

FINE STRUCTURE OF THE HUMAN OVUM IN THE PRONUCLEAR STAGE

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ABSTRACT

A penetrated ovum was recovered from the oviduct of a 33 year old surgical patient who had had sexual intercourse 26 hr before the operation. The ovum was in the pronuclear stage. The ooplasmic organelles were mainly represented by mitochondria, endoplasmic reticulum components, and Golgi elements. Small vesicles were found in the space between the two sheets of the pronuclear envelope. These vesicles appeared to be morphologically similar to the ER vesicles in the ooplasm and were considered to be involved in pronuclear development. Numerous annulate lamellae were seen in the ooplasm as well as in the pronuclei. Ooplasmic crystalloids were also observed. These were thought to represent cytoplasmic yolk. Remnants of the penetrating spermatozoon were found in close relation to one of the pronuclei. The fine structure of the first and second polar body is also described. The nuclear complement of the first polar body consisted of isolated chromosomes, whereas the second polar body contained a membrane-bounded nucleus. In consideration of the possibility that polar body fertilization may take place, these differences in nuclear organization could be of importance. Other recognizable differences between the two polar bodies were presence of dense cortical granules and microvilli in the first polar body, and absence of these structures in the second. These dissimilarities were considered to be related to the organization of the egg cytoplasm at the time of polar body separation.

INTRODUCTION

The ultrastructural changes that take place in the mammalian ovum in the early stages following penetration by the spermatozoon have been studied in the rat by Szollosi and Ris (47), Szollosi (45, 46), and Sotelo and Porter (40), and in the rabbit by Zamboni and Mastroianni (55). The fine morphology of the human penetrated ovum has never been investigated.

Until the present time, only two unequivocally penetrated¹ ova have been recovered from human oviducts. In 1959, Khvatov found a pronuclear

egg in a serially sectioned oviduct (29). In 1965, Dickmann, Clewe, Bonney, and Noyes studied, by means of phase microscopy, a pronuclear egg obtained by flushing the oviducts of a woman who had an intrauterine contraceptive device (11).

A third human ovum in the pronuclear stage has been recently recovered. Its fine structure is described in this article.

MATERIALS AND METHODS

The ovum was obtained from a 33 year old woman who had a vaginal hysterectomy for symptomatic

¹ Penetration refers to any stage after gamete plasma membranes have become confluent. Fertilization is

completed only at fusion of male and female pronuclei (*syngamy*).

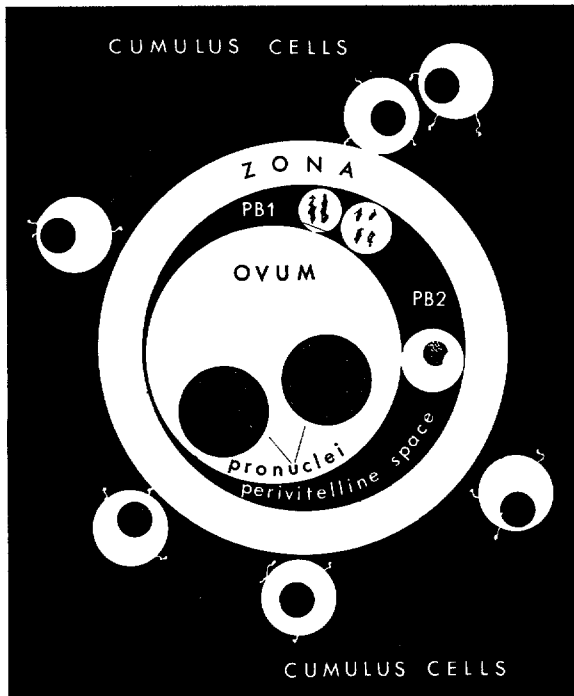


FIGURE 1 Diagrammatic representation of the ovum at the time of fixation.

pelvic relaxation. A bilateral salpingectomy was also performed. The patient had had sexual intercourse approximately 26 hr before the operation. The two oviducts were flushed with Krebs-Ringer phosphate solution in a retrograde fashion, from the uterotubal junction to the fimbriated end. The material obtained by the flushing was collected in a watchglass and immediately examined under a dissecting microscope. One ovum was easily visualized. It was removed with a Pasteur pipette and transferred into chilled 1% osmium tetroxide with salts added according to the formulation of Sjöstrand as modified by Zetterqvist (56). One hr was allowed for fixation. Dehydration was rapidly performed in increasing concentrations of alcohol. The ovum was embedded in Epon 812 (32) and serially sectioned. The sections were stained with lead hydroxide following the method of Karnovsky (21), and viewed with an Hitachi HU-11A electron microscope.

About 300 electron micrographs were taken,

ranging in magnification from 2,000 to 28,000 diameters.

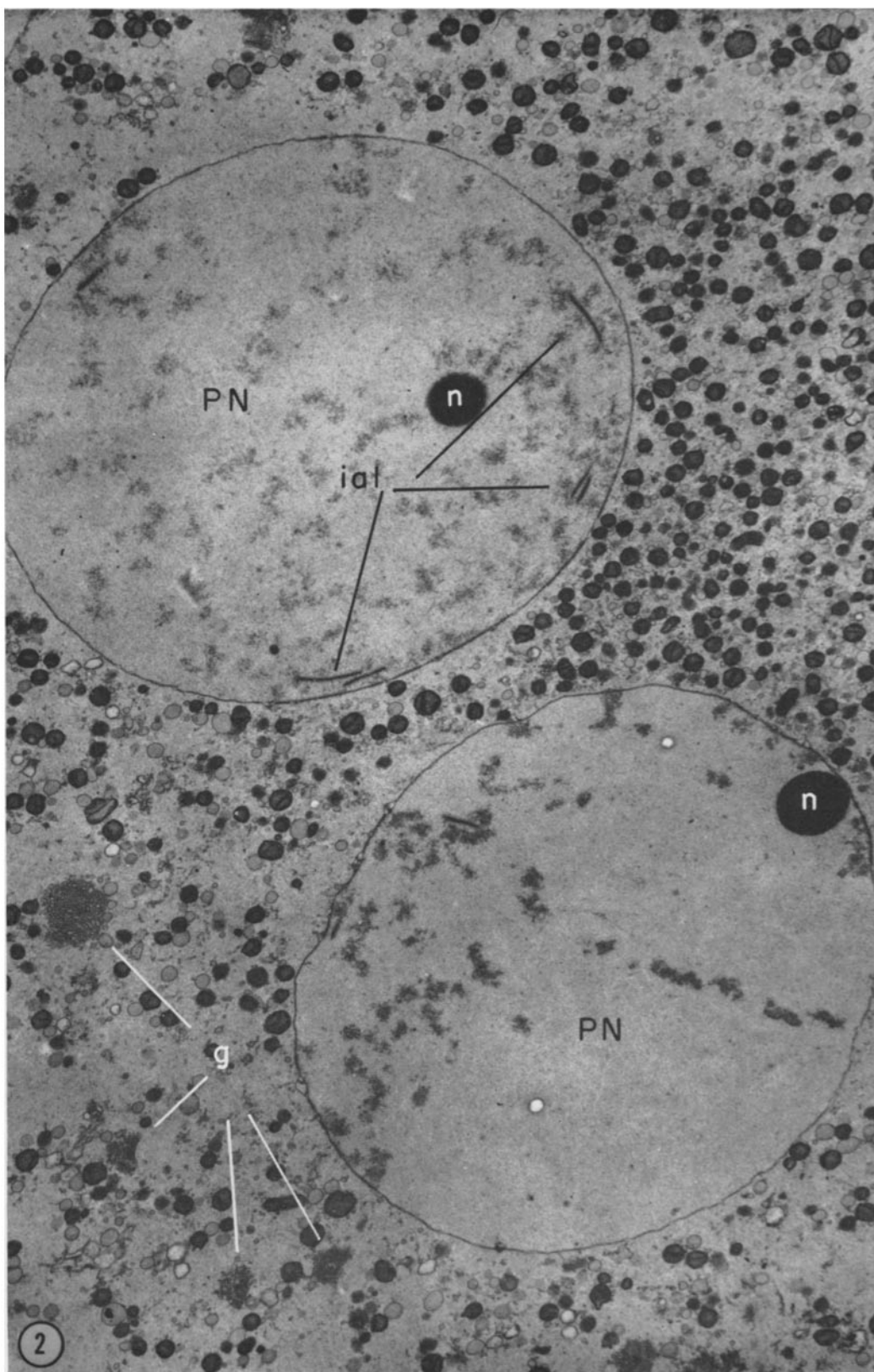
RESULTS

The ovum was separated from the encircling zona pellucida by an irregularly wide perivitelline space (Fig. 1). A few cells of the *cumulus oophorus* were present outside the zona pellucida. Three polar bodies were observed in the perivitelline space. Two of them were located adjacent to each other. The third was found at a considerable distance from the first two, outside the opposite hemisphere of the ovum.

The Ovum

The outline of the ovum was regular, with the exception of shallow indentations in those portions immediately adjacent to the polar bodies (Fig.

FIGURE 2 Region of the penetrated human ovum with male and female pronuclei (PN). The pronuclear material appears to be highly hydrated, although zonally condensed in patchy aggregates. Nucleoli (*n*) and intrapronuclear annulate lamellae (*ial*) are evident. Note the numerous organelles which populate the cytoplasm adjacent to the pronuclei. *g*, Golgi complex. $\times 5,400$.



