The Occurrence of Intercellular Bridges in Groups of Cells Exhibiting Synchronous Differentiation*

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ABSTRACT

A previous electron microscopic study of the cat testis revealed that spermatids derived from the same spermatogonium are joined together by intercellular bridges. The present paper records the observation of similar connections between spermatocytes and between spermatids in Hydra, fruit-fly, opossum, pigeon, rat, hamster, guinea pig, rabbit, monkey, and man. In view of these findings, it is considered likely that a syncytial relationship within groups of developing male germ cells is of general occurrence and is probably responsible for their synchronous differentiation. When clusters of spermatids, freshly isolated from the germinal epithelium are observed by phase contrast microscopy, the constrictions between the cellular units of the syncytium disappear and the whole group coalesces into a spherical multinucleate mass. The significance of this observation in relation to the occurrence of abnormal spermatozoa in semen and the prevalence of multinucleate giant cells in pathological testes is discussed.

In the ectoderm of Hydra, the clusters of cnidoblasts that arise from proliferation of interstitial cells are also connected by intercellular bridges. The development of nematocysts within these groups of conjoined cells is precisely synchronized.

Both in the testis of vertebrates and the ectoderm of Hydra, a syncytium results from incomplete cytokinesis in the proliferation of relatively undifferentiated cells. The intercellular bridges between daughter cells are formed when the cleavage furrow encounters the spindle remnant and is arrested by it. The subsequent dissolution of the spindle filaments establishes free communication between the cells. The discovery of intercellular bridges in the two unrelated tissues discussed here suggests that a similar syncytial relationship may be found elsewhere in nature where groups of cells of common origin differentiate synchronously.

INTRODUCTION

In histological sections of the germinal epithelium of the mammalian testis, spermatids occur in clusters of eight or sixteen and all of the cells of such a group develop into spermatozoa at the same rate. In the past, their synchronous differentiation has been attributed to the fact that they originate at about the same time from division of the secondary spermatocytes and they are exposed to the same local environmental conditions during their development. A more satisfactory explanation has been provided recently by electron microscopic studies of spermiogenesis (3, 6) which have shown that the spermatids within these groups are not simply in close proximity, as was formerly supposed, but are actually connected to one another by intercellular bridges. Thus, it is probable that the protoplasmic continuity between the cells is the basis for their synchronous development.

The present paper records the existence of a similar syncytial relationship of the developing germ cells in several additional vertebrate and invertebrate species. It describes the appearance of the intercellular bridges in isolated living cell clusters, and discusses their mode of formation, the

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number of spermatids involved, and the probable time of their dissociation into individual cells. The paper also reports the occurrence of intercellular bridges of the same type in the cnidoblast clusters of the fresh-water polyp, Hydra, and discusses the significance of this syncytial organization for the coordination of nematocyst differentiation and its implications for current theories of cnidoblast migration.

Materials and Methods

Studies on living spermatocytes and spermatids were limited to the guinea pig and rat. Short segments of seminiferous tubules obtained at testicular biopsy were teased in a drop of Tyrode solution, covered with a coverslip, sealed with petroleum jelly, and examined with oil-immersion phase contrast optics. Photomicrographs were made with a Leitz micro Ibso, ⅓ × tube, using a Wratten No. 58 (green) filter, and Adox KB 14 film processed with FR X-22 developer. The light source was an electronic flash tube mounted in a Spencer model 370 microscope lamp housing. An American Speedlight Corporation model B-501 power supply was used, which provides an intense flash with an effective duration of 1/500 second.

Thin sections of testis from a variety of invertebrate and vertebrate species were examined with the electron microscope. Small blocks of testis were fixed by immersion for 2½ hours in 1 per cent osmium tetroxide adjusted to pH 7.6 with veronal-acetate buffer (19). Observations on the cnidoblasts of Hydra were based upon examination of specimens of *Pelmatohydra oligactis* and *Chlorohydra viridissima*. Hydra cultivated by the methods of Loomis (13, 14), were fixed intact for 2½ hours in 1 per cent osmium buffered to pH 7.8. After fixation the tissues were dehydrated in graded concentrations of ethyl alcohol and infiltrated 3 hours in three changes of *n*-butyl methacrylate monomer containing luperco as catalyst. Polymerization was carried out in a 50° C. oven overnight. Sections exhibiting yellow interference colors were cut on a Porter-Blum microtome (21). Electron micrographs were made with an RCA model EMU-2E or EMU-3B or Siemens Elmiskop II at original magnifications of 2000 to 10,000 and enlarged to the desired final magnification.

Historical Background

In both of the tissue types discussed in this paper a syncytial relationship is established within groups of cells that arise by proliferation from a common cell of origin; in the one instance, the *spermatogonium*, and in the other, the *interstitial cell*. The protoplasmic connections observed appear to result from incomplete cytokinesis. It is pertinent, therefore, to review here certain early descriptions of the final stages of normal cell division which have a bearing upon a subsequent discussion of the mechanism by which persistent intercellular bridges may be formed.

In studying the dividing eggs of *Limax*, Mark (16) in 1881 observed that after formation of daughter nuclei and constriction of the cell body, the two new cells may remain united for some time by a bundle of filaments of the achromatic spindle which persists for a while as a bridge between the two cells. This structure received further attention from Carnoy (5). Working with arthropod cells, he distinguished the spindle plate (*plaque fusoriale*), which consisted of a transverse alignment of stainable swellings at the midpoint of the spindle filaments, and a cytoplasmic plate (*plaque complétive*) which appeared as a dark line connecting the spindle plate with the cell membrane in the cleavage furrow. Flemming (7) in 1891 drew attention to minute stainable bodies found in the middle of the stalk connecting two dividing cells in late anaphase, and described their fusion, in telophase, into a single elongated *intermediate body* (*Zwischenkorper*). Events leading to the formation of this structure are well described in Henneguy's (10) account of observations on the dividing blastomeres of the trout. At the time when the cleavage furrow reaches the bundle of connecting spindle filaments each of these shows at its midpoint, precisely in the plane of the furrow, a swelling which stains more intensely than the rest of the filament. These thickenings together constitute the *spindle plaque* of Carnoy that is encircled by the advancing margin of the cleavage furrow. As the furrow constricts the spindle plate, the elements of the latter seem to fuse and the filaments shorten so that the daughter cells are connected by only a short, slender strand that stains intensely. This is the *intermediate body* of Flemming. The furrow finally severs this strand and for a short time thereafter a minute dot, which represents the last vestige of half the spindle plate, can be identified on the membranes of the daughter cells, at the point of abscission.

In the classical cytological papers on spermatogenesis published around the turn of the century, spindle bridges between spermatocytes or spermatids were frequently illustrated, but these were invariably interpreted as transient structures of the kind described by Carnoy and Flemming. An excellent lithograph of human germinal epithelium illustrating a paper by Retzius (1902) (23), clearly depicts an open communication between two

spermatids, but no reference to this is found in the text. In an early electron microscopic study of thin sections of testis reported by Watson in 1952 (31), several examples of protoplasmic continuity between adjacent spermatids can be found in the illustrations, but again the author makes no mention of this. Conjoined spermatocytes and syncytial clusters of spermatids were first described as a *normal occurrence* by Burgos and Fawcett (1955) (3), in a paper on spermiogenesis in the cat.

OBSERVATIONS

Further Electron Microscopic Observations on Germinal Epithelium of the Testis.—In the course of comparative studies on the cytological events in spermatogenesis, a syncytial relationship of the developing germ cells has now been observed in the fresh water polyp, *Hydra oligactis*, the fruit fly, *Drosophila virilis*, and in the opossum, pigeon, rat, hamster, guinea pig, rabbit, cat, monkey, and man. In all of these species, open communication exists between spermatocytes, and between spermatids. The bridges are relatively short, cylindrical in form, and are surrounded by an annular thickening of that portion of the plasma membrane common to the two cells. The thickened appearance is due to a dense substance that forms a layer of uniform thickness on the cytoplasmic surface of the membrane and apparently reinforces and stiffens it (Figs. 8 to 10). After the disappearance of the spindle remnant the interior of the bridge is occupied by cytoplasm indistinguishable from that of the cell body, even including canalicular elements that clearly establish continuity of the endoplasmic reticulum of one cell with that of the other (Figs. 5 and 6).

