

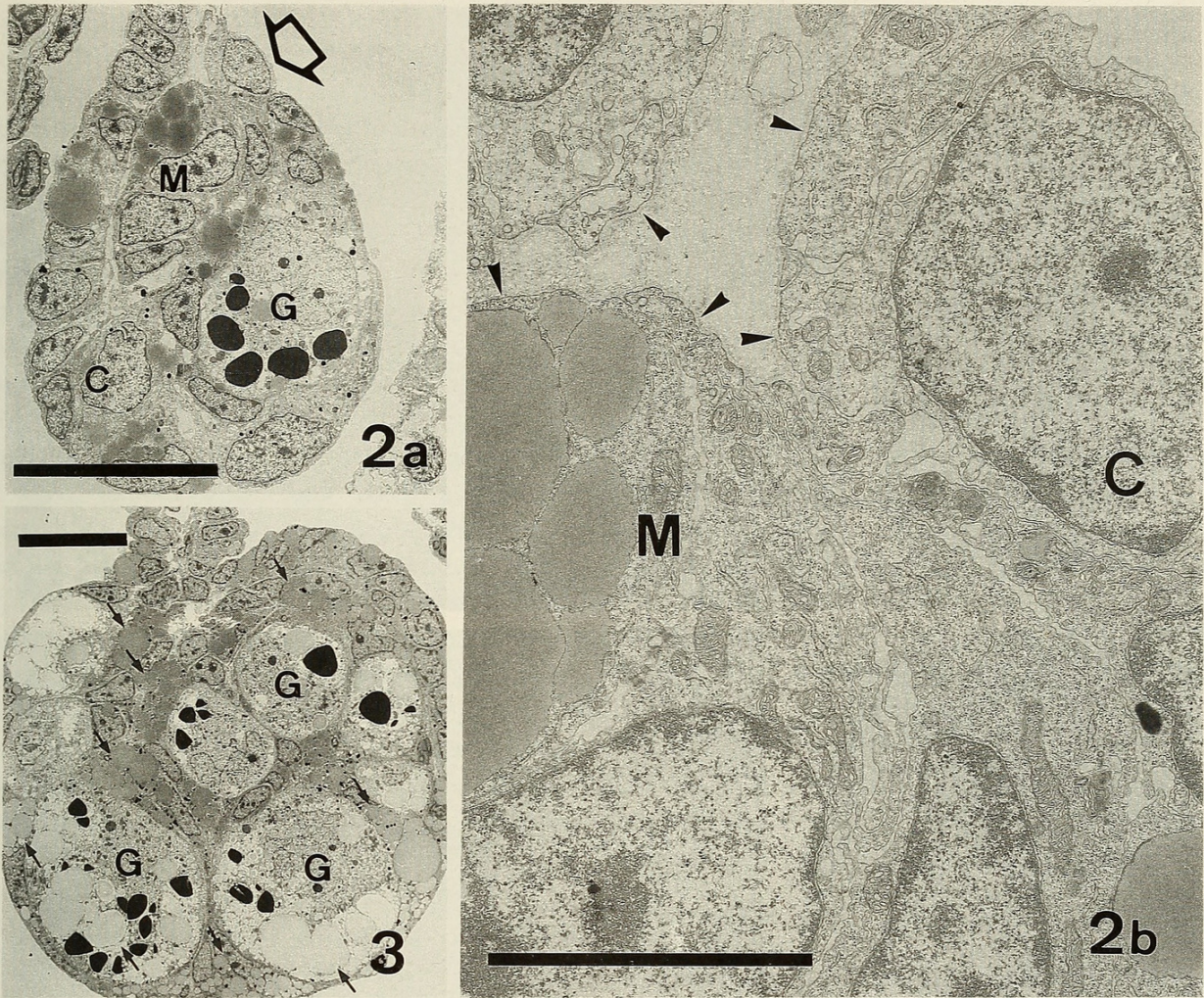


FIG. 2. Sexually indifferent gonad at stage 40. a: Cortex (C) and medulla (M) can be distinguished. G: germ cell. Scale bar: $20\ \mu\text{m}$. b: Higher magnification of the primordial gonad indicated by the open arrow in Fig. 2a. Cortex and medulla are tightly connected and are covered with a common basal lamina (arrow heads). Scale bar: $2\ \mu\text{m}$.

FIG. 3. Bidder's organ at stage 40. Germ cells (G) and somatic cells are filled with lipid droplets (arrows). Scale bar: $20\ \mu\text{m}$.

