INTERCELLULAR BRIDGES AND SYNCHRONIZATION OF GERM CELL DIFFERENTIATION DURING OOGENESIS IN THE RABBIT

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A certain degree of synchronization of germ cell differentiation is a general feature of mammalian oogenesis. In the rat (3, 10), mouse (4), and rabbit (15, 16), germ cell differentiation is highly synchronized in that there is a direct correspondence between fetal age and predominant stage of cellular activity. In the guinea pig (11), monkey (2), and man (1), the synchronized pattern is less apparent because of a considerable overlapping of mitosis, meiosis, and degeneration. Correspondence between age and stage of differentiation of a significant percentage of germ cells is found even in these species, however.

In the course of an electron microscopic study of ovarian development in the newborn rabbit, we have observed that the synchronization of the oogenetic process finds expression not only in the close relationship between fetal age and stage of cellular differentiation, but also in the maturation of the germ cells in groups and in the synchronous differentiation of all the cells in each group. Such synchronization appears to be related

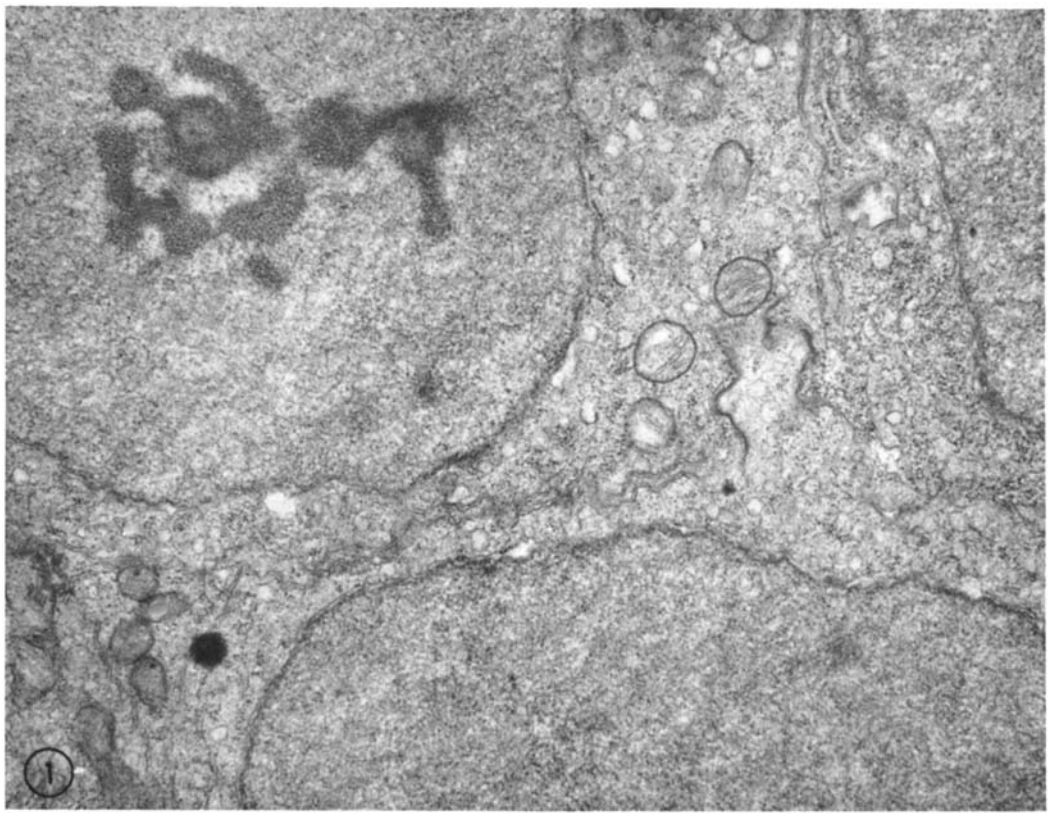

FIGURE 1 Two oogonia connected by an intercellular bridge. The bridge is limited by a plasma membrane of increased electron opacity. × 22,500.

to the presence of intercellular bridges connecting adjacent germ cells and resulting in a syncytial organization of the germ cells in the cords.

MATERIAL AND METHODS

This study was performed on the ovaries of 22 newborn New Zealand white rabbits, 1–15 days of age. The tissue fragments were fixed in 1% OsO_4 with salts added (17) and were embedded in Epon 812 (13). Sections were stained with lead hydroxide (12) and examined with Hitachi HU-11A and HU-11C electron microscopes.

