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ULTRASTRUCTURE AND PHYSIOLOGY OF CYTOKINESIS IN AN AMPHIBIAN EMBRYO (*Xenopus laevis*)

by

PAWAN K. SINGAL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled ULTRASTRUCTURE AND PHYSIOLOGY OF CYTOKINESIS IN AN AMPHIBIAN EMBRYO (*Xenopus laevis*) submitted by PAWAN K. SINGAL in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Abstract

First cleavage *Xenopus* embryos were studied to determine ultrastructural [illegible] of the furrow, its time related development, and to investigate the source of [illegible] membranes as well as the source and [illegible] of the cell surface material. The morphology and [illegible] of mitochondria and other organelles during cytokinesis has also been examined.

The cleavage furrow [illegible] the appearance of a single stripe of pigment granules. Thereafter the membrane started dipping [illegible] with the simultaneous appearance of furrow lines. The latter appeared as membrane folds in scanning electron micrographs and were accompanied by the presence of microfilaments in a corresponding area of the cortical cytoplasm. Furrowing accompanied the furrow tip only to a depth of about 300 Å. Thereafter they remained stationary and were present as a band (5 - 10 μ wide). First cleavage took 45 ± 5 minutes for its completion. Daughter cells showed contacts closer than 30 Å.

Two morphologically distinct types of vesicles were present in the cytoplasm close to the developing furrow. A large type (0.3 - 0.5 μ diameter) with fibrous contents, fused with the furrow membrane behind the tip, and apparently released a fibrous exudate onto the surface. This corresponded with increased lanthanum staining of this surface. Smaller vesicles (400 - 600 Å diameter) were present near the tip, where lanthanum staining was either patchy or uniform, and were associated with Golgi elements. The completed furrow entrapped small vesicles in the intercellular space. The variations in the lanthanum-

[illegible] this material in [illegible] the [illegible]

[illegible] Golgi bodies and [illegible] with [illegible] appear, indicating their probable [illegible] from the latter. Rough [illegible] reticulum, mainly [illegible] of tubular cisternae and vesicles, appeared [illegible] the Golgi body and occasionally showed [illegible] with [illegible]. The Golgi body showed several morphological forms. Different types of laminated figures were seen, some of which were noticed close to the Golgi body and the furrow membrane. These topographical associations among the cell constituents indicated a route which probably is followed for the assembly of the cell surface material.

The involvement of the Golgi body in furrow formation was investigated cytochemically. Thiamine pyrophosphatase was localized within the outermost cisternae of the Golgi body, in some associated vesicles, and in the furrow. Osmium tetroxide-zinc iodide reaction showed a reaction in the Golgi-associated vesicles and in the furrow membrane. This distribution indicated that some of the furrow membrane and surface material was supplied by the Golgi body via vesicles.

Embryos grown in fucose-H^3 solution were studied. Formation of the furrow was accompanied by fucose incorporation and the appearance of silver grains in the intercellular space.

In conclusion, the process of cytokinesis in *Xenopus* embryos involves membrane [illegible] intercellular contacts and membrane growth. Vesicle fusion activity is responsible for at least

[redacted]

I express my feelings of appreciation to

[illegible] on which this thesis is based. His support, encouragement and

[illegible] has gone a long way in the preparation of this thesis.

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INTRODUCTION

Reproduction, whether [illegible] cell [illegible] dependent upon [illegible] cellular [illegible] cell division [illegible] [illegible] for [illegible] consists [illegible] of complicated [illegible] anaphase and telophase, occurring [illegible] by cytokinesis. Mitosis and cytokinesis are [illegible] but [illegible] separately accelerated processes of cell division. At the end of cytokinesis the newly laid down membrane partitions becomes an [illegible] of the overall limiting membrane which [illegible] the daughter cells with their own internal environment. This membrane partition is complex in its role [illegible]. In addition to the above, it also provides a site for cell-cell interaction or communication and cell recognition. This property is vested upon the partitions membrane by the presence of the cell surface material. Thus, the problem of cytokinesis has two [illegible] aspects: a) formation and supply of new membranes and b) synthesis and transport of the cell surface material and its distribution on the cell surface. These aspects of cytokinesis have been studied in the dividing cells of *Xenopus laevis*.

