

PERMEABILITY OF THE OVARIAN FOLLICLE OF *Aedes aegypti* MOSQUITOES

WINSTON A. ANDERSON and ANDREW SPIELMAN

From the Department of Anatomy and Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02115, the Department of Anatomy, The University of Chicago, Chicago, Illinois 60637, and the Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts 02115

ABSTRACT

The passage of tracers of various molecular weights into resting and vitellogenic ovarian follicles of *Aedes aegypti* mosquitoes was studied ultrastructurally. The outermost layer of the follicular sheath (the basement lamina) is a coarse mechanical filter. It is freely permeable to particles with molecular weights ranging from 12,000 to 500,000 (i.e. cytochrome *c*, peroxidase, hemoglobin, catalase, ferritin, immunoglobulin (IgG)-peroxidase, iron dextran and Thorotrast) that have dimensions less than 110 Å. Molecules as large as carbon (300–500 Å) are totally excluded. Whereas proteins and polysaccharide tracers permeate the basement lamina with apparent ease, certain inert particles (e.g. Thorotrast, Fellows-Testager Div., Fellows Mfg. Co., Inc., Detroit, Mich.) penetrate more slowly. With respect to the tracers tested, resting follicles are as permeable as vitellogenic follicles. The follicle epithelium of resting or vitellogenic follicles is penetrated by narrow intercellular channels. Our observations suggest that these spaces are lined with mucopolysaccharide material. After permeating the basement lamina, exogenous tracers fill these channels, while the bulk of material accumulates in the perioocytic space. Within 3 hr after imbibing blood, the pinocytotic mechanism of the oocyte is greatly augmented. Pinocytosis is not selective with regard to material in the perioocytic space, since double tracer studies show that exogenous compounds are not separated, but are incorporated into the same pinocytotic vesicle. During later stages of vitellogenesis, 36–48 hr after the blood-meal, the pinocytotic mechanism of the oocyte is diminished. Simultaneously, the intercellular channels become occluded by desmosomes, and the vitelline membrane plaques separate the oocyte and follicle epithelium.

Oocytes of *Aedes aegypti* appear to acquire yolk by accumulating large molecules that have been synthesized at extra-ovarian sites (Roth and Porter, 1964), and this holds true for most insects that have been studied (Anderson, 1964, 1967; Favard-Séréno, 1969; Stay, 1965; Telfer, 1961, 1965). Some insects synthesize yolk precursors in other cells within the ovarian follicle (Anderson and Telfer, 1969, 1970; Hopkins and King, 1966), while other animals produce such material within

the oocyte itself (Beams and Kessel, 1962; Favard and Carraso, 1958; Lanzavecchia, 1961; Ward, 1962). Extrafollicular synthesis of yolk requires that large molecules be transported across a layer of cells and through the oocyte membrane.

Mosquitoes are extraordinary in that rapid vitellogenesis follows prolonged periods in which fully competent oocytes do not acquire yolk, and Roth and Porter (1964) suggested that the state of permeability of the follicle epithelium is a de-

terminant of this state of activity. A few hours after a female feeds upon blood, the follicle cells separate, allowing the oocyte full exposure to hemolymph. The follicle epithelium would, therefore, be functioning as a variable filter.

In the present study we measured the permeability of the ovarian follicle of *A. aegypti* and determined whether blood-feeding influenced the passage of particles through this tissue. In addition, we studied the ability of the follicle to separate molecules of diverse physical and chemical nature.

MATERIALS AND METHODS

TECHNIQUES

Entomological Techniques

Virgin *Aedes aegypti*, 4–7 days old, were used in these experiments. The mosquitoes were maintained in the laboratory at 24°C and were exposed to 16 hr of light/day.

For injection, mosquitoes were transferred, without anesthesia, onto a screen and immobilized by air suction. A calibrated glass needle inserted between the thoracic pleurae was used to inject approximately 0.001 ml of solution/mosquito.

In experiments requiring measurement of follicles, ovaries were disrupted in saline with a vibrating needle, and individual follicles were measured with an ocular micrometer. For electron microscopy, ovaries were either excised directly in fixative or in an insect Ringer's solution at pH 7.6 (Bodenstein, 1946) and immediately transferred to fixative.

Electron Microscope Techniques

The fixative employed for optimal preservation of ultrastructure was a formaldehyde-glutaraldehyde mixture containing trinitroresol, while formaldehyde-glutaraldehyde fixative was used when demonstrating peroxidatic activity (Ito and Karnovsky, 1968; Karnovsky, 1965). Additional fixatives were employed as indicated. Time of fixation varied from 5 min to 2 hr. All tissues were postfixed in 2% osmium tetroxide solution, dehydrated in acetone- or alcohol-water solutions, and embedded in an Epon-Araldite mixture (10 vol Epon 812, 8 vol Araldite 6005, 24 vol dodecenylsuccinic anhydride [DDSA]). Sections were cut with a diamond knife and stained with lead citrate (Venable and Coggeshall, 1965) or doubly stained in uranyl acetate and lead. Sections were examined with a Siemens Elmiskop I and a Hitachi HU 11C electron microscope.

Demonstration of Polysaccharides

Ovaries from blood-starved and blood-fed mosquitoes were fixed for 2 hr in formalin-glutaraldehyde

mixture containing 5–20 mg/ml ruthenium red (K & K Laboratories Inc., Plainview, N. Y.). The ovaries were postfixed for 2–3 hr in 2% osmium tetroxide which contained 10 mg/ml ruthenium red. Tissues were held in the dark during both periods of fixation.

In other studies, thin sections of ovaries that were fixed in glutaraldehyde and osmium tetroxide were treated with the periodic acid-thiosemicarbazide-silver proteinate procedure according to Thiéry (1967).

TRACER EXPERIMENTS

Enzymatic Tracers

HORSERADISH PEROXIDASE: Mosquitoes were injected with horseradish peroxidase (Type IV; Sigma Chemical Co., St. Louis, Mo.) at concentrations of 2, 5, 10, and 20 mg/ml insect Ringer's solution. This tracer was injected into blood-starved mosquitoes and into blood-fed mosquitoes at various intervals after feeding. Ovaries were excised immediately after injection and at frequent intervals until 50 hr thereafter. They were then fixed for 30–45 min in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 5% sucrose. After rinsing for approximately 12 hr in 0.1 M phosphate buffer (pH 7.4), they were incubated for 30–120 min in 10 ml of 0.05 M Tris-HCl buffer containing 0.01% H₂O₂ and 10 mg diaminobenzidine (DAB) tetrahydrochloride (Graham and Karnovsky, 1966). As controls, ovaries from mosquitoes that were injected with saline or Ringer's were treated similarly while ovaries from peroxidase-injected insects were incubated in the Graham-Karnovsky medium that lacked H₂O₂. In order to show possible endogenous peroxidase activity, ovaries from blood-starved and from blood-fed mosquitoes were incubated in the DAB oxidation medium at pH 6.0 and 9.0 according to Beard and Novikoff (1969).

CYTOCHROME *c*: Cytochrome *c* (Type II, Sigma Chemical Co.) at concentrations of 20–40 mg/ml Ringer's solution was injected into blood-fed mosquitoes. At 4, 16, and 24 hr after injection, ovaries were excised and immersed for 10 min in 2% depolymerized paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) containing 2% sucrose. Tissues were then rinsed for 9–19 hr in phosphate buffer at 5°C and incubated for 1 hr in 9.5 ml of 50 mM citrate buffer at pH 3.9 containing 0.5 ml 1% H₂O₂ and 5 mg DAB (Karnovsky and Rice, 1969).

CATALASE: Beef liver catalase (Sigma Chemical Co.) was dissolved by sonication for 5–20 min at 20 kc/sec and immediately injected into unfed and blood-fed mosquitoes. Ovaries were excised at 5 and 16 hr and fixed for 2 hr in formalin-glutaraldehyde fixative. Ovaries were incubated in the DAB oxidation medium after Venkatachalam and Fahimi

(1969). As controls, ovaries from catalase-injected mosquitoes were incubated in the same medium from which H_2O_2 was omitted.

IMMUNOGLOBULIN-PEROXIDASE CONJUGATES: Globulins from rabbit serum were purified by chromatography on diethylaminoethyl (DEAE) cellulose and by elution with phosphate buffers (pH 8.0) in increasing molarities. The immunoglobulin (IgG) was lyophilized and coupled to horseradish peroxidase according to Nakane and Pierce (1967) and Høedemacker and Ito (1970). The IgG-peroxidase complex was injected into blood-fed mosquitoes; after 4, 6, and 12 hr, ovaries were fixed in formalin-glutaraldehyde mixture and treated to demonstrate peroxidase activity.

HEMOGLOBIN: In some experiments, 10–20 mg/ml hemoglobin was injected into each of 12 blood-fed and unfed mosquitoes. At 3, 5, and 16 hr after the injection, ovaries were excised in formaldehyde-glutaraldehyde fixative or in insect Ringer's and then immersed in the fixative. The tissues were washed in cold 0.1 M cacodylate buffer (pH 7.4) and incubated in the Graham-Karnovsky medium. Ovaries were then osmicated. In other experiments, ovaries from hemoglobin-injected mosquitoes were fixed in for-

maldehyde-glutaraldehyde-trinitroresol fixative and postfixed in osmium tetroxide.

Nonenzymatic Tracers

FERRITIN AND IMFERON: Twice-crystallized horse spleen ferritin obtained as a cadmium-free solution (Miles Research Products, Kankakee, Ill.) was injected into blood-fed and blood-starved mosquitoes. In other experiments, an iron-dextran complex (Imferon; Lakeside Laboratories, Inc., Milwaukee, Wis.) was injected. Ovaries were excised at 3, 5, 16, and 24 hr and fixed in glutaraldehyde and OsO_4 .

ALCIAN BLUE-LANTHANUM: Ovaries from blood-starved and from blood-fed mosquitoes were fixed for 1 hr in formalin-glutaraldehyde fixative containing 0.5% Alcian blue and postfixed for 1 hr in 2% OsO_4 containing 1% lanthanum nitrate (Shea and Karnovsky, 1969).

THOROTRAST AND COLLOIDAL CARBON: In concurrent experiments, Thorotrast (Fellows-Testagar, Anaheim, Calif.) and a suspension of shellac-free, nontoxic carbon (Günther-Wagner, Hannover, Germany) were injected into nonblood-fed and into

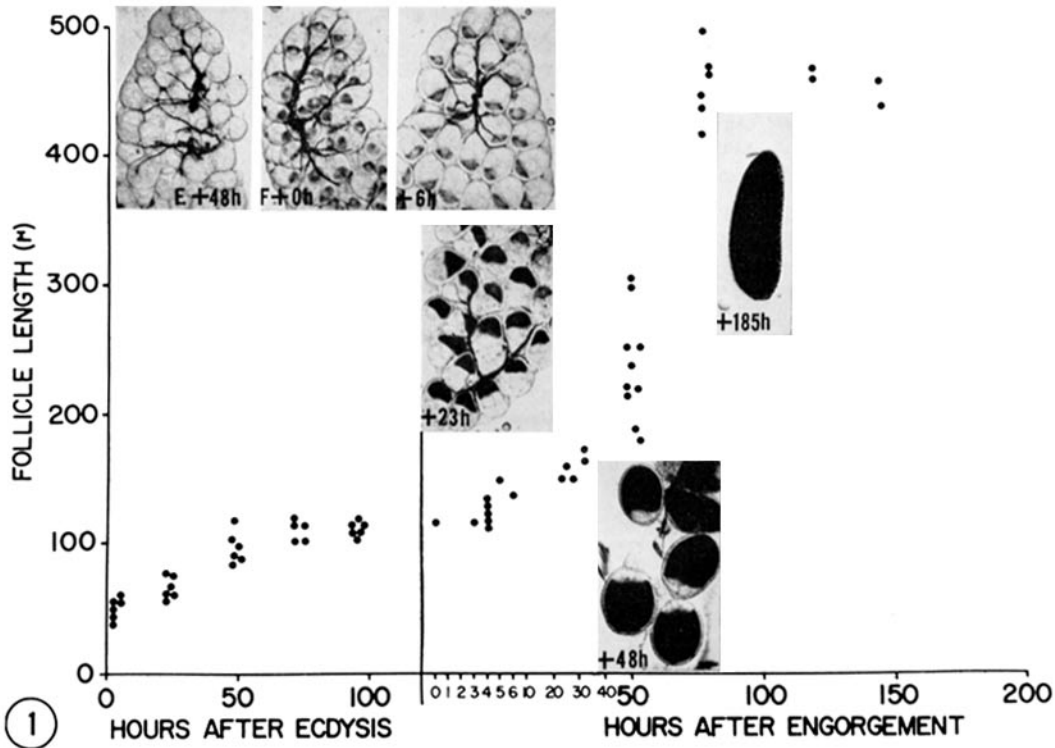


FIGURE 1 Rate of elongation of follicles containing developing oocytes of *A. aegypti*. Light micrographs show follicles at 48 hr after ecdysis (E + 48h) and at various hours after the blood-meal (F + 0h to 185h).

blood-fed mosquitoes. At various intervals after injection, the ovaries were excised and fixed in formalin-glutaraldehyde-trinitrocresol.

