NEXUS JUNCTIONS BETWEEN DIVIDING AND INTERPHASE GRANULOSA CELLS OF THE RAT OVARY

FREDERICK B. MERK and N. SCOTT McNUTT. From the Biology Department, Boston University, Boston, Massachusetts 02215, and the Mixter Laboratory for Electron Microscopy, and the Pathology Department, Massachusetts General Hospital, Boston, Massachusetts 02114. Dr. Merk's present address is the Department of Pathology, University Hospital, Boston, Massachusetts 02118.

INTRODUCTION

A type of intercellular junction known as the nexus or "gap" junction is found in many tissues where the outer leaflets of adjacent plasma membranes are in contact (15, 24). These areas of membrane specialization are thought to provide a limited form of cytoplasmic continuity by providing pathways for passage of ions and small molecules between the interiors of apposed cells (4, 13). Low resistance electrical coupling (by ion transfer) has been attributed to nexuses between adjacent cells in tissues such as heart muscle (5), smooth muscle (1) and hepatic epithelium (21). A tracer with mol wt of 500 has been reported to pass between cells joined by nexuses (20). These junctions may also play a role in the cell-to-cell coordination of growth and differentiation in developing tissues (14, 22).

Certain factors in the cytoplasm are important regulators of nuclear activity (3, 9), and they have been reported to reach high levels just before mitosis (26). When several nuclei share a common cytoplasm, the complete cytoplasmic continuity results in synchronous cell differentiation (7) and synchronous mitoses (12, 23). In this report we describe nexuses between dividing and interphase granulosa cells. Our observation documents the presence, at dividing cell surfaces, of the type of junction which provides low resistance ionic coupling and therefore might be capable of passing mitosis-inducing factors into adjacent interphase neighbors. Other cell contacts of the adherens (6) type are found in the granulosa (18), but they probably play no role in intercellular ion transfer (5).

MATERIALS AND METHODS

The ovaries of untreated sexually mature (6 month) Sprague-Dawley rats were studied. In addition, weanlings of the same strain were treated as follows: three daily injections of 30 IU pregnant mare serum gonadotrophin (Equinex, Ayerst Laboratories, New York) were administered subcutaneously to one group of rats, starting on the 18th day. The ovaries of these rats and another group of untreated animals were removed on the 21st day for ultrastructural examination. Other 21 day old rats were hypophysectomized at Hormone Assay Laboratories, Chicago, Ill., and shipped by air the same day. Upon arrival the animals were individually caged and divided into two equal groups. 30 hr after the operation a series of six daily injections was begun. One group received a total of 6.0 mg diethylstilbestrol (DES) (Sigma Chemical Co., St. Louis, Mo.) dissolved in sesame oil. The other group was given vehicle only and served as a control. Both groups were sacrificed 1 day after the final injection.

The ovaries of all animals were fixed for 6 hr in a 2% glutaraldehyde, 2% paraformaldehyde, and 0.04–0.2% picric acid solution (10). After a phosphate buffer rinse, the specimens were postfixed for 4 hr in a 2% OsO_4 solution. The blocks were embedded in Epon 812 and sections were stained with uranyl acetate followed by lead citrate.

RESULTS AND DISCUSSION

The joining of dividing and interphase granulosa cells by nexuses was observed in all groups of animals except the hypophysectomized controls. Nexuses and mitotic figures are much less frequent after hypophysectomy. Gonadotrophic hormone treatment increases proliferation of the follicular

epithelium (16). Estrogens in particular are responsible for increasing granulosa cell division (2, 27) and also for increasing the number of nexuses per cell (17, 18). Mitotic figures in prophase (Figs. 1–3), metaphase, anaphase (Figs. 4, 5), and telophase are found connected to one or more interphase neighbors by nexuses.

Recent investigations (5, 8, 20) have demonstrated that nexuses produce ionic and metabolic coupling of adjacent cells. In light of this evidence, our morphological observations have physiological implications. The presence of nexuses between dividing and interphase cells places certain limitations on the interpretation of nexus function during cell division.

Since the nexus may be considered to provide a limited form of cytoplasmic continuity, consideration of the effect on mitosis produced by complete cytoplasmic continuity (as in multinucleated cells) is relevant. Experiments with homokaryons reveal that when several nuclei share a common cytoplasm, nuclear synchrony is morphologically demonstrable (11). The explanation of synchrony in homokaryons is based on the concept that nuclei, which are synthesizing DNA or undergoing mitosis, probably generate factors (28) that pass through the cytoplasm and trigger events in other nuclei, drawing them into synchrony (12, 23).

