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ULTRASTRUCTURAL STUDIES ON THE SURFACE MEMBRANE OF THE MOUSE EGG

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SUMMARY

Fertilized and unfertilized mouse eggs were examined by scanning and transmission electron microscopy for evidence of mosaicism in the organization and concanavalin A-binding properties of their surface membranes. No obvious quantitative mosaicism in concanavalin A binding was noted. The egg membrane was microvillous over most of its surface, but was smooth in the region overlying the 2nd metaphase spindle of the unfertilized egg and on the polar body of the fertilized egg.

INTRODUCTION

We have previously reported a mosaicism in the organization of Concanavalin A (Con A) receptors on the surface of the unfertilized mouse egg that may be dependent on microfilament activity. The area of the egg membrane overlying the second metaphase spindle was found to be relatively deficient in binding sites for fluorescein-labelled Con A and was specifically excluded as the membrane of the second polar body. The region of Con A negativity has also been shown to bind uncapacitated mouse spermatozoa relatively poorly, if at all, which has lead us to suggest that the surface mosaicism might reflect a mechanism for avoidance of fertilization at the site of polar body extrusion (Johnson, Eager, Muggleton-Harris & Grave, 1975).

Two possible explanations were offered for the mosaicism. Either the relative deficiency of Con A binding on the membrane overlying the metaphase spindle could reflect a reduced density of Con A receptor molecules at that site, or the apparent density of such receptors might be reduced as a result of the absence of surface membrane corrugations. We have now undertaken a study by scanning and transmission electron microscopy in an attempt to distinguish these possibilities.

METHODS

Over 300 fertilized or unfertilized eggs were obtained from 3- to 6-month-old female outbred mice. Five international units of pregnant mare serum gonadotropin (Folligon from Intervet, Organon Laboratories, Surrey) were injected intraperitoneally between 17.00 and 18.00 hours followed 48 h later by injections of 5 I.U. of human chorionic gonadotropin (Chorulon from Intervet). Eggs were recovered 3–4 h after superovulation and cumulus cells were removed with hyaluronidase (Koch-Light 50 I.U./ml in phosphate-buffered saline with 10 % polyvinylpyrrolidone). Zonae pellucidae were removed by placing the eggs in Pronase (Calbiochem 0·5 %, Tris-citrate buffer, pH 7·0) for 5 min at 37 °C, in acidic Tyrode's solution, pH 2·5, with 0·4 % polyvinylpyrrolidone for 5–15 s at 20 °C, or in 0·1 M β-mercaptoethanol in 0·01 M Tris-HCl,

0·9 % NaCl, 0·4 % polyvinylpyrrolidone, pH 7·4 for 30–40 min at 20 °C. The eggs were immediately washed in medium PB1 before 30-min incubations at 37 ° or 4 °C in medium PB1 with or without reagents. The reagents used were $5{\cdot}0 \times 10^{-4}$ M sodium azide, 10 μg/ml cytochalasin B, 1 % dimethylsulphoxide (DMSO) and 3·6 μg/ml colcemid. In some cases, 0·86–1·72 mg/ml of ferritin-labelled concanavalin A (Miles Laboratories) were added to the incubations. Following incubation, the eggs were again washed briefly with medium PB1 before processing for scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

All eggs were fixed for 1 h at 4 °C in Karnovsky's fixative (Karnovsky, 1965) and washed 3 times in 0·1 M sodium cacodylate. Eggs for TEM were postfixed in 1 % osmium tetroxide in 0·1 M Na cacodylate for 1 h at 4 °C, transferred by mouth pipette through a graded acetone series (25, 50, 70, 95, 100 and 100 %) and embedded in Araldite. Fifty-nanometre thin sections, stained with lead citrate and uranyl acetate, were viewed in the Philips 300 electron microscope. Unstained sections were viewed at 60 kV. Eggs for SEM were transferred after fixation to small brass carriers designed by K. W. Thurley and R. Brignell. The eggs were held in these carriers by a very fine stainless steel mesh from N. Greening (London) Ltd. After dehydration through a graded acetone series and drying from 100 % acetone via CO_2 in a Polaron E3000 critical-point drying apparatus, the mesh containing the eggs was attached by double-sided tape to an aluminium stub and coated to a thickness of 40–50 nm in a Polaron Sputter Coater (E5000) using a gold target. The eggs were subsequently viewed in a Cambridge S600 scanning electron microscope operated in the secondary mode at 15 kV.

