ELECTRON MICROSCOPY OF GROWING OOCYTES OF RANA PIPIENS*

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Plates 56 to 60

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Deposition and utilization of cellular inclusions represent problems of general biological importance which the embryologist must face when he considers growth of the oocyte and development of the embryo. Cytological details of the origin, distribution, and fate of the inclusions in developing eggs or embryos have been recorded by many investigators (see Tyler, 1955), and in recent years chemical embryologists (*e.g.*, Needham, 1950; Brachet, 1950; Barth and Barth, 1954; Boell, 1955) have begun to study the biochemistry of the deutoplasmic inclusions and their utilization during embryonic development.

Prior to the first appearance of yolk platelets young oocytes carry on active synthesis of lipide granules (lipochondria of Holtfreter, 1946), which soon are dispersed throughout the cytoplasm (Brachet, 1950; Wittek, 1952; Grant, 1953). Through measurements of P^{32} uptake Grant has demonstrated that, correlated with the deposition of lipochondria, there is heightened incorporation of phosphorus into the phospholipide fraction of oocytes of *Rana temporaria*. Furthermore, he found that P^{32} was selectively incorporated into the phosphoprotein fraction in later stages when synthesis of yolk platelets was in progress. From the distribution of phosphorus in various fractions of mature oocytes, Grant deduces that yolk contains practically all (91.7 per cent) of the phosphoprotein, 34.1 per cent of the nucleic acid, and 52.8 per cent of the lipide of the egg. Adsorption of nucleic acid and difficulties encountered in separating lipochondria from yolk platelets may, however, account in part for his high values for nucleic acid and lipide. Synthesis of protein continues throughout the growth period of the oocyte (Osawa and Hayashi, 1953). Selective uptake of radioactive glycine at the periphery of

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the cell after the onset of yolk deposition suggests regional localization of active protein synthesis (Kemp, 1955 *a*) in the peripheral cytoplasm.

In order to interpret the physiological and biochemical data now being accumulated, it is important to have before us a clear picture of cellular architecture. Observations on the pattern of yolk deposition in developing oocytes of the frog (Kemp, 1953) have demonstrated the limitations of standard microscopy for resolving fine cytological details in these cases; hence the present investigation with the electron microscope was undertaken. Some of the results have previously appeared in short reports (Kemp, 1954, 1955 *b*, *c*).

Materials and Methods

To furnish the different stages of growing oocytes, summer frogs obtained from a dealer in Wisconsin were used. Ovaries were excised from pithed frogs and placed in Ringer's solution. With the aid of a stereobinocular microscope and iridectomy scissors, small groups of young, transparent oocytes or individual older, opaque oocytes, together with their follicular envelopes, were dissected from the ovary and transferred as quickly as possible to the fixative (isotonic 1 per cent osmium tetroxide buffered with acetate-veronal to pH 7.4) according to Palade's (1952) technique. The tissues were fixed for 2 hours, after which they were rinsed quickly in distilled water and dehydrated rapidly. Infiltration was accomplished with a mixture of 40 per cent ethyl–60 per cent *n*-butyl methacrylate containing 2 per cent luperco CDB. Embedding and polymerization were accomplished through exposure overnight to two General Electric sun lamps to provide heat (40–45°C.) and ultraviolet light. Sections were cut with a glass knife either with an International Minot rotary microtome or a Porter-Blum cantilever microtome set at 0.025 μ. After spreading for 20 minutes on 20 per cent alcohol beneath a sun lamp, the sections were mounted on formvar-coated Athene grids. Both an RCA EMU-2A and an RCA EMU-3 microscope were used for observation and photography.

OBSERVATIONS

Oocytes in Stage Y_0.—As defined in a previous study (Kemp, 1953), stage Y_0 includes all oocytes prior to the appearance of yolk platelets at the periphery of the cell. In Fig. 1 a young oocyte in stage Y_0 displays the typical morphology of nucleus, cytoplasm, and follicular cells. Since lipochondria precede the yolk platelets in order of appearance (Brachet, 1950, p. 53), lipogenesis must begin in late stage Y_0. Considerable growth and differentiation of oocytes occur, however, before lipochondria make their appearance; some of the fine structural changes accompanying early growth are illustrated in Figs. 1 to 4. Starting with a young oocyte estimated to be about 100 μ in diameter (*cf.* Kemp, 1953, Fig. 3), we note (Fig. 1) that the nucleus is approximately spherical and the nuclear membrane forms a relatively smooth boundary between nucleus and cytoplasm. Nucleoli (*n*) are assuming a position toward the periphery of the nucleus. Within the cytoplasm filamentous mitochondria (*m*) are abundant, and a few profiles of endoplasmic reticulum (*e*) are visible. The surface of the oocyte, bordering the inner follicle cell, is smooth at this stage. Inner follicle cells (*i*) are adherent to the oocyte on

their internal margins and externally are separated from outer follicle cells (*o*) by an intercellular space (*s*) containing connective tissue fibrils.

Fig. 2 illustrates the nucleocytoplasmic boundary and adjacent protoplasm of an oocyte probably measuring about 175 μ in diameter (*cf.* Kemp, 1953, Figs. 4 and 5). In this cell the nuclear membrane has begun to increase its area through the development of an irregular contour. Inside the nucleus the large nucleolus lacks a definite limiting membrane and looks here as though some of its substance were being sloughed into the karyoplasm. Close examination of the nuclear membrane reveals the occurrence of tiny striations and apparent pores (*p*, Fig. 2). Whether these ring-shaped structures actually are pores permitting direct continuity of cytoplasm and nucleoplasm, or instead merely indicate inhomogeneity in the structure of the membrane, has not been decided (*cf.* Watson, 1955). In the cytoplasm beyond the membrane we observe a concentration of tiny granules and short rods (*g*, *r*, Fig. 2) which are smaller in diameter than the regular mitochondria (*m*, Fig. 2). From the localization of these structures next to the nuclear membrane one is led to speculate (1) that they may come directly from nuclear materials passed into the cytoplasm, or (2) that they are newly synthesized in the cytoplasm as a result of stimulation by substances of nuclear origin. The extensive development of endoplasmic reticulum in an oocyte with slightly folded nuclear membrane is shown in Fig. 3.

As the nuclear membrane begins to fold or sacculate, "yolk nuclei" (Fig. 4) may be observed close to the periphery of the cell (*cf.* Kemp, 1953, Figs. 5 and 6). These bodies appear to consist of a cluster of mitochondria (see Fankhauser, 1948, p. 690) together with granules identified as lipochondria (see Brachet, 1950, p. 53). Although lipochondria thus appear to be closely associated with mitochondria in the yolk nuclei, they come to be distributed throughout the cytoplasm by the end of stage Y_0. The small lipochondria, which seem to emerge from the ground cytoplasm, undoubtedly enlarge somewhat; but the larger ones (Fig. 4) are probably formed by coalescence of smaller droplets.