RESULTS AND DISCUSSION

From days 1–3 postpartum the rabbit ovary is characterized by large numbers of mitotic oogonia which are consistently found in groups of two to six elements, all at the same stage of mitosis. In no instance could we find a dividing cell which was not in phase with the others in its group. At days 4 and 5 postpartum, when meiosis follows mitosis as the predominant activity of the germ cells, the meiotic cells are found in clusters consisting of oocytes which are all at the same stage of meiotic prophase. The waves of degeneration, which last until about day 15 and destroy great numbers of oocytes, also occur in a zonal pattern and involve groups of oocytes showing identical, regressive changes.

The structural basis for this synchronization appears to be a network of cytoplasmic bridges connecting adjacent germ cells to form syncytial groups. These intercellular bridges (Figs. 1–8), short and cylindrical in shape, are limited by a plasma membrane which is directly continuous with the plasma membranes of the connected cells but is distinguished from them by a greater electron opacity and an increased thickness.

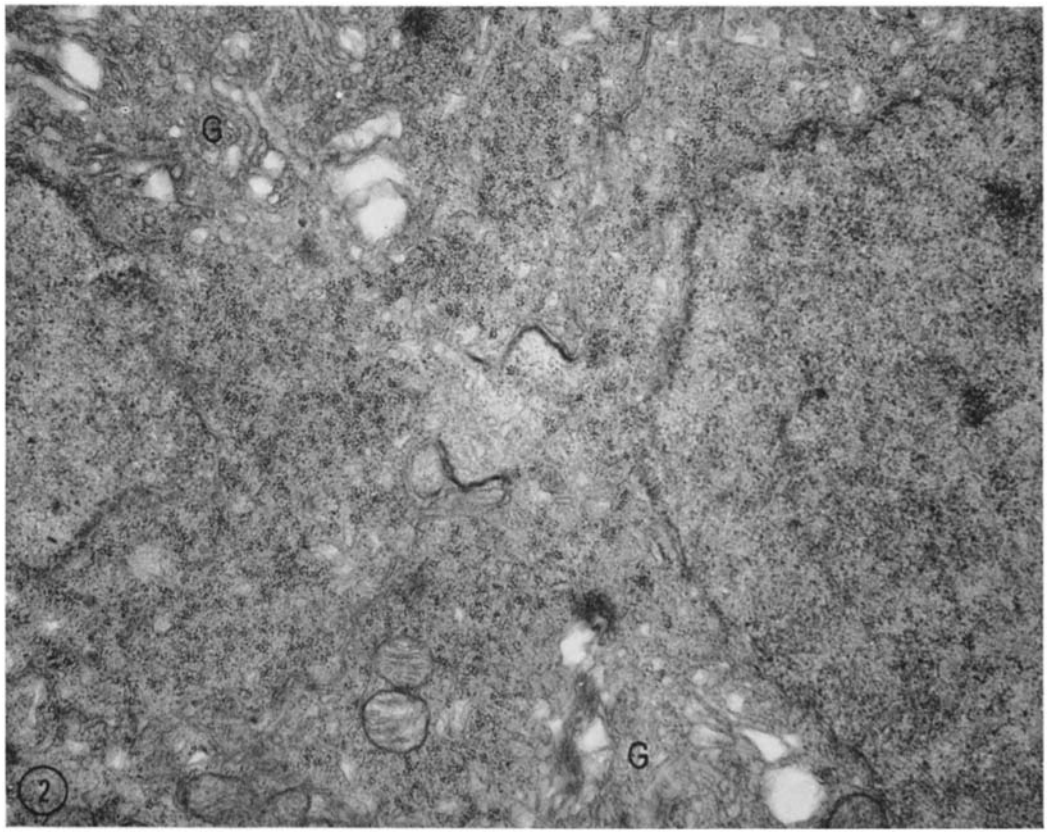

FIGURE 2 As in Fig. 1. Note the close morphological similarity of the two connected oogonia. A mirror-image configuration is indicated by the symmetrically opposite locations of the Golgi complexes (*G*) in the cells. × 19,500.