RESULTS

Unfertilized mouse eggs

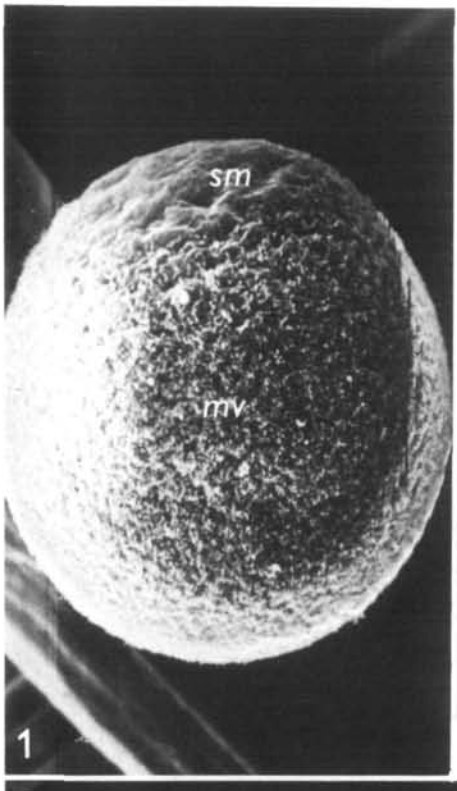
Clear mosaicism of surface ultrastructure was revealed by both scanning and transmission electron microscopy. A small polar region devoid of microvilli was consistently observed, and was shown in transmission electron microscopy to overlie that region of cytoplasm containing the second metaphase spindle and relatively devoid of cytoplasmic organelles (Figs. 1–3). Similar results were obtained whether the zona was removed by pronase or by acidic tyrode, but use of mercaptoethanol produced rather variable results.

Treatment of the egg with 10 μg/ml cytochalasin B resulted in a progressive change in increasing numbers of eggs over a period of 90 min as viewed by SEM. While the microvilli became shorter, stumped and ballooned, the region of previously smooth membrane became heavily corrugated with fine ridges (Figs. 4, 5). In addition microvillous-like structures started encroaching on the previously smooth area of egg membrane (Fig. 6). A similar, but not so marked effect was seen with azide treatment, but not with DMSO or colcemid.

Fig. 1. SEM of an unfertilized mouse egg showing the typical microvillous membrane (*mv*) and the polar region of smooth membrane (*sm*). × 9000.

Fig. 2. Higher-power SEM of a similar egg. Note the interface between microvillous (*mv*) and smooth membrane (*sm*) areas indicated by arrows. × 27 500.