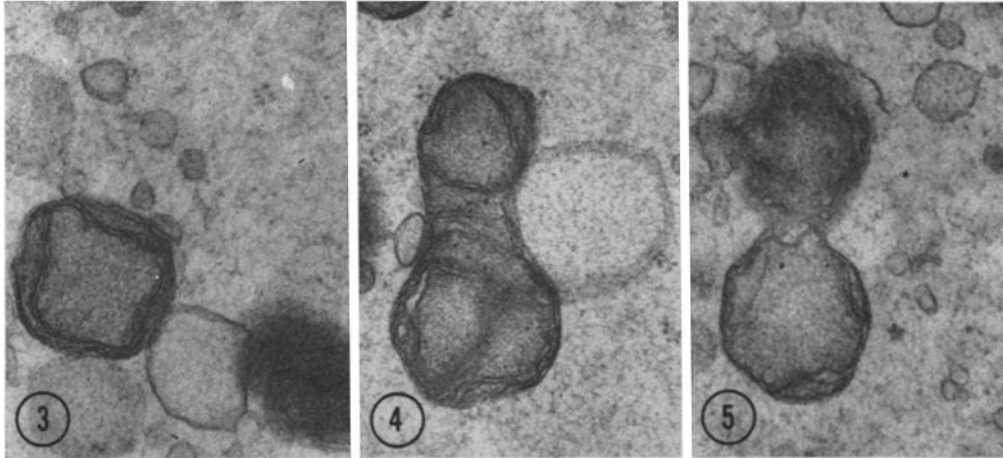


FIGURE 3 Spheroidal mitochondria represent the most common type of mitochondria to be encountered in the ovum (see also Fig. 5). They have limited numbers of cristae which are arranged roughly parallel to the outer mitochondrial membranes. $\times 46,000$.

FIGURE 4 Elongated mitochondria are less numerous than those of the spheroidal type. The elongated mitochondria are equipped with transversely oriented cristae and show symmetrical constrictions in the central region. $\times 40,000$.

FIGURE 5 Deepening of the indentations of the elongated mitochondria may lead to the formation of two mitochondria of the spheroidal type. The possibility exists, however, that the structures in the micrograph represent two independent, spheroidal mitochondria, one of which has been sectioned in a tangential plane. $\times 46,000$.

29). The plasma membrane was smooth and devoid of typical microvilli. A few blunt projections of the membrane, however, were observed protruding into the perivitelline space. A marked pinocytotic activity of the plasma membrane was seen.

Organelles were present in large numbers throughout the cell, with the exception of the cortical region, in which they were found in a considerably lesser amount.

The mitochondria were mostly concentrated around the pronuclei (Figs. 2 and 20) and were characterized by a discrete pleomorphism. The predominant type of mitochondria was spheroidal (Figs. 3, 6, and 20). They usually contained small numbers of cristae (one to three pairs) which were either arranged parallel to the outer mitochondrial membranes or oriented transversely in the matrix. Cristae with archlike orientation were also observed in proximity to one of the poles of the organelle (single arrows, Fig. 6). A few mitochondria were elongated and had transversely oriented cristae. These mitochondria frequently showed symmetrical indentations in their central

region (Fig. 4). The image in Fig. 5 suggests that deepening of these indentations toward each other could result in the formation of two mitochondria of the spheroidal type. All mitochondria were characterized by a highly dense matrix. In a few, the matrix appeared focally condensed into one or two areas of a greater electron density.

The endoplasmic reticulum (ER) was prominent and equally distributed throughout the cell. It was seen mostly as large and small vesicles of a spheroidal shape. The large ER vesicles (Figs. 3, 4, 6 to 9, and 20) were usually located in close proximity to the mitochondria. They measured 0.5μ in average diameter. The membranes of these vesicles were infrequently associated with ribosomes (double arrow, Fig. 6). The lumina of the large vesicles were occupied by a coarsely granular material of a low electron opacity. Either one or more elongated, tubular profiles were observed budding off from some of the vesicles (Figs. 7 to 9). These buds had dilated ends in the form of sacculi about $100 m\mu$ in diameter and contained a material of the same electron opacity as that present in the lumina of the

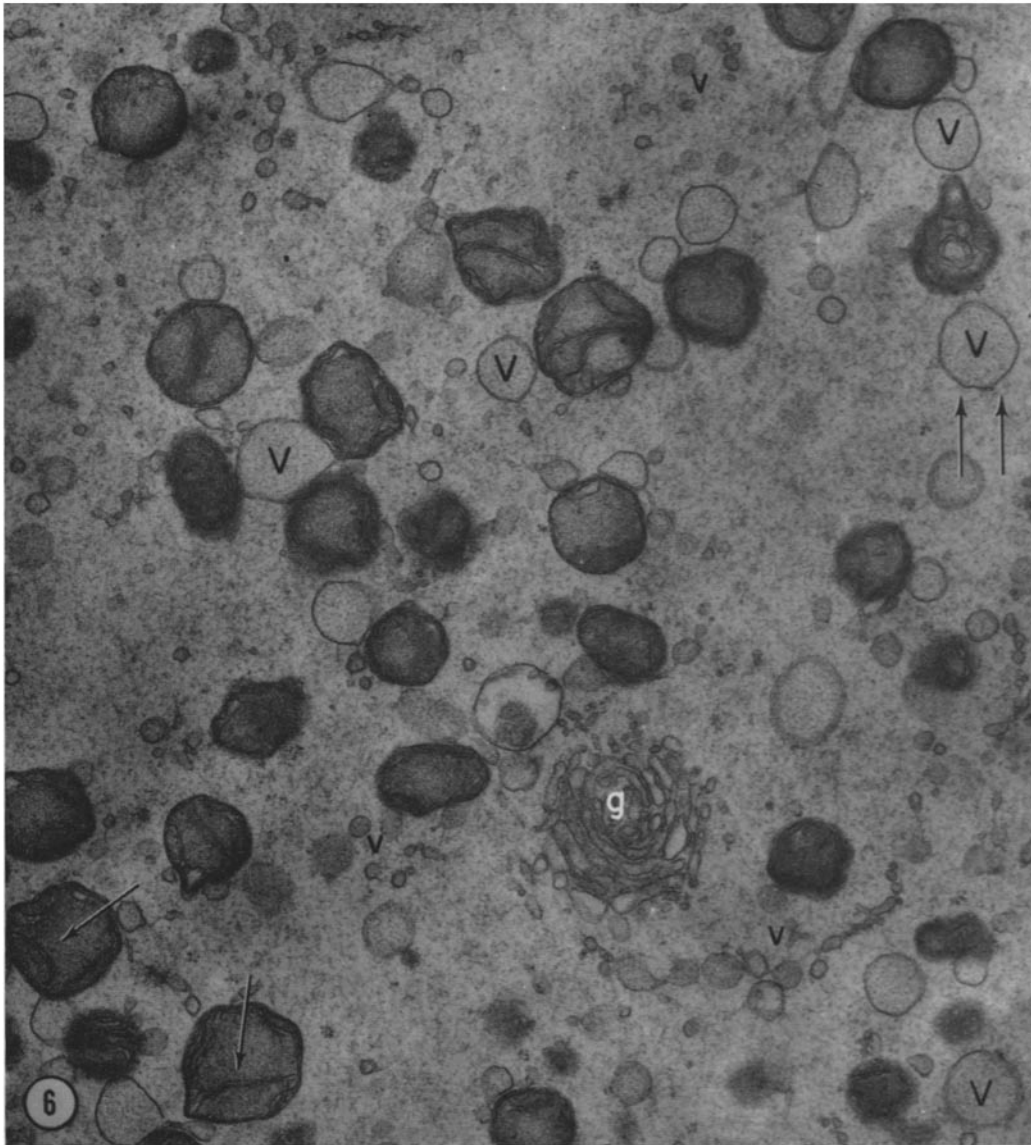


FIGURE 6 Region of the ooplasm of the penetrated ovum with typical complement of organelles. The mitochondria are numerous and mostly spheroidal. Two mitochondria with archlike arrangement of the inner cristae are indicated by the single arrows. The ER is present in the form of large (*V*) and small vesicles (*v*). The large vesicles are usually associated with mitochondria. The small vesicles are thought to originate by budding from the large ER vesicles (see Figs. 6 to 8). The double arrow indicates a large vesicle with a few ribosomes attached to its limiting membrane. *g*, Golgi complex. $\times 29,000$.

vesicles. The membranes of these appendages were never seen to be associated with ribosomes. The small ER vesicles (Figs. 3, 5, 6, 7, 9, and 20) were numerous throughout the cytoplasm, but appeared to be particularly concentrated around

the large ER vesicles. The average diameter of the small vesicles was 100μ . The appearance of the small ER vesicles was similar to that of the saccular terminations of the appendages of the large vesicles. The limiting membranes of the

small vesicles were not associated with ribosomes, and their lumina were filled with material of a low electron opacity.

The Golgi complex consisted of prominent clusters of closely packed vesicles and encircling arrays of parallel tubules (Figs. 2, 6, and 25). These clusters appeared to be distributed equally throughout the ooplasm. In the equatorial region of the ovum, 15 to 20 such clusters were observed in any given section.

Annulate lamellae (42) were present in a considerable number (Figs. 10 and 11). They were arranged in parallel stacks consisting of 5 to 15 units. The units were separated by a distance of about 100 m μ . The lamellae varied from 0.5 to 2.0 μ in length. In sections perpendicular to their surface, each lamella appeared to consist of two parallel, smooth membranes delimiting a flattened cisterna, about 20 m μ wide (Fig. 10). Both ends of the cisterna were dilated. The membranes of each lamella were seen to join each other at regularly spaced intervals. These junctions were characterized by an increased electron opacity. In oblique or tangential sections (Fig. 11), the areas of adhesions of the membranes were observed as regularly spaced annuli which were separated by a center-to-center distance of 110 to 130 m μ . The rims of the annuli appeared to consist of two concentric rows of extremely small, hollow, circular subunits. Dense granules were observed in the centers of the annuli.