At the surface of the oocyte in Fig. 4, one sees the beginning of an extraordinary structural relationship between oocyte and inner follicle cells. This is characterized by the extension, from the cortical cytoplasm of the oocyte, of tiny processes that will become microvilli (*cf.* Fawcett and Porter, 1954). In order to make room for the microvilli, however, the follicle cell must pull away, and we see in Fig. 4 an early stage in this process. The follicle cell remains attached to the oocyte at some regions, but conspicuous intercellular spaces have developed through localized withdrawal of portions of the follicular cell.

Oocytes in Stage Y_1.—By the time of appearance of the first yolk platelets (*y*, Fig. 5) in a narrow peripheral ring of cytoplasm, the cortex has undergone

extensive further differentiation. The microvilli (*v*, Fig. 5), measuring 0.08 μ in width by 1.67 μ in average length, now form a prominent layer (zona radiata of light microscopy) on the surface of the oocyte. They appear to be grouped in clusters attached to basal protrusions of the cortical cytoplasm. It is not easy to detect the extent of penetration of protoplasmic processes of the follicle cells among the microvilli, but it is clear that processes of the follicular cells (*f*, Fig. 5) form an interlacing network between the microvillous layer and the main body of the follicular cells. That some of the follicular processes penetrate deep into the microvillous layer is clearly demonstrated in Fig. 6. The region containing follicular processes, also including considerable intercellular space, was formerly considered the vitelline membrane, or chorion (Kemp, 1953). Electron microscopy permits one to demonstrate that this region arises primarily from modifications of the follicular cells rather than from the oocyte; hence it should probably not be called the vitelline membrane at stage Y_1. Just how the definitive vitelline membrane or chorion does develop as growth of the oocyte continues has not been followed in the present investigation.

Another conspicuous feature of cortical differentiation is the layer of cortical granules (*c*, Fig. 5) which forms just beneath the surface cytoplasm. These granules do not appear to be formed in direct association with mitochondria or other formed elements but apparently arise *in situ* from the peripheral ground cytoplasm. Likewise, yolk platelets (*y*, Fig. 5) appear to be synthesized from the ground cytoplasm with no obvious relationship to the mitochondria. At the stage illustrated (Fig. 5) cortical granules and yolk platelets have attained approximately equal maximum sizes of 1 μ in diameter. Most of the lipochondria are smaller than this, but one (*l*) in Fig. 5 is slightly larger.

Oocytes in Stages Y_2–Y_4.—As oocytes grow, yolk platelets continue to be synthesized peripherally while those previously formed become larger and are displaced inward toward the nucleus. At stage Y_2 (oocytes 500 to 600 μ in diameter), platelets fill the outer half of the cytoplasm; at stage Y_3 (600 to 900 μ in diameter), they fill the outer three-fourths of the cytoplasm; and at stage Y_4 (900 to 1400 μ in diameter), the cytoplasm is completely filled with platelets (Kemp, 1953). Full grown oocytes (stage Y_5) are not included in the present study. Pigment granules first appear peripherally during stage Y_3 and shortly become dispersed throughout the cytoplasm.

As oocytes enlarge, growth of the cortical layer is apparent from a comparison between Figs. 5 and 7. In Fig. 7 the largest cortical granules (1.75 μ × 2.0 μ) in an oocyte at stage Y_3 are twice as large as those in an oocyte at stage Y_1. By stage Y_3 the cortical cytoplasm has undergone such extensive folding that it appears in section to be thrown into ridges alternating with deep valleys. The microvilli are now slender processes about the same size as at stage Y_1, and extend outward from the peaks of the ridges (Figs. 7 and 8). It will

be observed that the cortical cytoplasm peripheral to the cortical granules now is packed with circular droplets light in the center and dark around the rim. Some of the droplets contain a dark central body (*cb*, Fig. 8). Since the droplets do not appear either in the layer of follicular processes or in the microvilli, it is most reasonable to hypothesize that they are newly synthesized in the cortical cytoplasm and that they reflect absorption of nutrients through the microvilli.

Figs. 9 and 10 show the appearance of the inclusions in the subsurface cytoplasm just below the cortical granules in oocytes of stages Y_3 and Y_4. Yolk platelets and lipochondria at this level do not differ markedly in size, although the largest platelet in Fig. 9 measures approximately 2.3 $\mu \times$ 3.0 μ as compared with 2.6 $\mu \times$ 3.7 μ for the largest in Fig. 10. The largest platelets observed in a section of the interior cytoplasm at stage Y_4 measured 3.7 $\mu \times$ 5.8 μ. Growth of pigment granules from about 0.15 μ to about 0.3 μ in diameter is indicated in Figs. 9 and 10.

DISCUSSION

Synthesis of Inclusions.—It was hoped when this work was initiated that electron microscopy might yield clues to the immediate source of the inclusions of the frog oocyte, particularly the yolk platelets. Previous workers have attributed the yolk to a variety of precursors (references in Kemp, 1953): nucleoli; yolk nuclei; mitochondria; Golgi apparatus; a combination of Golgi apparatus, ground cytoplasm, and nuclear substances; and microsomes. The roles which each of these proposed sources plays in vitellogenesis have certainly not been finally determined in the present study, but, with the exception of the ground cytoplasm and possibly microsomes, I believe they should be ruled out as direct precursors of the yolk platelets, or for that matter of lipochondria, cortical granules, or pigment granules.

In electron micrographs one can observe that nucleoli appear to liberate nucleolar fragments (Fig. 2; see also Porter, 1954) into the karyoplasm, and there is evidence that these or other nuclear substances may traverse the nuclear membrane (Sparrow and Hammond, 1947; Pollister, Gettner, and Ward, 1954; Watson, 1955). The small granules and rods first appearing in the cytoplasm just outside the nuclear membrane of the frog oocyte may be unchanged nuclear substance, or they may be particles synthesized from the cytoplasmic ground substance under the influence of nuclear material. In addition to these particles in the perinuclear cytoplasm, one finds endoplasmic reticulum and mitochondria throughout the cytoplasm prior to the appearance of the four types of microscopically identified inclusions; yet the physiological relationships among these various components are at present unknown. Lipochondria, the first of the larger inclusions to appear, may be associated with clusters of mitochondria in the yolk nuclei (Fig. 4; see also Brachet,

1950, p. 53) but later they are also found scattered throughout the cytoplasm. Cortical granules and yolk platelets, and later the pigment granules, appear to emerge from the ground cytoplasm at the periphery of the cell. All the inclusions increase in size as the oocyte enlarges, although coalescence of smaller droplets undoubtedly accounts for some of the larger lipochondria.