Fig. 3. Low-power TEM of an unfertilized mouse egg similar to that seen in Fig. 1. The boundary between the microvillous and smooth membranes is indicated by arrows. Note that the orientation is the same as in Fig. 1 and that smooth membrane overlies the organelle-free cytoplasm containing the metaphase chromosomes (*). × 1250.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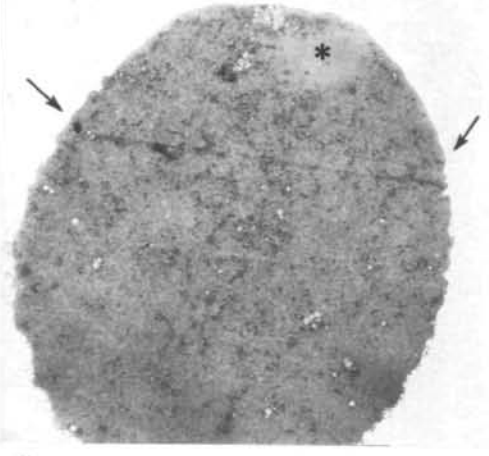
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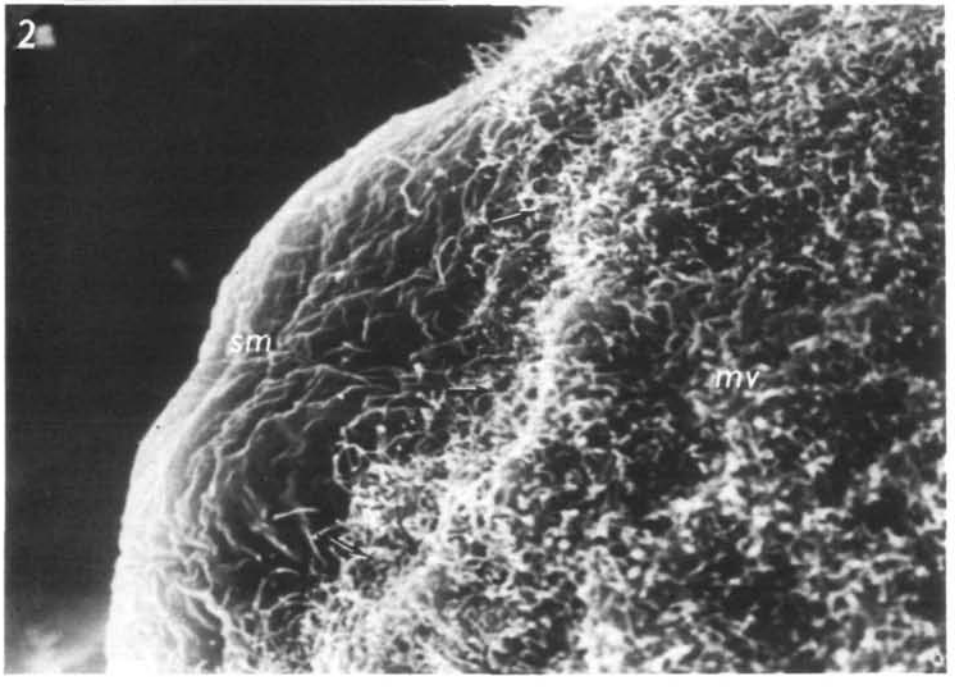