Crystalline inclusions were also observed (Figs. 12 and 13). These formations were few and they appeared to be located haphazardly in the cytoplasm. In one plane of sectioning (Fig. 12), they were seen as parallel arrays of 10 to 25 rods which were separated one from the other by a distance

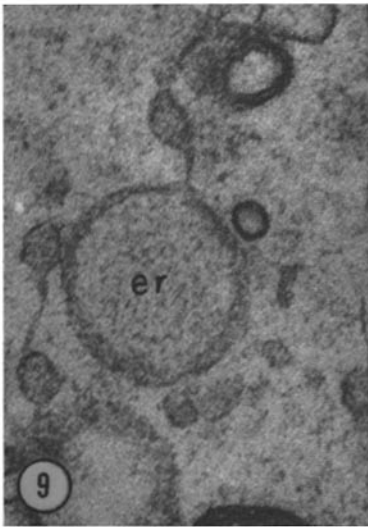
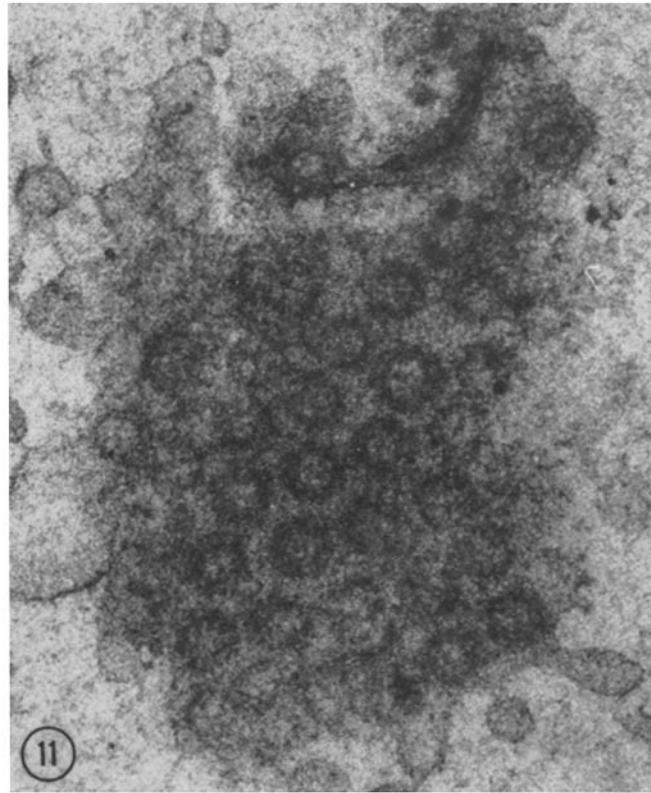
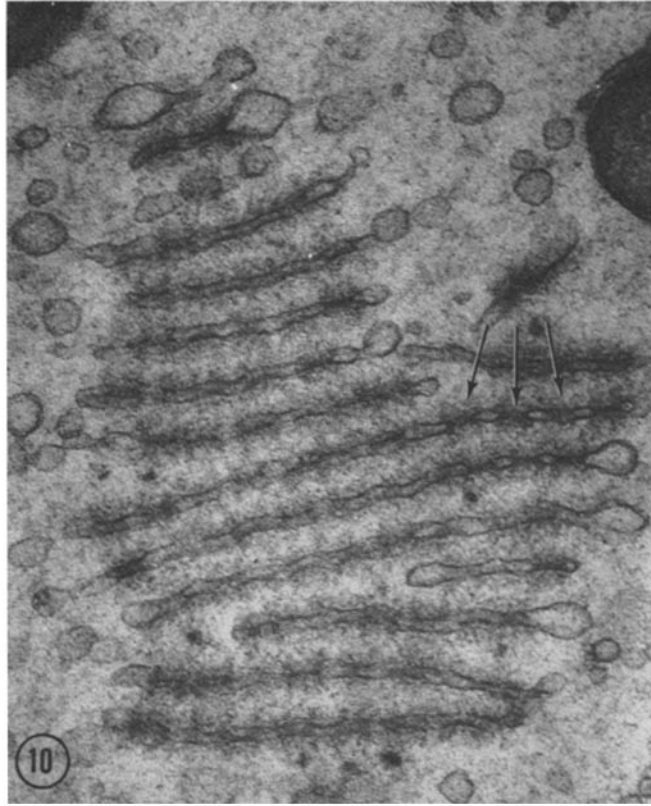
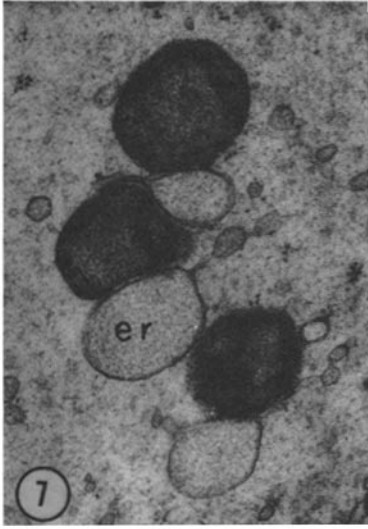
of about 150 A. Each rod showed a faint periodicity. In other planes of sectioning (Fig. 13), the rods appeared to be oriented at 65° angles with each other to form an hexagonal crystalline pattern. These crystalline formations often had pointed outlines.

The two pronuclei were located eccentrically and close to each other (Fig. 2). They appeared to be of about equal size (largest diameter 30 μ) and showed identical characteristics. They were ovoid in shape and had a regular outline. The pronuclear envelope consisted of two membranous sheets separated by a space 20 m μ in width (Fig. 17). In oblique or cross-sections, the so called pores of the membrane were readily apparent (Fig. 18). The space between the pronuclear membranes appeared considerably enlarged in places because of outpocketings of the outer sheet toward the cytoplasm (Figs. 14 to 16). Small round or elongated vesicles containing material of a low electron opacity were observed in these dilated areas (Figs. 14 to 16). The membranes limiting these vesicles appeared distinct from the pronuclear envelope. The pronuclear chromatin gave the impression of being highly hydrated (Figs. 2, 17, and 20). It was zonally condensed in irregular patches of dense filaments, most of which were located close to the pronuclear membrane and were separated by lakes of low density pronucleoplasm (Figs. 2 and 14). One nucleolus was present in each pronucleus (Fig. 2). The nucleoli, spheroidal in shape and remarkably compact in appearance, consisted of aggregated granular material or compacted fibrils of a high electron opacity (Fig. 19). A few lamellar structures were observed also in the pronuclei (Figs. 2 and 17). These lamellae were similar to the

FIGURES 7 to 9 Large ER vesicles (*er*) in budding activity. The buds have tubular profiles and are provided with saccular terminations which are similar to the small ER vesicles scattered in the ooplasm (compare with Fig. 6). Fig. 7, \times 31,000. Fig. 8, \times 88,000. Fig. 9, \times 60,000.

FIGURE 10 Cytoplasmic annulate lamellae as seen in a section perpendicular to the surface of the membranous sheets. The majority of the cisternae have dilated, ampullar ends. Some of the lamellae join at regularly spaced intervals (arrows). In other lamellae, the areas of membrane adhesions are less numerous. This appearance is probably due to the plane of sectioning. \times 56,000.

FIGURE 11 Cytoplasmic lamellae in oblique or longitudinal section. The characteristic, regularly spaced annuli are evident. \times 88,000.



annulate lamellae in the ooplasm. The pronuclear lamellae, however, were isolated and not arranged in stacks.

Remnants of the penetrating spermatozoon were observed close to one of the pronuclei. Figs. 20 and 21 show the sperm axial filament complex encircled by the outer row of nine dense fibers. Since these structures were neither surrounded by the mitochondrial sheet nor by the fibrous sheet, it was impossible to establish whether they belonged to the middle or principal piece of the sperm tail. Other components of the sperm were observed and followed in serial sections (Figs. 22 to 24). Again, exact identification of these components was not possible. It is thought, however, that they were part of the neck region of the spermatozoon and possibly represented the connecting piece (14) seen in an oblique plane of sectioning.

The Perivitelline Space

Three polar bodies were present in the perivitelline space. Two of them were located close to one another and showed identical morphologic characteristics. They were assumed to represent divided portions of the first polar body (see Discussion). Their plasma membranes were provided with a considerable number of microvilli. The nuclear complement consisted of chromosomes which were mainly clustered together and connected with one another by thin chromatin bridges (Fig. 27). A few chromosomes were isolated and randomly scattered in the cytoplasm. All chromosomes were in a close topographic relation with the microtubules of the meiotic spindle (Fig. 27) which were present in large numbers and formed a complex network extending throughout the cytoplasm (Fig. 28).