The early cleavage *Xenopus* embryo is good experimental material for such a study because of its relatively large size (1.2 mm average diameter) and the observation of the time related development of the furrow is relatively easy. Moreover, partition in a large size

egg apparently requires the growth of a large amount of new membrane, a phenomenon which lends itself to ultrastructural analysis. Further, the site of the cleavage furrow in this material can be precisely localized using a dissecting microscope and also a large number of embryos can be readily obtained at one time. The system can therefore be used as a model for studying membrane assembly and sources.

It is becoming increasingly apparent that cytokinesis in embryonic cells from a number of species is a result of the integrated effect of several events such as: a contractile process (Marsland and Landau, 1954), the formation of stable intercellular contacts between the dividing cells (Bluemink, 1971a, b) and a phenomenon involving membrane growth, whereby new membrane is added to the surface to accommodate the two daughter cells (Selman and Perry, 1970). The precise interrelationships of these events are as yet unclear.

In a recent report (Kalt, 1971b), attention has been focused on the importance of surface features and interblastomeric junctions as early as the first cleavage stage in the formation of the blastocoel in amphibian embryos. Also, Bluemink (1971a, b), studying the effect of cytochalasin B on early amphibian embryos, has emphasized the importance of the interblastomeric surface in holding the two blastomeres together. While the relationship between the plasma membrane of adjacent blastomeres has been examined by Sanders and Zalik (1972a) by transmission electron microscopy, there is no account of the three-dimensional relationship between such cells, as offered by scanning electron microscope (SEM). This is due, in part, to the fact that methods which are suitable for examining the outer surface of the cells

by SEM cannot be readily applied to the interblastomeric zone.

Membrane growth is important in dividing as well as in non-dividing cells. In the latter case new membranes are required for cell repair, in intracellular transport and in membrane turnover. While the importance of membrane growth in the furrow region has been emphasized by work on amphibian embryos (Selman and Waddington, 1955; Zotin, 1964; Selman and Perry, 1970; Bluemink, 1971b), it is probably the least understood of the phenomena associated with cleavage. The appearance of unpigmented surface in the furrow of the normally pigmented animal pole has been considered to be evidence for the addition of new membrane in this region (Selman and Perry, 1970; Bluemink and De Laat, 1973). While discussing formation of the blastocoel in early *Xenopus* embryos, Kalt (1971b) has suggested that vesicles transporting material from the cytoplasm to the blastocoel may fuse with the cell membrane, thus providing a possible mechanism for the appearance of the additional membrane required in cleavage. However, ultrastructural demonstration of any precise location for such fusion activity has heretofore been lacking.

Regarding the supply of new membrane in *Xenopus* there have been two suggestions which are probably not mutually exclusive. First, preassembled membranes in the form of vesicles may be inserted into the existing membranes (Bluemink, 1971b; Singal and Sanders, 1974a). Second, precursor molecules may be interpolated into the plasma membrane (Bluemink, 1971b; Bluemink and De Laat, 1973). The preassembled membranes in *Xenopus* may either be derived from the Golgi bodies (Sanders, 1973; Singal and Sanders, 1974a) or from the endoplasmic

reticulum (Bluemink, 1971b). In addition to the Golgi bodies and the endoplasmic reticulum, lipid droplets are given attention here as a possible source of the new membranes. From *in vitro* experiments (Stoeckenius, 1962a, b) and *in vivo* studies (Mercer, 1962; Norrenvang, 1968) it has been shown that lipids and lipid droplets are capable of forming lamellar structures and ordered membranes. However, no report is available that describes the morphology and interrelationship of Golgi bodies, endoplasmic reticulum and lipid droplets in the first cleavage *Xenopus* embryos.

The Golgi body and its associated structures have been implicated in the synthesis, intracellular transport and transport of macromolecules to the exterior in several cell types (Wise and Flickinger, 1970a; Bennett and Leblond, 1971; Northcote, 1971; Whaley *et al.*, 1972). In *Xenopus* embryos, its association with the plasma membrane and the probable transport of Golgi-derived vesicles to the cell surface has been documented (Sanders, 1973; Singal and Sanders, 1974a). How the Golgi body maintains itself in *Xenopus* embryos is still an unanswered question.

This thesis presents some hitherto undescribed features associated with the process of first cleavage in *Xenopus* embryos, and correlates results from several techniques such as scanning and transmission electron microscopy coupled with the use of lanthanum as a marker for cell surface material. Application of two main methods for the SEM study of these embryos is described which makes it possible to examine both the outer and interblastomeric surfaces and thereby build up a composite topographical picture of the entire blastomeric surface.