Double Tracer Experiments

PEROXIDASE AND FERRITIN: Horseradish peroxidase at concentrations of 1, 2, 5, and 10 mg/ml were dissolved in cadmium-free, horse spleen ferritin solution. This mixture was injected into unfed mosquitoes and into blood-fed mosquitoes at various intervals after feeding. At 4, 16, and 24 hr after the injection, ovaries were excised and fixed in 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min, rinsed for 12 hr in phosphate buffer, and incubated in the DAB oxidation medium of Graham and Karnovsky (1966).

PEROXIDASE AND IMPERON: A mixture containing 10 mg peroxidase/ml Imperon was injected into unfed and blood-fed mosquitoes. 4, 16, and 24 hr later, the tissues were fixed in formalin-glutaraldehyde or in 2% depolymerized paraformaldehyde in phosphate buffer (pH 7.4) and processed to determine peroxidase activity.

OBSERVATIONS

The paired, spindle-shaped ovaries of *A. aegypti* each contain approximately 100 polytrophic ovarioles. Such ovarioles include a series of follicles, of which the basal is the most advanced. Normally, these primary ovarian follicles develop synchronously and become mature before more distal follicles begin to deposit yolk. There are five trophocytes within each follicle and they appear to be without cytoplasmic connections.

Changes in Oocyte Fine Structure after Imbibition of Blood

At the time of adult ecdysis, primary follicles were approximately as large as secondary follicles and the oocyte was devoid of yolk. Primary follicles grew slowly during the next 50 hr (Fig. 1), but thereafter ceased growth. At this time, the

ooplasm, which was rich with ribosomes, contained occasional plaques of yolk. Scattered microvilli, pits, and pinocytotic vesicles were present on the surface of the oocyte (Fig. 2).

Following imbibition of blood, the surface of the oocyte changed dramatically. Within 3 hr that portion of the oolemma that was apposed to the follicle layer developed numerous microvilli and pinocytosis vesicles (Fig. 3). Both coated and naked vesicles were present.

Follicle cells remained in close apposition throughout this period of yolk deposition (Figs. 2 and 3). Nor did the basement lamina encompassing the follicle epithelium (Fig. 4) change in structure. This membrane was divided into two distinct zones: an inner network of fine fibrils embedded in an amorphous matrix, and an outer lamina comprised of several fenestrated sheets.

The space between follicle cells was approximately 200 Å wide (Fig. 5). Desmosomes were generally present at follicle cell boundaries adjacent to the oocyte, but tight junctions (zonulae occludentes) were apparently absent. Occasionally, we observed local expansions of the interspace between follicle cells in the region next to the basement lamina.

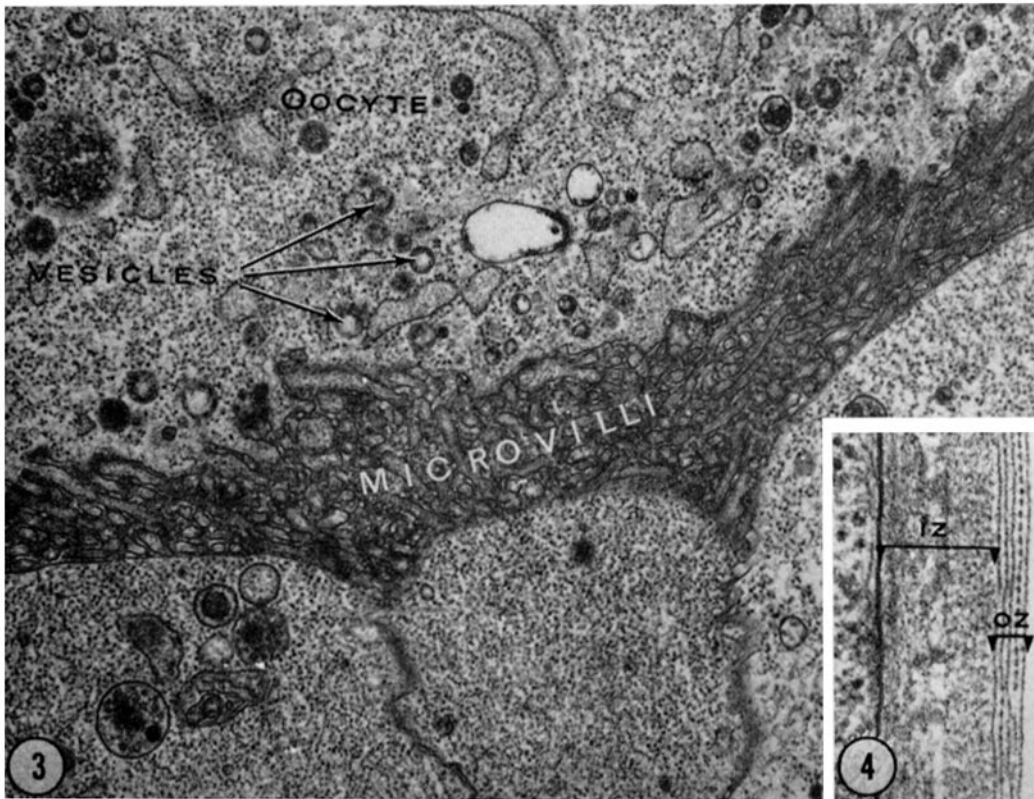
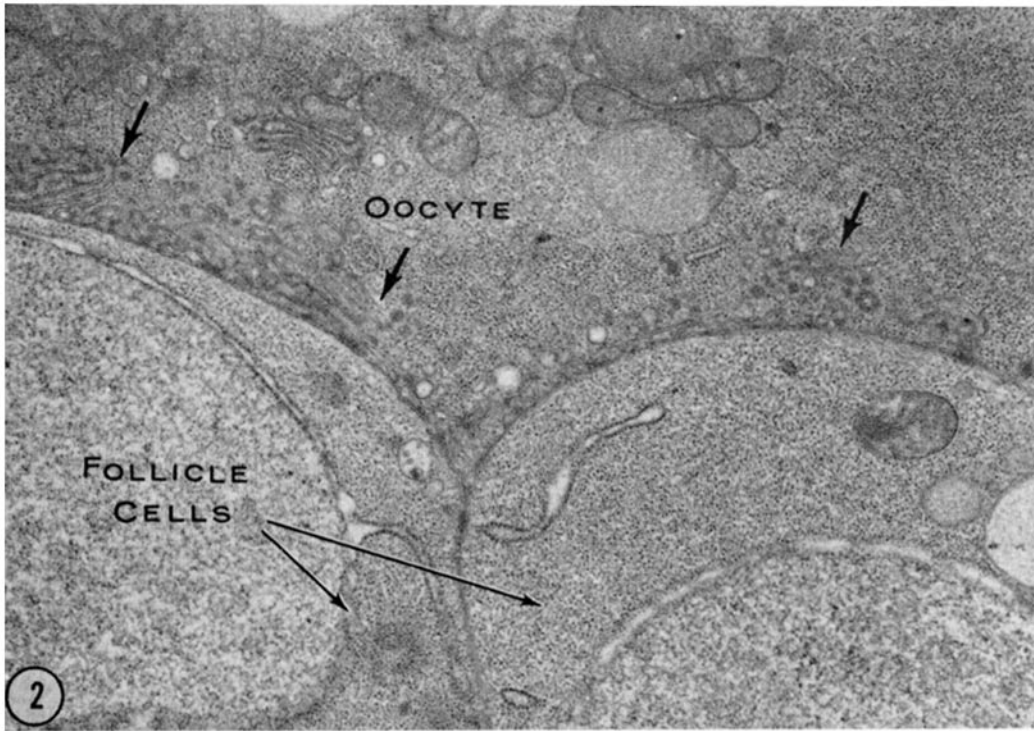
Penetration of Peroxidase into the Developing Oocyte

Horseradish peroxidase was employed as a tracer to determine the route by which exogenous proteins reach the oocyte and the selectivity of the pinocytotic mechanism for exogenous material. 10 µg were injected into blood-starved mosquitoes and the ovaries were excised 1 hr later. Tracer filled the basement lamina, the channels between follicle cells, and coated the surface of the enclosed oocyte and trophocytes (Fig. 6) but was absent from those pinocytotic vesicles that did not have contact with the surface of the oocyte. Thus, the overlying cell layer was permeable to peroxidase

FIGURE 2 A portion of an oocyte and adjacent follicle cells of an ovary from a blood-starved mosquito. The oolemma shows minimal proliferation, and sparse vesicles (arrows) are present in the ribosome-rich ooplasm. A desmosome is present between follicle cells. $\times 20,000$.

FIGURE 3 Cortex of an oocyte from a mosquito that had fed upon blood 3 hr previously. The surface of the oocyte has proliferated, forming numerous microvilli and pinocytotic vesicles. Follicle cells are tightly juxtaposed and desmosomes are present at their apices. $\times 22,000$.

FIGURE 4 Section through the basement lamina beneath the follicle epithelium. A series of fenestrated sheets comprises the outer zone (oz) and thin filamentous material makes up the inner zone (iz). $\times 25,000$.