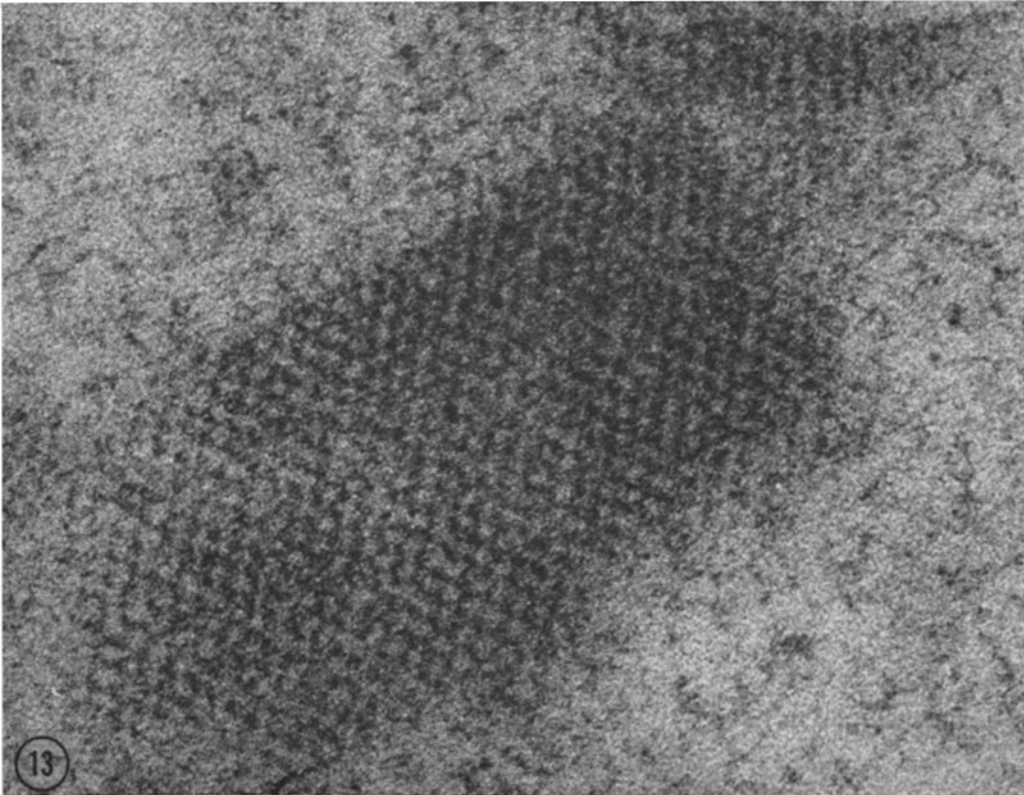
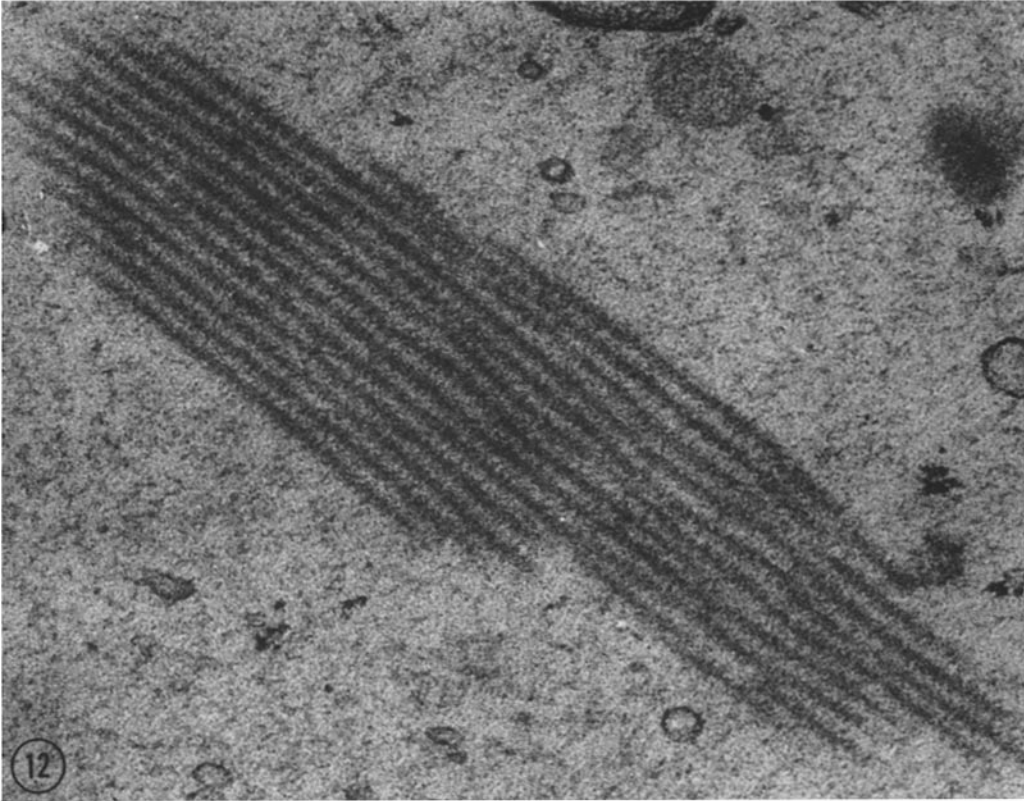
The organelles of the first polar body consisted of mitochondria, vesicles of the endoplasmic reticulum, and dense cortical granules. The mitochondria (Fig. 26) were few and all of the spheroidal type.

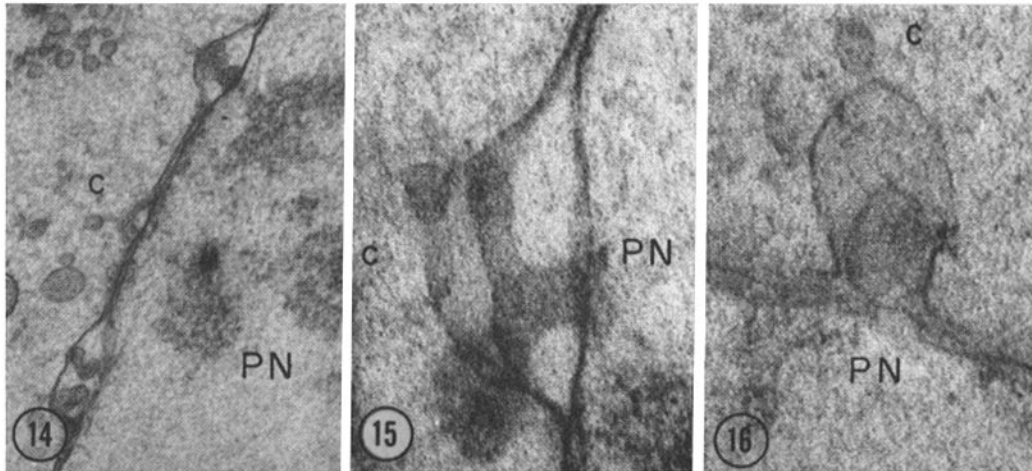
They were similar to the spheroidal mitochondria of the ovum, but seemed to possess relatively fewer cristae. The vesicles of the endoplasmic reticulum (Figs. 26 to 28) were numerous. Although equal in size to the large ER vesicles in the cytoplasm of the ovum, these vesicles had greatly irregular outlines. There were no ribosomes on their limiting membranes, and no budding activity was observed. Dense cortical granules (Figs. 26 and 28) were arranged in a single row a few microns beneath the plasma membrane. The granules were spheroidal and measured about 1μ in diameter. They were limited by a single, smooth membrane and consisted of a granular matrix of a uniformly high electron opacity.

The isolated polar body which was located outside the opposite hemisphere of the ovum conceivably represented the second polar body, normally extruded after sperm penetration. As indicated by the presence of an elongated cytoplasmic bridge extending toward the ovum (Fig. 30), this polar body had recently separated. The cytoplasmic bridge was almost totally occupied by a dense midbody, consisting of the stretched remnants of the meiotic spindle.

The second polar body was considerably larger than the first. It had a spherical nucleus consisting of regular aggregates of dense chromatin which were surrounded by extensive nucleoplasmic lacunae of a low electron density (Fig. 29). The nucleus was limited by a continuous double membrane. Isolated lamellar structures were contained in the nucleus (asterisks and insert, Fig. 29). The density of the surrounding chromatin did not allow a detailed observation of these structures. A few microtubules were present in the cytoplasm around the nucleus. The mitochondria of the second polar body were few and all of spheroidal configuration. Some of them showed regressive changes consisting of vacuolization of the matrix and fragmentation of the cristae (Fig. 29). The structural components of the endoplasmic reticu-

FIGURES 12 and 13 Crystalline inclusions such as those shown in these micrographs are present in limited numbers throughout the cytoplasm of the penetrated ovum. They are thought to represent ooplasmic yolk. When observed in cross-section (Fig. 12), the inclusions appear to be composed of 10 to 25 parallel, faintly striated rods. In an oblique or tangential plane (Fig. 13), the rods form hexagonal crystalline arrays. Note the pointed profile of one of these inclusions in the lower left corner of the micrograph. Fig. 12, $\times 89,000$. Fig. 13, $\times 133,000$.





FIGURES 14 to 16 The space between the two sheets of the pronuclear envelope is zonally enlarged due to blebbing of the outer sheet toward the cytoplasm (*c*). Spheroidal or elongated vesicles are contained in these dilated regions. The vesicles are bound by membranes which are always distinct from the pronuclear envelope. The vesicles are similar to the small ER vesicles present in the cytoplasm. They may represent endoplasmic reticulum components which have become associated with the pronuclei (*PN*). Fig. 14, $\times 28,500$. Figs. 15 and 16, $\times 104,000$.

lum appeared identical to those seen in the cytoplasm of the ovum. They consisted of large and small vesicles (Figs. 29 and 30). Some of the large vesicles were budding. Dense cortical granules were not observed. The plasma membrane of the second polar body was smooth and devoid of microvilli.

The Zona Pellucida

The zona pellucida was regular in outline. A few cytoplasmic processes of the cells of the *cumulus oophorus* were embedded in the zona. These processes showed degenerative changes consisting principally of vacuolization of the cytoplasm and presence of myelinlike whorls.

The cells of the *cumulus oophorus* were morphologically similar to the *cumulus* cells which are present around follicular oocytes (3, 9, 31, 37, 40, 41, 54). These cells were provided with fewer cytoplasmic processes, however, and showed modest degrees of regressive changes, including increased granularity of the cytoplasmic ground substances and swelling as well as vacuolization of mitochondria. These regressive changes were morphologically similar to those induced in *cumulus* cells by incubation in tubal fluid *in vitro* (53).

DISCUSSION

The penetration of the spermatozoon into the ovum initiates a series of transformations which involve the nuclear and cytoplasmic components of both ovum and sperm (5). These transformations characterize the so called stage of activation which culminates in the fusion of the pronuclei (*synkaryon*).

As shown by comparative oologic studies, the penetration of the spermatozoon into the ovum triggers a second meiotic division which is followed by release of the second polar body. Upon completion of the meiotic process, the haploid chromosomes of the ovum begin to migrate from the cortex of the cell toward the center. During migration they clump, and a nuclear membrane becomes reconstituted around them (55). At the end of this process, a female pronucleus is formed. Meanwhile, the sperm head swells and the chromatin loses compactness. The penetrated portion of the sperm moves toward the center of the ovum where the head completes its transformation into a male pronucleus. The sperm organelles, particularly the mitochondria, eventually disorganize and undergo regressive changes (46).

In the ovum described in the present study, most of the changes leading to pronuclear transformation had already occurred.