The study also provides a morphological description of Golgi bodies, endoplasmic reticulum and lipid droplets and reveals an interrelationship among these organelles. Evidence is provided for a common source of the Golgi bodies and the endoplasmic reticulum. A better insight regarding the role of the Golgi body during cleavage has been obtained by the demonstration of thiamine pyrophosphatase activity and by fixation of embryos with an osmium tetroxide - zinc iodide reagent. Preliminary information obtained from fucose-^{3}H uptake into the embryos has also been included. An assembly route followed by the cell surface material has been proposed.

Since the commencement of this project in 1971, a number of reports of ultrastructural investigations have appeared, both from our laboratory (Sanders and Zalik, 1972a; Sanders, 1973; Sanders and Singal, 1973; Singal and Sanders, 1973a, b, 1974a, b, c) and from others (Kalt, 1971a, b; Bluemink, 1971a, b, 1972; Bluemink and De Laat, 1973; De Laat *et al.*, 1973) devoted to the understanding of the phenomenon of cleavage in *Xenopus* embryos.

LITERATURE REVIEW

Several theories have been proposed to explain the formation of the cleavage furrow in a dividing cell (see reviews by Swann and Mitchison, 1958; Wolpert, 1960; Rappaport, 1971). These can be roughly categorized as those involving the mitotic apparatus, a surface force or membrane growth. According to the *spindle elongation theory* (Dan, 1947, 1958) spindle tubules push the centres apart and as a result the astral rays attached to the equator pull in the surface. The evidence provided concerns the relative movement of kaolin particles attached to the surface of an egg membrane. This, and other, theories involving the role of mitotic apparatus have been disproved, since division occurs even after the spindle is removed or modified (Hiramoto, 1956; Swann and Mitchison, 1958).

The *expanding surface theory*, based on birefringence measurements, suggests that expansion of the surface at the poles provides the necessary force for cleavage and is caused by a nuclear liberated substance during early telophase (Mitchison, 1952). Although this theory also assumes a surface contraction in a broad band-like area encircling the equator, occurring concomitantly with the expansion of the surface, the latter is considered as the primary part of the cleavage process. Marsland and Landau (1954) raised the objection to this theory that: "Mitchison's (1952) experiments were carried out on cells from which the fertilization membranes and hyaline layers had been removed, and for such unconstrained cells it seems certain that sus[illegible]xpansion of the

surface would merely lead to [illegible]
of fragile or varying consistency [illegible]
Marsland and Landau (1954) put forward a [illegible]
involved *contractile ring* or *cortical gel* [illegible]
to this theory, a passive increase in surface occurs [illegible]
as a result of active contraction or folding of [illegible]
the furrow region. The latter concept has been supported by Selman
and Waddington (1955) to explain furrow formation in *Tubifex*
embryos. According to these workers (Selman and Waddington, 1955)
a sheet of gel appears in the furrow region and the former contracts
immediately after its formation resulting in 'dipping in' of the new
furrow. When the furrow reaches completion the gel could undergo
solation to clear the way for the approach and final fusion of the
cell membrane thereby forming daughter cells (Marsland and Landau,
1954).

The concept of active contraction has been supported by the demonstration of oriented microfilaments and is discussed later. However, the evidence against these theories is that cleavage can occur without chromosomes, also eggs can cleave while under tension (Hiramoto, 1968). The *Astral relaxation theory* of Rappert (1960, 1963) suggests that when the asters reach the poles the surface tension at the poles is lowered. This allows the furrow region, where the surface tension is maintained, to contract while the polar regions expand. The suggestion includes the concepts of *equatorial surface* and *cortical gel contraction*.

The *growth theory* assumes that the furrow formation is

Schechtman, 1937). Schechtman (1937) has compared the increase in the cleavage furrow to [illegible] necessary new material [illegible] local *cortical growth* which corresponds to the [illegible] and yolk "granules" (Schechtman, 1937). The presence of [illegible] vacuoles splitting the blastema in the plane of the cleavage furrow in sections, has been related to the formation of the surface of daughter blastomeres in amphibian (Zotin, 1964) and sea urchin embryos (Motomura, 1966). Electron microscope studies (Selman and Perry, 1970; Bluemink, 1970; Kalt, 1971b) have shown that these vacuoles represented a series of dilations of the intercellular space and were, in fact, already a part of the furrow. The role of membrane growth in furrow formation is reviewed below.