The number of cells which may be connected in this fashion cannot be ascertained from the study of thin sections, but inasmuch as one finds pairs of primary spermatocytes in prophase of the first meiotic division joined in this manner (Fig. 13), it is reasonable to expect that their division would yield four conjoined secondary spermatocytes, whose division would, in turn, produce a syncytial cluster of eight spermatids. It is a rather common occurrence to obtain electron micrographs showing three (Fig. 5) or even four spermatids connected by bridges (Fig. 7). It is not unlikely that there are as many as eight cells in these groups for if one considers that different planes of cleavage are involved in their formation, it is highly improbable that more than half of the connections would ever fall in the plane of a single thin section.

According to traditional descriptions of spermatogenesis, the germ cells develop to a certain stage independently and then secondarily establish their characteristic relationship to the sustentacular cells of Sertoli. Whether the heads of the immature spermatozoa are actually embedded in the Sertoli cell cytoplasm was, until recently, a subject of dispute. Electron microscopic studies have clearly shown that the spermatozoa are extracytoplasmic, occupying deep recesses in the highly irregular surface of the Sertoli cells (3). Moreover, this relationship is not fundamentally different from that found earlier in spermatogenesis. From the outset, the Sertoli cells are extraordinarily irregular in shape, surrounding the germ cells and filling nearly all of the interstices between them, however small. When the spermatocytes divide, a thin process of the associated Sertoli cell extends into the cleavage furrow. There is some indication from observations on living cells that the presence of this Sertoli cell process in the furrow may be necessary to maintain the constriction between daughter cells after the furrow has been arrested at the spindle remnant and the active movements of cytokinesis have subsided.

Inasmuch as spermatozoa are ultimately released as free swimming individual cells, the question arises as to how and when they normally separate from the syncytial groups in which they develop. No observations have been made bearing directly upon their mode of separation, but for the mammalian species studied, there is evidence that this takes place quite late in spermiogenesis. In the guinea pig, for example, when the condensed nucleus has already assumed its definitive discoid shape, and the flagellum projects into the lumen of the seminiferous tubule, the spermatids are still connected by bridges (Fig. 12). At this late stage, little remains in spermiogenesis but to cast off the caudally projecting mass of excess cytoplasm where the spermatids are joined. It is highly probable then, that abstriction of the residual bodies of Regaud is the process which finally separates spermatids. It would not be surprising to find that bridges persist for a while between these anuclear fragments, but such connections have not been encountered in electron micrographs studied to date. Thus the observations reported here suggest that the syncytial clusters resulting from incomplete cytokinesis in the spermatocyte divisions remain intact throughout the ensuing period of spermatid differentiation, up to the time

when the spermatids cast off their excess cytoplasm and become spermatozoa.

Observations on Living Testicular Cells.—When the living cells of the germinal epithelium are mechanically dissociated for examination by phase contrast microscopy, the Sertoli cells are almost invariably disrupted. Many of the conjoined germ cells are also broken or separated from their fellows, while others flow together to form conspicuous multinucleated masses (Figs. 3 and 4) not normally seen in sections of the testis. Occasionally groups of two to four cells are observed that have partially withstood the isolation procedure and remain connected by visible intercellular bridges corresponding to those seen in electron micrographs (Fig. 2). The syncytial clusters may be caused to separate further into individual cells by gently tapping the coverslip with a needle. This manipulation does not produce a sudden break of the intercellular bridges, but results in a gradual separation of the cells so that the protoplasmic connection between them becomes more and more attenuated, especially at its midpoint where it ultimately gives way. The sundered halves of the bridge slowly retract but often continue to be recognizable as rounded irregularities on the otherwise smooth surface of the separated cells.

When such groups of cells connected by bridges are not subjected to added disruptive forces but are simply observed for some time after isolation, it is found that they are not able to maintain this relationship. The constrictions between the cells gradually disappear and a spherical multinucleate mass is formed that contains as many nuclei as there were conjoined cells in the original cluster. This process of coalescence is illustrated in the three photomicrographs comprising Fig. 1. Fig. 1 *A* shows a group of four rat spermatids that survived the isolation procedure still connected by narrow intercellular bridges. The condition of the same group a few minutes later is depicted in Fig. 1 *B* where the constrictions between three of the cells have been obliterated. In Fig. 1 *C* the coalescence is farther advanced and a short time later was completed when the group had rounded up into a spherical quadrinucleate mass. These observations provide an explanation for the occurrence of multinucleate giant cells in smears or teased preparations of living testicular cells. The alternative possibility that they arise by fusion of cells that were originally independent is extremely unlikely in view of the fact that nuclei of different types are not found in the same cytoplasm. Instead, one finds either multinucleate spermatocytes with all nuclei in the same stage of prophase or multinucleate spermatids with all of the acrosomes in exactly the same stage of differentiation (Fig. 4). It is apparent therefore, that the multinucleate giant cells which abound in teased preparations of seminiferous tubules arise by obliteration of the constrictions between cells of the same type, which are normally joined by intercellular bridges.

The Occurrence of Intercellular Bridges in Hydra.—The small fresh-water coelenterate, Hydra, obtains its food by means of nematocysts, small projectiles which are ejected to poison or entangle its prey. The nematocysts are lost in great numbers in feeding and are constantly replaced by special cells called cnidoblasts. These, in turn, arise from *interstitial cells*, undifferentiated cells in the ectoderm which proliferate to give rise to clusters of cnidoblasts that then go on to form nematocysts. The divisions of interstitial cells which produce groups of four to sixteen cnidoblasts are similar to the divisions of spermatocytes in the testis, in that cytokinesis is incomplete and the spindle bridges persist as open communications between cells. Within any one group of conjoined cnidoblasts all of the nematocysts are in exactly the same stage of differentiation. Ectodermal musculo-epithelial cells in Hydra bear much the same relationship to the cnidoblast clusters as Sertoli cells do to the syncytial groups of spermatids in the testis. However, they do not send processes into the intercellular clefts. Therefore, the plasma membranes of adjacent cnidoblasts are usually in apposition except in the immediate vicinity of the bridge where there is a small intercellular space (Figs. 14 and 16) often filled with extracellular dense granules that are believed to be a particulate form of glycogen (26).

Electron micrographs of interstitial cells that have recently divided show a long cylindrical spindle bridge between the daughter cells (Figs. 14 and 15). This is occupied by a closely packed bundle of extremely thin filaments that seem to be poorly preserved by osmium-containing fixatives. There is a dark transverse band precisely at the middle of the bridge which consists of an extrafibrillar dense material (Figs. 14 and 15). This appears to correspond to the spindle plate (*plaque fusoriale*) of Carnoy and is doubtless also the stainable intermediate body (*Zwischenkörper*) observed by Flemming in the middle of the strand connecting daughter cells in telophase.