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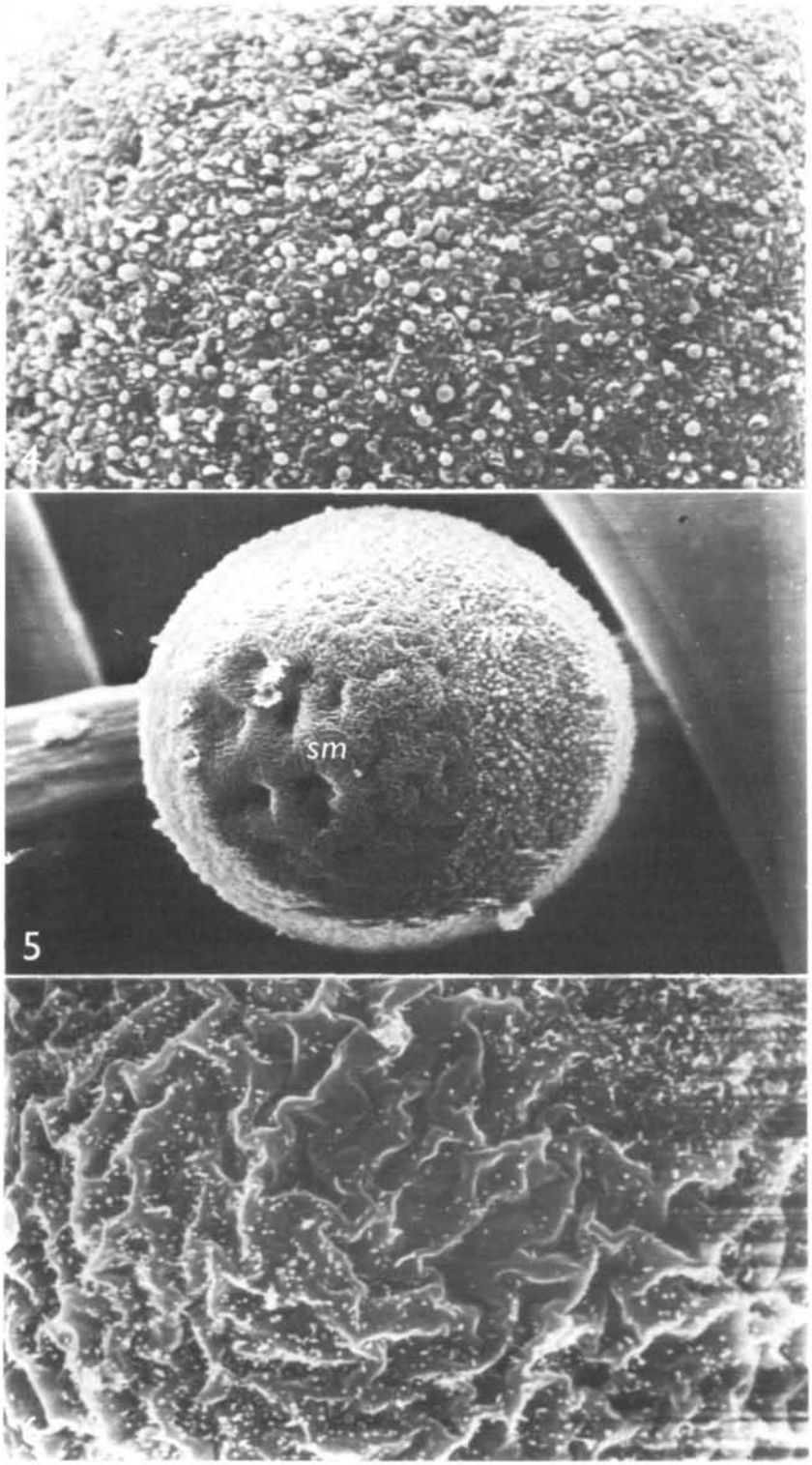
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Treatment of normal eggs with ferritin-labelled Con A prior to fixation resulted in staining of the egg membrane. Staining was not uniform, but occurred in clumps. No unequivocal quantitative difference in the density of ferritin particles could be detected in the various parts of the membrane (Figs. 7, 8), except at points of contact between adjacent eggs, when dense deposits were evident.