In spite of all the suggestions or theories a generalized description of cytokinesis is still lacking. However, more recent studies have indicated the involvement of several processes during cleavage, such as contraction of microfilaments, formation of stable interblastomeric contacts and the supply of additional plasma membrane.

From the study of furrow formation in various marine eggs Marsland and Landau (1954) concluded that the cleavage involves contraction of a ring of gelated cytoplasm in the furrow region. In the case of axolotl and sturgeon eggs, Zotin (1964) suggested that the surface is able to undergo contractile responses through

[illegible] distant [illegible] (1971) a narrow zone of altered cytoplasm located at the base of the furrow.

In *Loligo pealii* the surface contraction has been attributed to the active contraction of microfilaments (Arnold, 1969). Recently the "contractile ring" hypothesis (Marsland and Landau, 1954) has drawn support from the demonstration of oriented microfilaments subjacent to the cell membrane in the furrow region of eggs from different sources such as *Arbacia punctulata* (Goodenough *et al.*, 1968; Schroeder, 1969a, Tilney and Marsland, 1969), *Loligo pealii* (Arnold, 1969), *Stomatopsa atra* (Schroeder, 1968), *Armandia brevis* and *Aequorea aequorea* (Szollosi, 1970), *Triturus alpestris* (Selman and Perry, 1970). A good account of microfilaments, together with the speculation that they might constitute a contractile system in *Xenopus* embryos, is also available (Bluemink, 1971a, b; Katt, 1971b). Such a contractile system would require anchoring as well as contraction of the microfilaments. According to Bluemink (1970, 1971b) one end of the microfilaments is attached to the plasma membrane and the other end is anchored into the cytoplasm through randomly oriented 65 Å filaments which are not involved in furrow formation. The actual contraction mechanism in each filament has not been described, although an increase in thickness of the filamentous layer in the region of constriction in the zygote has been attributed to contraction of filaments (Bluemink, 1970). The relationship of microfilaments with the contraction of epidermal cells in *Amaroucium constellatum* and *Distaplia occidentalis* has been demonstrated using electron microscopy, time lapse cinemicrography

and birefringence techniques (Cloney, 1966).

With regard to the intercellular contacts, it has been suggested (Bluemink, 1971a, b; Eggenson, 1971) that in the absence of stable cell-to-cell ligands the cleavage furrow fails to complete. Such contacts play a significant role during the development of many different species, for example movements of the primary mesenchyme cells in the developing sea urchin larva are dependent upon the formation of stable contacts between the cell pseudopods and blastocoel wall (Gustafson and Wolpert, 1961). During early morphogenesis in the chick embryo the contacts are present between cells of epiblast and hypoblast, mesoblast and hypoblast, mesoblast and epiblast and within migrating mesenchyme of the mesoblast, and the presence of contacts has been correlated with the morphogenetic movements during development (Trelstad *et al.*, 1967). Similarly, the occurrence of cell contacts in *Fundulus* blastoderms has been correlated with the cell movements during gastrulation (Trinkaus and Lentz, 1967).

Intercellular contacts in *Xenopus* embryos have been characterized by using the transmission electron microscope (Kalt, 1971b; Sanders and Zalik, 1972a). Some such contacts are formed through membrane protuberances - a surface feature that can be observed with a scanning electron microscope. The latter has proved to be a valuable technique for studying the surface features of a great variety of biological specimens (see reviews by Carr, 1971 and Hollenberg and Erickson, 1973). However, relatively few reports are available that describe surface features of amphibian embryos as

seen through a scanning electron microscope. These examples are on relatively late stages (gastrula and neurula) of development (Tamm, 1973).

Another important aspect of the cleavage is the resultant increased surface area. In amphibian embryos the two blastomeres formed at the end of first cleavage are more or less hemispheres and together have 50% more surface area than that of the uncleaved embryo (Wolpert, 1960). In the *Xenopus* embryo the additional membrane required is in the range of 3.5 mm^2 (Singal and Sanders, 1974b). On the other hand, in sea urchins the daughter blastomeres are two similar spheres and there is only 28% increase in surface area (Hiramoto, 1968). The increased surface area in dividing cells could arise either by (a) uniform stretching of the existing membrane (Hiramoto, 1968); (b) by insertion of new material into the existing membrane (Selman and Perry, 1970; Bluemink, 1971b); (c) by the unfolding of a pre-formed pleated membrane (Gwynn and Jones, 1972); or (d) it could be brought about by molecular re-orientation (Swann and Mitchison, 1958). The first three possibilities have also been raised by Prothero and Rockfeller (1967) to explain the movement of surface particles. However, in the amphibian egg and certain other cells the evidence is accumulating in favour of the process (b) mentioned above, i.e. additional membrane is provided by new materials (Tehmisian *et al.*, 1967; Selman and Perry, 1970; Bluemink, 1971b; Sanders, 1973). Further, the supply of new materials could either be through the interpolation of precursor molecules into the existing membrane (Bluemink, 1971b;