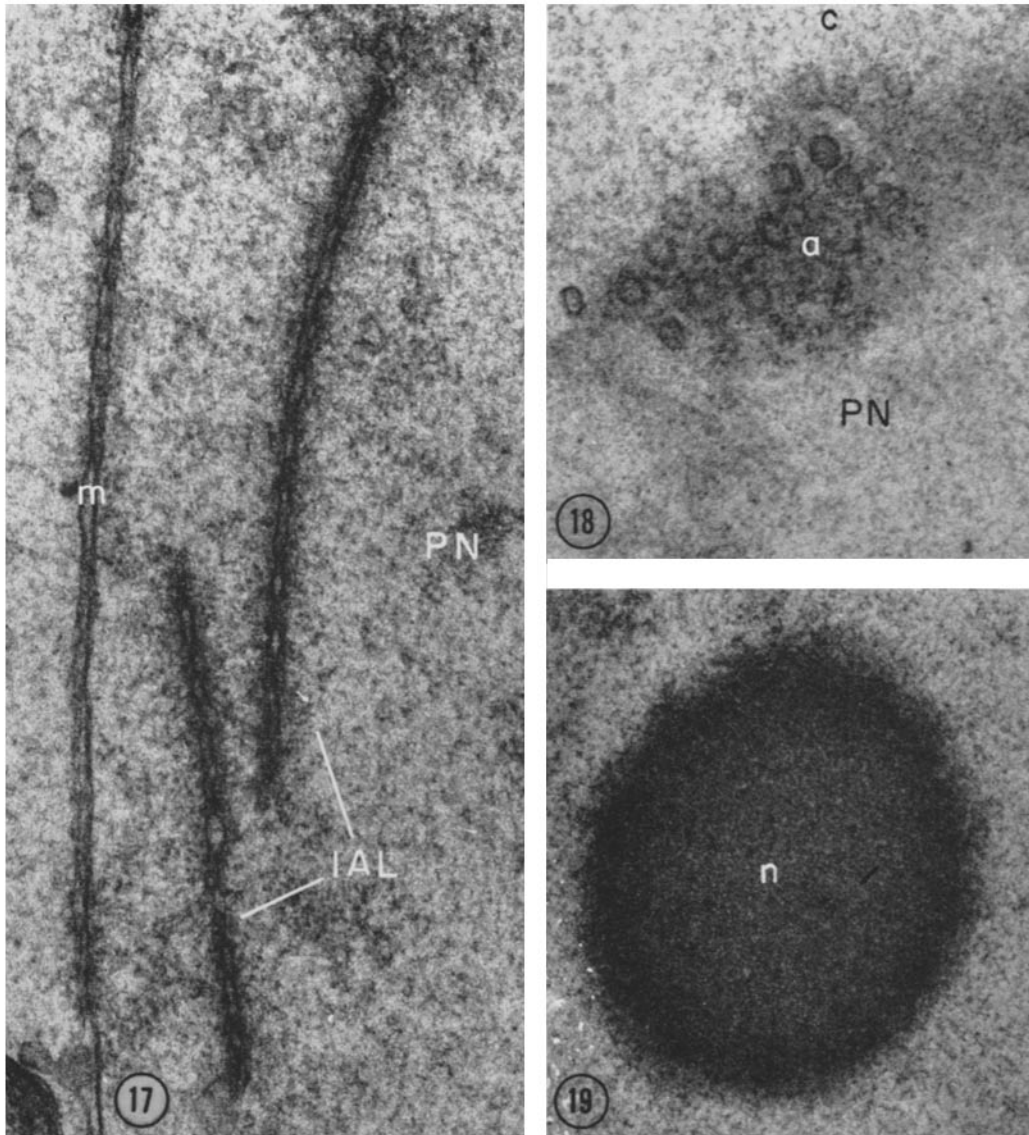


FIGURE 17 The annulate lamellae (IAL) which are present inside the pronuclei (PN) are morphologically identical to those observed in the ooplasm (compare with Fig. 9). Note also the similarity between the annulate lamellae and the pronuclear envelope (m). $\times 62,000$.

FIGURE 18 Grazing section of a pronucleus (PN). The characteristic annuli (a) present on the surface of the pronuclear envelope are similar to those of the annulate lamellae when the latter are observed in face-on view. c, cytoplasm. $\times 45,000$.

FIGURE 19 The nucleoli (n) consist of a highly dense and compact material. $\times 40,500$.

The presence of numerous organelles in the ooplasm indicates a high metabolic rate of this recently penetrated cell. The largest concentration of organelles was found in the cytoplasm around the pronuclei, at this stage the most likely epicentrum of the cell activity (pronuclear de-

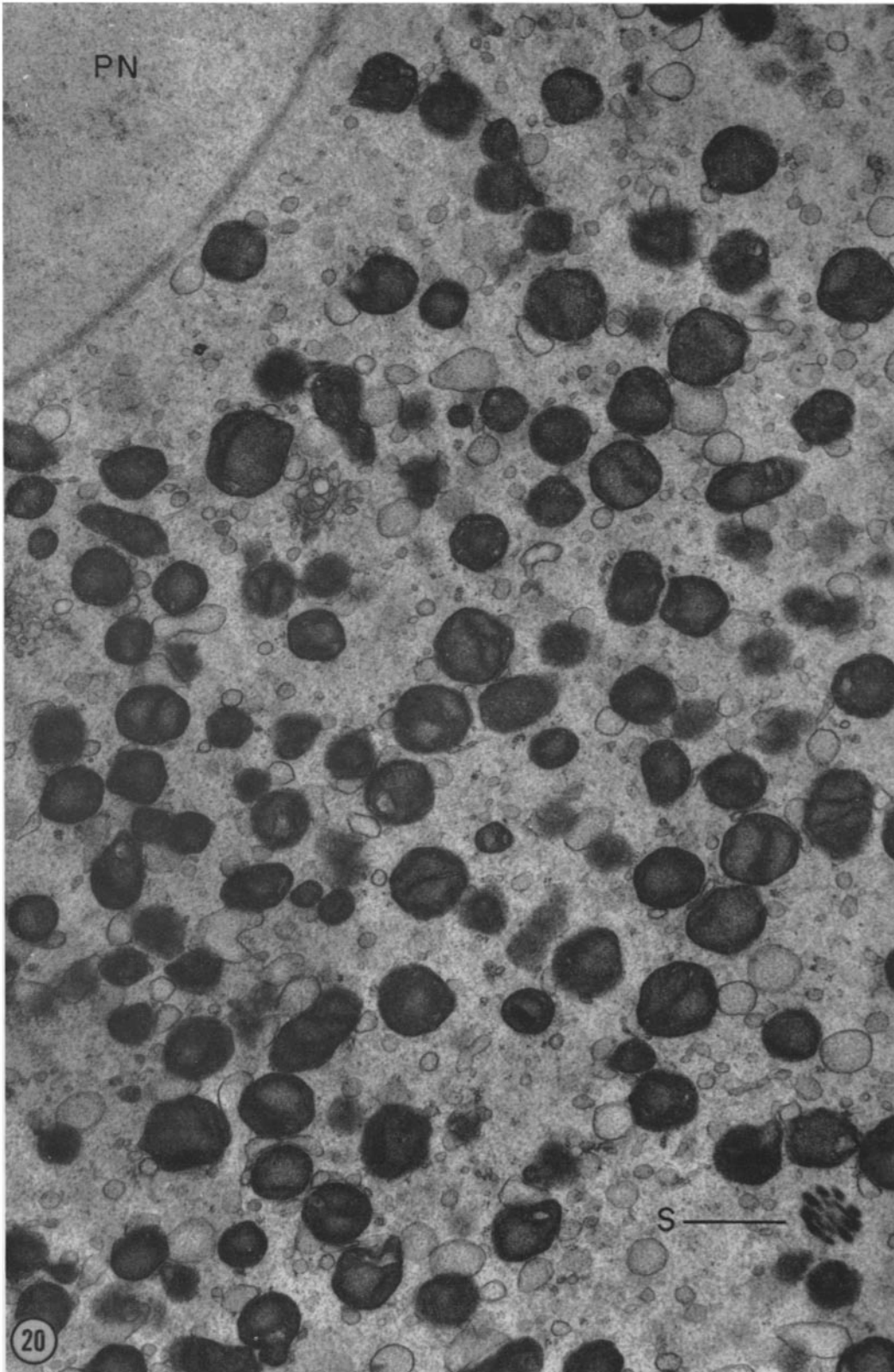


FIGURE 20 A portion of the penetrating spermatozoon (*S*) in the cytoplasm of the ovum and adjacent to one of the pronuclei (*PN*). $\times 23,000$.

velopment, accumulation of biochemical energy required for pronuclear fusion, and cell cleavage).

Spheroidal mitochondria with limited numbers of cristae have been observed to be typical of follicular oocytes and fertilized ova of a large variety of species (1, 7, 18, 31, 37, 38, 40, 54, 55) including man (6, 50). Predominance of the



FIGURE 21 Higher magnification of the sperm structures shown in Fig. 19. The outer row of nine dense fibers and the axial filament complex are easily recognized. $\times 55,000$.

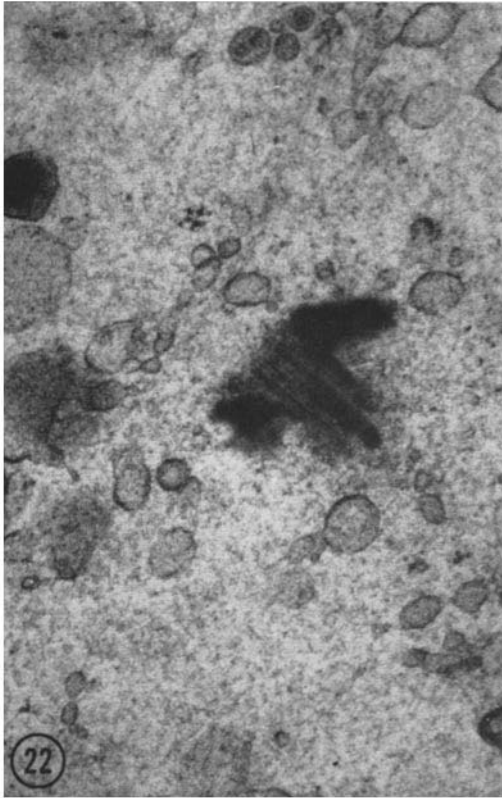
spheroid form also occurred in the penetrated ovum of the present study. It was observed, however, that the number of elongated mitochondria in this ovum was higher than in human follicular oocytes (6, 50). It is conceivable that, in order to maintain a constant number of mitochondria in the daughter cells, mitochondrial duplication has to precede the stage of cell cleavage. In consideration of this hypothesis, the elongated mitochondria observed in the penetrated ovum were assumed to represent those about to divide. Although no

definite proof can be provided, this assumption seems to be supported by (1) the finding that the central portion of the elongated mitochondria frequently showed symmetrical indentations of the outer membranes, and (2) images suggesting that deepening followed by fusion of the indented membranes led to mitochondrial duplication. Dividing forms of mitochondria, similar to those observed here, were described by Fawcett in the hepatic cells of the rat (13), by Blanchette in oocytes of the rabbit (7), and by Odor in those of the hamster (38). Dividing mitochondria were not observed in the polar bodies, as these were likely to regress.