At a slightly later stage, the connection between

cells is shortened and the filaments are no longer identifiable. In their place is a clear area devoid of granules or organelles (Fig. 16). It is likely that this empty appearance does not reflect a real absence of structure in the living cell, but rather represents the site of the spindle remnant that has been dissolved out in the course of specimen preparation. The ease with which the spindle filaments are extracted at this stage may reflect a real change in their solubility as they begin to undergo dissolution. Even after the filaments are no longer discernible, the transverse band of dense substance in the middle of the bridge persists for some time. This may be taken as further evidence that it is an entity distinct from the filaments which pass through it (Fig. 16). Where this dense band makes contact with the plasma membrane the latter bends outward to form a distinct ridge which marks, on the surface, the midpoint of the intercellular bridge. This particular feature is characteristic of the bridges of the cnidoblasts of Hydra, and has not been observed on the bridges connecting the germ cells in the testis (Fig. 11). Ultimately all trace of the spindle remnant disappears and the bridge becomes filled with cytoplasm indistinguishable from that of the neighboring cell bodies (Fig. 17). Later in the differentiation of the cnidoblasts, when their endoplasmic reticulum develops, some of its canalicular elements pass from cell to cell through the bridges (Fig. 18). The annular thickening of the encircling membrane, which is a conspicuous feature of the intercellular bridges in the testis, is less prominent in those of the cnidoblasts. Bridges are seen between cells that contain nematocysts in an advanced stage of development. Thus, the connections are present throughout the period of cnidoblast differentiation. Just how long they persist thereafter or how they are finally severed is not known, but inasmuch as mature nematocysts are distributed on the body wall with one, or at most two, associated with each epithelial cell (25), it is clear that at some stage the clusters of four to sixteen conjoined cnidoblasts must break up into individual cells which migrate to their definitive position at the epithelial surface. The details of this process have yet to be elucidated.

DISCUSSION

In recent years electron microscopic studies have disclosed that cardiac muscle is not a syncytium, as formerly supposed, but is composed of discrete cellular units which meet end-to-end at the intercalated discs (20). The individuality of smooth muscle fibers (4) and of mesenchymal cells has been reaffirmed, and the so called "intercellular bridges" of stratified squamous epithelia have been shown to be closed processes that meet end-on (22). Thus, several of the classical examples of syncytia have been removed from this category as a result of the application of the electron microscope to the study of tissue organization. Most of the remaining examples of syncytia, such as osteoclasts, multinucleate giant cells, placental trophoblast, and the like, develop by coalescence of originally separate cells. The same mechanism may also apply to the origin of skeletal muscle fibers, although some contend that these arise by elongation of myoblasts and multiplication of their nuclei without accompanying cytoplasmic division. The present paper describes two examples of a new type of syncytium which escaped detection with the light microscope—a syncytium which does not arise by fusion of cells, but which results from incomplete cytoplasmic division that leaves groups of cells connected by sizeable intercellular bridges. These are formed in telophase when the advancing margin of the cleavage furrow is arrested by the spindle remnant. With the subsequent dissolution of the spindle filaments, an open communication is established between the daughter cells which persists throughout the ensuing period of cell differentiation. It follows from the mode of formation of this type of syncytium that there is only one bridge between any two cell bodies, but one cell may have more than one bridge. In the examples discovered to date, the number of units associated in this manner is estimated to be between eight and sixteen.

In the development of the germ cells of the testis and the cnidoblasts of hydra an initial period of proliferation is followed by one of rapid cell differentiation. Within the groups of conjoined cells, both the divisions and the subsequent events in cell differentiation are precisely synchronized. The discovery of intercellular bridges in both of these situations has led us to assume that protoplasmic continuity is the structural basis for the observed synchrony. It is well known that in the syncytial germinal disc of certain elasmobranch fish (*e.g. Scyllium canicula*) the nuclei divide in unison (24). Also, in the early development of insect eggs, karyokinesis proceeds without cytokinesis and the mitoses are synchronous; the first sign of asynchronism occurring only after the nuclei have become separated from each other by cell membranes (27). In these examples, the synchrony has

usually been attributed to the influence of a uniform cytoplasmic environment upon a population of structurally similar nuclei. It should be pointed out, however, that syncytial organization does not necessarily insure coordination within a multinucleate cell or organism. In the multinucleate protozoa *Opalina*, *Cepedea*, *Actinosphaerium*, and in *Myxosporidia* the nuclei are more or less evenly distributed, and there is very little movement of the cytoplasm. In these forms nuclear division is not simultaneous and one must presume that the condition causing division is localized to a particular area. On the other hand, in *Pelomyxa*, *Plasmodiophora*, and related organisms, where there is a continuous streaming movement of the cytoplasm, all nucelei divide simultaneously (11). It is supposed that factors causing nuclear division are widely and uniformly distributed throughout the cytoplasm by the protoplasmic streaming. While there is no reason to believe that there is any such movement of the cytoplasm in the spermatid or cnidoblast clusters described here, it is tempting to think that coordination of development is achieved by uniform distribution, in the syncytium, of chemical factors controlling differentiation. The intercommunicating system of channels of the endoplasmic reticulum might conceivably provide a preferential pathway for the diffusion of such an hypothetical regulatory agent, but this appears somewhat unlikely for, in some of the examples studied, simultaneous nuclear division and coordinated differentiation occur in the absence of a well developed endoplasmic reticulum.

The observations reported here now provide a reasonable explanation for the frequent occurrence of multinucleate cells in smears and teased preparations of seminiferous tubules. Guyer (9) described numerous multinucleate cells in stained smears of fowl testis, and Stroganova (28) reported similar findings in smears of rat, mouse, and rabbit testis. Tillman and others (30), studying freshly isolated living germ cells of a variety of domestic animals, concluded that multinucleate spermatocytes were a normal occurrence in the healthy testis. The rarity of multinucleate cells in histological sections of testis is in marked contrast to these observations on smears and teased preparations of living cells. This discrepancy has puzzled a number of investigators. Lake (12) has recently reinvestigated this problem in fowl testis and concluded that the multinucleate condition is normal and that failure to observe it in sections is due to slow or inadequate penetration of the fixative. Although Lake's interpretation was incorrect in invoking fixation artifact to account for the discrepancy in appearance of living preparations and histological sections, nevertheless his supposition that spermatocytes undergo nuclear division without cytoplasmic division was as close to the fact as it was possible to come by light microscopy alone. The correlated phase contrast and electron microscopic studies reported here finally bring the observations on isolated living cells into accord with the findings in sections. The sequence of changes observed in freshly isolated testicular cells clearly demonstrates that the multinucleate cells in such preparations arise by coalescence of groups of germ cells originally connected by protoplasmic bridges. It is suggested that the maintenance of narrow bridges between germ cells in the intact epithelium requires the support of the surrounding Sertoli cells and when this normal relationship is disturbed, the conjoined germ cells flow together in multinucleate masses.

A relatively high incidence of multinucleate giant cells has been reported in pathological conditions of the testis resulting from influenzal pneumonia and other febrile illnesses (18), low atmospheric pressure (1), x-radiation (8) and dietary deficiency (17, 29). In interpreting this finding it has often been assumed that germ cells in various stages of differentiation have coalesced to form the multinucleate masses. Although this may well occur in instances of severe damage, a clear distinction should be drawn between giant cells of this kind and those in which all of the nuclei are the same. In the latter case, damage to the germ cells or the supporting Sertoli cells may simply have caused a change in form of a preexisting syncytium.

It has already been pointed out by Lake (12) that the development of spermatozoa from syncytia offers a more feasible explanation for the occurrence of mature spermatozoa in clusters, than the previously postulated chemotactic effect of Sertoli cytoplasm, which was believed to cause spermatozoa to congregate and affix themselves to the sustentacular cells for nourishment. It is appropriate also to consider what implications the syncytial nature of spermatids may have for the interpretation of abnormal spermatozoa. Retzius (23), von Wiedersperg (32), and Maddox (15) in their early studies of the development and normal structure of spermatozoa, all observed giant forms, double-tailed sperm, and sperm with two heads. However, Broman (2) in 1902 appears to have published the first paper specifically devoted to a description of abnormal human spermatozoa. He

distinguished four principal classes: (*a*) those that differ from normal in size, *i.e.*, giant and dwarf sperm; (*b*) sperm with one head and two or more tails; (*c*) sperm with two or more heads; and (*d*) sperm that are single and of normal size, but otherwise atypical in shape. He speculated that two-tailed giant sperm resulted from multipolar mitosis in which the chromosomes failed to separate and, during restitution, became enclosed in a common nuclear membrane while the cytoplasm remained undivided. In fact, Broman believed that all giant or double forms developed from spermatids that were the product of abnormal mitosis, and in the sixty odd years since his paper no more satisfactory explanation has been advanced. The recent demonstration that cytoplasmic division is *normally* incomplete and the further suggestion that the support of the Sertoli cell may be required to maintain the usual degree of separation of the spermatids during differentiation, provide a better basis for understanding the genesis of double abnormalities of spermatozoa than existed heretofore. Indeed, in the light of these new findings, it is surprising that such abnormalities are not far more common.