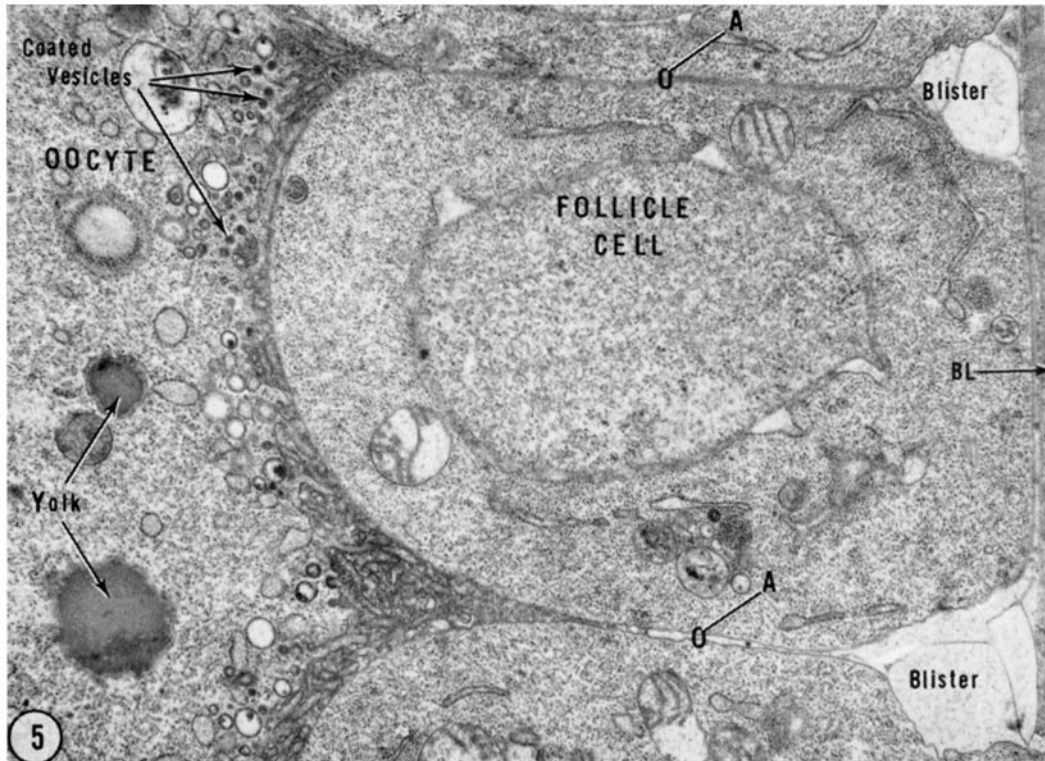


FIGURE 5 A portion of the oocyte and follicle epithelium of a blood-fed mosquito that was injected with insect Ringer's. At 5 hr after feeding, follicle cells remain close (*A*) and there is no apparent accumulation of yolk precursors in the intercellular spaces. Local blister-like expansions of the space next to the basement lamina (*BL*) are sometimes observed. $\times 12,500$.

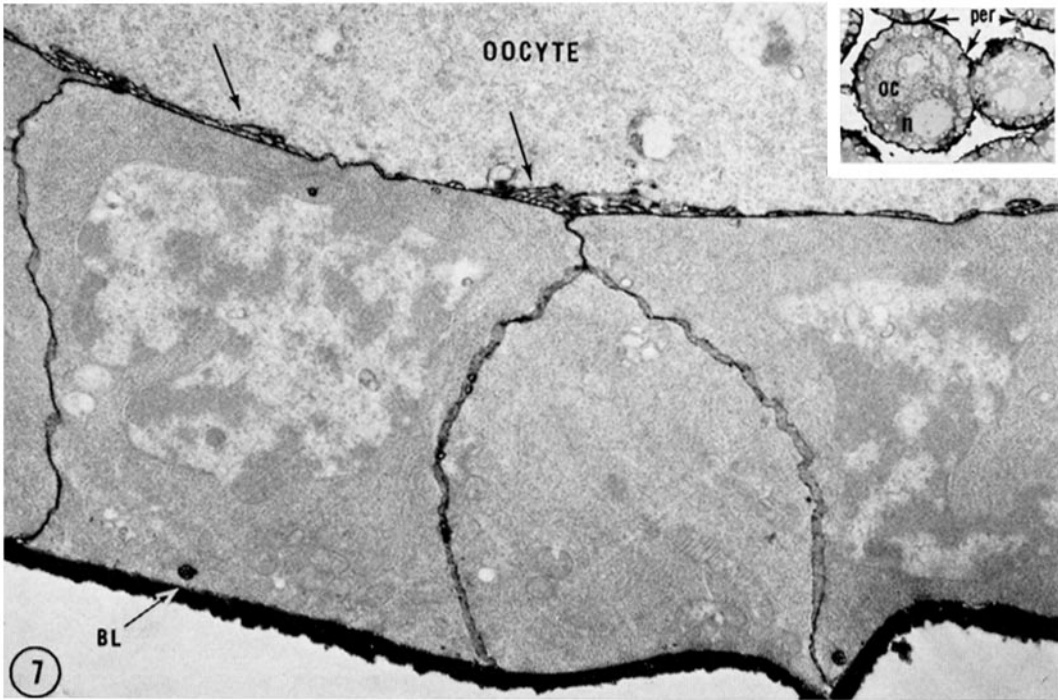
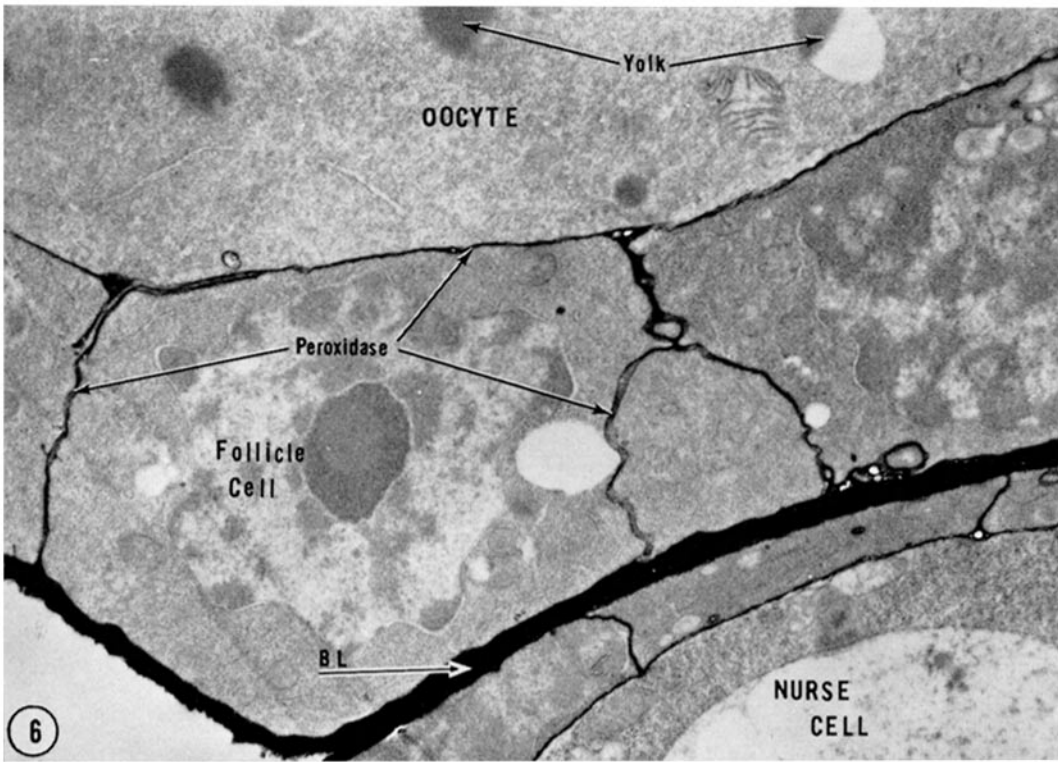
but the enclosed "resting" oocyte was not penetrated.

Ovaries were excised from peroxidase-injected mosquitoes 1 hr after blood-feeding, but these were essentially similar to the ovaries of blood-starved females (Fig. 7). At 2 hr after the blood-meal, oolemmal microvilli had become more numerous and filled the perioocytic space at sites opposite the junctions of adjacent follicle cells (Fig. 8). Significant quantities of tracer first appeared in the oocyte at 3 hr after feeding (Fig. 9). Microvilli

were numerous except on that portion of the oocyte apposed to the trophocytes. Many pits and cortical vesicles contained the tracer, some of which had already fused to form yolk plaques. At 7-24 hr, pinocytosis vesicles were densely packed within the cortex of the oocyte and most of them contained peroxidase (Figs. 10 and 11). Larger cortical vesicles had a clear central core. They enlarged by fusion; only rarely did ooplasmic cisternae fuse with the vesicles. Peroxidase activity was present in fully mature yolk plaques (Fig. 11).

FIGURE 6 Ovary excised from unfed mosquito that received an injection of peroxidase. After 1 hr, peroxidase is present within the basement lamina (*BL*), in the narrow interfollicular cell space, and between the surfaces of follicle cells and the oocyte. $\times 10,000$.

FIGURE 7 Ovaries from mosquitoes inoculated with peroxidase and fixed 1 hr after feeding upon blood. Tracer has infiltrated the perioocytic space and around projections of the oolemma (arrows). The *inset* is a light micrograph showing the presence of peroxidase (*per*) in the basement lamina in follicles. *BL*, basement lamina; *oc*, oocyte; *n*, nurse cell. $\times 10,000$; *inset*, $\times 400$.



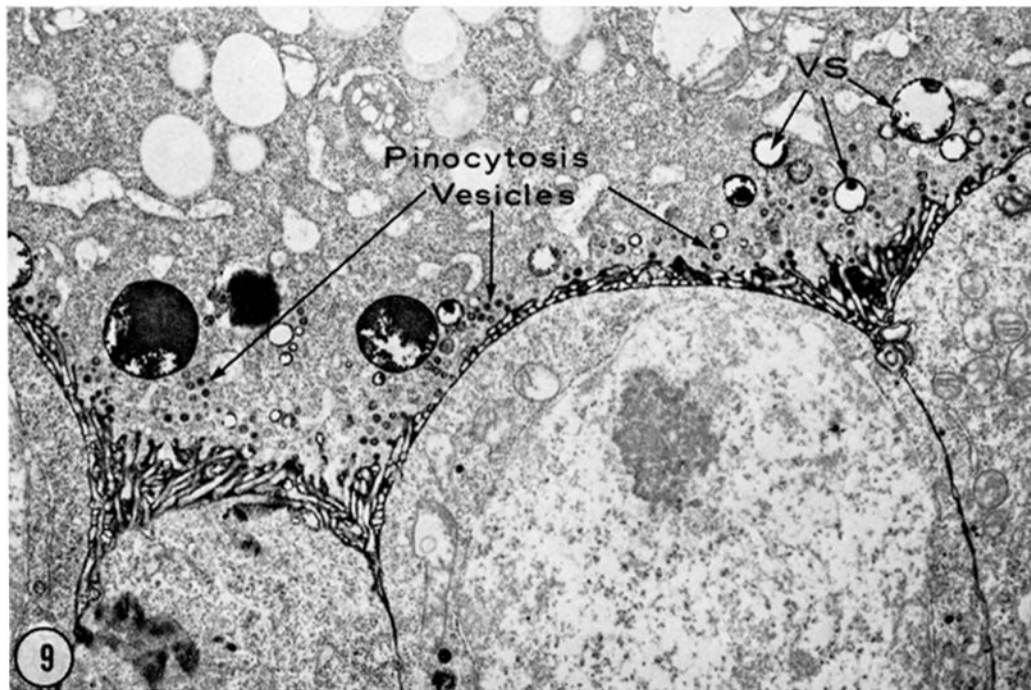
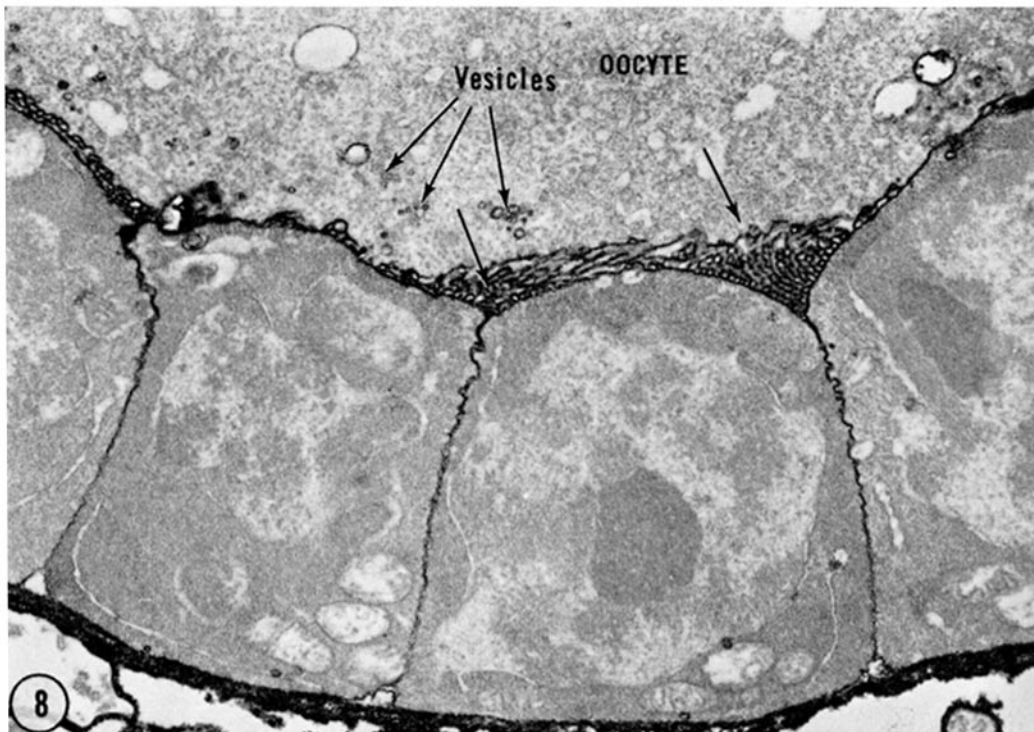


FIGURE 8 Ovaries from mosquitoes inoculated with peroxidase and fixed 2 hr after feeding upon blood. Projections of the oolemma are most prominent facing the junctions between follicle cells. $\times 10,000$.