Identification of the bridges is thus possible even when the bodies of the joined cells do not appear in the section (Fig. 5). The cytoplasm of the bridges is identical with that of the connected cells (Fig. 4), and never contains spindle remnants. In many instances organelles, such as mitochondria, extend the whole length of the intercellular bridge and thus are shared by both cells (Fig. 3). Sets of double lamellae, similar to those described by Nagano (14) in bridges connecting avian meiotic spermatocytes, are occasionally present (Fig. 7). These lamellae are arranged in parallel rows which are oriented perpendicular to the long axis of the bridge.

The intercellular bridges are found between oogonia in interphase (Figs. 1 and 2), as well as between mitotic oogonia (Fig. 7). Bridges occur also between oocytes in meiosis (Fig. 8), as well as in early degeneration.

These bridges, thus, are true intercellular bridges and differ considerably from the intermediate body of Flemming (mid-body, Zwischenkörper) characteristic of mitotic telophase. However, the frequent finding of a germ cell with multiple bridges connecting it to two or more adjacent elements (Fig. 6) indicates that the bridges derive from intermediate bodies in the course of successive mitotic division in which karyokinesis is not followed by cytokinesis.

No attempts were made at this stage to correlate the incidence of the intercellular bridges with the maturation stages during the period of development studied. However, the intercellular bridges were found in significant numbers in all sections. If one considers the thinness of the sections and the fact that the bridges are obviously oriented on different planes, the actual number of these bridges must certainly be much greater than is apparent on the sections. Such a high number of cellular interconnections clearly results in the organization of the germ cells into multiple syncytial groups. The presence of organelles in the cytoplasm of the intercellular bridges indicates that material is exchanged from one cell to the other, with the result that each cell must share the developmental activity and suffer the fate of those to which it is connected. This explains not only the occurrence of mitotic, meiotic, and degenerating cells in groups but also the observation that all cells in any given group are at the same stage. The intimate cellular connections and common differentiation process also explain the close morphological similarity noted between adjacent cells and those frequently observed in the form of a mirror-image configuration (Fig. 2).

Bridges identical with those reported here have been observed by Franchi and Mandl between oogonia and oocytes of the developing rat ovary

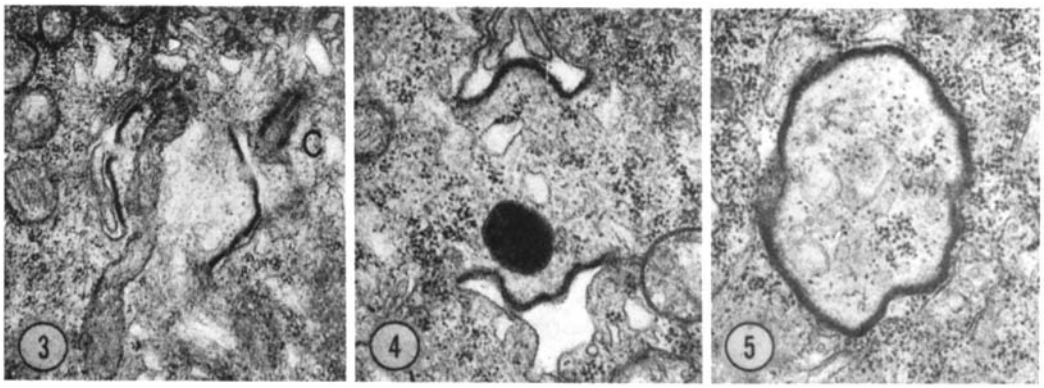

FIGURE 3 An intercellular bridge in which an elongated mitochondrion extends from one of the connected cells to the other. *C*, centriole. × 30,000.

FIGURE 4 Ribosomes, ergastoplasmic vesicles, and cytosomes in the cytoplasm of a bridge between two oogonia. × 20,500.

FIGURE 5 An intercellular bridge in cross-section showing the characteristic cylindrical configuration. Increased density of the plasma membrane permits identification of the bridge even when continuity with the connected cells is not evident. × 30,000.