The consequence of nexus persistence throughout division in a rapidly proliferating epithelium is not clear. However, if nexuses freely pass mitosis-inducing factors, then a high frequency of synchronous mitoses (manifest by many clusters of dividing cells) would be expected. Since paired mitotic figures are almost never observed, synchrony is presumably not imposed by dividing granulosa cells even on nexus-coupled neighbors.

Another possible interpretation is that cytoplasmic controls over mitosis do slowly pass through nexuses but are not morphologically expressed in neighboring cells until the dividing cell has passed into interphase. This possibility is difficult to eliminate since it requires exact knowledge of the cell cycle stage for each cell in the population.

Anatomically normal nexuses could conceivably become nonfunctional during DNA synthesis or mitosis. Evidence against this is provided by the observation of O'Lague et al. (19) that electrical coupling between cultured interphase and dividing fibroblasts remains intact during mitosis. The low resistance between cultured fibroblasts is probably accounted for by small nexuses (8, 25).

In the present investigation granulosa tissue of intact and hypophysectomized rats has been studied. In addition, hormonal stimulation has been used as a tool to increase cell division and the incidence of nexuses. The occurrence of these junctions between mitotic and interphase cells, a phenomenon not previously reported, may explain the results of O'Lague et al. (19). Our interpretation, while based only on morphological evidence, is consistent with the hypothesis that cells can remain coupled throughout the cell cycle. If granulosa cell nexuses are functional during division, they apparently do not readily pass factors responsible for the initiation of mitosis.

The authors are grateful to Drs. Lynn Margulis and Linda P. Merk for valuable criticisms and suggestions.

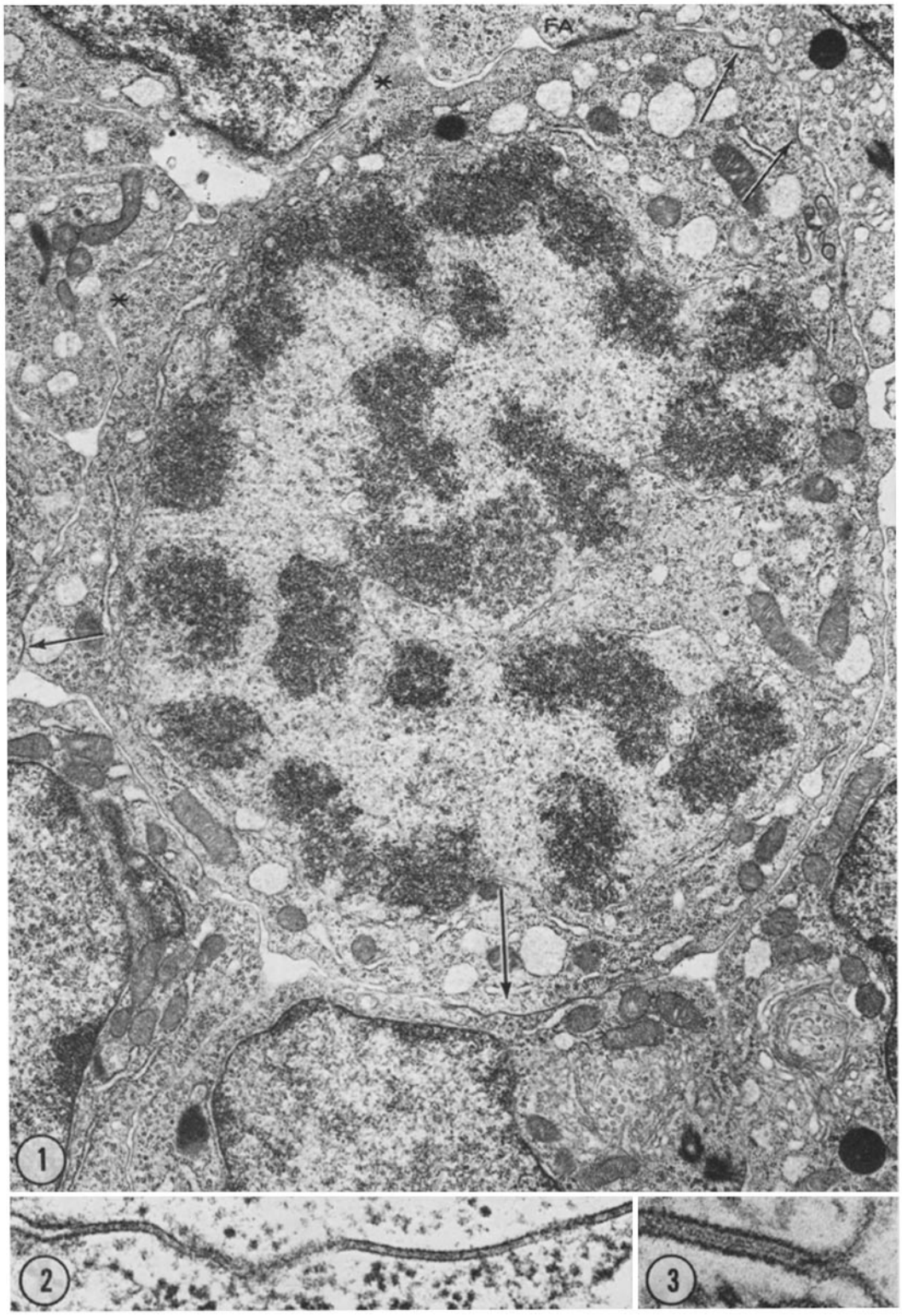
This investigation was supported by grants CH-HDTOI-00217, and CA-07368 from the United States Public Health Service.