FIGURE 9 At 3 hr after the blood-meal, injected tracer is observed within many pinocytotic vesicles and in larger vacuoles (VS) within the ooplasm. $\times 14,000$.

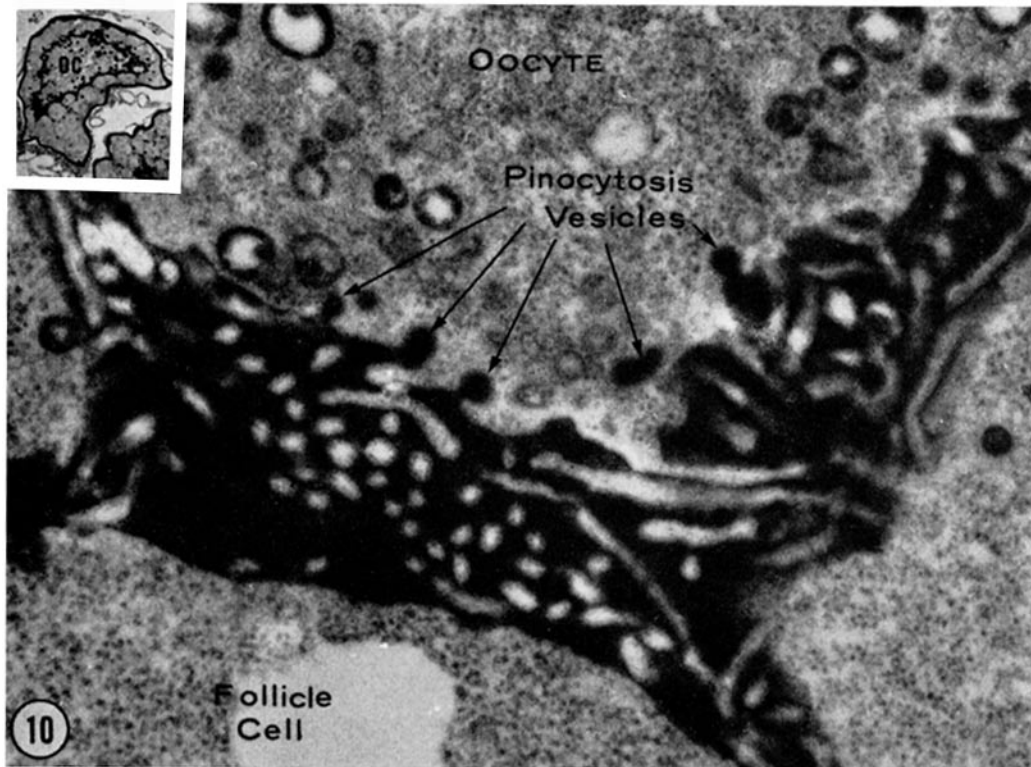


FIGURE 10 Ovary from mosquito inoculated with peroxidase and fixed 7 hr after feeding upon blood. Tracer fills the perioocytic space and the pits within the oocyte. The *inset* shows peroxidase within yolk plaques; *oc*, oocyte. $\times 25,000$; *inset*, $\times 400$.

Follicles did not enlarge significantly until the second day after blood-feeding (Fig. 1). Elongation was a function of yolk deposition and, thus, vitellogenesis proceeded most rapidly at about the 50th hr.

Paradoxically, pinocytosis declined during the second day after blood-feeding and ceased by the beginning of the third (Fig. 12). At 48 hr, the developing vitelline membrane obscured most of the oolemma. Microvilli were present solely in the spaces in the "pavement-like" membrane (Fig. 12). Concurrently, the follicle cells became flattened, and desmosomes appearing at their apices appeared to obliterate the interfollicle cell channels. Although peroxidase infiltrated the basement lamina, none appeared between follicle cells and none reached the surface of the oocyte. By 54 hr, vitelline membrane plaques began to fuse and appeared to separate the oocyte from the ribosome-rich follicle cells (Figs. 13 and 14).

Nature of the Channels between Follicle Cells

In order to determine whether the spaces between follicle cells were patent, ovaries were fixed in solutions containing Alcian blue and lanthanum. Lanthanum generally penetrated the follicle and was present within the basement lamina, in the interfollicle-cell spaces, and in the perioocytic space (Fig. 15). Lanthanum passed as freely into resting follicles as into those accumulating yolk and penetrated those vesicles that had not yet separated from the surface of the oolemma.

The presence of mucopolysaccharide within the follicle was demonstrated by ruthenium red and periodic acid-thiosemicarbazide-silver proteinate techniques. Both methods produced well-defined staining of the spaces between follicle cells (Fig. 16). In addition, pits, microvilli, and other exposed portions of the oolemma were stained.

Permeability of the Follicle during Vitellogenesis

We studied the penetration of exogenous material into actively developing follicles. Tracers of varied dimensions and molecular weight were employed (Table I) in an attempt to determine the size of the pores in the follicular membrane. Mosquitoes were injected at 3–12 hr after blood-feeding and sacrificed 1 hr later.

Cytochrome *c*, peroxidase, iron dextran, hemoglobin, and ferritin readily penetrated the follicle layer and were incorporated into the oocyte (Table I). Each of these tracer compounds was evident in advanced yolk plaques, in the spaces between follicle cells, and in the basement lamina (Figs. 17–20).

Uptake of catalase was variable, as it was incorporated by oocytes in some follicles but not in others. In those follicles in which tracer was identified, enzymatic activity was intense and the pattern of incorporation was similar to that of peroxidase.

A conjugate of IgG and peroxidase was readily taken up. However, this tracer was not incorporated into yolk, being limited to large vacuoles and smooth-surfaced cisternae in the cortex of the oocyte.

Thorotrast appeared to penetrate the follicle layer more slowly than did other molecules. However, once in the perioocytic space it was readily incorporated into yolk.

Only colloidal carbon failed to penetrate the follicle layer (Fig. 21). The basement lamina served as an absolute barrier to its passage.

In a series of mixed-tracer experiments, peroxidase was injected with ferritin, with Thorotrast, and with iron dextran. These “doubled” tracers were taken up by the oocyte simultaneously and incorporated into yolk plaques (Figs. 22–25). Staining by peroxidase was intense and its con-

centration was, therefore, reduced to 1/10 that of the other tracer in order to avoid masking.

DISCUSSION

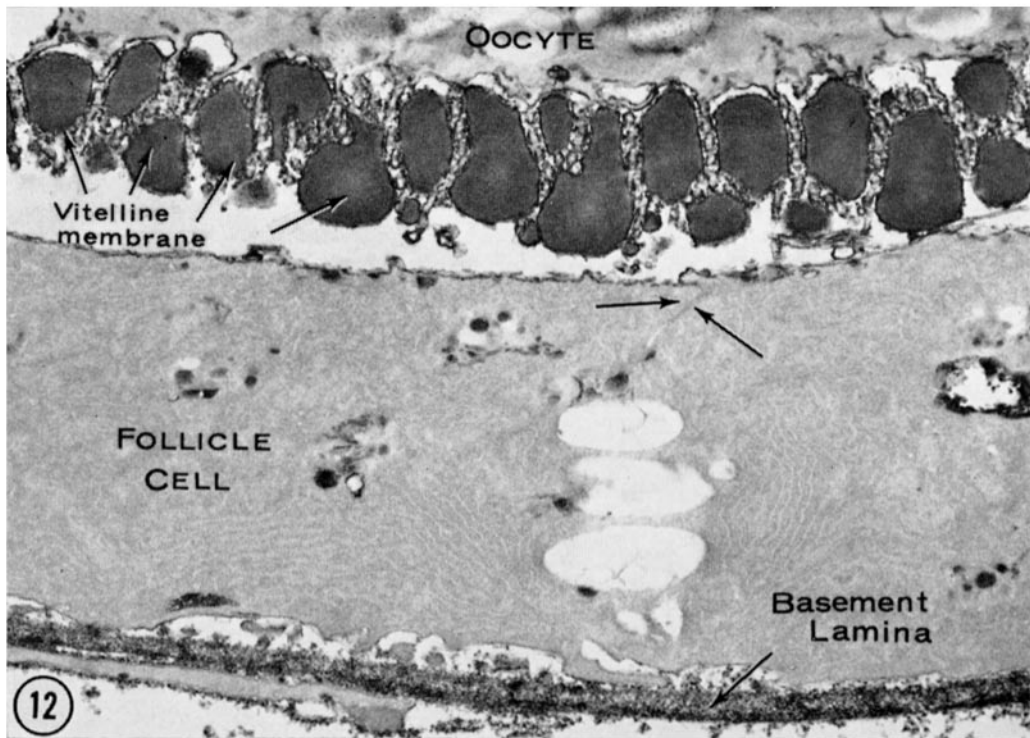
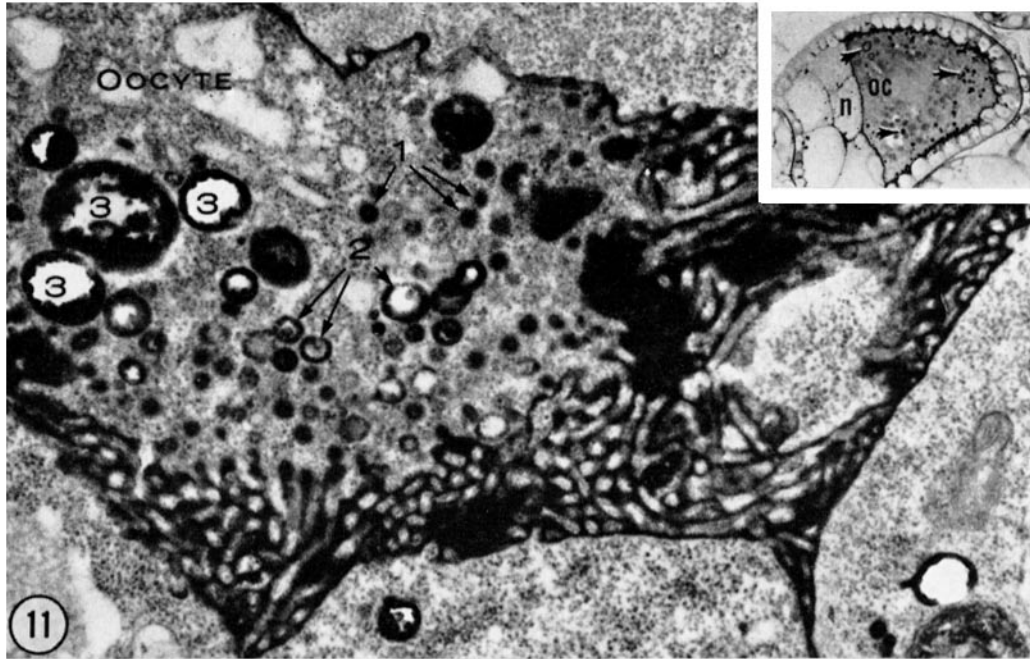
Passage of Material through the Follicle Epithelium