As in the case of spermatogenesis, the observation of intercellular bridges connecting the cnidoblasts of Hydra sheds new light upon the mechanism of coordination of their differentiation. At the same time it casts some doubt upon widely accepted theories concerning cnidoblast migration. Since the latter part of the 19th century, investigators of Hydra have noticed that the ectoderm of the body column contains many clusters of cnidoblasts but has relatively few mature nematocysts, whereas, the tentacles are richly provided with nematocysts but seem to have few nests of cnidoblasts. To explain this paradox several authors have contended that the cnidoblasts migrate. It is believed that they penetrate the mesoglea and enter the endoderm. From there, they are reported to become free in the gastrovascular cavity and later to reenter the endodermal epithelium at a higher level, from whence they make their way into the tentacles. Although the microscopic observations on which this theory is based were made on related forms that are more transparent, the same explanation is generally believed to apply to the distribution of nematocysts in Hydra. It is not clear in the literature at what stage of the development of the cnidoblasts their remarkable peregrinations occur, but the demonstration that they are connected in syncytial clusters throughout most of the period of nematocyst formation would indicate that if any such amoeboid migration of separate cells occurs, it must take place quite late in their differentiation. In our studies thus far we have seen no evidence of further constriction of the intercellular bridges and eventual separation of the cnidoblasts into individual cells, but this may well take place. Recent autoradiographic studies seem to bear out the contention of earlier workers that the principal site of nematocyst formation is in the column, but much further work is needed to establish whether these structures reach the tentacles by individual migration or by a slow upward displacement of the epithelium as a whole, as it moves away from a zone of proliferation in the column. The whole subject is in need of reinvestigation with modern labelling methods and taking into account the newly discovered syncytial nature of the cnidoblast clusters.

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Explanation of Plates

Legend

Ac, Acrosome.
Ch, Chromosomal core.
Er, Endoplasmic reticulum.
G.C., Golgi complex.
Gl, Granules presumed to be glycogen.
I.B., Intercellular bridge.
M, Mitochondrion.
Nc, Nucleus.
Ncl, Nucleolus.
Nm, Nematocyst.
S.C., Sertoli cell.
Sp, Spermatid.
S.R., Spindle remnant.
St, Spermatocytes.

Plate 201

Fig. 1. Phase contrast photomicrographs of a group of rat spermatids illustrating the progressive changes after isolation from the germinal epithelium. × 1000.

Fig. 1. *A*. Four spermatids are joined by intercellular bridges at the sites indicated by arrows.

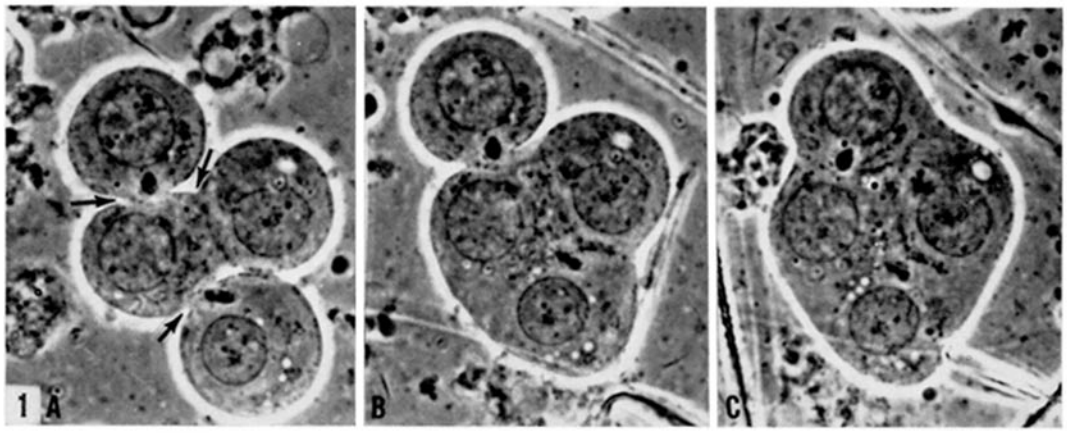
Fig 1. *B*. A few minutes later, the constrictions between three of the cells have disappeared and the bridge connecting them with the fourth is distinctly wider.

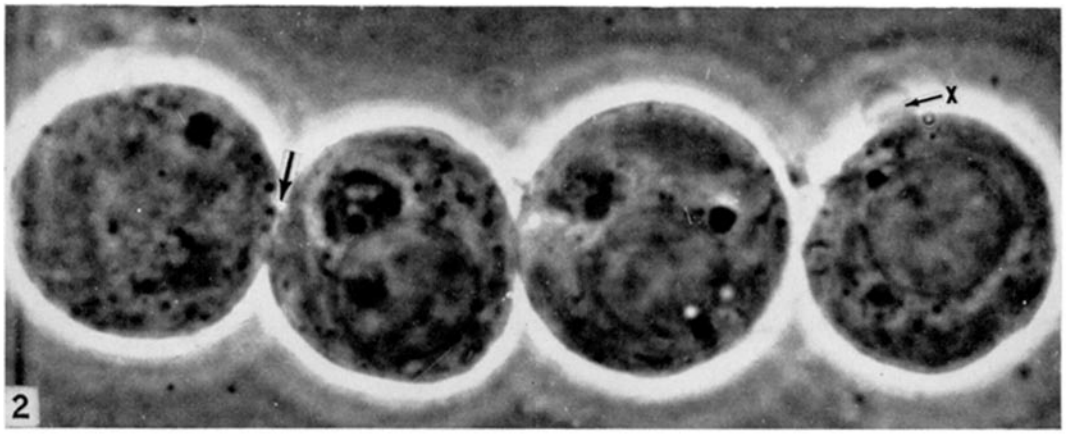
Fig. 1. *C*. All four have coalesced to form a quadrinucleate mass.

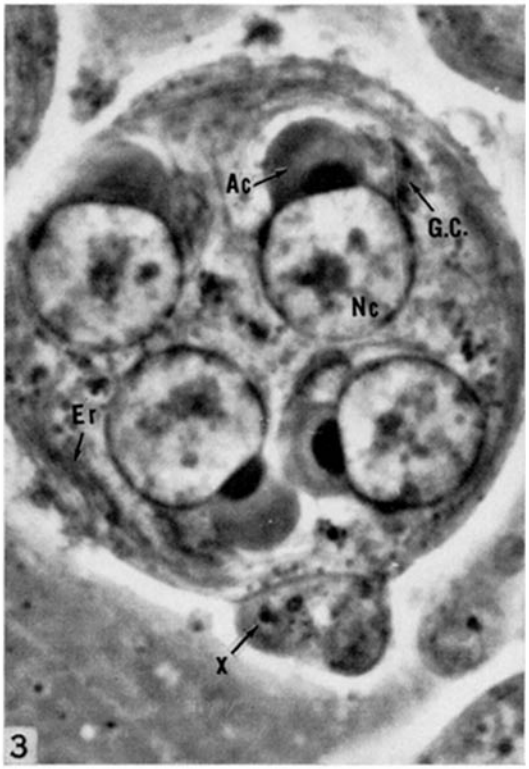
Fig. 2. Four conjoined guinea pig spermatids that have withstood the isolation procedure without being broken or separated into individual cells. One of the three connections is visible (see arrow), the others could be seen in the original preparation at slightly higher or lower levels of focus. Compare this group with the electron micrograph of a similar group in Fig. 7. The small bleb at *X* is the site where a bridge connecting this cell with another was broken. There were probably eight spermatids in the original cluster. × 2500.