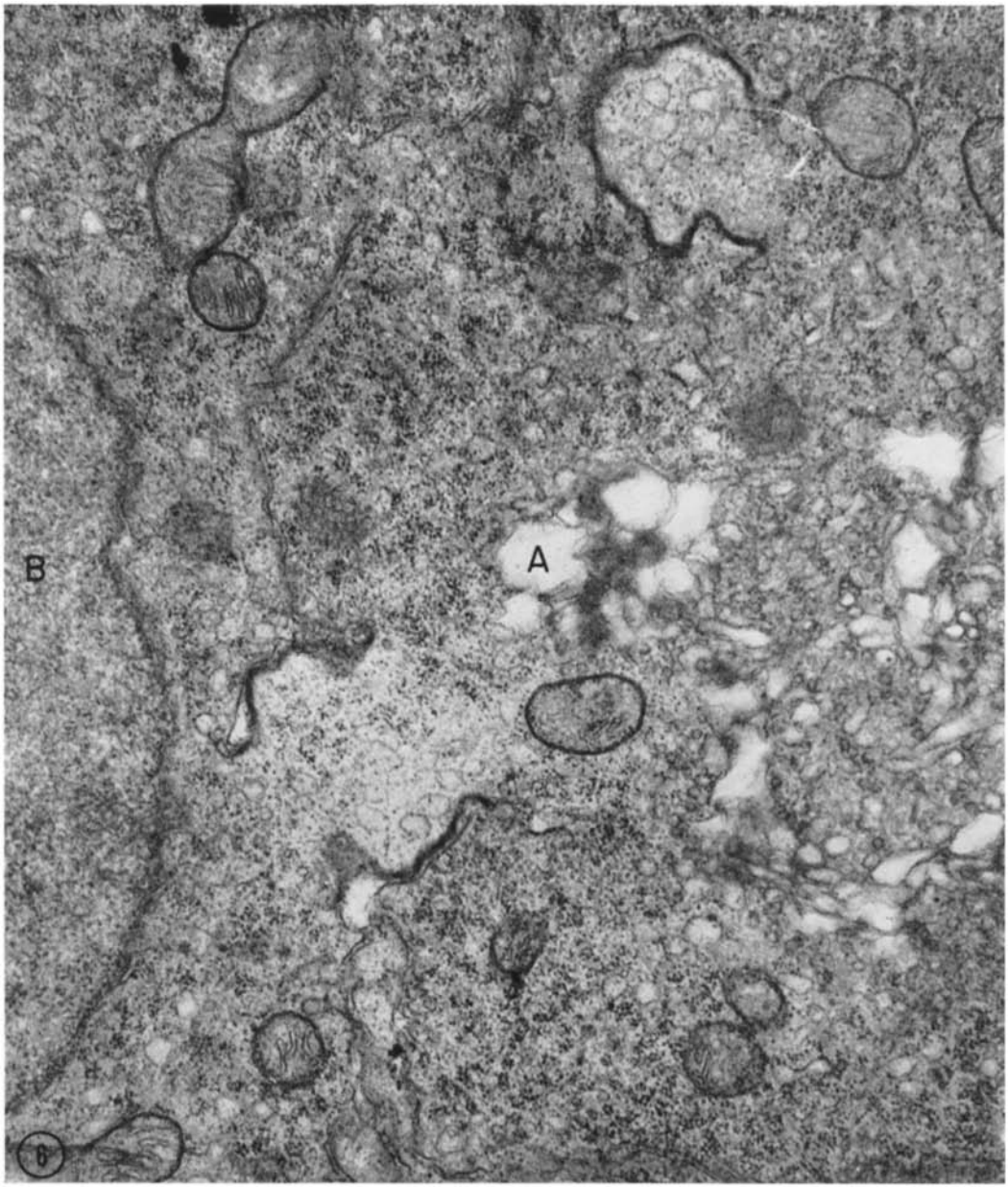

FIGURE 6 An oogonium (*A*) connected to adjacent cells by two bridges. Only one of the connected cells (*B*) is shown in the picture, since the oblique sectioning of the upper bridge prevents visualization of the other. × 25,000.

(10). Although the possible significance of this finding was not commented upon, the observation made by these authors is important in that it indicates that syncytia of germ cells may be common to all mammalian species. A syncytial organization identical with that observed by us in the ovary has been described in the seminiferous tubules of the testis by Fawcett and his collaborators (5–8). These authors have demonstrated that the spermatogenetic cells are connected by