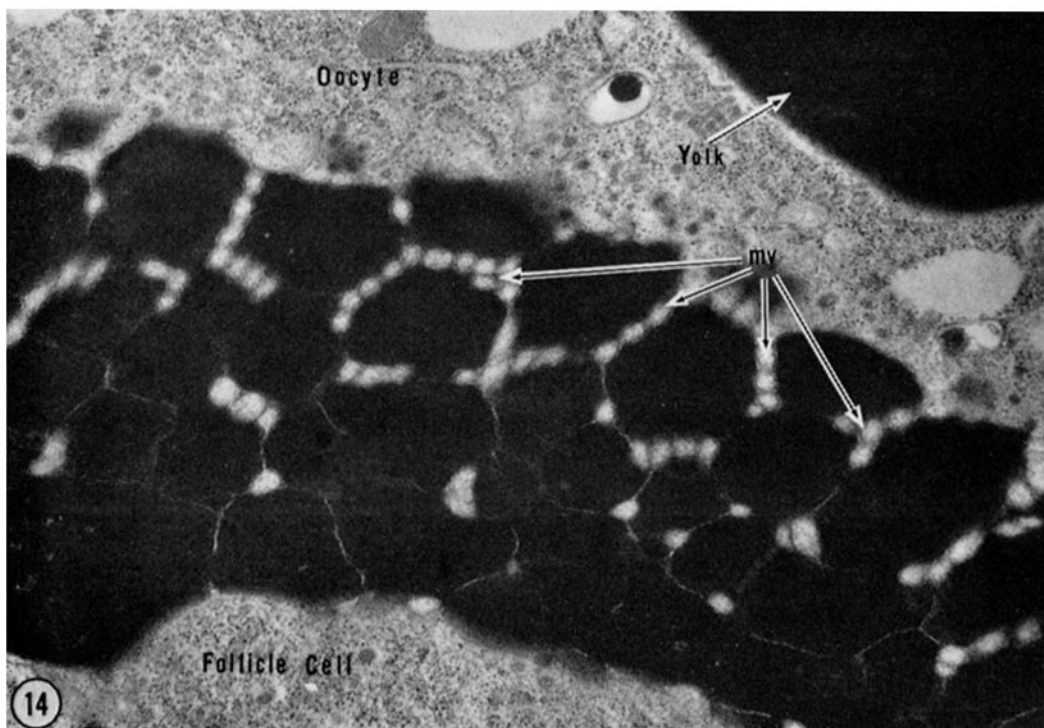
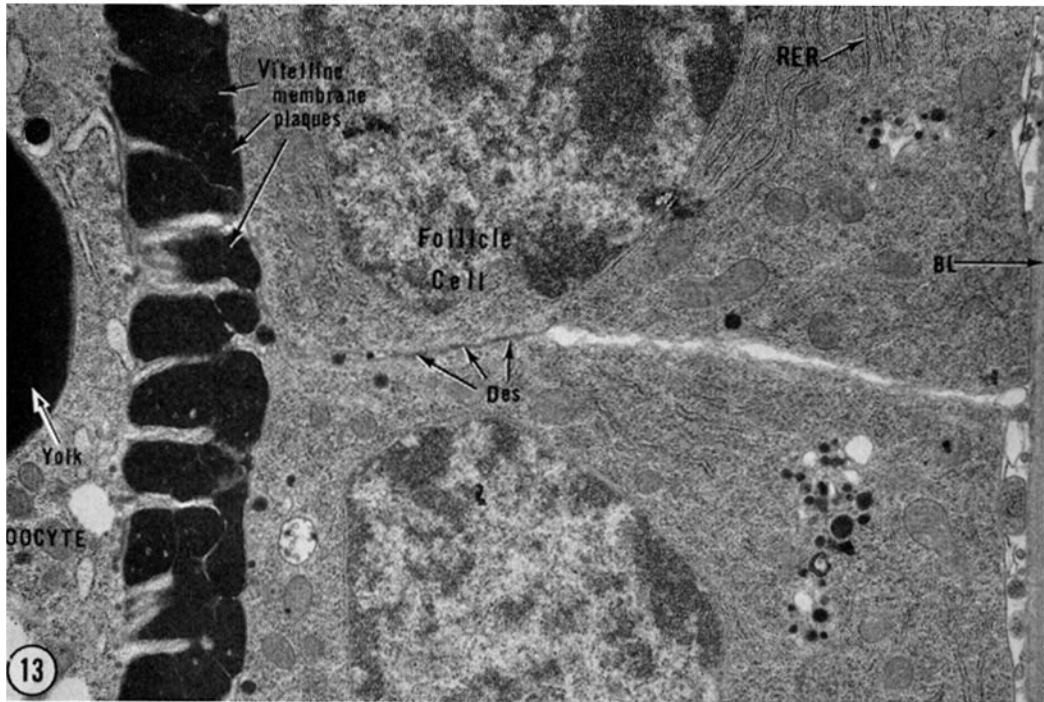
Deposition of yolk within oocytes of *A. aegypti* requires that the surrounding tissues be permeable to the precursors from which yolk is formed. The basement lamina and the enclosed layer of follicle cells ensheath the oocyte in such a manner as to present a potential barrier to the passage of this material. Indeed, Roth and Porter (1964) suggested that ovarian activity is determined by the state of permeability of the follicular sheath.

The outermost layer of this sheath, the basement lamina, is a coarse mechanical filter. It is freely permeable to particles with molecular weights ranging from 12,000 to 500,000 (cytochrome *c*, peroxidase, hemoglobin, ferritin, catalase, IgG-peroxidase, iron dextran, and Thorotrast) that have dimensions less than 110 Å. Molecules as large as carbon (300–500 Å) are totally excluded. Nor is the lamina highly selective of molecules with differing chemical properties, since both protein and polysaccharide tracers pass with apparent ease. However, certain inert particles (Thorotrast) seem to penetrate more slowly. Furthermore, with respect to the tracers tested, resting follicles are as permeable to injected materials as are actively vitellogenic follicles. Thus, in addition to its supportive role as a scaffolding beneath the follicle epithelium, the basement lamina plays a functional role as a molecular sieve, excluding large particles and perhaps retarding passage of inert particles. Our results are consistent with those based on *Cecropia* which conclude that proteins and other yolk precursors in the interfollicle-cell space have already undergone

FIGURE 11 At 25 hr after blood feeding, the tracer is present within all pits and cortical vesicles. Small cortical vesicles (1) fuse to form progressively larger vacuoles (2, 3) containing peroxidase and unstained material. *Inset*, some mature yolk plaques contain peroxidase (arrows); *oc*, oocyte; *n*, nurse cell. $\times 15,000$; *inset*, $\times 800$.

FIGURE 12 Ovary fixed 48 hr after blood-feeding (2 hr after peroxidase injection). Tracer is confined to the basement lamina. Some endogenous peroxidase is present in smooth cisternae of the oocyte, on the oolemma of microvilli projecting between the vitelline membrane plaques. The apices of adjacent follicle cells appear to be partially fused by a desmosome (arrows). $\times 15,000$.





FIGURES 13 and 14 Portions of the oocyte and follicle epithelium are illustrated here. At 54 hr after feeding upon blood, dense plaques forming the vitelline membrane occupy the space between the oocyte and follicle cells. Microvilli (*mv*) from the oocyte project between the dense plaques. Pinocytosis vesicles are few and prominent desmosomes (*Des*) appear to seal the apices of adjacent follicle cells. *RER*, rough endoplasmic reticulum; *BL*, basement lamina. $\times 22,000$ and $\times 33,000$, respectively.

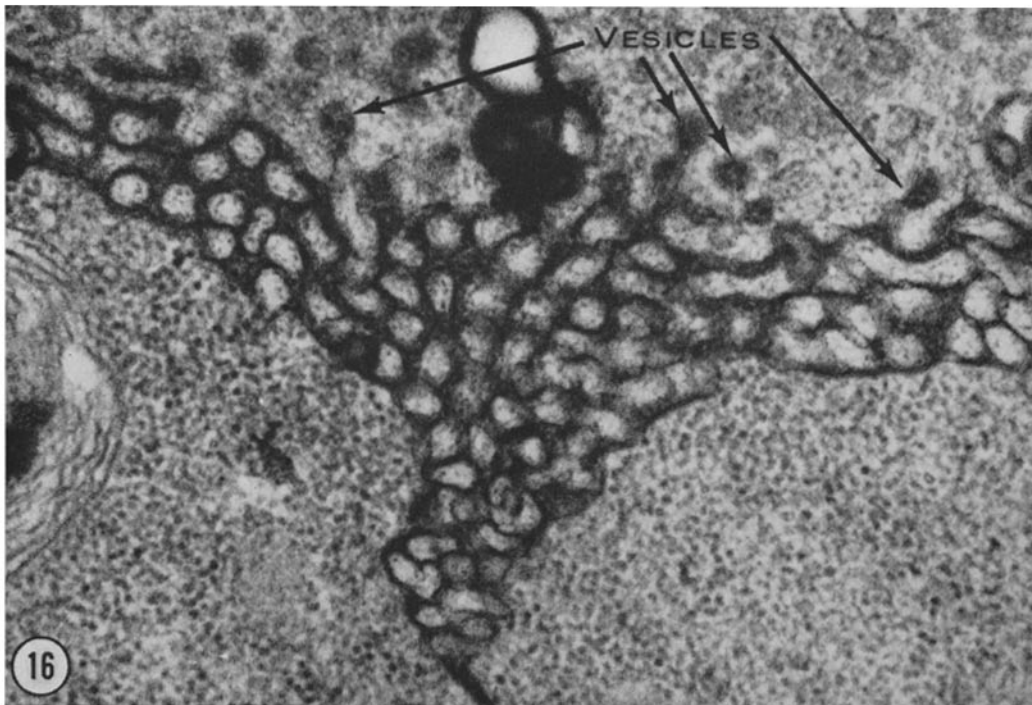
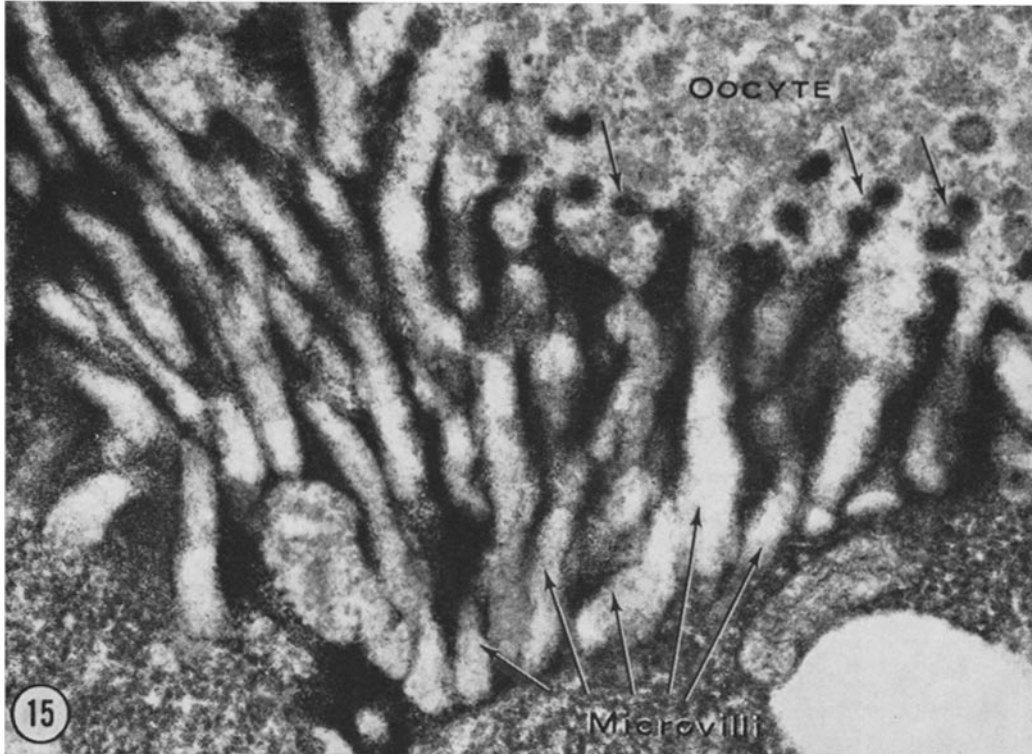