Bluemink and De Laat, 1973), and/or by the invagination of plasma membranes (Balinsky and Devis, 1963; Karasaki, 1963; Karasaki, 1971; Bluemink and De Laat, 1973). The Golgi complex (Katz, 1971b; Sanders, 1972) as well as the endoplasmic reticulum (Karasaki, 1971b) have been suggested as the source of the preaxolotl membranes. However, there is little literature concerning the details of these cytomembranes in first cleavage *Xenopus* embryos. These membranes along with other cell constituents have been described in amphibian ectoderm cells (Karasaki, 1959) and in a variety of oocytes, for example *Rana pipiens* (Kemp, 1956), *Rana temporaria* and *Triton alpestris* (Wartenberg and Schmidt, 1963), *Xenopus laevis* (Wartenberg, 1962; Balinsky and Devis, 1963; Wischnitzer, 1966), *Rana temporaria*, *Rana esculenta*, *Triton alpestris* and *Ambystoma mexicanum* (Wartenberg, 1962), *Triturus viridescens* (Hope *et al.*, 1964).

The basic characteristic morphological element of a Golgi apparatus is a disc-like saccule or cisterna which may be flat or slightly concavo-convex and is usually fenestrated at the edges. A typical Golgi apparatus is composed of stacks of such saccules and numerous associated vesicles.

The Golgi apparatus, since it discovery by Camillo Golgi in 1898, has been reported in nearly all the cells of animals and plants, however, controversy about the existence of the organelle prevailed for a number of years. This was partly because of the empirical nature of the techniques, which involved metallic impregnation, available to demonstrate the organelle, and partly

1969; Bennett and [illegible] 1971; Bennett et al., 1971).

The structure, function and [illegible] of this characteristic [illegible] organelle has been reviewed [illegible] by several workers (e.g., Dalton and Felix, 1956; Northcote, 1971; Morre et al., 1971; Whaley et al., 1972). Structural and chemical details of the organelle may vary in different cells, but all the various reports cited above converge on the point that the Golgi body plays an important role in intracellular transport and in the secretory activity of the cell. The importance of secretory activity in *Xenopus* embryos for the formation of the blastocoel has been emphasized (Kalt, 1971b).

It has become apparent that a cell surface material is present on the surface of the limiting membrane of all animal cells (see reviews by Nicolson, 1970; Winzler, 1970; Rambourg, 1971). The general influence of the cell surface on many cellular functions has been discussed by Winzler (1970) and by Weiss et al. (1972). Conversely, cell differentiation during embryonic induction and tissue morphogenesis correlates with changes in the properties of the cell surface macromolecules (Moscona, 1971).

Performance of specialized functions such as cell-to-cell recognition and cell antigenicity is dependent upon the nature of the cell surface material. An important role for cell-cell recognition is accepted for many phenomena such as gamete recognition, selective cellular adhesion and aggregation and homing of lymphocytes. Specific carbohydrate groups on the cell surface are responsible for the recognition by gametes of sea urchin (Anderson, 1968; Glabe

recognition and adhesion in the gametes of *Chlamydomonas* (Wiese and Shoemaker, 1970). Studies on aggregation of cells from *Microciona prolifera*, a bright red sponge, and *Haliclona occulata*, a light purple-brown sponge, have shown that the presence of cell surface material endows the cells with selective adhesiveness (Moscona, 1963). Another interesting example of cell recognition through specific surface carbohydrate is provided by the phenomenon of homing of lymphocytes. Small lymphocytes have a unique circulatory route, selectively emerging from the blood stream in lymphoid tissue and then circulating back to the blood via the lymphatic system. Gesner and Ginsburg (1964) showed that lymphocytes, treated *in vitro* with glycosidase and then re-injected, went primarily to the liver instead of the spleen and lymph nodes, indicating that surface sugars are important in proper homing of the cells. Antigenicity of blood cells is also determined by the sugar moieties in the surface glycoproteins and glycolipids (see review by Winzler, 1970).