Fertilized mouse eggs

The structural mosaicism seen in the unfertilized eggs was preserved following fertilization. The area of membrane devoid of microvilli was specifically excluded in the second polar body (Figs. 9–12), the mid-body membrane becoming intensely corrugated and the membrane of the egg having an enhanced microvillous appearance.

DISCUSSION

The mosaicism of membrane conformation under different conditions described for both fertilized and unfertilized mouse eggs exactly parallels that mosaicism of binding sites for fluorescent-Con A reported previously (Johnson *et al.* 1975). In other words, the presence or absence of fluorescent staining correlates positively with the presence or absence of microvilli. In contrast, the density of ferritin-labelled Con A on smooth-membraned surfaces was not seen to be significantly different from the density of ferritin stain on microvillous surfaces. We therefore suggest that the membrane mosaicism could be more readily explained on the basis of structural variation than on the basis of an unequal molecular distribution. Our observations do not preclude minor quantitative variation in the density of Con A receptors at different sites nor do they preclude qualitative differences in, for example, the mobility of such receptors in different regions of the membranes. However, no such variation has been reported by other workers (Nicolson, Yanagimachi & Yanagimachi, 1975).

This interpretation of the results would also readily explain the much greater incidence of sperm binding to the highly microvillous portion of egg membrane, since it has been demonstrated that a microvillous structure favours cell contact and agglutination (Nicolson, 1974; Willingham & Pastan, 1975). Furthermore, binding molecules might preferentially localize on the apices of microvilli, thus strengthening

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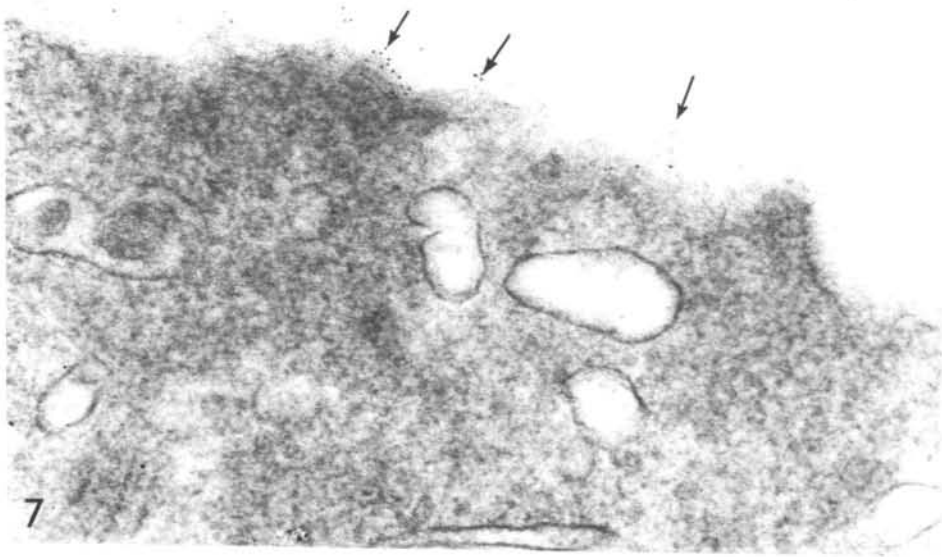
Fig. 4. SEM of the microvillous membrane of an unfertilized egg incubated in 10 μg/ml cytochalasin B for 30 min. The 'ballooning' microvilli are typical of eggs treated with cytochalasin B and clearly differ from the microvilli of the untreated egg seen in Fig. 2. × 20000.

Fig. 5. Lower-power SEM of a cytochalasin B-treated egg. Ballooning microvilli are again seen and fine ridges have formed in the smooth polar region. Note that the interface between the smooth (*sm*) and microvillous (*mv*) regions is not as distinct as in the untreated egg (see Figs. 1 and 2). × 9000.

Fig. 6. SEM of the smooth membrane of another fertilized mouse egg treated with cytochalasin B. The entire polar region has been invaded by stubby microvilli. × 22500.