FIGURE 15 Oocyte from mosquito that fed upon blood 7 hr earlier. Lanthanum deposits are present between oocytic microvilli, in pits, in connected vesicles, and in vesicles that appear to be separated within the ooplasm (arrows). $\times 20,000$.

FIGURE 16 Ovary excised 7 hr after mosquito had fed upon blood, then incubated in ruthenium red. Stained material is present on the surface of follicle cells, in the space surrounding the microvilli, in pits, and in coated vesicles. $\times 20,000$.

TABLE I
Incorporation of Exogenous Tracers into Oocytes

Tracer	Approximate particle size	Approximate molecular weight	Degree of incorporation	Technique references
	A			
Cytochrome <i>c</i>	30	12,000	free	Karnovsky and Rice, 1969
Peroxidase	40-60	40,000	free	Graham and Karnovsky, 1966
Hemoglobin	60	64,500	free	Pietra et al., 1969
Iron dextran	70	70,000	free	Wartenberg, 1962
Thorotrast	50-80	—	limited	Revel, 1964
IgG-peroxidase	—	200,000	free	Nakane and Pierce, 1967
Catalase	100	240,000	limited	Venkatachalam and Fahimi, 1969
Ferritin	110	500,000	free	Farquhar and Palade, 1961 Bruns and Palade, 1968
Carbon	300-500	—	none	Cotran, 1967
Peroxidase + iron dextran	—	—	free	
Peroxidase + Thorotrast	—	—	limited	
Peroxidase + ferritin	—	—	limited	

some degree of selection by the basement lamina (Anderson and Telfer, 1970). The lamina of *A. aegypti* differs somewhat in that it appears to be largely a mechanical rather than a chemical filter.

The follicle epithelium surrounding the resting oocyte of *A. aegypti* is penetrated by narrow intercellular channels. Contrary to previous findings with this insect (Roth and Porter, 1964), we have found that the spaces between follicle cells remain constant, even when vitellogenesis is most prominent. Occasional dilatations do not traverse the entire layer of follicular epithelium. Instead, the channels are of highly uniform dimension, being approximately 200 Å wide. Our observations suggest a mucopolysaccharide lining, although we could not distinguish such an extraneous coat from polysaccharides in transit to the oocyte. Even in fixed ovaries containing resting oocytes these channels are permeable to Alcian blue-lanthanum.

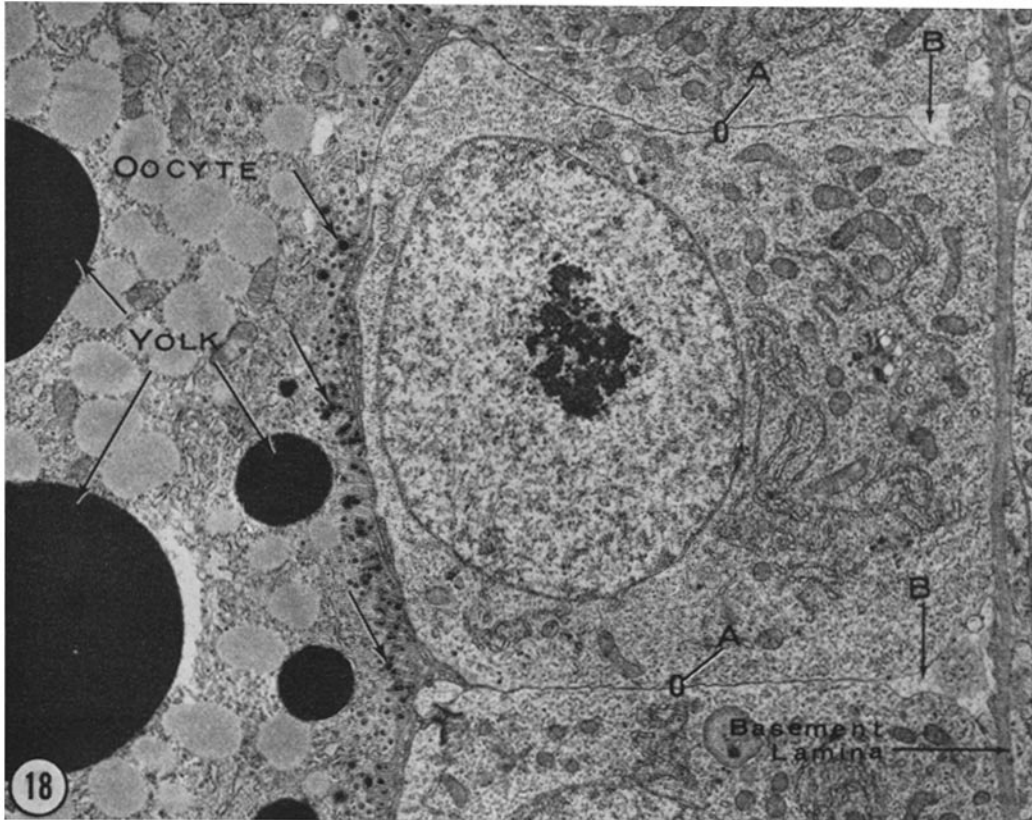
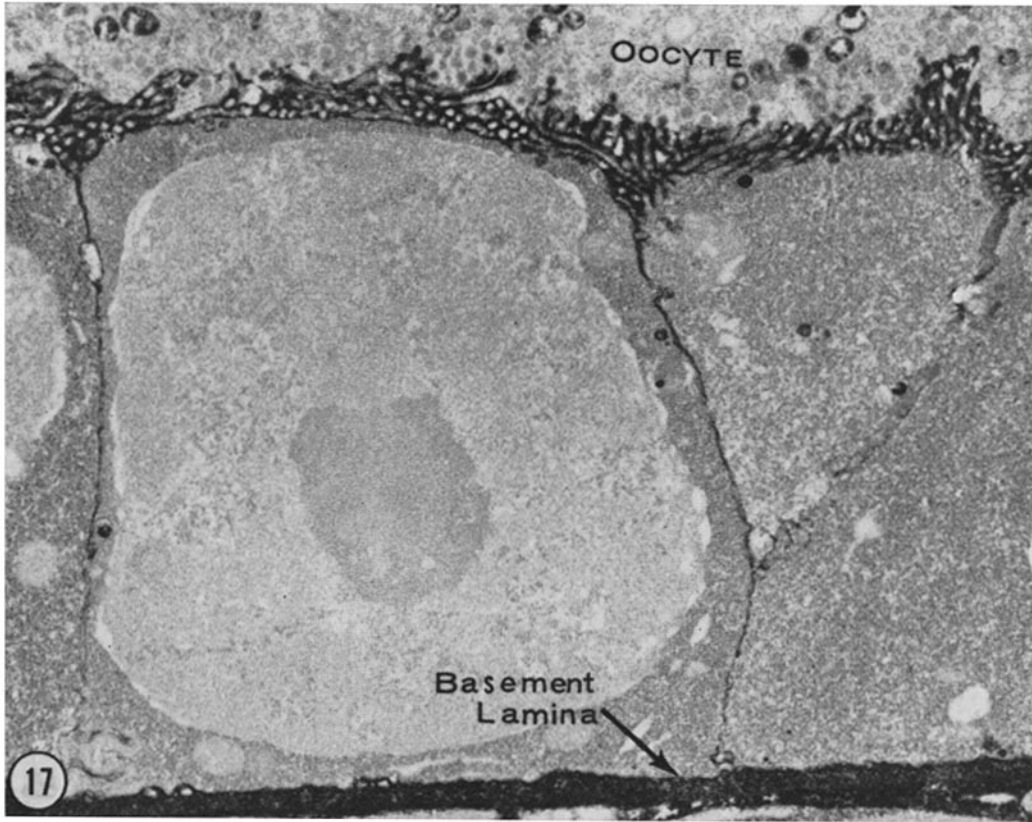
Narrow junctions obstructing the intercellular clefts are rare and occur with equal frequency in resting and in vitellogenic follicles.

After permeating the basement lamina, exogenous tracers fill the narrow follicle cell channels, but appear not to accumulate at these sites within the follicle. In contrast, follicles of *Cecropia* (Telfer, 1965; Melius and Telfer, 1969; Anderson and Telfer, 1970) and of *Nasonia* (King and Richards, 1969) contain intercellular expansions that contain reservoirs of vitellogenin. In *Aedes*, the bulk of exogenous tracer accumulates in the perioocytic space that is enlarged to accommodate it.

The uniform "tightness" of the follicle cell layer suggests that it may limit the flow of yolk precursors to the oocyte. That the follicle epithelium is an effective seal is apparent during later stages of vitellogenesis 36-48 hr after the blood-meal when intercellular channels become occluded by desmo-

FIGURE 17 Ovary of blood-fed mosquito injected with cytochrome *c*. Tracer is present in the basement lamina, in interfollicle cell spaces, in pits, and vesicles within the ooplasm. $\times 14,000$.

FIGURE 18 A portion of an oocyte from a blood-fed mosquito injected with hemoglobin. 5 hr after feeding, the space between adjacent follicle cells is narrow (A), although a blister-like expansion (B) is present near the basement lamina. Electron-opaque material interpreted as hemoglobin appears in vesicles (arrows) and in yolk. $\times 14,000$.



somes. The concomitant formation of dense plaques and their fusion to form the "vitelline membrane" further coincides with the cessation of micropinocytosis by the oocyte and with the loss of permeability of the follicle epithelium. In *Aedes*, as in other insects, the follicle epithelium appears to be involved in the synthesis of this "vitelline membrane" (Beams and Kessel 1969).

Uptake of Material by the Oocyte

Mosquitoes present an unusually favorable subject for the study of vitellogenesis because the onset of it is well defined, commencing within a few hours after the blood-meal. The primary event is a proliferation of microvilli and of pinocytotic invaginations of the oolemma. These amplifications of surface favor the uptake of yolk precursors.