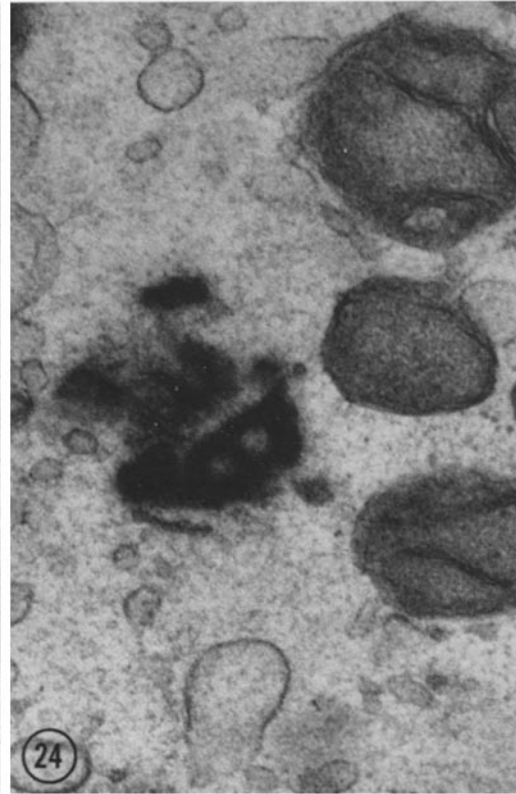
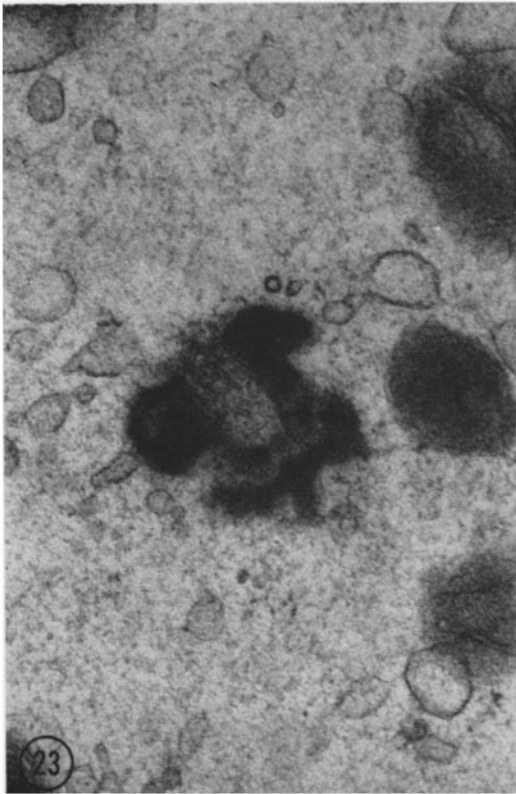
It is generally reported that the endoplasmic reticulum of follicular oocytes is meagerly developed (7, 37, 40, 50, 51). Although a few investigators disagree (1, 54), it is certain that ER becomes substantially more developed in penetrated and in fertilized ova. In the rabbit, considerable development of the ER occurs simultaneously with pronuclear formation (55). In the rat, differentiation of a prominent ER begins early in the two-cell stage (40).

The penetrated human ovum was characterized by a well developed endoplasmic reticulum. It was observed mainly in the form of large spheroidal vesicles limited by mostly smooth membranes. These vesicles were located in close proximity to mitochondria. The other component of the ER consisted of smaller vesicles which were present in large numbers throughout the cytoplasm. It is highly probable that the small vesicles were formed by budding from the large ER vesicles. This hypothesis is based upon the finding that the small ER vesicles were morphologically similar to the saccular terminations of the appendages of the large components of the ER and contained material which was identical in appearance to that present in the lumina of the large vesicles. It would seem that the function of the small vesicles was to carry, to target areas in the cell, material which had been synthesized in the large ER vesicles. This concept will be examined in more detail below.

The two pronuclei were of about equal size. Dickmann et al. (11) also found pronuclei of equal size in the human ovum they recently studied. On the other hand, Khvatov reported the human male pronucleus to be smaller than its female counterpart (29). In other mammalian species, the male pronucleus is usually observed



FIGURES 22 to 24 Serially sectioned components of the penetrating sperm. Although these structures could not be identified with certainty, it is assumed that they represent the connecting piece of the spermatozoon. $\times 49,500$.



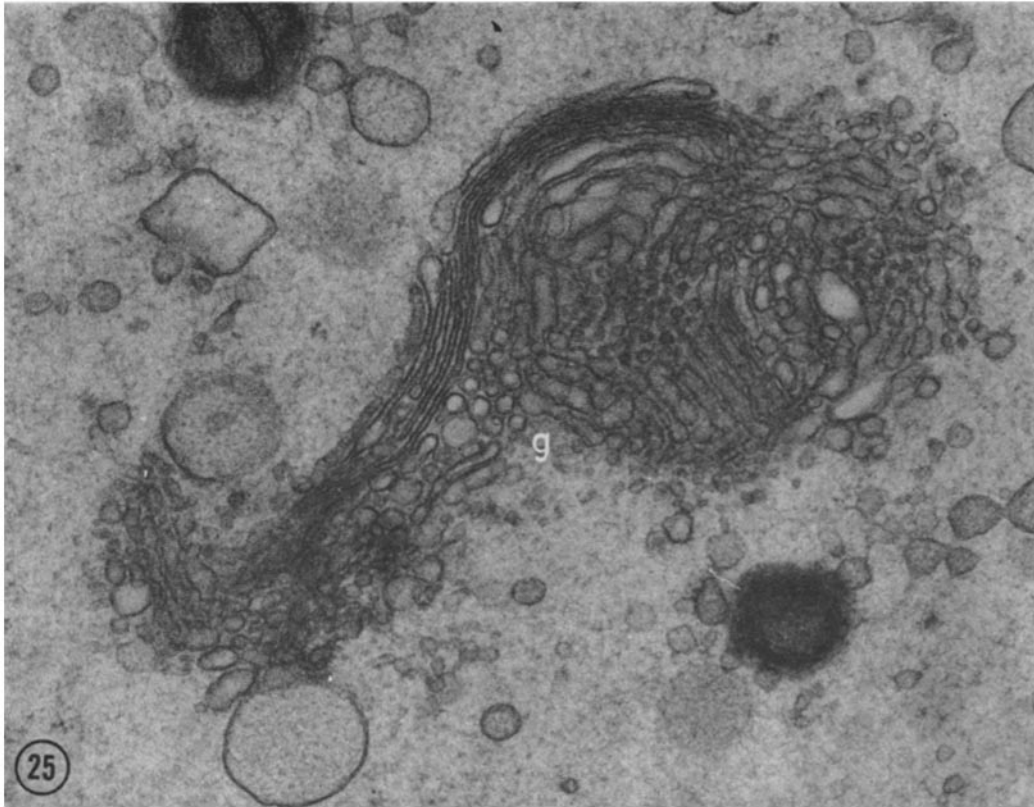


FIGURE 25 The Golgi complex (*g*) of the penetrated ovum is dispersed into prominent clusters of vesicles and tubules. These clusters are profusely distributed throughout the cytoplasm. $\times 40,000$.

to be of a larger size than the female pronucleus (5, 8, 10, 39). These conflicting observations are probably of little significance. Identification of the male pronucleus is generally made on the basis of its proximity to the tail of the penetrating spermatozoon. This method of identification is unreliable because of possible dislocation of the sperm midpiece and tail during the handling of the ovum.

The pronuclei of the human ovum were morphologically similar to the pronuclei of penetrated rat (40) and rabbit ova (55). The human pronuclei, however, did not exhibit the numerous outpocketings and deep indentations which characterize the pronuclear profile in the rabbit ovum (55). This dissimilarity may be related to different phases of pronuclear development. It is likely that the human pronuclei observed in this study were of a recent formation. This assumption is supported by the finding that the second polar body

had recently separated from the ovum, by the presence of only one nucleolus in each pronucleus, and by the observation of remnants of the penetrating sperm in the ooplasm.

The observed focal dilations of the space between the two sheets of the pronuclear envelope appeared to be due to outpocketings of the outer sheet toward the ooplasm. These dilated areas contained membrane-bounded vesicles filled with a material of relatively high electron opacity. The membranes limiting these vesicles were always found to be distinct from the pronuclear membranes. It is difficult to establish the exact nature of these vesicles. Similar structures have been observed between the two sheets of the pronuclear envelope of the rabbit ovum (55). In that case, the presence of ribosomes associated with their membranes provided enough convincing evidence that these vesicles represented components of the endoplasmic reticulum. Similar evidence could

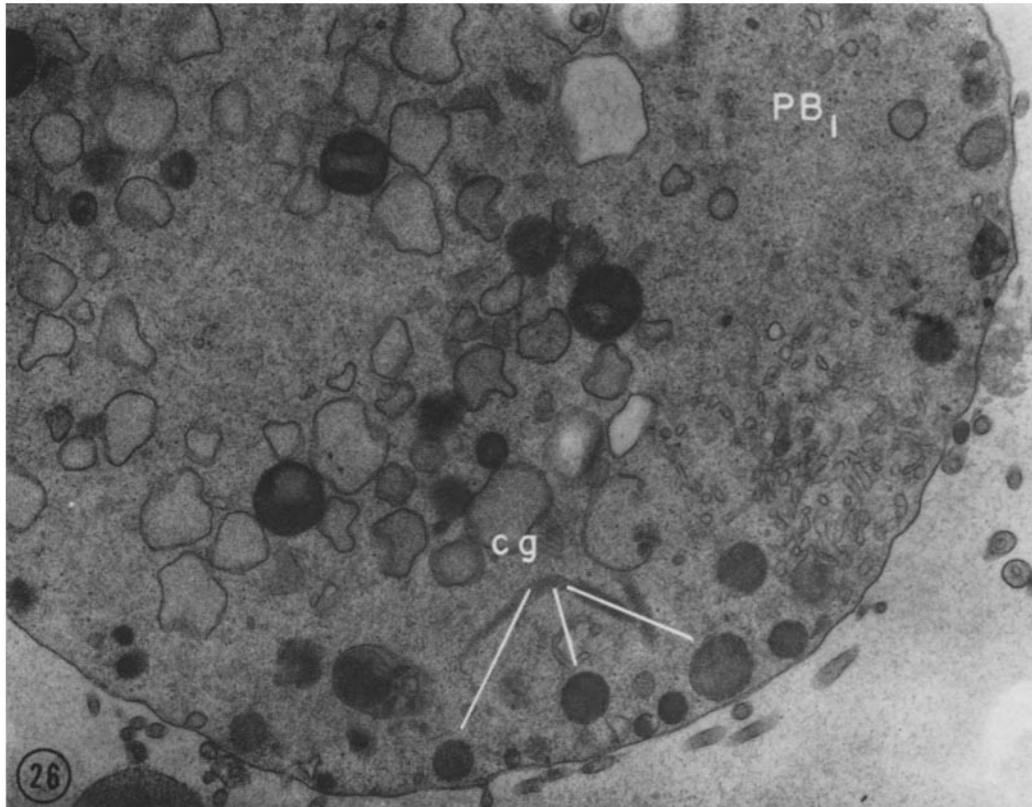


FIGURE 26 Portion of the cytoplasm of the first polar body (PB_1). The mitochondria are few. The large ER vesicles are collapsed. Note the absence of the small ER vesicles. Numerous dense cortical granules (cg) are present beneath the plasma membrane of the polar body. $\times 24,000$.