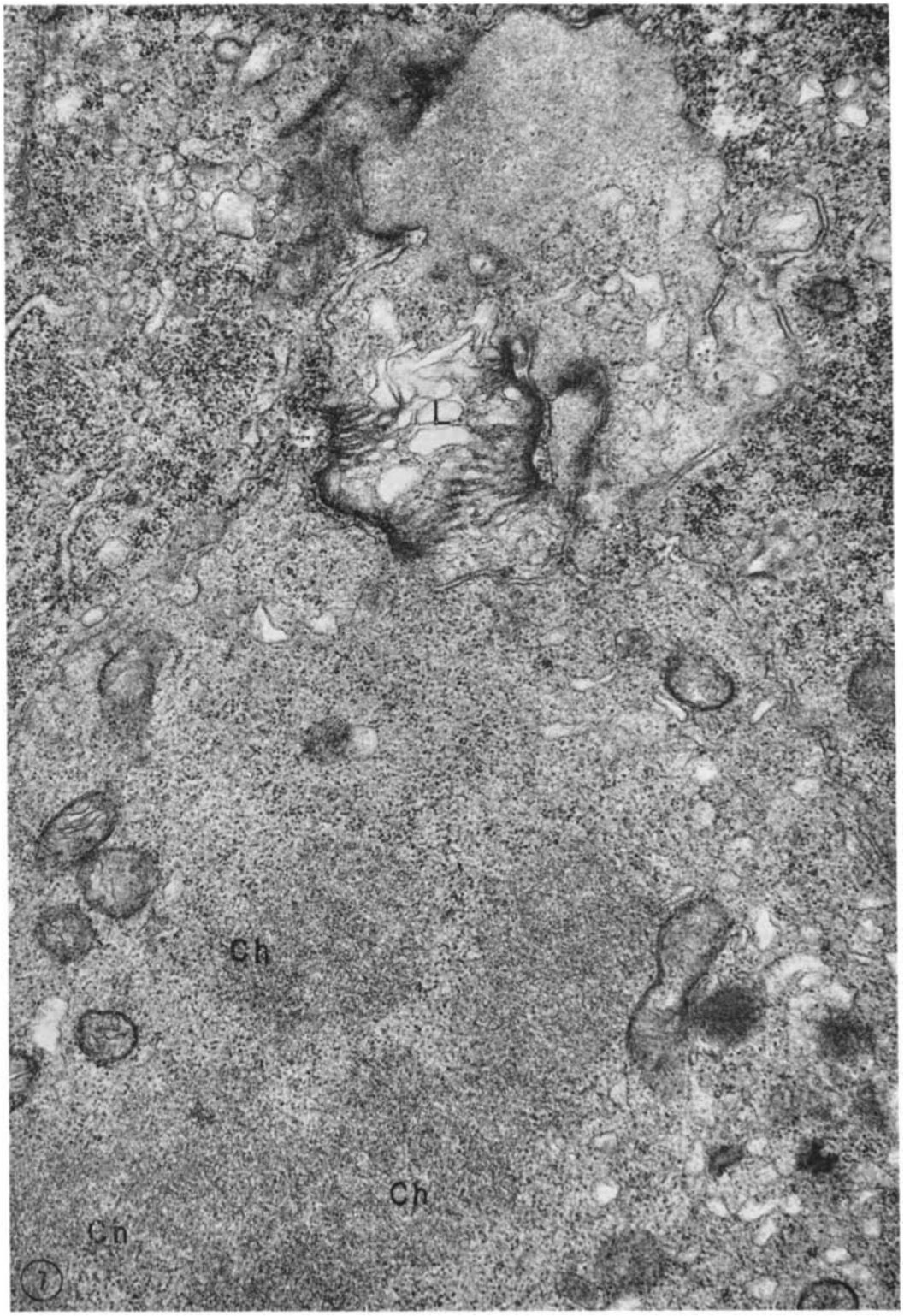

FIGURE 7 Oogonium in mitotic metaphase with intercellular bridge connecting it to another cell partially included in the section. Note the lamellar structures (*L*) which appear in the cytoplasm of the bridge and attach to the bridge plasma membrane. *Ch*, chromosomes. × 32,000.