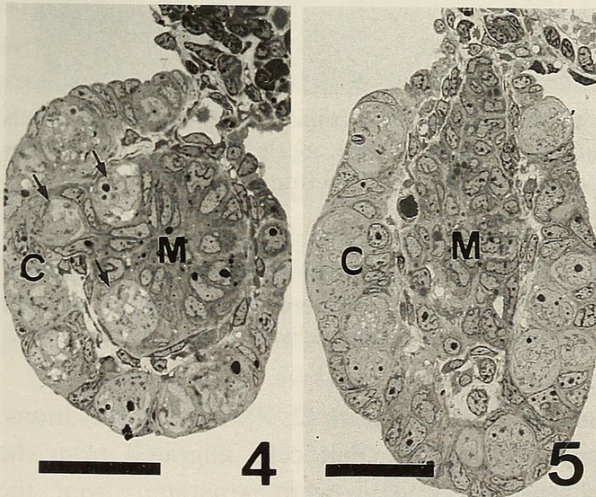


FIG. 4. Cross section of testis of larva at stage 43. Germ cells (arrows) are migrating from the cortex (C) to the medulla (M) through their connection. Scale bar: $30\ \mu\text{m}$.

FIG. 5. Cross section of ovary of larva at stage 45. The medulla (M) develops as a somatic cell mass. All germ cells are situated in the cortex (C). Scale bar: $30\ \mu\text{m}$.