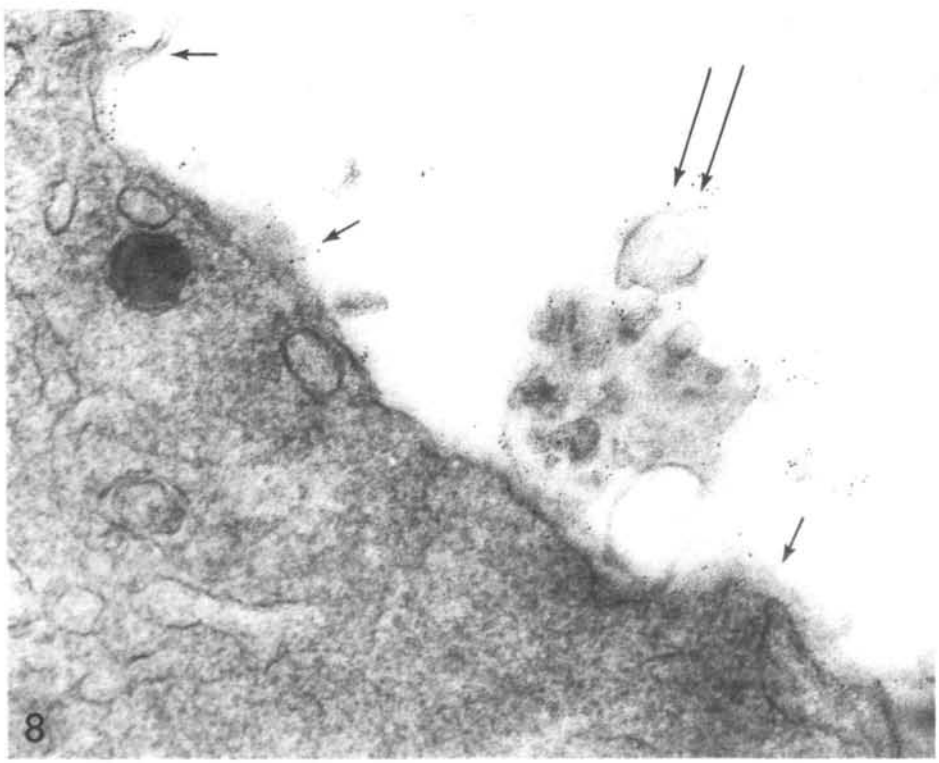


Fig. 7. TEM of the smooth membrane of an unfertilized mouse egg treated with ferritin-labelled Con A. Arrows indicate ferritin staining. Unstained. × 65 000.

Fig. 8. TEM of the microvillous membrane of an unfertilized mouse egg treated with ferritin-labelled Con A. Ferritin staining is indicated by arrows. Paired arrows indicate extensive microvilli formation. Unstained. × 58 500.

the degree of cross-linking to adjacent cells. Since binding of spermatozoa is presumably a necessary prelude to fusion, sperm penetration would thus be favoured distant from the site of polar body extrusion.

The organization of surface mosaicism seems to be at least partly dependent on a mechanism sensitive to cytochalasin B. A very clear polarity of the cytoplasmic content of the egg is also evident (Odor & Renninger, 1960) and this presumably is maintained by the cytoskeletal system of the cell. It is plausible to consider that the cytoskeletal elements that maintain most organelles away from the spindle area of the egg, perhaps directly or indirectly via anchorage to the surface membrane, might also be involved in the corrugation of this adjacent membrane. This possibility is currently under investigation.

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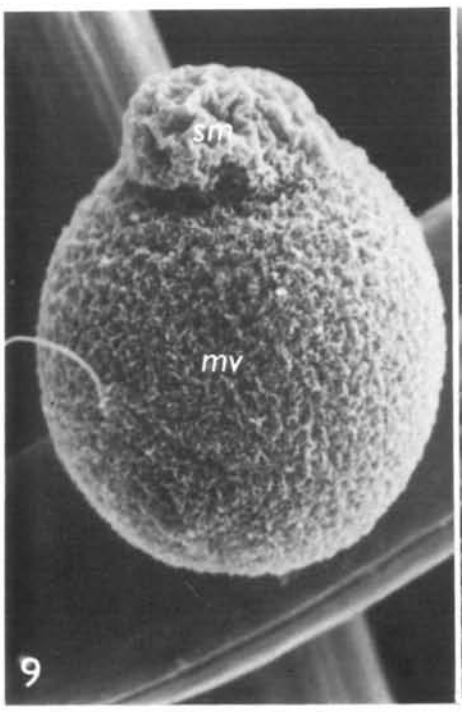
Fig. 9. SEM of a fertilized egg extruding a polar body. Note the smooth membrane (*sm*) on the polar body and the sharp line of division following the interface of the smooth and microvillous (*mv*) membranes. × 8000.

Fig. 10. Higher-power SEM of Fig. 9, showing the smooth membrane (*sm*) of the extruding polar body and the microvillous (*mv*) surface of the egg. × 20000.

Fig. 11. A fertilized egg is shown with an extruded polar body. Note the microvillous surface of the egg and mid-body (*mb*) and the smooth membrane on the polar body (*pb*). × 7000.