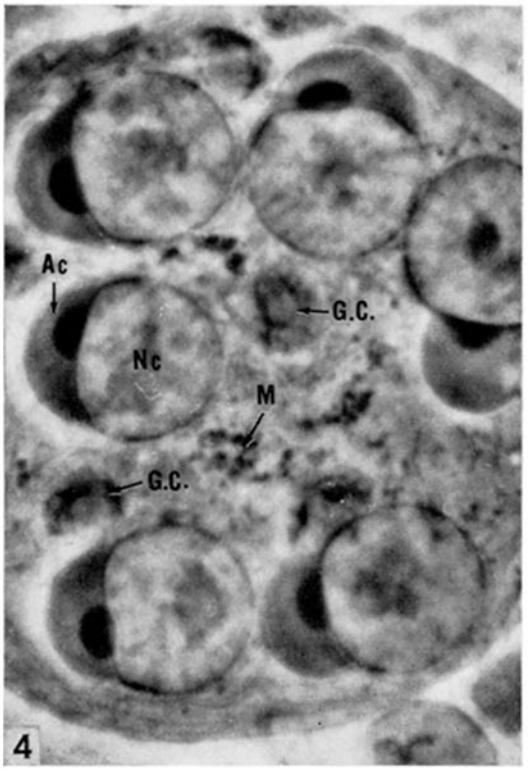
Fig. 3. A multinucleate mass formed by the coalescence of four guinea pig spermatids. The irregularity at *X* probably represents the site where other spermatids of the original group were broken off. × 2500.

Fig. 4. A syncytial mass containing at least six spermatid nuclei with their associated acrosomes in precisely the same stage of differentiation. × 2500.







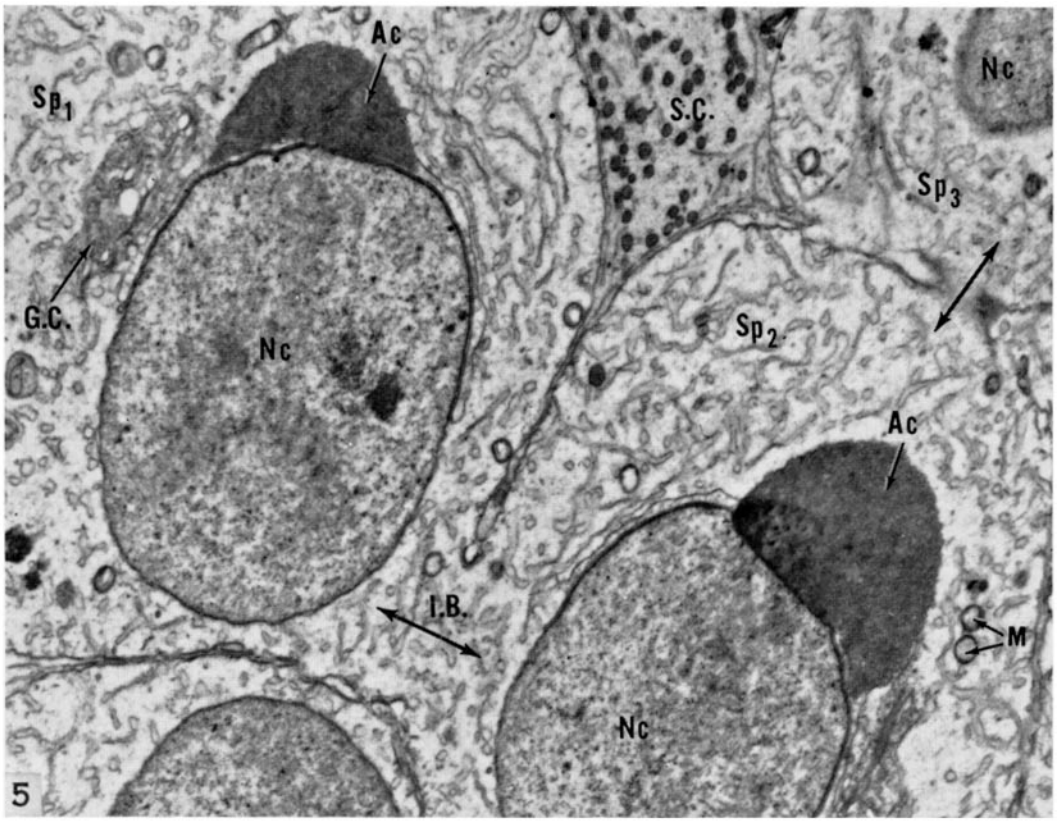


(Fawcett *et al.*: Occurrence of intercellular bridges)

PLATE 202

FIG. 5. An electron micrograph of guinea pig testis showing parts of three spermatids (Sp_1, Sp_2, Sp_3) connected by intercellular bridges (*I.B.*). The plane of section passes tangential to the bridge at the upper right. Notice that a narrow process of the associated Sertoli cell (*S.C.*) extends deep into the furrow between the conjoined cells (Sp_1 and Sp_2). × 13,000.

FIG. 6. Portions of four guinea pig spermatids. The bridges (*I.B.*) connecting three of them are included in this field (see arrows). Notice at the asterisks the characteristic concave shape and the local thickening of the membrane surrounding the bridge. Observe also that the endoplasmic reticulum (*Er*) of one cell is in communication with that of the adjacent cell via numerous canalicular elements that pass through the bridges. × 22,500.