The status of microsomes as discrete components of intact cells is still in question. There is, in fact, evidence that these particles which are isolated by centrifugation (references in Watson, 1955; Schmitt, 1955) represent more than one cellular component. Slautterback (1953), for example, reports that microsomes from mouse liver fall into three size groups. The smallest of these, according to Palade (1955), may be composed of the granules called "cytoplasmic particles;" the larger microsomes may, in Palade's view, consist of fragments of the endoplasmic reticular membranes. The microsomes are known to be rich in ribonucleic acid (Palade, 1955) and thus, if Caspersson's (1950) theories are correct, they are probably implicated somehow in protein synthesis. The link between protein synthesis and the presence of cytoplasmic ribonucleic acid, however, has not been ascertained. If this link is discovered, it would be interesting to determine whether ribonucleic acid also accompanies the synthesis of stored products other than protein.

With respect to the special problem of synthesis of the protein-rich yolk platelets, one is tempted to speculate on the factors which account for their first appearance and continued deposition in the peripheral endoplasm. That this is an area active in protein synthesis seems evident from the pattern of uptake of C^{14}-labelled glycine (Kemp, 1955). It is thus logical to assume that here are assembled, perhaps localized, the enzymes capable of synthesizing yolk. The raw materials for the platelets include amino acids and phosphate for phosphoproteins, possibly also ribonucleic acid and phospholipides (see Grant, 1953). Of these, ribonucleic acid is known to be abundant peripherally (Brachet, 1950; Wittek, 1952; Kemp, 1953), and presumably phosphate and the other constitutents of phospholipides are in good supply, since synthesis of the lipochondria is already underway when the yolk platelets first appear. Amino acids, however, are probably in short supply within the oocyte. The tremendous increase in surface area resulting from the development of microvilli and folds of the cortex of the oocyte could most easily be explained as a structural response to the physiological demand for amino acids and other nutrients essential for growth.

Another cause for speculation is the mechanism of growth of individual platelets as they move away from the periphery. Not only do they grow but they assume characteristically different shapes in different groups of Amphibia, *e.g.*, rectangular in *Rana pipiens*, triangular in *Amblystoma punctatum* (Holtfreter, 1946). Apparently the enzymes responsible for yolk deposition are localized in the peripheral endoplasm during the primary (centripetal) wave

of deposition of platelets (Wittek, 1952) which continues until stage Y_3, when secondary (centrifugal) deposition begins in the perinuclear cytoplasm. Continued growth of the platelets as they come to lie deeper within the cytoplasm, however, implies continued proximity of the enzyme complex responsible for synthesis of yolk. The necessary enzymes must persist in the cytoplasm immediately surrounding each growing platelet and may even be adsorbed on its surface. Two enzymes, phosphoprotein phosphatase and phosphotransferase, are known to be closely associated with yolk platelets (Harris, 1946; Barth and Jaeger, 1950). That the peripheral endoplasm remains a site of continued renewal of the yolk-synthesizing enzymes throughout the period of growth of the oocyte is evident from the cytological observation that small yolk platelets continue to form there. If the site of synthesis of these enzymes is localized chiefly at the periphery, the interior endoplasm may become progressively more depleted of enzymes as the platelets grow at the expense of surrounding cytoplasm. Eventually both the concentration of enzymes and of substrates may reach a level so low that continued growth of the larger platelets is no longer possible. *À propos* of these speculations, it is revealing to observe that autoradiograms of stage Y_4 oocytes show high peripheral uptake of C^{14}-labelled glycine but very low uptake in the interior (Kemp, 1955, *a*, and unpublished observations).

Significance of the Cortex.—The term cortex when applied to cells usually refers to an outer gelated zone of cytoplasm which surrounds a more fluid endoplasm. According to Holtfreter (1948) "the architecture of any cell from early amphibian embryos closely resembles the general organization found in *Amoeba proteus*." There is in these embryonic cells an inner plasmasol (equivalent to endoplasm) containing the nucleus, organoids, structural cytoplasm, and granular inclusions. Outside this is the cortex, consisting of the viscous plasmagel surrounded by a hyaline layer of ectoplasmic fluid delimited externally by the plasma membrane. Holtfreter (1943) has described for amphibian eggs and embryos an elastic surface layer, the surface coat, which accounts for such fundamental phenomena as cell aggregation, permeability, osmotic regulation, mass movements of cells, and wound healing. The exact nature of the surface coat has not been determined, but it is clearly either gelated cortical cytoplasm or a product of the cortex. Trinkaus (1951) has demonstrated that a similar layer, which he calls the surface gel layer, is responsible for wound healing and for epiboly in *Fundulus*.

The significance of the cortex in cell division has been discussed by Schechtman (1937) and Marsland (1951). Visible changes in the cortex, including a breakdown of cortical granules or alveoli, are known to follow fertilization in echinoderms, fishes, and amphibians (Runnström, 1949; Motomura, 1952; Kusa, 1954; Yamamoto, 1954; Allen and Hagström, 1955). Evidence for localization of the structural basis for polarity in the cortex of eggs of certain

invertebrates has recently been reviewed by Watterson (1955). Observations on the cortical layer of the egg of the frog and of other vertebrates led Dalcq (Dalcq and Pasteels, 1937; Dalcq, 1940) to formulate the hypothesis of *potential morphogenetique*, which proposes an interaction between two metabolic gradients, one (*c*) in the cortex and another (*v*) associated with the yolk in the interior cytoplasm, to account for differentiation.

Because of the obvious importance of the cortex for a variety of functions, its structure should be better understood. In the present study differentiation of the cortical cytoplasm has been shown to coincide with the onset of lipogenesis and vitellogenesis in the young oocyte. The cortex is divided into three zones: an outer one composed of microvilli, a middle layer consisting of the folded basal cytoplasm, and an inner layer containing cortical granules. Possibly one should consider the latter layer in the peripheral endoplasm, since Yamamoto (1954) has shown that cortical alveoli of fish eggs may be displaced by light centrifugation. On the other hand, the cortical granules in the sea urchin are not displaced by moderate centrifugation (Moser, 1939). Whether the cortical granules of the frog oocyte may be moved by centrifugal force has yet to be determined. Behavior of the granules of frogs' eggs after fertilization is currently under investigation. The region between follicle cells and zona radiata, previously considered vitelline membrane or chorion (Kemp, 1953), is seen with the electron microscope to contain protoplasmic processes from the follicle cells. Probably the vitelline membrane is laid down as intercellular material between the follicular processes, which are eventually withdrawn. My observations thus far do not permit a decision as to whether the vitelline membrane is derived primarily from material contributed by the oocyte or by the follicle cells, although it is usually considered to be derived from the oocyte.