not be obtained in the present study. The pronuclear-associated vesicles, however, were morphologically similar to the small vesicles, which were profusely distributed throughout the ooplasm and which were assumed to represent ER components originating by budding from the large vesicles of the endoplasmic reticulum. If these morphological similarities reflect identity of structures, the functional bases for the association of ER components with the pronucleus may be explained considering the possibility that the ER plays an important role in pronuclear formation and growth. This hypothesis is in agreement with the conclusions drawn from the above-mentioned study of the penetrated rabbit ovum (55). In that study, formation of a double membrane envelope around the developing female pronucleus seemed to be brought about by the ER which became directly associated with the naked chromo-

somes shortly after completion of the second meiotic division. The hypothesis is also indirectly supported by the finding that, whereas small and large vesicles of the ER (the large in budding activity) were numerous in the second polar body in which reconstitution of a nuclear envelope had occurred, they were absent in the first polar body which had chromosomes deprived of nuclear membranes.

The possibility that the vesicles which were present in the dilated regions of the space within the pronuclear envelope could have contained nucleolar material to be released into the ooplasm must also be considered, in view of the numerous studies which have been recently devoted to extrusion of nucleoli from nuclei (17, 25) and pronuclei (43, 45) of invertebrates and mammalian oocytes. The concept that nucleolar material may be released into the ooplasm is es-

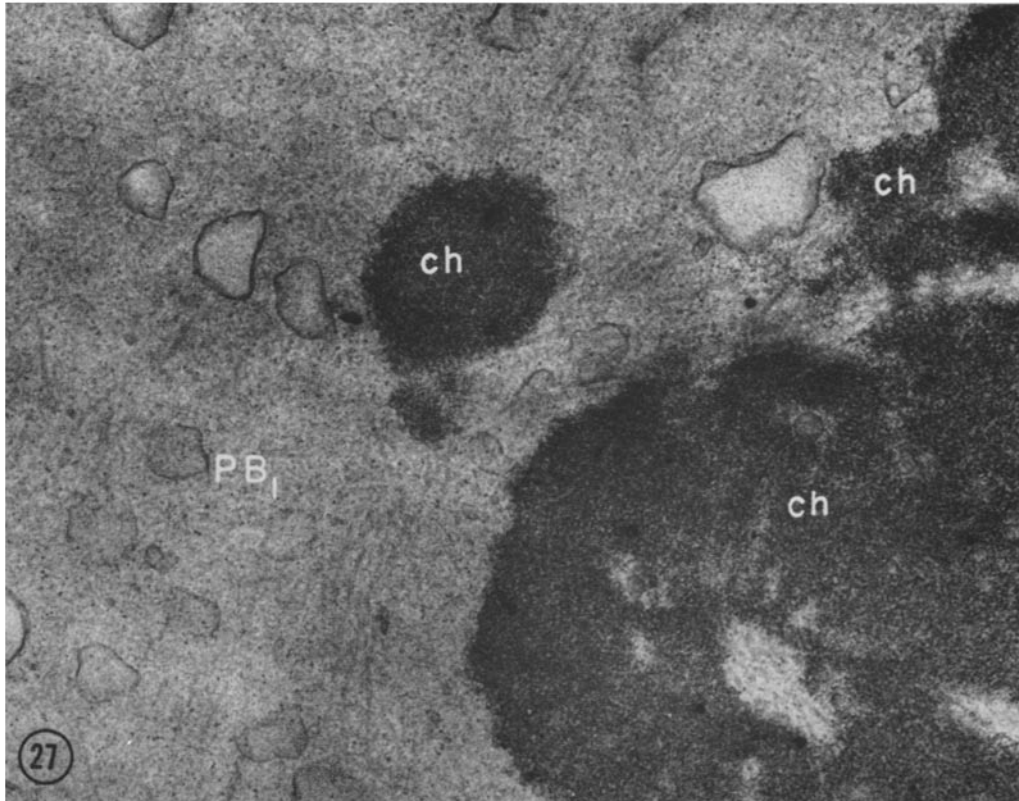


FIGURE 27 The nuclear complement of the first polar body (PB_1) consists of chromosomes (ch) associated with the numerous microtubules of the spindle of the first meiotic division. $\times 27,000$.

essentially based upon the occasional finding, in the spaces within nuclear and pronuclear envelopes, of membrane-bounded vesicles containing a granular material which appears to be morphologically and histochemically similar to that in the nucleolus. This concept needs further screening for two main reasons: (1) Observation of fixed material is not an ideal method for the determination of the direction along which structures were moving in the living state; and (2) the demonstrated proteinaceous structure of the material contained in the vesicles, as well as its marked RNA content and loss of stainability after RNase treatment (2, 45), does not necessarily indicate its nucleolar origin. These histochemical findings, in fact, are equally acceptable with the above discussed possibility that the vesicles observed in the space within the pronuclear envelope may belong to the endoplasmic reticulum and may contain material synthesized in the ER components.

Studies performed on oocytes of *Necturus maculosus* and *Thyone briareus* have indicated that the cytoplasmic annulate lamellae form through fusion of detached vesicular blebs of the outer sheet of the nuclear envelope (23, 24, 26). The observations made in the present study allow us neither to confirm nor to deny this hypothesis, although blebbing of the outer sheet of the pronuclear envelope was frequently observed, and striking morphological similarities existed between annulate lamellae and pronuclear envelope (compare Figs. 10 and 17).

The finding of lamellar structures inside the pronuclei is also difficult to evaluate. The presence of annulate lamellae inside oocyte nuclei has been reported in a few invertebrates (19, 27, 28, 33). To our knowledge, intranuclear annulate lamellae have never been previously described in mammalian eggs, although, in view of the present finding, the annular formations observed by Zamboni and Mastroianni within the pro-

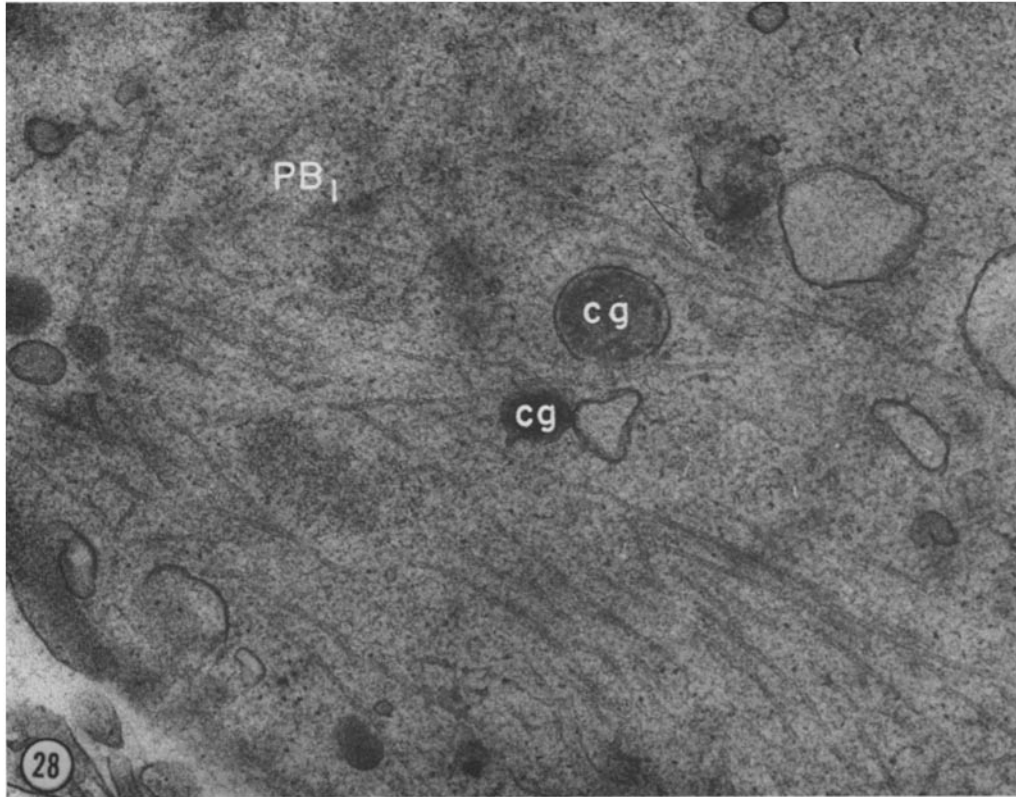


FIGURE 28 An extensive network of microtubules pervades the cytoplasm of the first polar body (PB_1). *cg*, dense cortical granules. $\times 41,000$.

nuclei of the penetrated rabbit ovum (55) could possibly have represented annulate lamellae seen in tangential or oblique section. The morphogenesis of the intranuclear annulate lamellae has been recently studied by Kessel (28), who indicated that they form by fusion of detached blebs of the inner sheet of the nuclear envelope. In the human ovum, blebbing activity of the inner pronuclear membrane was not observed, although isolated vesicles were occasionally found in the pronucleoplasm. The functional significance of the presence of annulate lamellae inside the pronuclei is not known at the present time.

The crystalline inclusions in the ooplasm are an entity which, to our knowledge, has never been previously described in mammalian ova, although similar crystalloids were observed by Enders and Schlafke in rabbit and mouse blastocysts (12). It is tentatively proposed that the crystalline inclusions found in the human ovum represent ooplasmic yolk.