Cytochemical techniques, such as the localization of thiamine pyrophosphatase or fixation with osmium tetroxide - zinc iodide reagent, by virtue of their special affinity for the Golgi body, can be useful tools for the better understanding of the role of the organelle in dividing or secretory cells.

The enzyme thiamine pyrophosphatase (TPPase) in a cell can be reliably localized by cytochemical procedures (Allen, 1963; Novikoff *et al.*, 1971). In most cells the enzyme is localized in the Golgi membranes, as a result of which it is usually considered

as a marker enzyme specific for the Golgi body (Novikoff and Goldfischer, 1961; Shanthaveerappa and Bourne, 1965; Goldfischer *et al.*, 1971). However, universality of the statement is debatable, as the enzyme has been localized in other cell organelles, for example, in hepatic cells and neurons of rat nodose ganglion the enzyme is present in rough endoplasmic reticulum (Holtzman *et al.*, 1967; Cheetham *et al.*, 1971). TPPase has also been reported in nerve endings (DeIraldi *et al.*, 1970; Seizo and DeTores Arnaiz, 1970; Griffith and Bondareff, 1973). The role of phosphatases in Golgi bodies and other cytomembranes has been speculated upon by Novikoff *et al.* (1962): "Since TPP is an essential coenzyme in pyruvate and α-ketoglutarate decarboxylations is it possible, even without a direct role, that the TPP-hydrolysing enzyme(s?) influence how much acetyl-CoA is funnelled to phospholipid or other substances of prominence in the Golgi apparatus?"

A complex of osmium tetroxide with zinc iodide (OZI) has been employed by several authors as fixative-stain, and in most cases, a preferential deposition of metal (OZI) on the Golgi region is observed (Ebner and Niebauer, 1967; Niebauer *et al.*, 1969; Elias *et al.*, 1972; Dauwalder and Whaley, 1973; Martin and Spicer, 1973). Complexes of osmium tetroxide with iodides of other divalent cations such as calcium and cadmium (Maillet, 1968), and sodium have also been tried (Elias *et al.*, 1972). Of all the complexes, OZI has been used most frequently and the technique has yielded reproducible results. The chemistry of the reaction is still not clear, inasmuch as in *Hydra* staining of the surface with OZI has been attributed to

mucopolysaccharides (Elias *et al.*, 1972) and in epidermal Langerhans cells lipids have been held responsible for the metal deposits (Maillet, 1968; Niebauer *et al.*, 1969).

Regardless of the nature of the actual source of new membranes during cleavage, whether it be Golgi bodies, endoplasmic reticulum or other cell constituents, studies on cell division in amphibian embryos recognize the fact that increased surface area in cytokinesis is largely accounted for by the supply of new materials (Selman and Perry, 1970; Bluemink and De Laat, 1973).

MATERIALS AND METHODS

1. Collection of Embryos

Fertilized embryos of *Xenopus laevis* were obtained from mature animals by injecting chorionic gonadotrophin (Antuitrin 'S', Parke-Davis). Females were injected with 1,000 international units (IU) and males with 500 IU. Embryos were always handled in Steinberg's physiological salt solution (SPSS) made up as follows: 17 g NaCl was dissolved in 100 ml of distilled water; 250 mg KCl in 50 ml; 400 mg $Ca(NO_3)_2$ 4 H_2O in 50 ml; 1,025 mg $MgSO_4$ in 50 ml; 2,800 mg Tris was added to the mixture of four different solutions described above. The final volume was made up to 5,000 ml with distilled water and the pH of the final solution was adjusted to 7.4 with 1 N HCl. The jelly was removed chemically in fresh Papain-cysteine solution (Brown, 1967) made up by dissolving 1 g of L-cysteine hydrochloride hydrate in 3 ml of 10% NaOH; final volume was made up to 100 ml with SPSS; 1 g Papain was added to obtain the final reagent. Whenever necessary the vitelline membrane was removed with fine forceps. Embryos at different developmental stages of the first cleavage (Table I) comprised the experimental material throughout the present study.

2. Scanning Electron Microscopy (SEM)

Two methods were employed for preparing the material for SEM study. In the first method the embryos were chemically fixed and air dried. In the second the embryos were frozen dried.