The oolemma is the ultimate site of selection of yolk precursors, and chemical selectivity would, presumably, be exercised there by a mucopolysaccharide coat. Our preliminary observations suggest that such a glycocalyx may be present on the oocytes of mosquitoes. If the oolemma does have a mucopolysaccharide coat similar to that present on the oolemma of a cockroach (Anderson, 1969) and of a cricket (Favard-Séréno, 1969), and to the glycocalyx of vertebrate cells (Luft, 1966; Rambourg, 1969; Rambourg and Leblond, 1967; Thiéry, 1967), it may selectively bind certain molecules and concentrate them preliminary to their uptake by pinocytosis. As in amoebae, the act of binding to this extraneous coat might itself induce pinocytosis (Christiansen and Marshall, 1965). In the case of the mosquito, however, it is not yet clear whether cytochemical techniques show a glycocalyx or a polysaccharide component in transit to the oocyte.

Exogenous proteins and polysaccharides are incorporated into the oocyte more rapidly than are inorganic tracers. This suggests some degree of

selectivity by pinocytosis. However, no material reaching the surface of the oocyte was rejected. Size of the protein molecule does not greatly influence pinocytosis, as reported for other animal cells where larger proteins are favored (Ryser, 1970). In oocytes of *Aedes*, peroxidase (mol wt 40,000) and ferritin (mol wt 500,000) are taken up with similar avidity. Our double tracer studies demonstrate that exogenous compounds are not separated, but are incorporated within the same pinocytotic vesicle, thereby supporting Ryser's (1970) conclusions. Tracers along with endogenous yolk precursors are stored in the same yolk plaque. Moreover, there is no evidence of breakdown of the tracer following its uptake by the oocyte.

It is curious that pinocytosis by the oolemma is prominent solely in the early stages of vitellogenesis. Most yolk is deposited after the micropinocytosis has ceased, after formation of the vitelline membrane, and after the follicle cell layer has become impermeable to small protein molecules. This suggests that the site of yolk synthesis may shift from extra- to intra-oocytic sites during vitellogenesis.

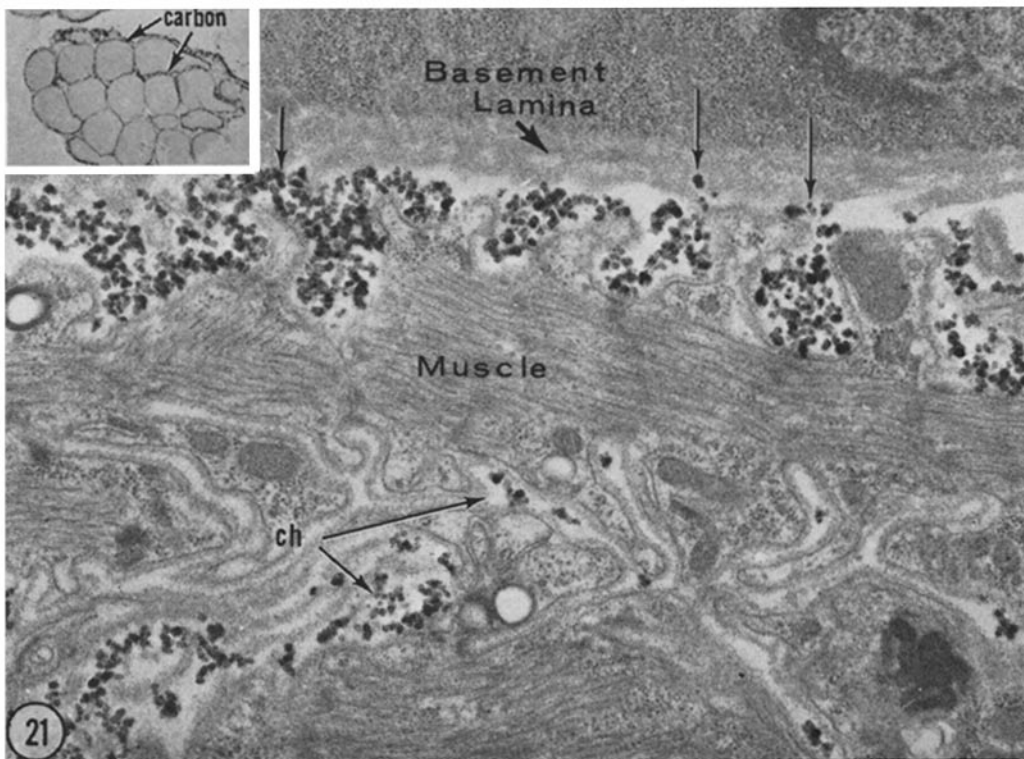
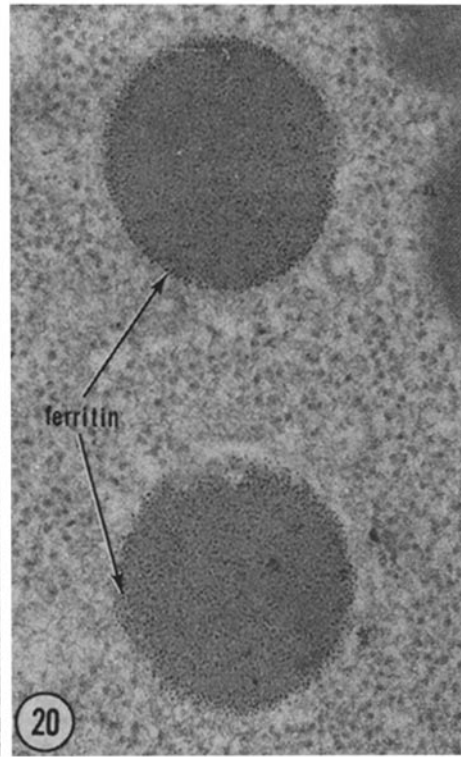
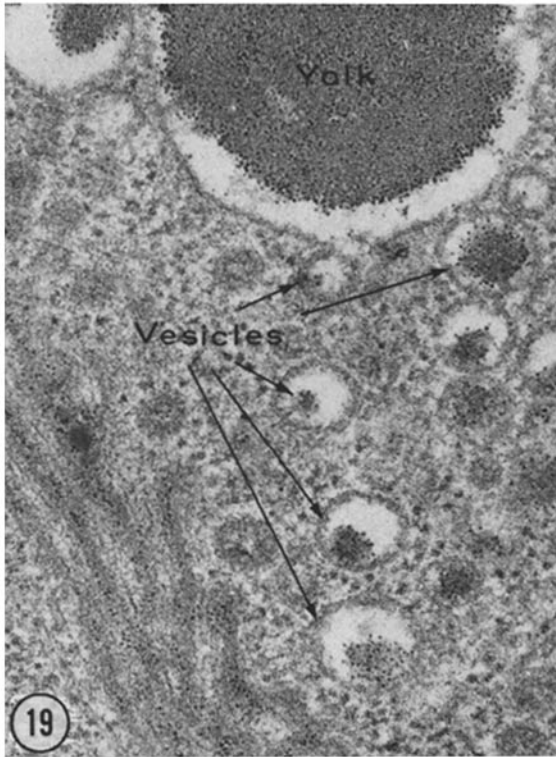
We thank Doctors M. J. Karnovsky and S. Shea, Department of Pathology, Harvard Medical School, for their encouragement and advice on this subject; Dr. P. Hoedemaeker, University of Gronigen, The Netherlands, for his assistance in preparing IgG-peroxidase conjugates; and Doctors Don Fawcett and J. P. Revel, Department of Anatomy, Harvard Medical School, and Dr. W. Massover, The Whitman Laboratory, University of Chicago, for reading this manuscript.

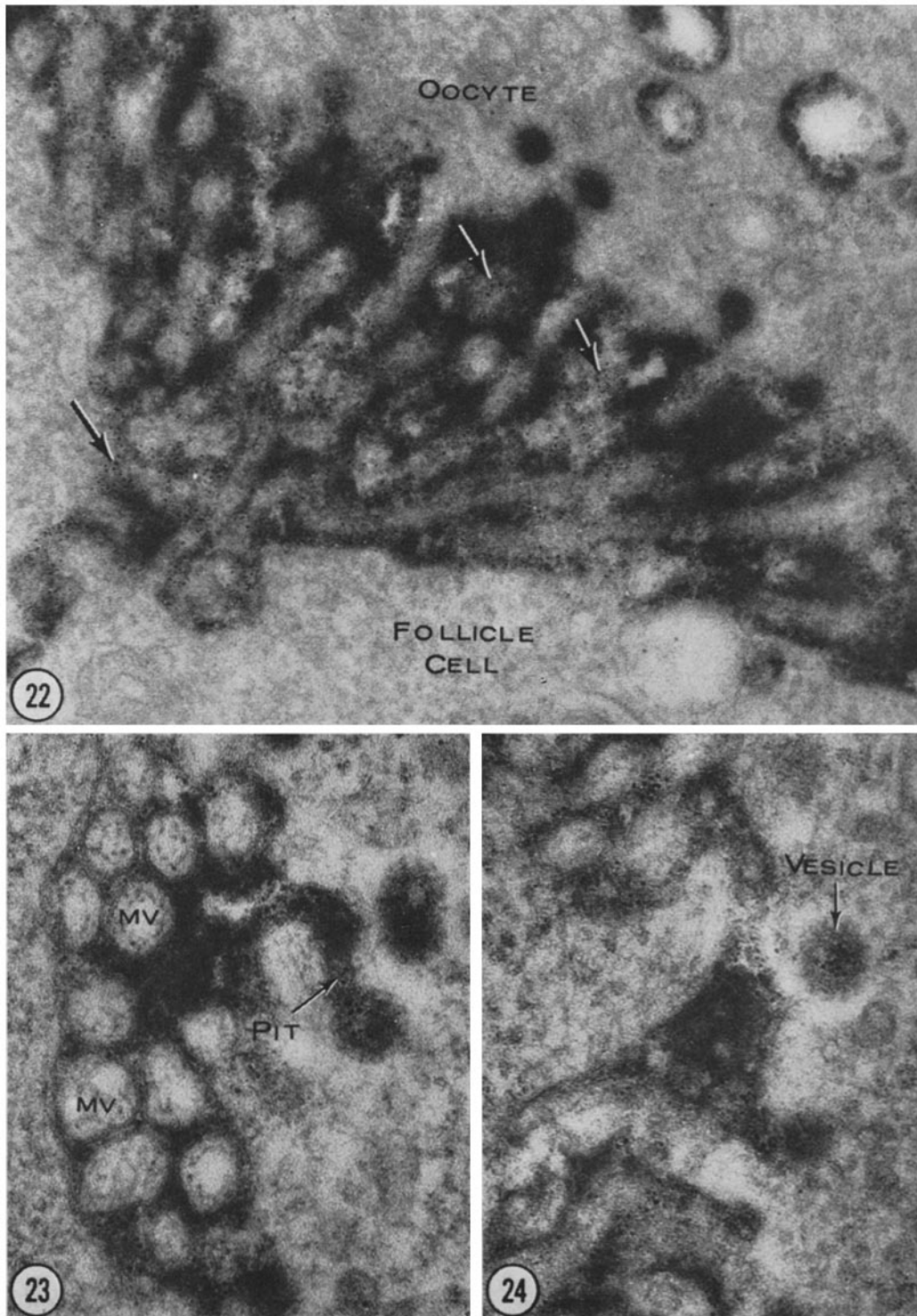
This work was supported in part by Research Contract No. NIH-69-2107 from the Center for Population Studies, by Public Health Service Grant No. AI-00046, and by Block Fund Grant No. 8, University of Chicago.

Received for publication 14 July 1970, and in revised form 3 February 1971.

FIGURES 19 and 20 Ovary of blood-fed mosquito injected with ferritin. Ferritin is present between microvilli, in cortical vesicles, and in larger storage granules. Within the vesicles, ferritin granules are associated with less dense amorphous material. $\times 30,000$ and $\times 20,000$, respectively.