Fig. 12. This TEM shows another fertilized mouse egg and extruded polar body. The surface of the egg and mid-body (*mb*) are microvillous while the polar body (*pb*) surface is smooth. Double stained with lead citrate and uranyl acetate. × 11250.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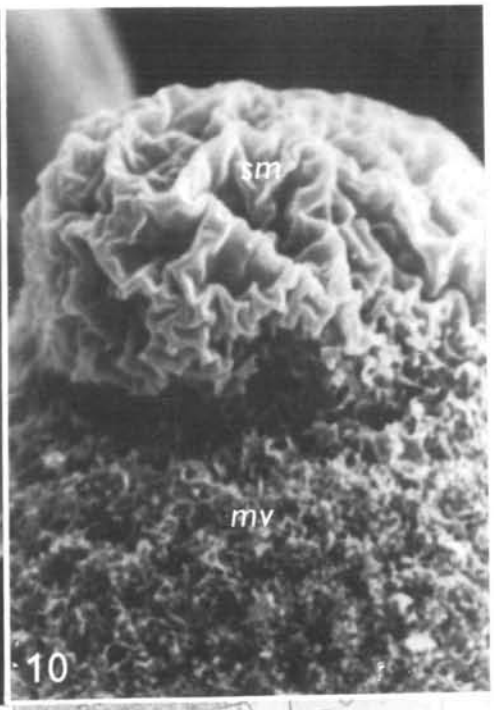
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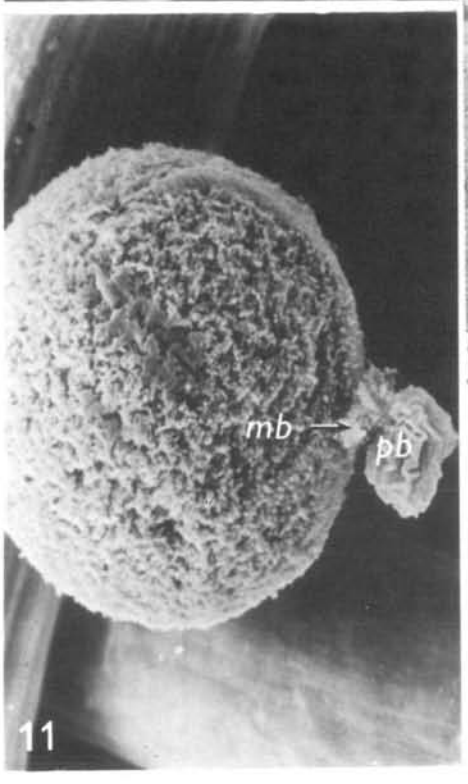
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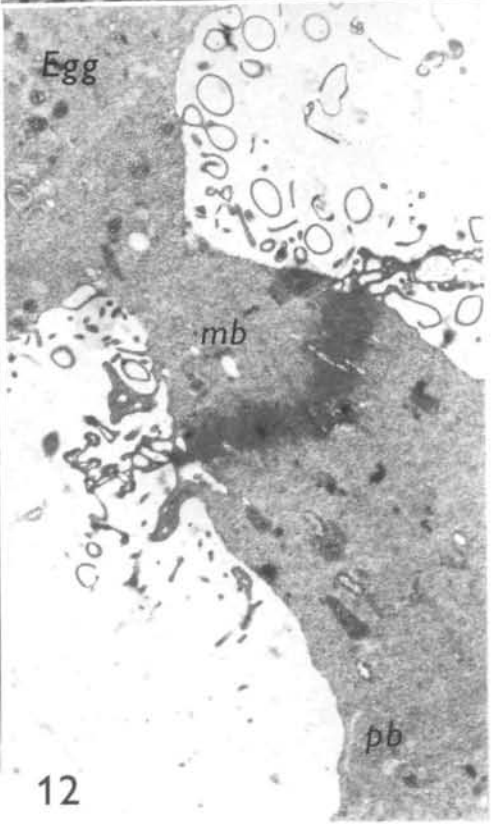
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