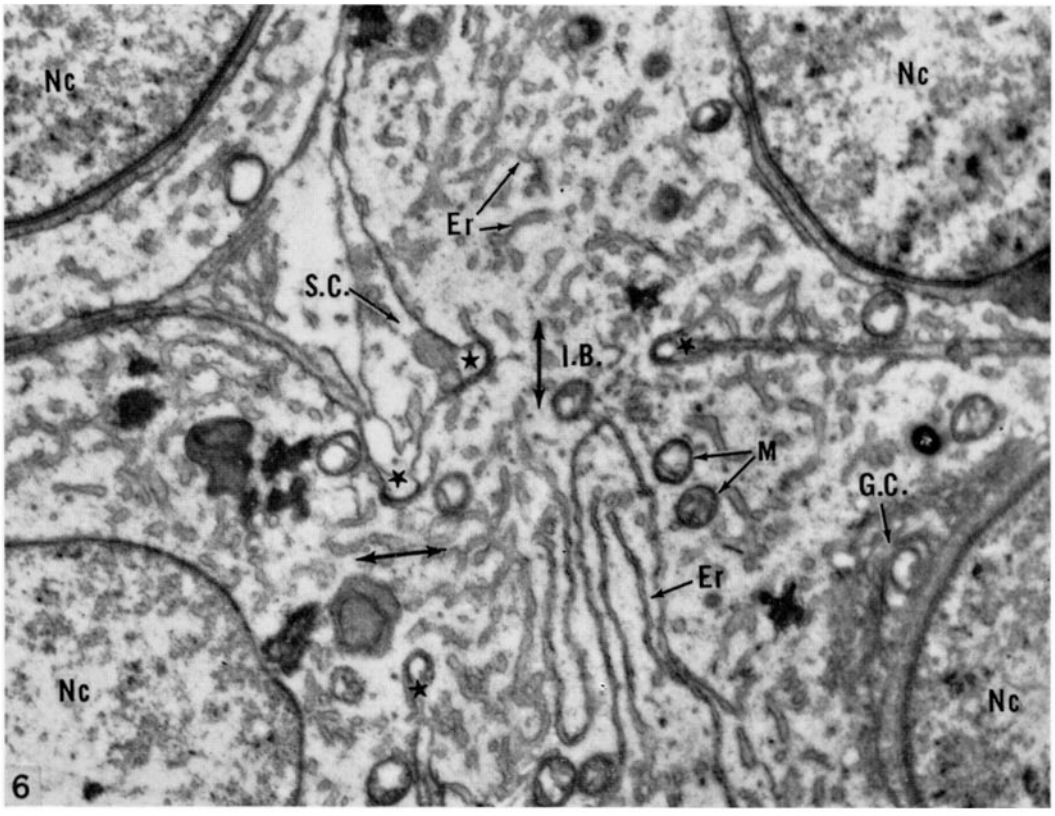


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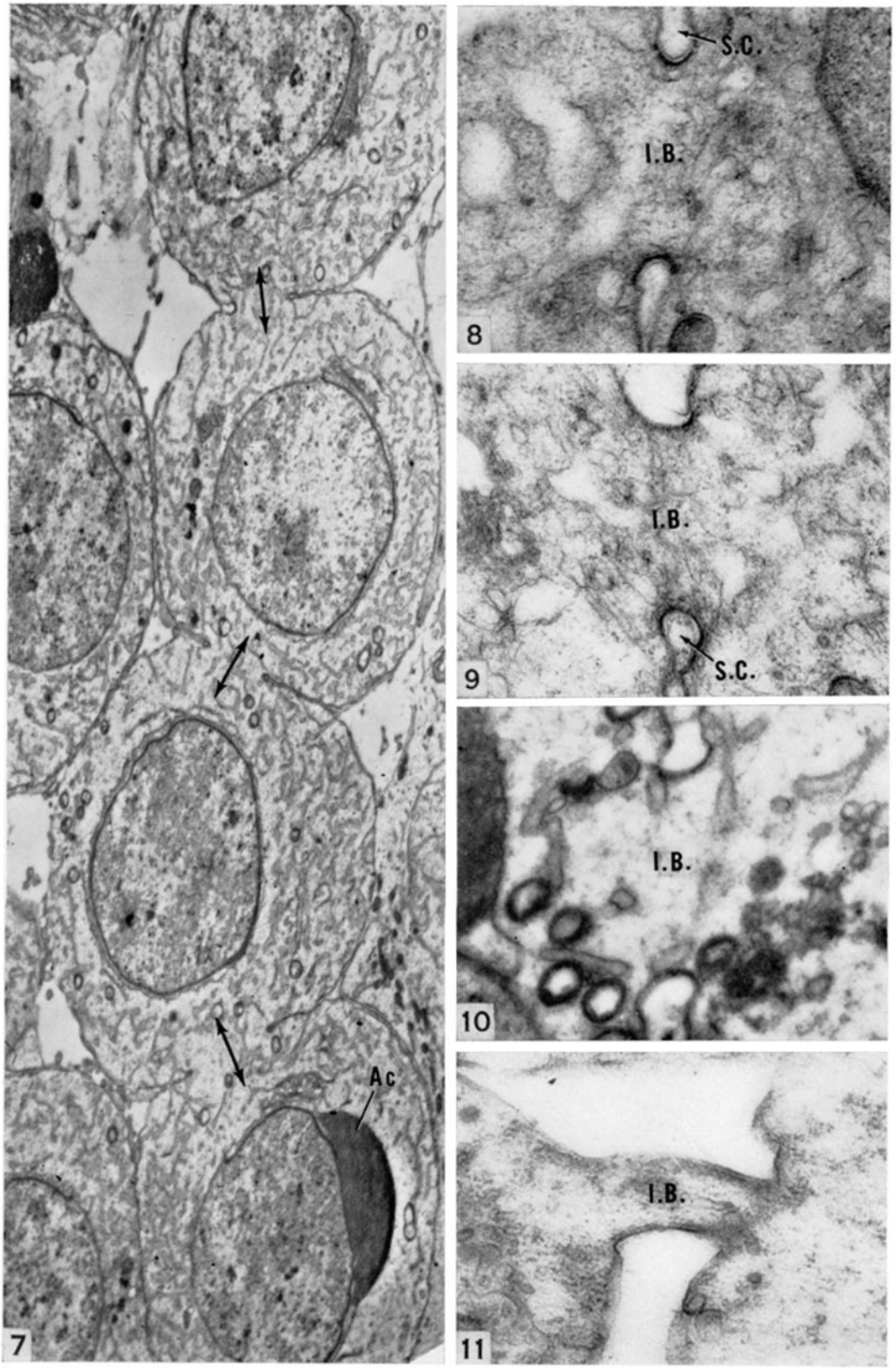
PLATE 203

FIG. 7. A low power electron micrograph of guinea pig testis showing four spermatids joined by three bridges that happened to lie in the same plane of section (see arrows). It is assumed that four other cells were connected to these in other planes of section. × 4,500.

FIGS. 8 and 9. The protoplasmic connections (*I.B.*) between cat spermatids showing the annular thickening of that portion of the membrane common to the two conjoined cells. The thin membrane outside of this is the plasma membrane of a slender Sertoli cell process (*S.C.*) that extends into the intercellular cleft. × 35,000.

FIG. 10. Intercellular bridge (*I.B.*) between two guinea pig spermatids. It has essentially the same structure as in the cat. × 35,000.

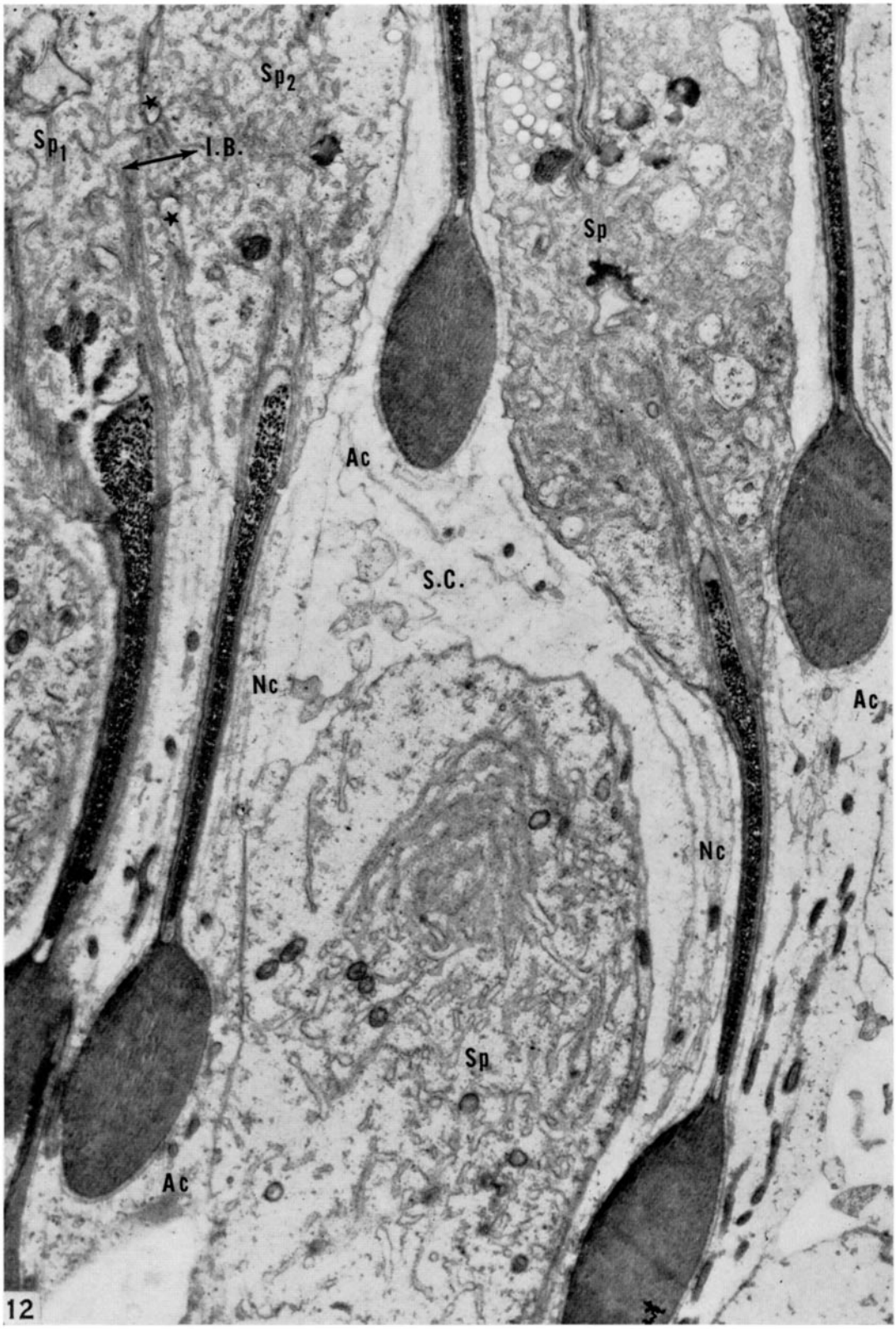
FIG. 11. A long cylindrical bridge between two spermatids of the fresh-water polyp, Hydra. × 48,000.