The proteinaceous yolk of amphibian eggs (20, 30, 48, 49) consists of laminae separated from each other by distances ranging from 60 to 85 Å. These laminae make 65° angles with one another and have an hexagonal configuration. In mature amphibian eggs, the proteinaceous yolk forms platelets where the centrally located protein is surrounded by at least three different layers, each having a distinct structure and appearance (49). Although the yolk platelets of amphibian eggs have often been observed to be contained inside mitochondria, the role played by the mitochondria in the synthesis and demolition of yolk has yet to be clearly defined (30, 49).

In the human ovum, the crystalloids appeared to consist of rods having an average spacing of about 150 Å and making 65° angles with one another. They appeared to be arranged in an hexagonal crystalline pattern. The observation that the rods were forming platelets, often with pointed outlines, indicates that the platelets them-

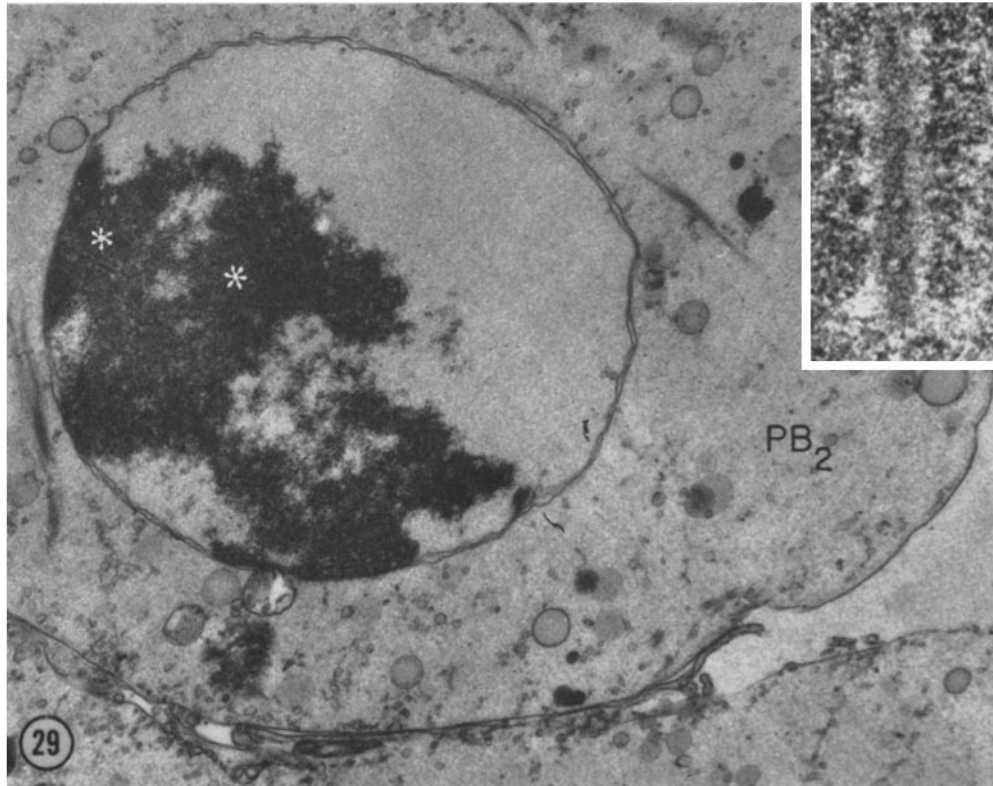


FIGURE 29 The second polar body (PB_2) has a spheroidal nucleus consisting of dense chromatin aggregates and a highly hydrated nucleoplasm. A double nuclear membrane is present. The lamellar structures (*) embedded in the chromatin clumps may represent either nuclear annulate lamellae or the chromosomal axial complex. The lamella toward the left of the micrograph is shown at higher magnification in the insert. Note the regressive changes of the mitochondria. Fig. 29, $\times 15,000$. Inset, $\times 114,000$.

selves had the same hexagonal configuration as those in the amphibian egg. The finding that the crystalloids were present in limited numbers agrees with the notion that the eggs of most mammals are miolecithal; i.e., they possess only meager amounts of yolk (5). It is apparent, however, that the nature of these structures could not be determined with certainty. Further investigation is needed to elucidate precisely the nature of these entities.

A precise identification of the remnants of the penetrating spermatozoon which were observed within the ooplasm was not possible. Easily recognized as such were the axial filament complex and the outer dense fibers. The absence of both mitochondrial and fibrous sheets, however, made it impossible to establish whether these structures were part of the middle or principal piece of the

sperm tail. If they belonged to the middlepiece, the absence of a surrounding mitochondrial sheet would imply that the latter had undergone disorganization and regression. It is known that regression of sperm mitochondria occurs in rat as well as rabbit ova. In the rat ovum, the mitochondria of the penetrating sperm disorganize after the first cleavage and totally degenerate in the four-cell stage (46). Unpublished observations made in this laboratory have shown that, in the penetrated rabbit ovum, degeneration of sperm mitochondria occurs at a much earlier stage, during pronuclear growth (52). This phenomenon is extremely important because it implies that the mitochondrial endowment of the embryo is exclusively maternal in derivation.

The two polar bodies which were located close to one another represented a divided first polar

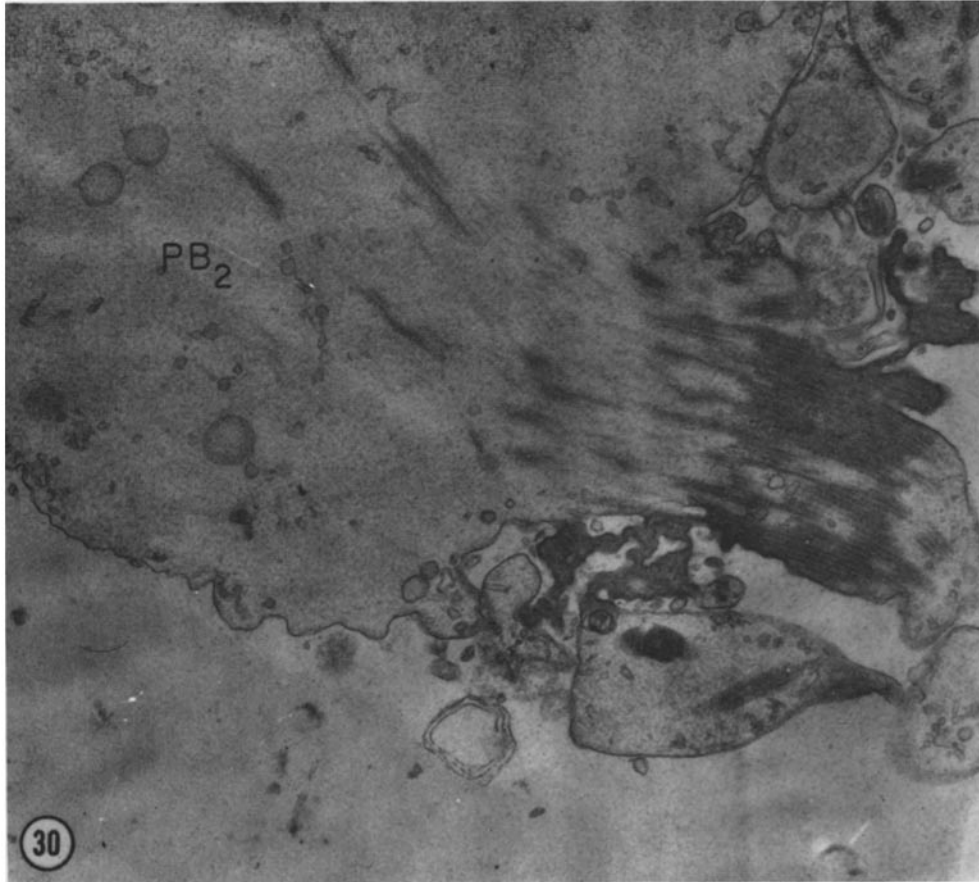


FIGURE 30 The second polar body (PB_2) was provided with an elongated cytoplasmic bridge extending toward the ovum. The bridge was occupied by remnants of the meiotic spindle microtubules. $\times 22,000$.

body. They were morphologically identical to each other, but conspicuously different from the second polar body. The dissimilarities included presence of microvilli and dense cortical granules as well as organization of nuclear material. Whereas the plasma membrane of the first polar body was ruffled and provided with numerous microvilli, the cell membrane of the second polar body was smooth throughout. The presence of microvilli in the plasma membrane of a polar body is a safe criterion to use for the identification of the first polar body, since the microvilli of the plasma membrane of the ovum decrease considerably in number, or totally disappear, shortly after sperm penetration (55).

The dense cortical granules were abundant in the first polar body and totally absent in the second. These granules are known to be charac-

teristic of large follicular oocytes and unpenetrated ova, where they are located a few microns beneath the plasma membrane (1, 4, 16, 44, 54). These granules are usually thought to play a fundamental role in the "zona reaction" against penetration of supernumerary spermatozoa (5). The granules are liberated into the perivitelline space immediately after penetration of the fertilizing sperm (22, 52). As a consequence, the first polar body, which is released prior to sperm penetration, is likely to have numerous cortical granules in its cytoplasm, whereas the second polar body should have none. Thus, the presence of dense granules in the cortical cytoplasm of a polar body should be employed as another criterion to differentiate the first polar body from the second.

Whereas the nuclear complement of the first polar body consisted of isolated chromosomes, the

second polar body had a membrane-bounded nucleus composed of chromatin aggregates similar to those present in the pronuclei of the ovum. On the basis of the observation of one ovum, it is difficult to establish whether the different organization of the nuclear material in the two polar bodies represents the usual pattern. The functional implications of the reconstitution of a complete nucleus in the second polar body may prove to be important when considering the possibility that fertilization of a polar body, followed by embryo development, may take place (57).

The lamellar structures present in the chromatin clumps of the second polar body could not be identified. These structures were similar to the annulate lamellae observed in the pronuclei of the ovum. The possibility, however, that they could have represented the chromosomal axial

complex must also be considered, although their morphology appeared to be distinct from that of this complex as described by numerous investigators (15, 34-36).

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