Fixation was done for 8 hours in a mixture of 3% glutaraldehyde,

2% formaldehyde, 1% acrolein and 2.5% dimethyl sulphoxide in 0.1 M cacodylate buffer, pH 7.2 (Kalt and Tandler, 1974). While the embryos were still in the fixative, the vitelline membrane was removed from some of them. A fresh change of the fixative was given after 4 hours. Following a thorough wash in the above buffer containing 1 M sucrose, the embryos were dehydrated in graded series of alcohols. From absolute alcohol they were transferred to propylene oxide for 1 hour with one change. Finally the embryos were left in a dust-free area to dry by evaporation at room temperature.

For freeze-drying, embryos were plunged into liquid isopentane which had been precooled in a liquid nitrogen bath. Care was taken to transfer the embryos with a minimum amount of water. The isopentane was used at a temperature just high enough to avoid freezing (Isopentane m.p. -160°C). Embryos were dried at low temperature (-40°C) under vacuum. In this method it was not possible to remove the vitelline membrane. The frozen embryos were also dried at room temperature, again under vacuum, to check for ice crystal damage of the cell surface.

Following these methods, attempts were made to break the CF stage (Table I) dried embryos along their cleavage plane by different procedures, to be described later. The material was then coated with a carbon and gold conducting layer and examined in a stereoscan S4 SEM (Kent Cambridge Ltd.). To ensure even application of the metallic film the carrying base and the specimen stubs were rotated continuously during the vacuum coating.

For TEM several fixatives were tried and the glutaraldehyde-osmium aldehydes described above (Kalt and Tandler, 1971) was found most suitable. Embryos were fixed as for SEM. After the first fixation in the aldehyde mixture the material was washed five times for a total of 90 minutes in the same cacodylate buffer containing 1 M sucrose. Embryos were post-fixed in 2% osmium tetroxide (OsO_4) solution made in the 0.1 M cacodylate buffer (pH 7.2) containing 0.2 M sucrose. The post-fixation was carried out for 4 hours at 4°C. After a thorough buffer wash the material was dehydrated in graded ethanol solutions and embedded in Epon.

Trialdehyde fixed embryos were also processed without post-osmication. Embryos were fixed in 6% glutaraldehyde made up in 0.1 M phosphate buffer, pH 7.3, followed by a second fixation in 1% osmium tetroxide made up in the same buffer. Buffered 1% osmium tetroxide alone was also used as a fixative.

Silver colour sections were picked up on Formvar and carbon-coated copper or steel grids. Sections were routinely stained with 5% aqueous uranyl acetate and lead citrate. In some of the embryos uranyl acetate staining was done en-block during dehydration in 70% ethanol solution. The grids were examined with a Philips 300 electron microscope.

4. Incubation of Embryos in Fucose-^{3}H Solution

Dejellied embryos with vitelline membrane intact, prior to the start of the cleavage, were grown at room temperature in SPSS containing 50 µC/ml of L-fucose-1,5,6-^{3}H (Sp. Act. 1.025 C/mM, 99% radiochemical

purity, purchased from New England Nuclear, Boston). Embryos were left in this solution for 2, 4, 6, 9, 12 and 15 minutes. After treatment with fucose the material was washed five times in fresh SPSS. Uncleaved embryos then were grown in SPSS for different lengths of time (0, 5, 20, 40 and 80 minutes) followed by a 4 hour fixation in the trialdehyde mixture described above.

a. Liquid Scintillation Counting

Counting was done on trialdehyde fixed embryos. The samples were run in triplicate, each lot containing 10 embryos picked up at random. In each batch the embryos were placed on a Whatman filter paper and were burnt in a Packard oxidizer followed by a quantitative collection of activity in scintillation vials. All samples were counted with a liquid scintillation system. All counts were converted to disintegrations per minute (dpm) using appropriate correction for spillover/quenching and the final values were obtained by subtracting background.

b. Autoradiography

Trialdehyde fixed embryos were post-osmicated and embedded in Epon as described above. 1 μ thick serial sections were cut and picked up on clean and dry slides prepared by dipping in a solution of 0.1% gelatin and 0.01% chromium potassium sulphate (Caro and Van Tubergen, 1962). Prior to their exposure to the emulsion, sections mounted on slides were treated to bleach pigment granules (Brunet and Small, 1959). The bleaching reagent was made by adding 0.2 ml concen-

crushed HgCl2 to 0.25g of potassium chloride in a 100 ml glass cylinder. After 30 seconds, 1 ml of distilled water was added and the gases (mostly chlorine) were retained by loosely covering the cylinder. After 2 minutes the final volume was made up to 100 ml with distilled water, while swirling to dissolve the gases (Brunet and Small, 1959). Slides were left in this solution for 45 minutes.