(Fawcett *et al.*: Occurrence of intercellular bridges)

PLATE 204

FIG. 12. An electron micrograph of guinea pig testis showing several advanced spermatids (*Sp*) occupying deep recesses in the irregular surface of a Sertoli cell (*S.C.*). The spermatid nuclei (*Nc*) have condensed and flattened in a plane perpendicular to the page. The large acrosomes (*Ac*) are beginning to acquire their definitive shape. Even at this late stage of spermiogenesis, the spermatid cell bodies (Sp_1 and Sp_2) are still connected by intercellular bridges (between asterisks at the upper left of the figure). It is believed that they remain joined until the excess cytoplasm is cast off. × 6500.

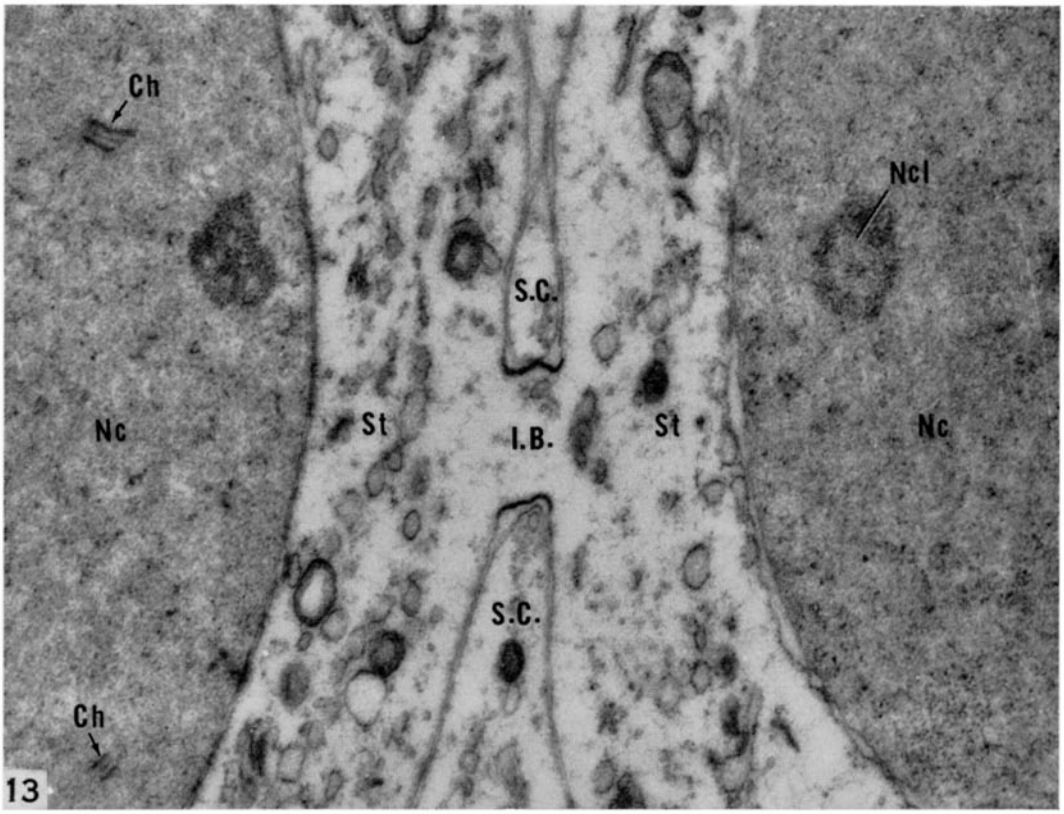


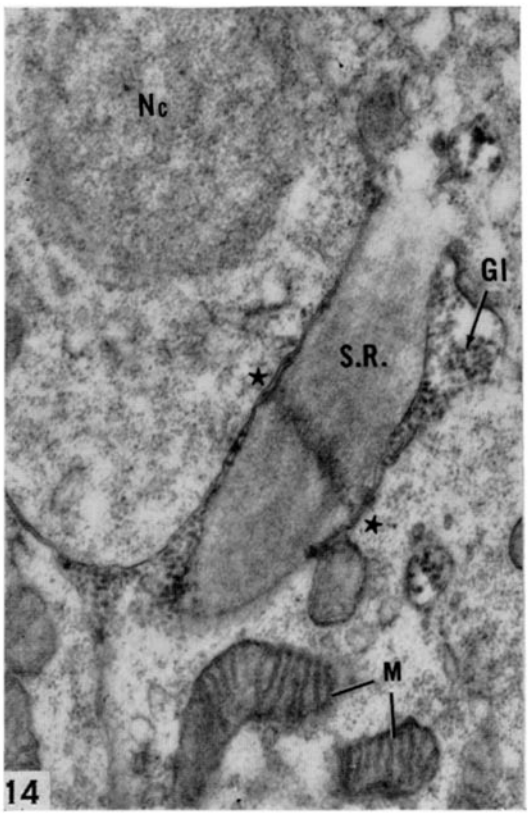
(Fawcett *et al.*: Occurrence of intercellular bridges)

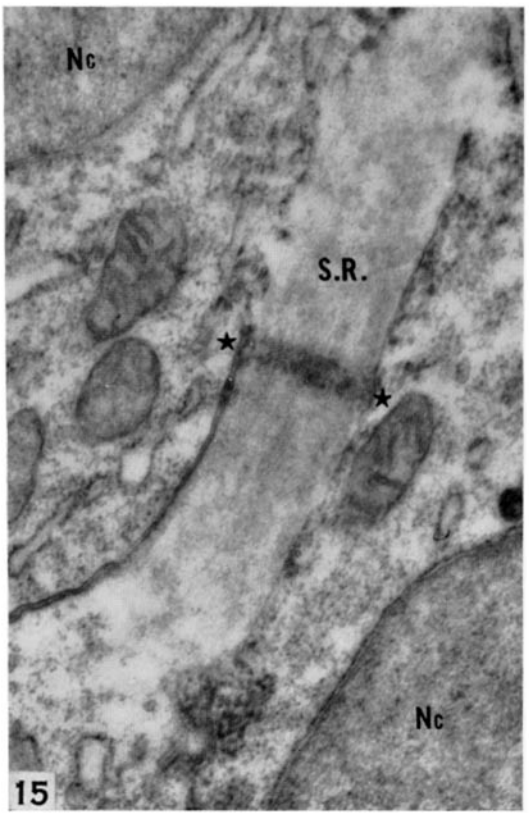
PLATE 205

FIG. 13. An electron micrograph of two human primary spermatocytes (*St*) joined by a bridge (*I.B.*) which shows, especially well, the annular thickening of the surrounding membrane. The cells are recognizable as primary spermatocytes by their size and by the presence in the nucleus (*Nc*) of chromosomal cores (*Ch*) that are found only in prophase of meiosis. Notice the processes of the Sertoli cell (*S.C.*) extending deep into the cleft between the two cells. × 20,000.