The zone of microvilli described above is especially interesting. It corresponds to the zona radiata described by light microscopists (Kemp, 1953) in the frog, and it is highly probable that similar microvilli will be found in the zona radiata of the eggs of other vertebrates. A preliminary study (unpublished) indicates that they occur in the mouse. Duryee (1954) pictures human oocytes with outpouchings of the vitelline membrane, called vitelline cones, connecting with protoplasmic processes of cells of the corona radiata, which he calls coronal canals. The resemblance between the folded cortical cytoplasmic layer of the frog's oocyte and Duryee's diagram of vitelline cones, and also between his "coronal canals" and the follicular processes around the frog's oocyte, is indeed striking. If the relationships between oocyte and follicle cells in the human being are analogous to those in the frog, however, there should not be a direct continuity between processes from the oocyte and those from the follicle cells. Electron microscopy is needed to resolve the true relationships in mammals. Among invertebrates, Runnström and Monné (1945) report the occurrence of protoplasmic filaments extending from the

cortex through the jelly coat of the oocyte of a number of species of echinoderms. It seems likely that these filaments are homologous to the microvilli or to the elevations of the folded cortical cytoplasm of the frog oocyte.

Filamentous projections analogous to those of oocytes are found at the free surface of a variety of types of cells specialized for absorption or secretion (Dalton, 1953; Fawcett and Porter, 1954; Yamada, 1955). These include epithelial cells in molluscs, amphibians, and chicks, as well as in mammalian intestine, stomach, kidney, gall bladder, choroid plexus, oviduct, and epididymis. Microvillous projections increase the absorptive or secretive surface tremendously. By making a few reasonable assumptions, one can calculate roughly the increase in absorptive area resulting from the presence of microvilli at stage Y_1 of growth of the frog oocyte. At this time the oocyte would measure about 400 μ in diameter. A simple sphere of this size would have a surface area of approximately 502,650 sq. μ. Assuming that half of this area is occupied by the bases of the microvilli, each of which has a diameter of 0.08 μ and thus occupies an area of 0.005 sq. μ, there are a total of 42,065,600 microvilli present at stage Y_1. If each microvillus were a cylinder measuring 0.08 $\mu \times 1.67$ μ, it would have a surface area of about 0.42 sq. μ. The total surface exposed by the microvilli thus would be 17,650,725 sq. μ, or about thirty-five times the surface area of a simple sphere. As oogenesis proceeds, folding of the basal cortical layer (Figs. 7 and 8) would still further increase the absorptive area.

SUMMARY

1. In the cytoplasm of oocytes of stage Y_0, prior to the appearance of yolk, one observes a few scattered profiles of endoplasmic reticulum and numerous filamentous mitochondria, usually distributed at random but sometimes clustered. As the nuclear membrane begins to bulge outward, small granules and short rods appear in the perinuclear cytoplasm and endoplasmic reticulum becomes more prominent throughout the cytoplasm.

2. Coincident with the appearance of the first yolk platelets, which are deposited in a narrow peripheral ring within the endoplasm at stage Y_1, protoplasmic processes, the microvilli, push out all over the surface of the oocyte. At the same time follicle cells pull away but remain attached to the oocyte at some points through finger-like processes which interdigitate with neighboring microvilli. It is estimated that the microvilli increase the absorptive area of the surface to about thirty-five times that of a simple sphere. Just beneath the microvillous layer is the basal protoplasm of the cortex, now containing tiny granules probably synthesized from newly absorbed raw materials. Cortical granules appear and become aligned below the basal layer on the external border of the endoplasm. Both the cortical granules and the yolk platelets measure up to 1 μ in diameter at this stage.

3. By stage Y_3 (yolk filling peripheral three-fourths of cytoplasm), the

basal layer of the cortex is folded so that it appears in section as alternating ridges and valleys. The microvilli now extend from the summits of the cortical ridges. Small, ring-shaped granules are abundant in the cortex. Cortical granules have increased to 2 μ in diameter.

4. Yolk platelets continue to be synthesized around the cortical granules and in the subjacent endoplasm. The largest platelets measured in the interior cytoplasm at stage Y_4 (cytoplasm filled with yolk) were 3.7 μ wide by 5.8 μ long. Pigment granules increase in size from 0.15 μ in diameter at stage Y_3 to 0.30 μ in diameter at stage Y_4.

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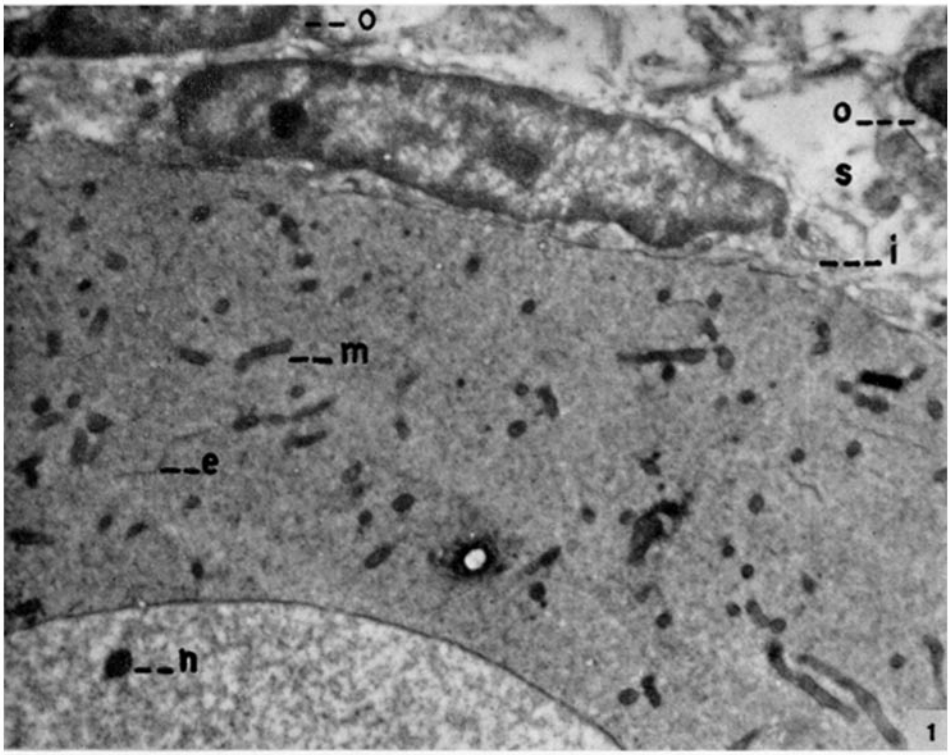
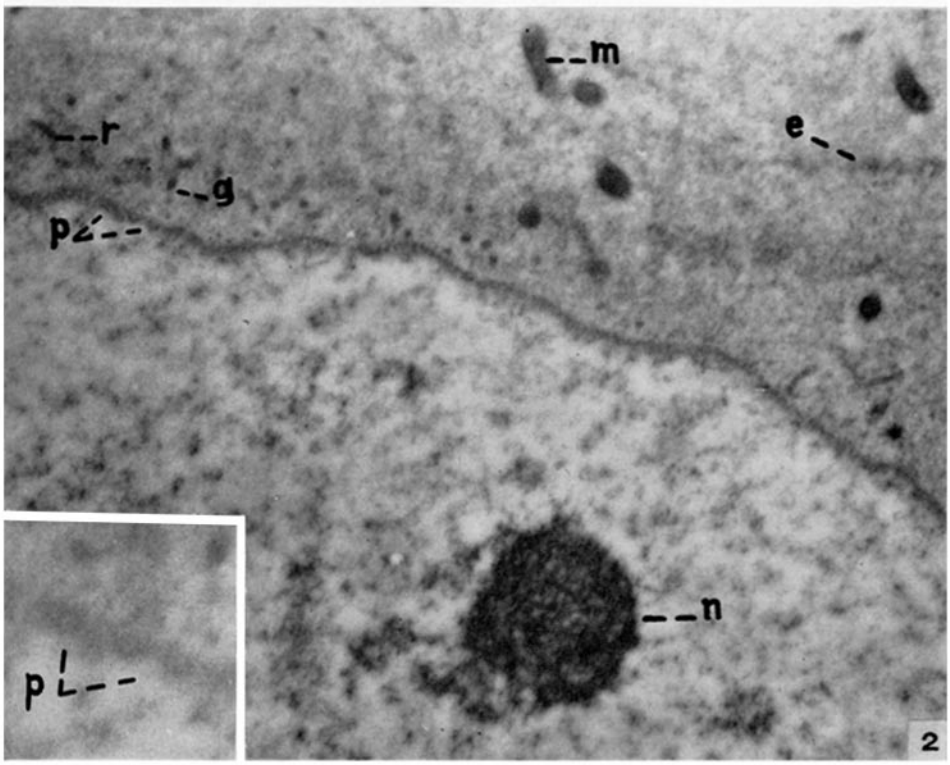
EXPLANATION OF PLATES