Received for publication 1 May 1972, and in revised form 20 June 1972.

FIGURE 1 Granulosa cell in prophase is joined to interphase neighbors by at least four nexuses (arrows). A fascia adherens (*FA*) is also present. Asterisks indicate regions where oblique sectioning of closely apposed plasma membranes gives a false impression of cytoplasmic continuity between cells. There is no distinct membrane-to-membrane continuity between cells at these regions. This specimen is from a hypophysectomized DES-treated rat. × 15,000.

FIGURE 2 Detail of the nexus in Fig. 1 indicated by the large outlined arrow. × 88,000.

FIGURE 3 High magnification of a granulosa cell nexus (from a gonadotrophin-treated animal). *En bloc* staining with uranyl acetate has enhanced the visibility of the central "gap", giving this junction a seven-layered appearance. × 220,000.


FA
1
2
3

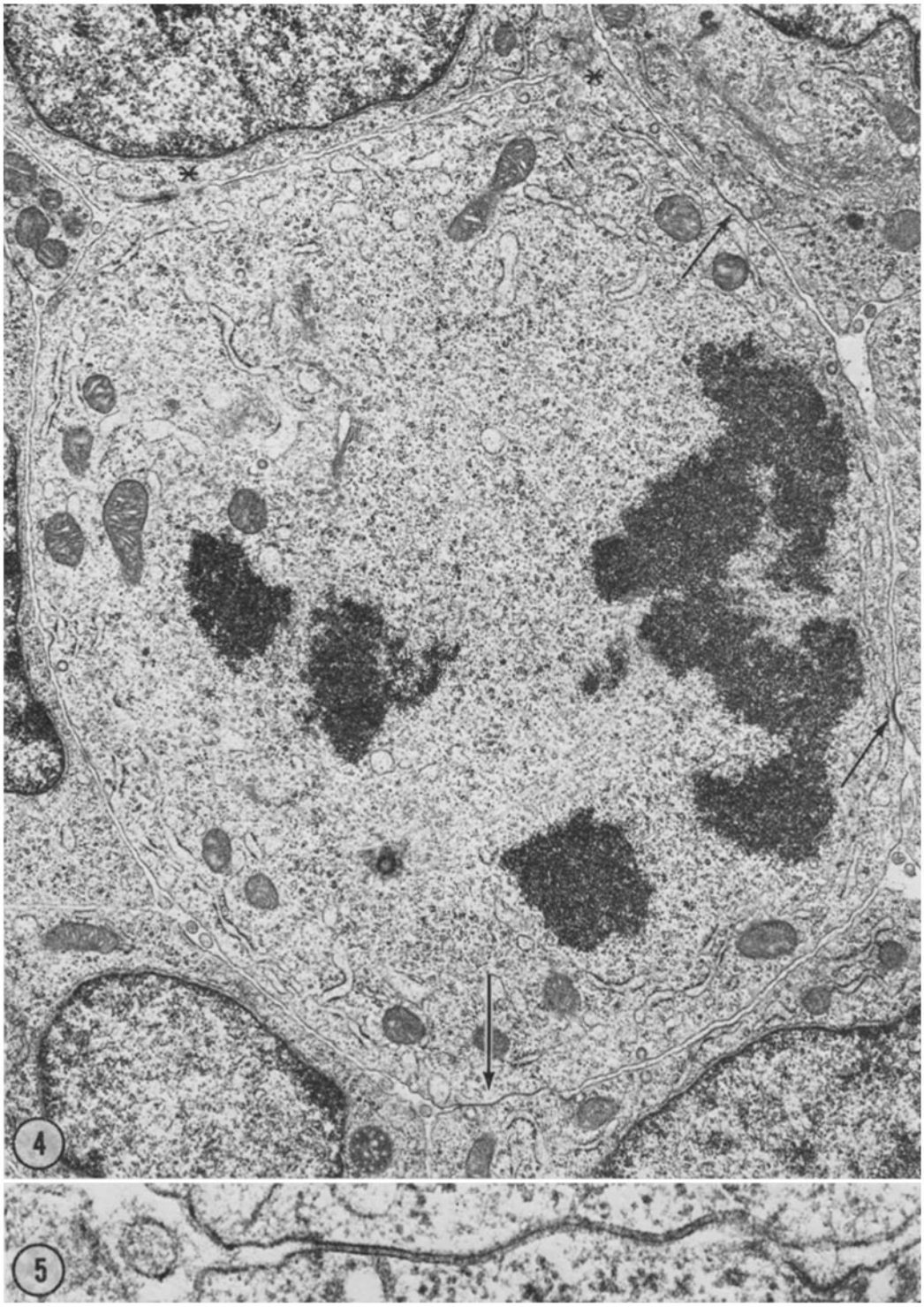


FIGURE 4 This specimen is from a hypophysectomized DES-treated rat. Nexuses (arrows) join an anaphase granulosa cell to three interphase neighbors. Asterisks indicate regions where closely apposed membranes have been obliquely sectioned (see legend to Fig. 1). × 16,000.

FIGURE 5 Detail of the nexus in Fig. 4 indicated by the large outlined arrow. × 75,000.

BIBLIOGRAPHY

1. BARR, L., W. BERGER, and M. M. DEWEY. 1968. *J. Gen. Physiol.* **51**:347.
2. BULLOGH, W. S. 1942. *J. Endocrinol.* **3**:150.
3. DETERRA, N. 1969. *Int. Rev. Cytol.* **25**:1.
4. DEWEY, M. M., and L. BARR. 1962. *Science (Wash. D. C.).* **137**:670.
5. DREIFUSS, J. J., L. GIRARDIER, and W. G. FORSSMANN. 1966. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **292**:13.
6. FARQUHAR, M. G., and G. E. PALADE. 1963. *J. Cell Biol.* **17**:375.
7. FAWCETT, D. W., S. ITO, and D. SLAUTTERBACK. 1959. *J. Biophys. Biochem. Cytol.* **5**:453.