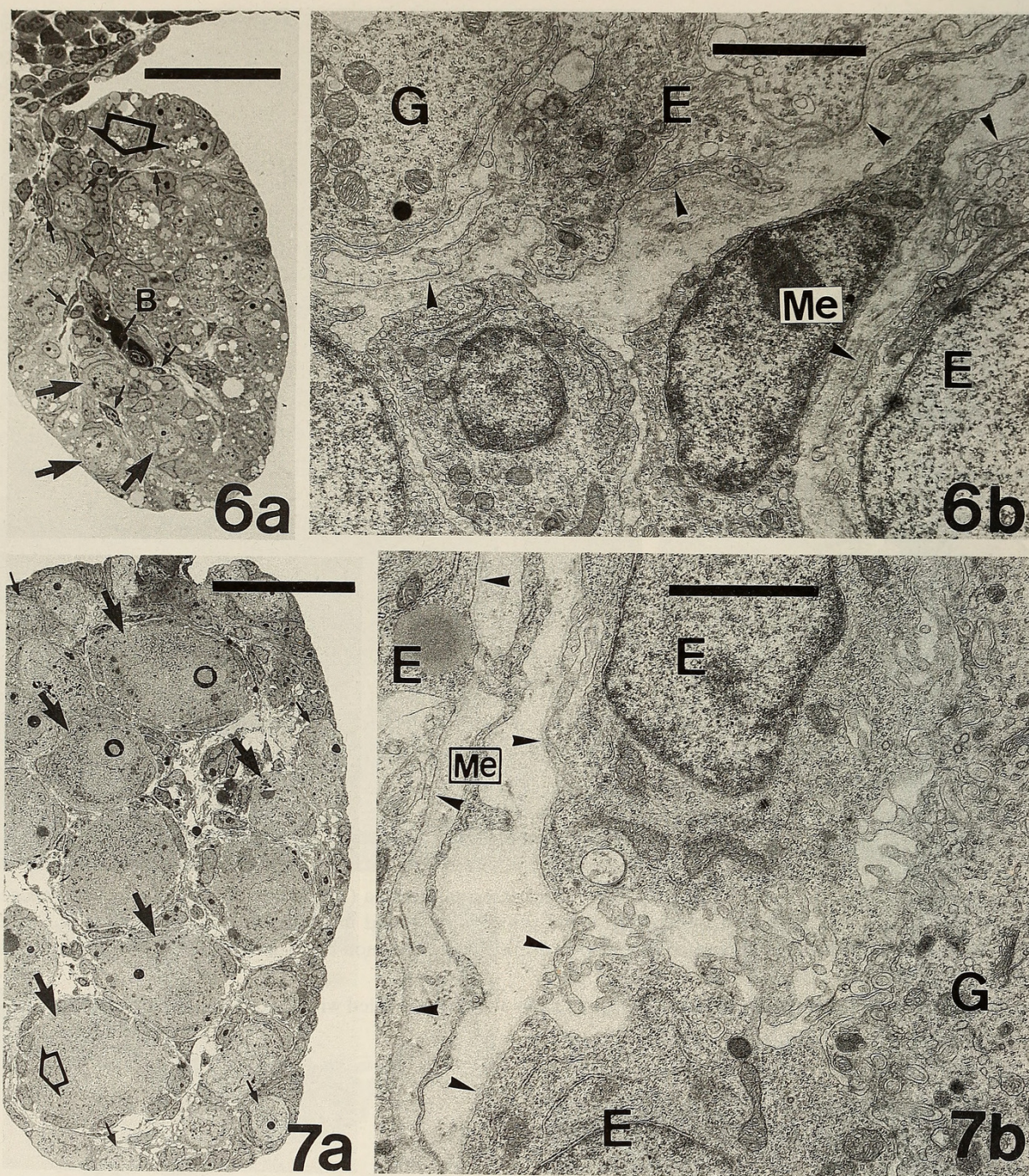


FIG. 6. Bidder's organ of female larva at stage 42. a: Invasion of mesenchymal cells (small arrows) and blood vessels (B) is seen. Some gonial cells are enlarging in the epithelial tissue (large arrows). Scale bar: $50\ \mu\text{m}$. b: Higher magnification at the open arrow in Fig. 6a. Mesenchymal cells (Me) are separated from the epithelial tissue (E) by the basal lamina (arrow heads). G: germ cell, scale bar: $2\ \mu\text{m}$.

FIG. 7. Bidder's organ of female larva at stage 45. a: Follicles (large arrow) consist of enlarged germ cells and a layer of epithelial cells. A lobular nucleus is seen in the enlarged germ cell. Normal gonial cells (small arrows) are seen in the peripheral region. Scale bar: $50\ \mu\text{m}$. b: A layer of epithelial cells (E) is covered with a continuous basal lamina (arrow head) and is in contact with germ cells (G) via microvilli. Me: mesenchymal cell, scale bar: $2\ \mu\text{m}$.

medulla has been regarded as a mesonephric-derived male inductor [11, 19], and the ovarian nature of Bidder's organ is interpreted to show a

lack of male inductors [2, 8]. Our observations, however, indicate that cells migrated from the mesonephric region were separated from the

epithelial tissue within the gonad proper by the basal lamina and were not included in the medulla. The cortex and medulla were directly connected and were covered with a common basal lamina, which strongly suggests the coelomic epithelial origin of the medulla. This is consistent with findings obtained in previous ultrastructural studies on several anuran species [13–18]. It is indicated that the origin of the somatic cells in Bidder's organ is identical to that of the cells in the gonad proper, and there is no contribution of the cellular origin to the ovarian nature of Bidder's organ. Lepori deduced that the most medial part of the coelomic epithelium has masculinizing capabilities, and that the absence of this part in Bidder's organ causes the ovary-like development [4]. Although this hypothesis fits with current opinions concerning the origin of gonadal somatic cells, the presence of masculinizing capabilities of such parts is difficult to demonstrate.

Our observations indicate that the invasion of the mesenchyme into the gonad proper occurred at the hindlimb stage simultaneous with the development of the medulla, whereas the invasion into Bidder's organ occurred with the formation of follicles at the metamorphosing stage. It is possible that the delay in the invasion of mesenchymal tissue can be attributed to the agenesis of the medulla in Bidder's organ. In *B. japonicus formosus*, the medulla differentiated into seminiferous cords and the inner epithelium of the ovarian cavity in the testis and ovary, respectively. Therefore, Bidder's organ should have no seminiferous cord or ovarian cavity, since the medulla was not developed. In the early testicular development, germ cells were incorporated within the seminiferous cord and did not enter into meiosis. In Bidder's organ, oogenesis occurs at the peripheral region [7], and no particular structure was formed around germ cells, like in the ovary. Thus, the agenesis of the seminiferous cords which is likely due to the lack of a medulla contributes to the female type differentiation of germ cells in Bidder's organ.

It is reported that follicles observed in Bidder's organ at the metamorphosing stage are formed without meiosis [4–7, 21]. We show clearly that these follicles were formed by a direct enlargement

of gonial cells and subsequent enclosure of the germ cell within a layer of epithelial cells. Surprisingly, contacts *via* microvilli were observed among the enlarging gonial and follicular cells, which appear in normal oocytes at the diplotene stage in the meiotic prophase [17, 18, 22]. This is an important phenomenon in understanding the changes in the interaction between germ cells and somatic cells during oogenesis.

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