PLATE 56

FIG. 1. Section of a young oocyte in stage Y_0, estimated to be about 100 μ in diameter. Nucleus, lower left, contains peripheral nucleoli (*n*). Nuclear membrane forms smooth boundary between nucleus and cytoplasm, which contains abundant mitochondria (*m*) and a few profiles of endoplasmic reticulum (*e*). An inner follicle cell (*i*), containing an elongated nucleus, borders the outer margin of the oocyte. Portions of nuclei of two outer follicle cells (*o*) can be seen in upper corners of the micrograph. Intercellular space (*s*) contains connective tissue fibrils. × 8,750.

FIG. 2. In this section of an oocyte still in stage Y_0 the nuclear membrane has begun to bulge outward. Nucleus, below, is less dense than perinuclear cytoplasm. Nucleolus (*n*) appears to be fragmenting. Nuclear membrane contains pores (*p*) shown at higher magnification in the insert at the lower left corner of the micrograph. Small granules (*g*) and rods (*r*) are now concentrated in the perinuclear cytoplasm. Mitochondria (*m*) and endoplasmic reticulum (*e*) occur throughout the cytoplasm. × 15,300; insert × 41,300.



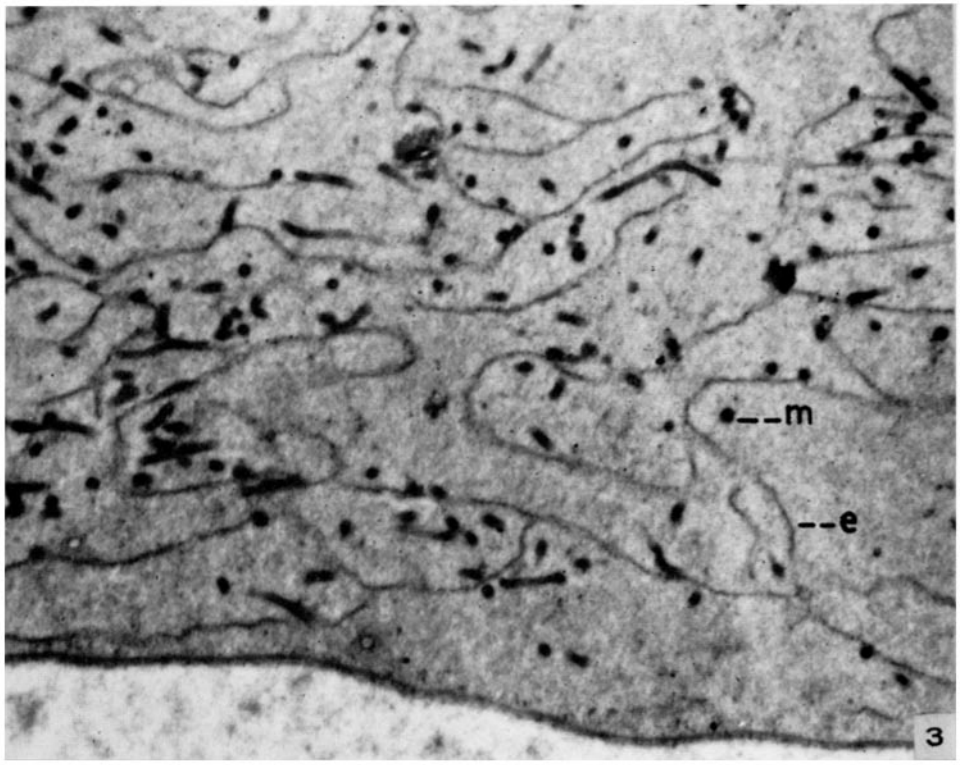


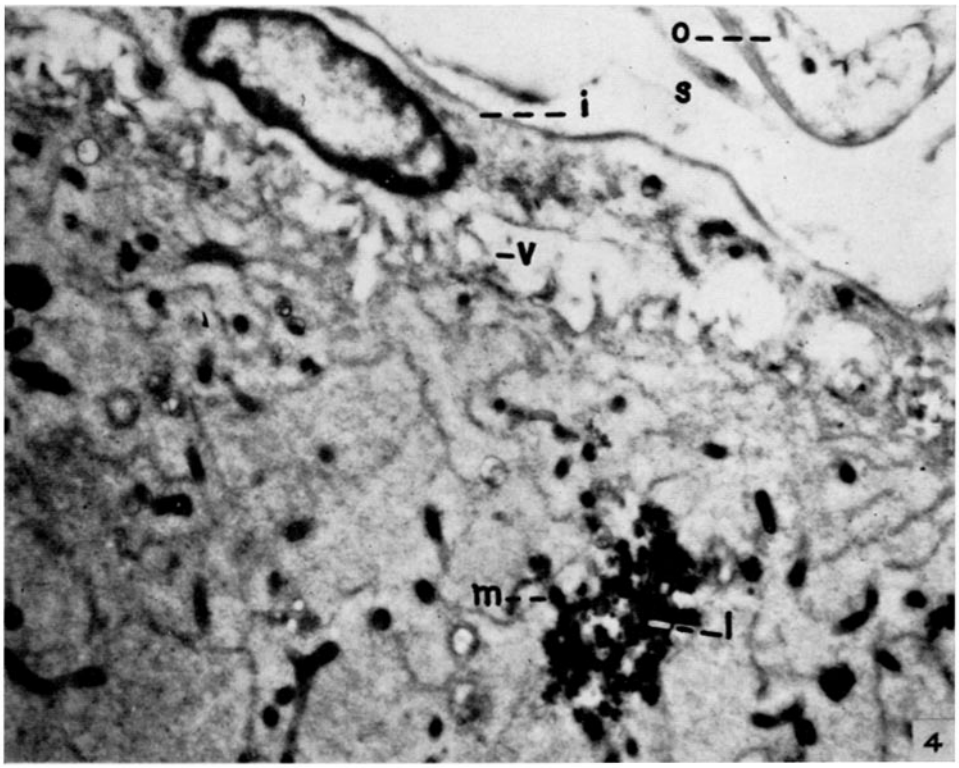
(Kemp: Electron microscopy of *Rana pipiens* oocytes)

PLATE 57

FIG. 3. Section of edge of nucleus, below, and perinuclear cytoplasm of an oocyte in stage Y_0. Endoplasmic reticulum (*e*) and mitochondria (*m*) are dispersed randomly in the cytoplasm. × 8,750.

FIG. 4. Surface of an oocyte in late stage Y_0. In the peripheral cytoplasm a "yolk nucleus" appears to consist of an aggregate of mitochondria (*m*) together with lipochondria of various sizes. It is believed that the larger lipochondria (*l*) are formed by coalescence of smaller ones, which seem to come from the ground cytoplasm. Microvilli (*v*) are beginning to protrude from the surface of the oocyte. Inner follicle cell (*i*) is pulling away from the oocyte, leaving conspicuous spaces into which microvilli from the oocyte protrude. Between inner follicle cell and outer follicle cell (*o*) is intercellular space (*s*). × 8,750.



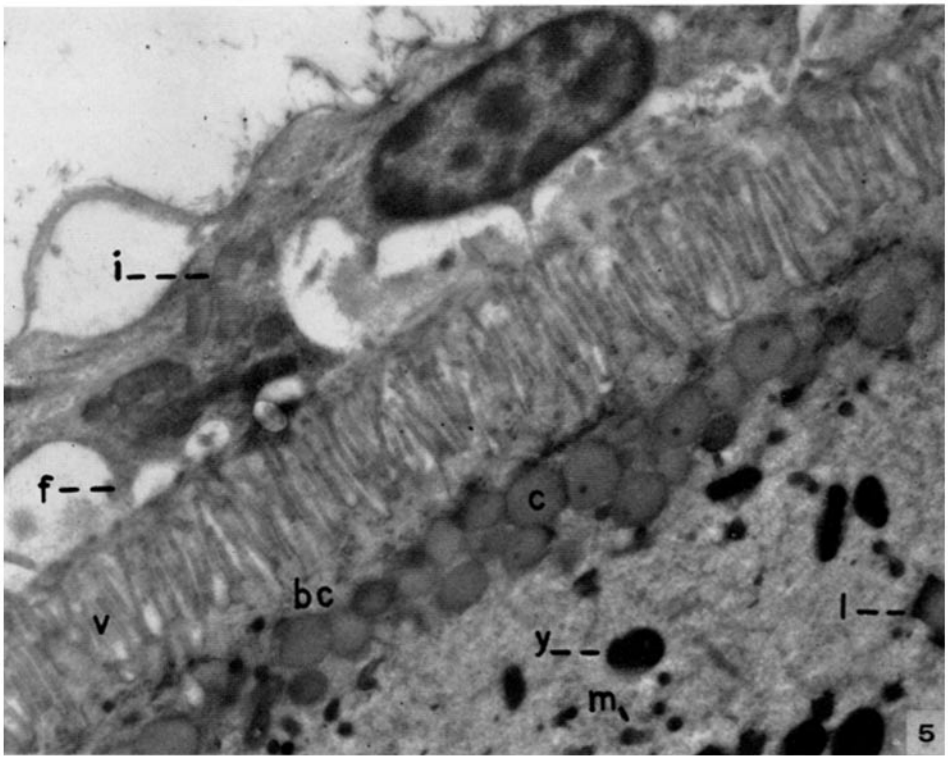


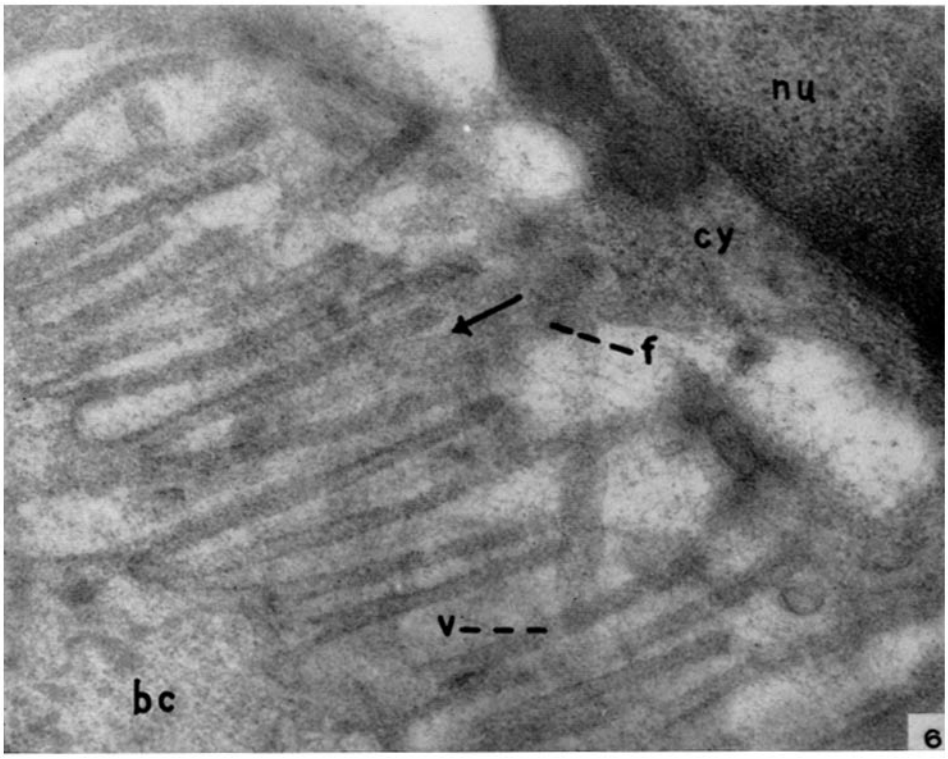
(Kemp: Electron microscopy of *Rana pipiens* oocytes)