FIGURE 21 Ovary of blood-fed mosquito injected with colloidal carbon. Carbon particles pervade the narrow channels (*ch*) of the muscular perifollicular sheath. Particles concentrate against the basement lamina (arrows) but do not penetrate. $\times 20,000$; *inset*, $\times 200$.





FIGURES 22-24 Ovary of a blood-fed mosquito that was injected with a mixture of peroxidase and ferritin. Ferritin appears as black punctate deposits (arrows) against the homogeneously opaque background of peroxidase. Both deposits appear together within the interfollicle cell space, in the perioocytic space (arrows), in pits, and in pinocytotic vesicles. *mv*, microvilli. Fig. 22, $\times 20,000$; Figs. 23 and 24, $\times 50,000$.

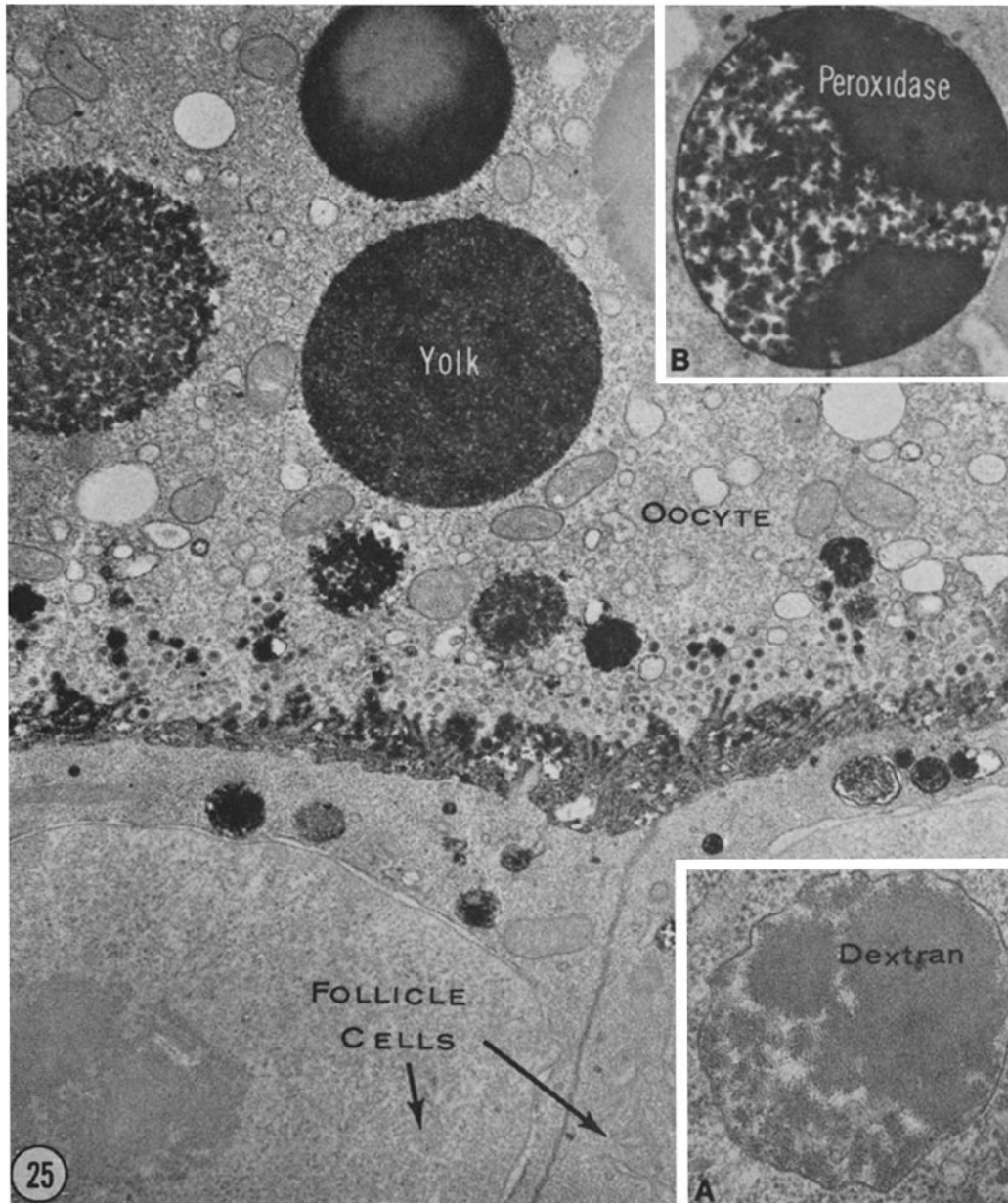


FIGURE 25 Ovary of blood-fed mosquito that was injected with a mixture of peroxidase and iron dextran. Both tracers are present within the perioocytic space, in pits, and in large accumulation droplets. The fine granular iron dextran deposits appear between the coarser peroxidatic reaction product. When iron dextran and peroxidase are injected separately, the iron dextran appears as fine granular material (*Inset A*), while the peroxidase reaction product appears coarser (*Inset B*). $\times 24,000$.

REFERENCES

- ANDERSON, E. 1964. Oocyte differentiation and vitellogenesis in the roach *Periplaneta americana*. *J. Cell Biol.* **20**:131.
- ANDERSON, E. 1967. A study of the specialization of the oolemma during oocyte differentiation in the roach *Periplaneta americana*. *J. Cell Biol.* **35**:(2, Pt. 2): 3A. (Abstr.)
- ANDERSON, E. 1969. Oogenesis in the cockroach, *Periplaneta americana*, with special reference to the specialization of the oolemma and the fate of coated vesicles. *J. Microsc.* **8**:721.
- ANDERSON, L. M., and W. H. TELFER. 1969. A follicle cell contribution to the yolk spheres of moth oocytes. *Tissue and Cell.* **1**:633.
- ANDERSON, L. M., and W. H. TELFER. 1970. Trypan blue inhibition of yolk deposition—a clue to follicle cell function in cecropia moth. *J. Embryol. Exp. Morphol.* **23**:35.
- BEAMS, H. W., and R. G. KESSEL. 1962. Intracisterna granules of the endoplasmic reticulum in crayfish. *J. Cell Biol.* **13**:158.
- BEAMS, H. W., and R. G. KESSEL. 1969. Synthesis and deposition of oocyte envelopes (vitelline membrane, chorion) and the uptake of yolk in the dragonfly (*Odonata: Aeschnidae*). *J. Cell Sci.* **4**:241.
- BEARD, M. E., and A. B. NOVIKOFF. 1969. Distribution of peroxisomes (microbodies) in the nephron of the rat. A cytochemical study. *J. Cell Biol.* **42**: 501.
- BODENSTEIN, D. 1946. Investigation on the locus of action of DDT in flies (*Drosophila*). *Biol. Bull.* **90**: 148.
- BRUNS, R. R., and G. E. PALADE. 1968. Studies on blood capillaries. I. General organization of blood capillaries in muscle. *J. Cell Biol.* **37**:244.
- CHRISTIANSEN, R. G., and J. M. MARSHALL. 1965. A study of phagocytosis in the amoeba *Chaos chaos*. *J. Cell Biol.* **25**:443.
- COTRAN, R. S. 1967. The fine structure of the microvasculature in relation to normal and altered permeability. In *Physical Basis of Circulatory Transport*. E. B. Reeve and A. C. Guyton, editors. W. B. Saunders Company, Philadelphia, Pa. 249.
- FARQUHAR, M. G., and G. E. PALADE. 1961. Glomerular permeability. II. Ferritin transfer across the glomerular capillary wall in nephrotic rats. *J. Exp. Med.* **114**:699.
- FAVARD, P., and N. CARASSO. 1958. Origine et ultrastructure des plaquettes vitellines de la planorbe. *Arch. Anat. Microsc. Morphol. Exp.* **47**:211.
- FAVARD-SÉRÉNO, C. 1969. Capture de polysaccharides par micropinocytose dans l'ovocyte du grillon en vitellogenèse. *J. Microsc.* **8**:401.
- GRAHAM, R., and M. J. KARNOVSKY. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**:291.
- HOEDEMAEKER, P. J., and S. ITO. 1970. Ultrastructural localization of gastric parietal cell antigen with peroxidase-coupled antibody. *Lab. Invest.* **22**:184.
- HOPKINS, C. R., and P. E. KING. 1966. An electron-microscopical and histochemical study of the oocyte periphery of *Bombus terrestris* during vitellogenesis. *J. Cell Sci.* **1**:201.
- ITO, S., and M. J. KARNOVSKY. 1968. Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. *J. Cell Biol.* **39**:(2, Pt. 2):168A. (Abstr.)
- KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**(2):137A. (Abstr.)
- KARNOVSKY, M. J., and D. F. RICE. 1969. Exogenous cytochrome c as an ultrastructural tracer. *J. Histochem. Cytochem.* **17**:751.
- KING, P. E., and J. G. RICHARDS. 1969. Oogenesis in *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). *Proc. Roy. Entomol. Soc. London Ser. A, Gen. Entomol.* **44**:143.
- LANZAVECCHIA, G. 1961. The formation of the yolk in frog oocytes. *Proc. Eur. Reg. Conf. Electron Microsc.* **2**:746.
- LUFT, J. H. 1966. Fine structure of capillary and endocapillary layer as revealed by ruthenium red. *Fed. Proc.* **25**:1773.
- MELIUS, M. E., and W. H. TELFER. 1969. An autoradiographic analysis of yolk deposition in the cortex of the cecropia moth oocyte. *J. Morphol.* **129**:1.
- NAKANE, P., and G. B. PIERCE. 1967. Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. *J. Cell Biol.* **33**:307.
- PIETRA, G. G., J. P. SZIDON, M. M. LEVENTHAL, and A. P. FISHMAN. 1969. Hemoglobin as a tracer in hemodynamic pulmonary edema. *Science (Washington)*. **166**:1643.
- RAMBOURG, A. 1969. Localisation ultrastructurale et nature du matériel coloré au niveau de la surface cellulaire par le mélange chromique-phosphotungstique. *J. Microsc.* **8**:325.
- RAMBOURG, A., and C. P. LEBLOND. 1967. Electron microscope observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. *J. Cell Biol.* **32**:27.
- REVEL, J. P. 1964. A stain for the ultrastructural localization of acid mucopolysaccharides. *J. Microsc.* **3**:535.
- ROTH, T. F., and K. R. PORTER. 1964. Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* **20**:313.
- RYSER, H. J.-P. 1970. Transport of macromolecules, especially proteins, into mammalian cells. *Proc. Int. Congr. Pharmacol.* **4th**. 96.

- SHEA, S., and M. J. KARNOVSKY. 1969. The cell surface and intercellular junctions in liver as revealed by lanthanum staining after fixation with glutaraldehyde and added Alcian blue. *J. Cell Biol.* 43(2, Pt. 2):128A. (Abstr.)
- STAY, B. 1965. Protein uptake in the oocytes of the *Cecropia* moth. *J. Cell Biol.* 26:49.
- TELFER, W. H. 1961. The route of entry and localization of blood proteins in the oocytes of saturniid moths. *J. Biophys. Biochem. Cytol.* 9:747.
- TELFER, W. H. 1965. The mechanism and control of yolk formation. *Annu. Rev. Entomol.* 10:161.
- THIÉRY, J. P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.* 6:987.
- VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* 25:407.
- VENKATACHALAM, M. A., and H. D. FAHIMI. 1969. The use of beef liver catalase as a protein tracer for electron microscopy. *J. Cell Biol.* 42:480.
- WARD, R. J. 1962. The origin of protein and fatty yolk in *Rana pipiens*. *J. Cell Biol.* 14:309.
- WARTENBERG, H. 1962. Elektronenmikroskopische und Histochemische Studien über die Oogenese der Amphibieneizelle. *Z. Zellforsch. Mikrosk. Anat.* 58:427.