8. GILULA, N. B., O. R. REEVES, and A. STEINBACH. 1972. *Nature (Lond.).* **235**:262.
9. GURDON, J. B. 1967. *Proc. Natl. Acad. Sci. U. S. A.* **58**:545.
10. ITO, S., and M. J. KARNOVSKY. 1968. *J. Cell Biol.* **39**:168 a (Abstr.).
11. JOHNSON, R. T., and H. HARRIS. 1969. *J. Cell Sci.* **5**:603.
12. JOHNSON, R. T., and P. N. RAO. 1970. *Nature (Lond.).* **226**:717.
13. LOEWENSTEIN, W. R. 1966. *Ann. N. Y. Acad. Sci.* **137**:441.
14. LOEWENSTEIN, W. R., and R. D. PENN. 1967. *J. Cell Biol.* **33**:235.
15. MCNUTT, N. S., and R. S. WEINSTEIN. 1970. *J. Cell Biol.* **47**:666.
16. MERK, F. B. 1969. *J. Cell Biol.* **43**:90 a (Abstr.).
17. MERK, F. B. 1971. *In* Proceedings of the 29th Meeting of the Electron Microscopy Society of America. Claitor's Publishing Division, Baton Rouge, La. 554.
18. MERK, F. B., C. R. BOTTICELLI, and J. T. ALBRIGHT. 1972. *Endocrinol.* **90**:992.
19. O'LAGUE, P., H. DALEN, H. RUBIN, and C. TOBIAS. 1970. *Science (Wash. D. C.).* **170**:464.
20. PAYTON, B. W., M. V. L. BENNETT, and G. D. PAPPAS. 1969. *Science (Wash. D. C.).* **166**:1641.
21. PENN, R. D. 1966. *J. Cell Biol.* **29**:171.
22. POTTER, D. D., E. J. FURSHPAN, and E. S. LENNOX. 1966. *Proc. Natl. Acad. Sci. U. S. A.* **55**:328.
23. RAO, P. N., and R. T. JOHNSON. 1970. *Nature (Lond.).* **225**:159.
24. REVEL, J. P., and M. J. KARNOVSKY. 1967. *J. Cell Biol.* **33**:C7.
25. REVEL, J. P., A. G. YEE, and A. J. HUDSPETH. 1971. *Proc. Natl. Acad. Sci.* **68**:2924.
26. RUSCH, H. P., W. SACHSENMAIER, K. BEHRENS, and V. GRUTER. 1966. *J. Cell. Biol.* **31**:204.
27. SMITH, B. D., and J. T. BRADBURY. 1961. *Proc. Soc. Exp. Biol. Med.* **107**:946.
28. THOMPSON, L. R., and B. J. MCCARTHY. 1968. *Biochem. Biophys. Res. Commun.* **30**:166.