PLATE 58

FIG. 5. Surface of an oocyte in stage Y_1. Yolk platelets (*y*), lipochondria (*l*), and mitochondria (*m*) are seen in the peripheral endoplasm. The cortex contains the layer of cortical granules (*c*), the basal cortical cytoplasm (*bc*), and the zone of microvilli (*v*), which now protrude prominently. Protoplasmic follicular processes (*f*) from an inner follicle cell (*i*) extend toward the microvillous layer and apparently intertwine to form an interlacing network around the outer ends of the microvilli. × 8,750.

FIG. 6. Highly magnified view of the surface of an oocyte and adjoining portions of an inner follicle cell at stage Y_1. Basal cortical layer (*bc*) and microvilli (*v*) of oocyte occupy lower left half of photograph. Nucleus (*nu*), cytoplasm (*cy*), and follicular processes (*f*) of inner follicle cell are at upper right. Note interdigitation of microvilli with follicular process extending downward from arrow. × 33,510.



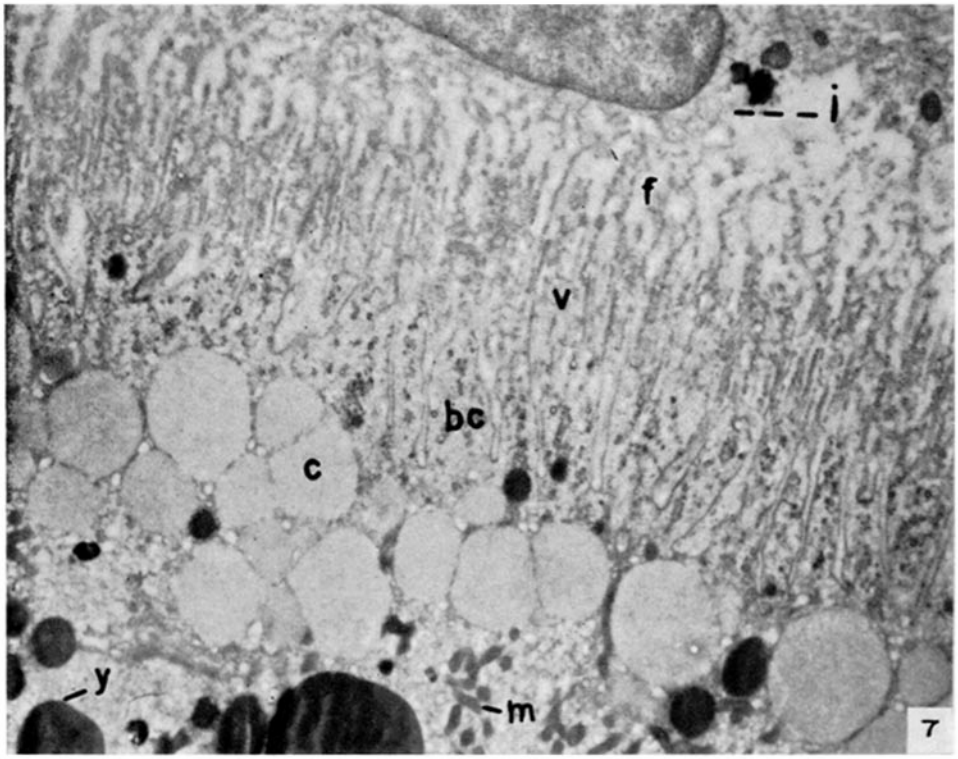


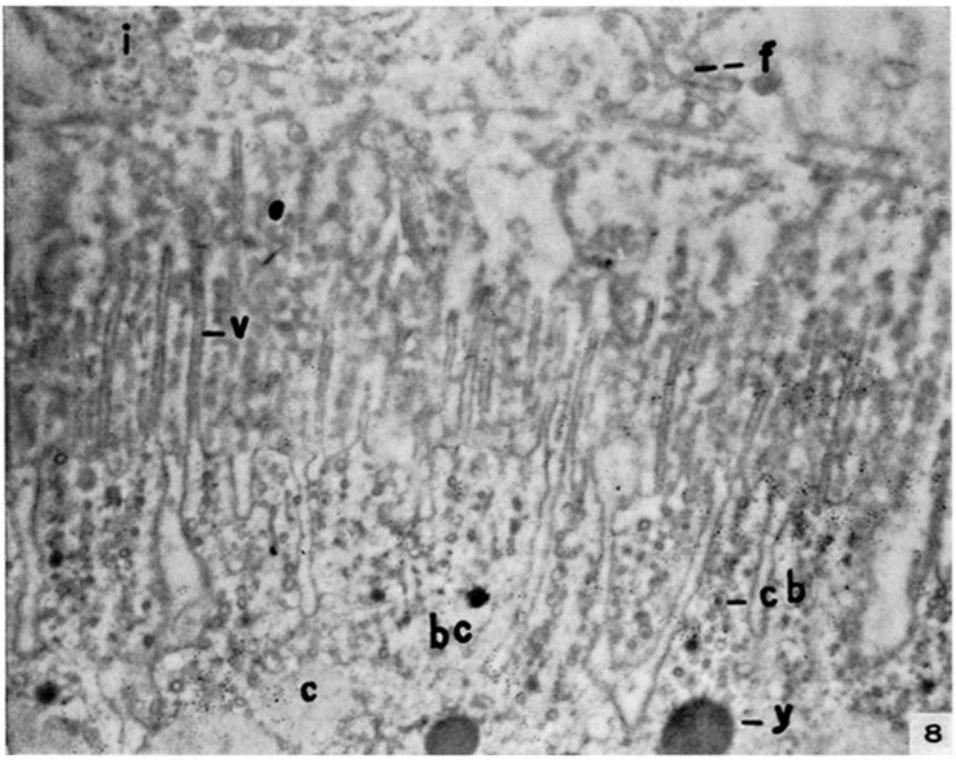
(Kemp: Electron microscopy of *Rana pipiens* oocytes)

PLATE 59

FIG. 7. Surface view of oocyte at stage Y_3. Yolk platelets (*y*) and mitochondria (*m*) are seen in the peripheral endoplasm. A few small platelets also are seen in the cortex. In the cortex are the layer of cortical granules (*c*), basal cortical layer (*bc*), and layer of microvilli (*v*). The basal cortical cytoplasm is now folded into alternating ridges and valleys, and the microvilli extend from the peaks of the ridges. Note also that small ring-shaped droplets are abundant in the basal cortical cytoplasm. Follicular processes (*f*) traverse the space between oocyte and inner follicle cell (*i*). × 8,750.