FIG. 14 and 15. Long spindle bridges between early cnidoblasts of Hydra. For a short time after their formation, the bridges are quite long and are occupied by a bundle of exceedingly thin filaments which are apparently remnants of the spindle (*S.R.*). At the midpoint of the bridge (between asterisks) there is a dark transverse band which seems to consist of an extrafilamentous dense substance. × 24,000.







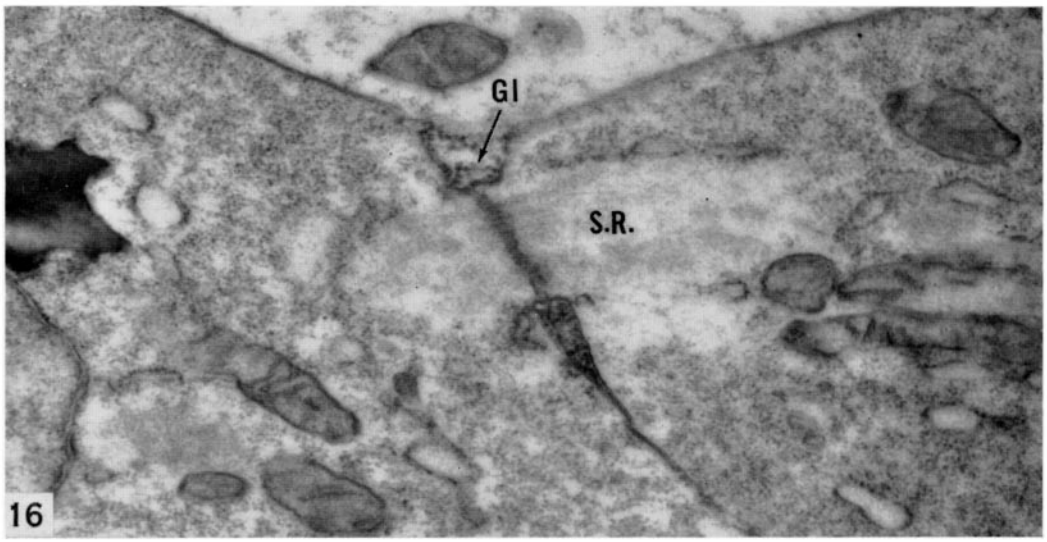
(Fawcett *et al.*: Occurrence of intercellular bridges)

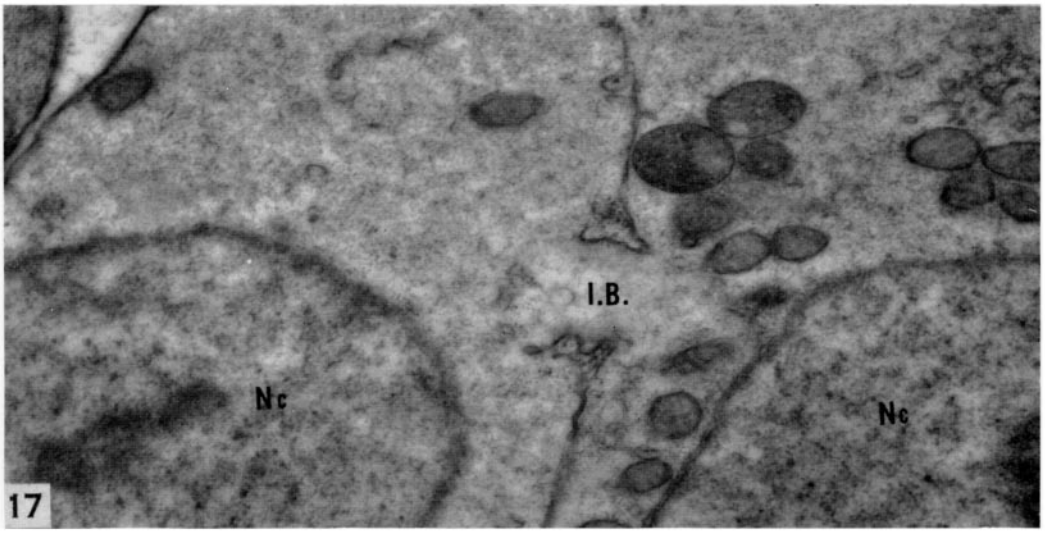
PLATE 206

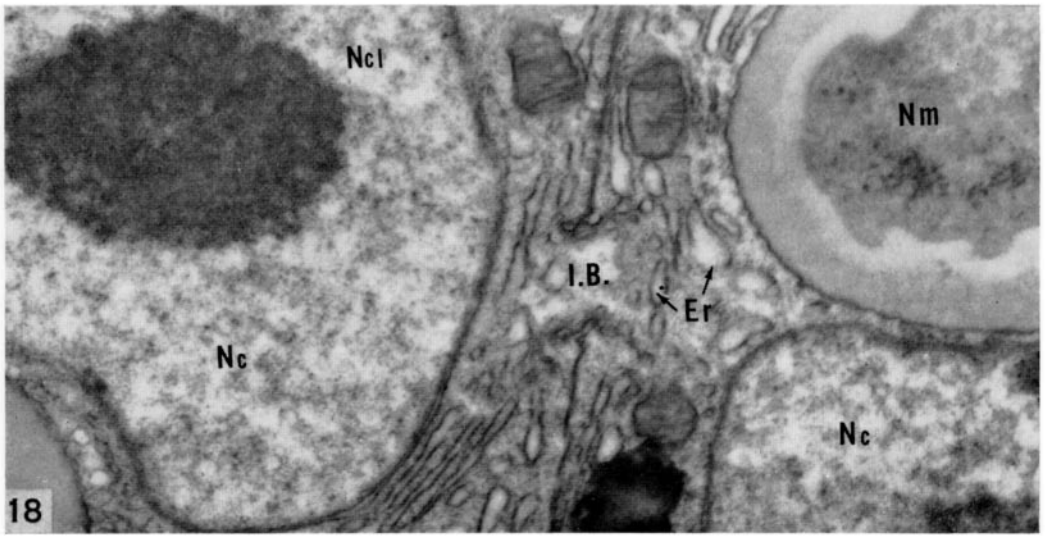
FIG. 16. A stage in the formation of a bridge between early cnidoblasts of Hydra. The spindle filaments (*S.R.*) have been largely resorbed or have dissolved in specimen preparation. In their place is a light area devoid of granules. The dense band across the middle of the bridge persists. The surrounding membrane bends outward opposite this band to form a ridge that encircles the bridge at its midpoint. Angular spaces between cells in the neighborhood of the bridges are filled with granules believed to be particulate glycogen (*GI*). × 24,000.

FIG. 17. A later stage in the evolution of a bridge (*I.B.*). All traces of spindle filaments have disappeared and the interior of the bridge is filled with cytoplasm only slightly less dense than that of the adjacent cell bodies. × 20,000.

FIG. 18. Two cnidoblasts at an advanced stage of differentiation still joined by a typical bridge containing elements of the endoplasmic reticulum (*Er*) that probably pass from one cell to the other. × 24,000.







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