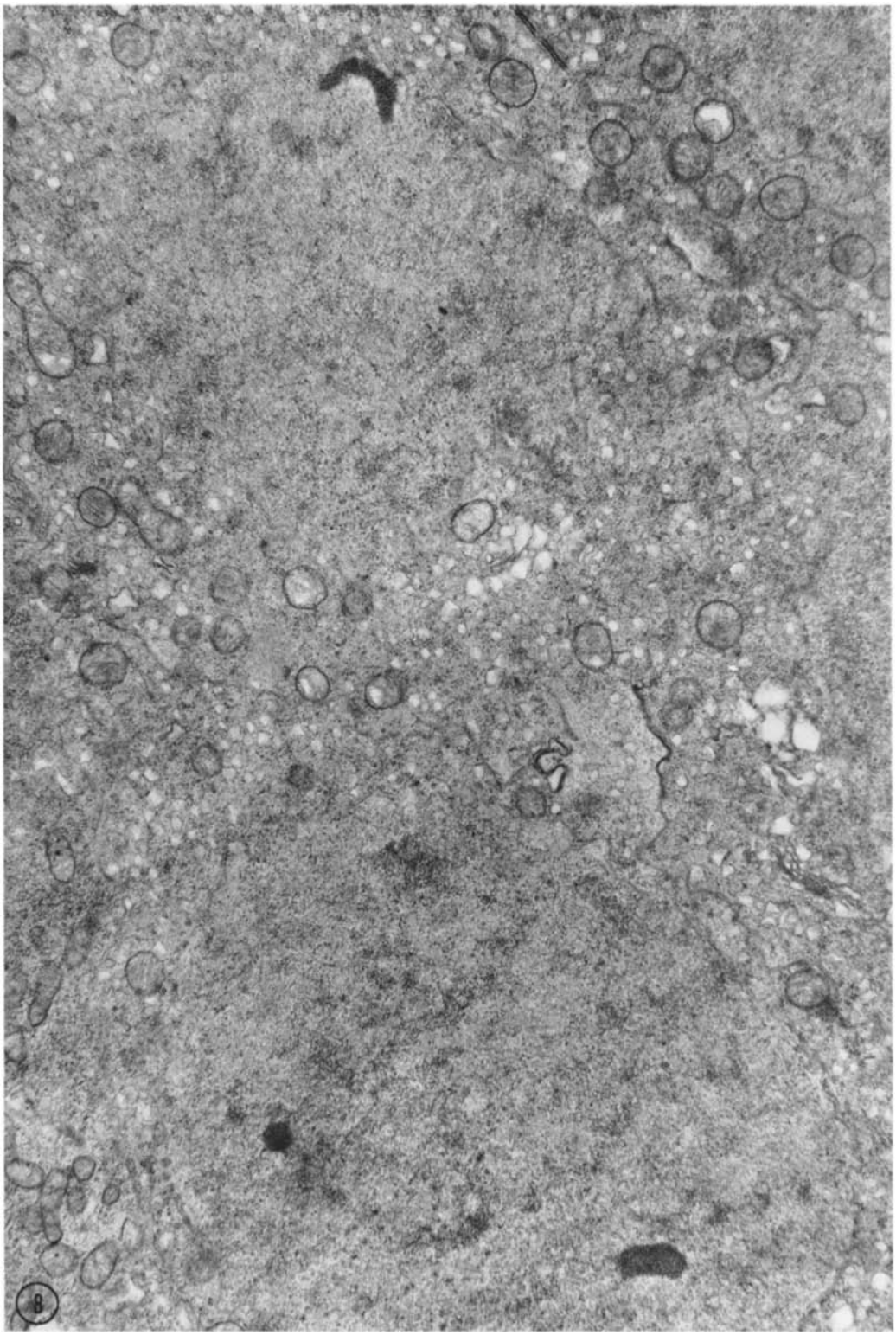

FIGURE 8 Two meiotic oocytes in leptotene conjoined by an intercellular bridge. Note the great similarity in the appearance of the two cells. × 14,000.

intercellular bridges to form cellular groups, each consisting of elements at the same stage of differentiation. These bridges, which do not include spindle remnants, were identified by Fawcett as true intercellular bridges which result in the formation of syncytia of spermatogenetic cells and represent the basis for the synchronization of cellular activity during spermatogenesis and spermiogenesis (6).

The syncytial organization of the germ cells in the developing ovary may have additional significance. The manner by which only a relatively small percentage of the total number of germ cells completes differentiation, escapes degeneration, and survives as oocytes in the follicle has long remained obscure (9). The key to survival may be complete cell division. In a relatively small number of germ cells karyokinesis may be followed by cytokinesis. These cells would then remain independent and, therefore, be able to escape the degeneration which affects the syncytial population of germ cells.

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Note Added in Proof: Since the submitting of this publication there has appeared a report by Weakley on the morphology of developing germ cells in the golden hamster (1967. *J. Anat.* **101:** 435.) in which the author describes intercellular bridges between germ cells and relates them to the synchronization of germ cell differentiation.

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