FIG. 8. Highly magnified view of surface of oocyte in stage Y_3. Cortex includes: cortical granules (*c*), along lower border; folded basal cortical layer (*bc*); and microvilli (*v*) extending from summits of cortical ridges. A few small yolk platelets (*y*), as well as many ring-shaped droplets, are in the basal cortical layer. Some of the droplets contain a dark central body (*cb*). Tiny black dots are an artifact resulting from the coarseness of the carbon particles in the carbon membrane supporting this section. Inner follicle cell (*i*) is at upper margin of photograph. Follicular processes (*f*) traverse space between microvillous layer and follicle cell. × 15,300.



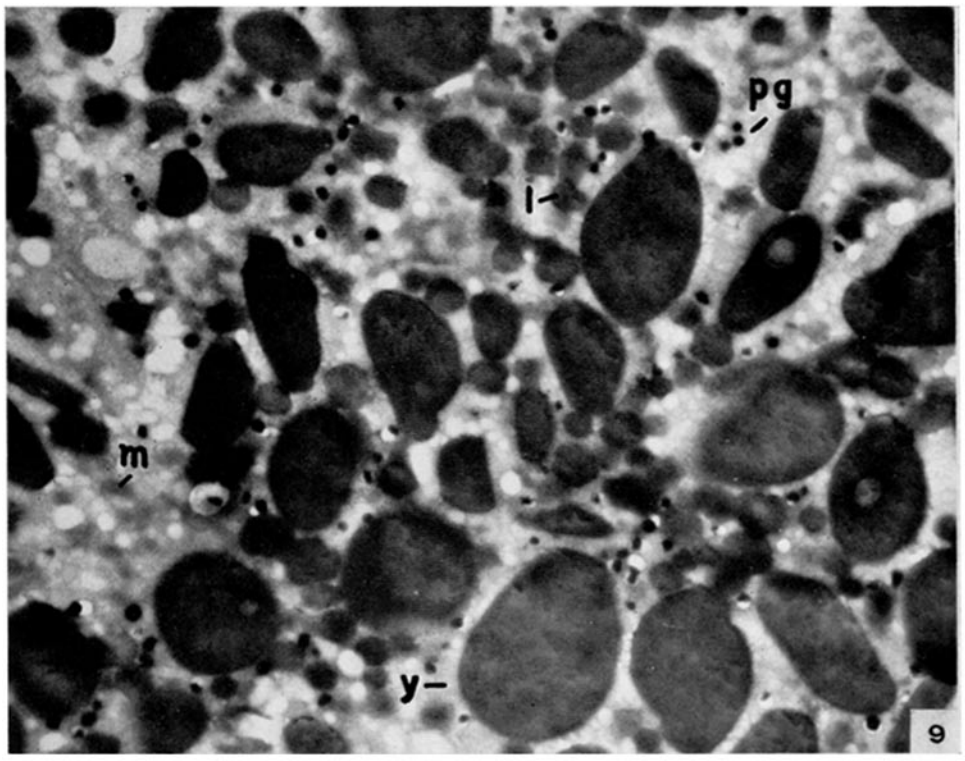


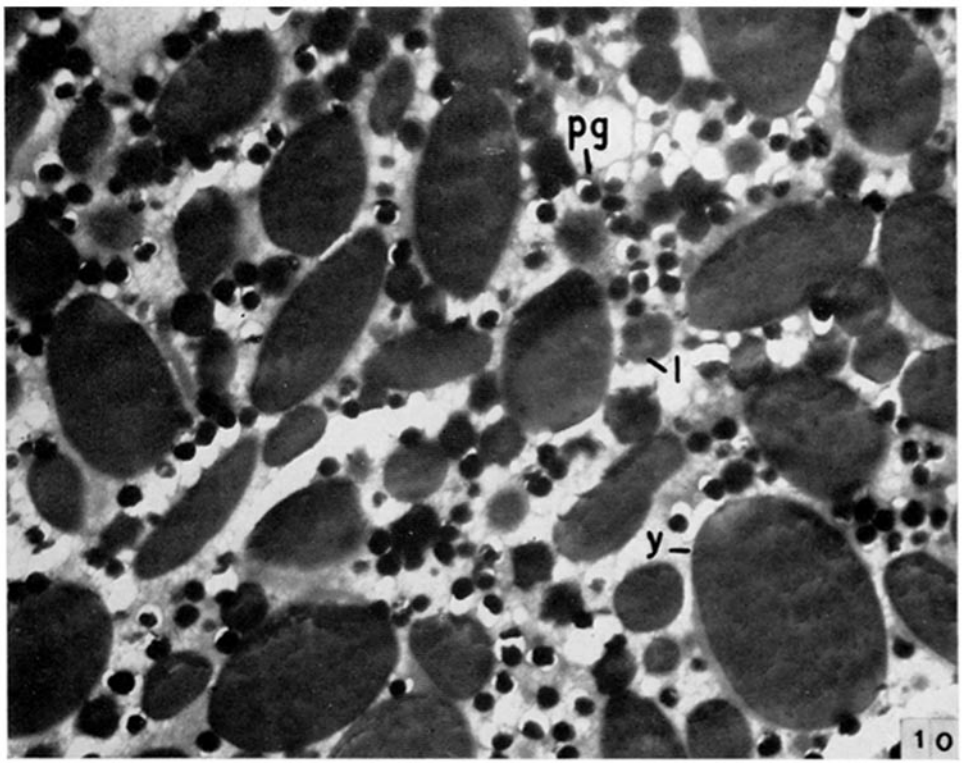
(Kemp: Electron microscopy of *Rana pipiens* oocytes)

PLATE 60

FIG. 9. View of inclusions in subsurface endoplasm of oocyte in stage Y_3. Pigment granules (*pg*) are interspersed among lipochondria (*l*), mitochondria (*m*), and yolk platelets (*y*) in various stages of growth. × 8,750.

FIG. 10. View of inclusions in subsurface endoplasm of oocyte in stage Y_4. Pigment granules (*pg*), lipochondria (*l*), and yolk platelets (*y*) are abundant. Mitochondria not preserved, presumably because of poor penetration of osmic acid into larger oocytes. × 8,750.





(Kemp: Electron microscopy of *Rana pipiens* oocytes)