Ilford L4 emulsion was prepared according to Caro and van Tubergen (1962). Essentially, 10 g of the emulsion were melted in 20 ml of distilled water in a beaker kept in a liquid water bath (temperature 45°C) for 15 minutes. While stirring the contents, the beaker was placed in an ice-bath for 3 minutes, and was then left at room temperature for 30 minutes before use. For emulsion coating the slides were dipped in the emulsion and withdrawn slowly (Caro and van Tubergen, 1962). Slides were dried in the dark under a gentle stream of air prior to their storage under anhydrous conditions. Test slides were developed (Caro and van Tubergen, 1962) every week between 3 and 9 weeks.

Ultrathin sections were also processed for EM autoradiography but erratic results were obtained.

5. Lanthanum Treatment

For lanthanum staining 1% lanthanum nitrate was added to the trialdehyde mixture and also to the post-fixative solutions (Sanders and Zalik, 1972a). The material was processed for transmission electron microscopy as described above.

6. Osmium Tetroxide - Zinc Iodide Fixation

Embryos were fixed in a solution containing OsO_4 - ZnI_2 in water. The procedure of Mallier (1968) as modified by Niebauer and associates (1969) was followed. Essentially, 5 g of metallic iodine and 10 g of metallic zinc were mixed as powders. To this powder mixture, 200 ml of distilled water was added slowly with a constant stirring. The resulting reaction is exothermic and is therefore performed with care. The solution was filtered after 5 minutes. For the final fixative, 8 ml of the above solution were combined with 2 ml of 2% aqueous OsO_4. The fixation was carried out in the dark for 24 hours at 4°C. Two changes of fresh fixative were given after 8 and 16 hours respectively. The material was then rinsed in water, dehydrated and embedded in Epon (Luft, 1961). Silver colour sections were studied with or without uranyl acetate and lead citrate staining.

From the poor quality of fixation it was felt that the osmolarity (190 m.osmols) of the final solution was too low for *Xenopus* embryos. Accordingly, the fixative was modified by adding 100 mg of sucrose to 10 ml of the final solution, giving a new osmolarity (222 m.osmols). The washing solution after fixation was also replaced with 0.1 M cacodylate - HCl buffer (pH 7.4) containing 0.4 M sucrose. The above modifications improved the quality of fixation.

7. Demonstration of Enzymes

For both the enzymes (thiamine pyrophosphatase and acid phosphatase) embryos were prefixed in Karnovsky's (1965) fixative diluted to contain 1.33% paraformaldehyde and 1.67% glutaraldehyde in 0.1 M

sodium cacodylate - HCl buffer (pH 7.4). The fixation was carried out for 4 hours at 4°C with one change. Subsequently the material was rinsed in several changes of 0.1 M cacodylate - HCl buffer for 24 hours. Frozen, 30 µ thick, sections of fixed embryos were cut with a Lipshaw microtome cryostat at -20°C. Whole embryos, denuded after fixation and cut into two halves with a sharp razor, were also used. The prefixed material was handled in the above buffer.

a. Thiamine Pyrophosphatase

The prefixed material was incubated at 35 - 37°C for 1 hour in a medium containing 0.5 ml thiamine pyrophosphate chloride (0.01 M, Sigma Chemical Co.), 0.2 ml distilled H_2O, 1.0 ml Tris-maleate buffer (0.2 M; pH 7.2), 0.3 ml lead nitrate (0.03 M), and 0.5 ml manganese chloride (0.25 M) (Novikoff and Goldfischer, 1961). All solutions and embryos were brought to the incubation temperature before use. Every time the medium was freshly prepared and filtered before use. Control pieces were incubated in a thiamine pyrophosphate-free medium.

b. Acid Phosphatase

The incubation medium of Gomori (1952) as modified by Barka and Anderson (1962) was used. The substrate mixture contained 1.25% β-glycerophosphate (Fisher Chemical Co.) in 0.05 M Tris-maleate buffer (pH 5.0) and 0.2% lead nitrate. The material was incubated in a manner similar to that used for the localization of thiamine pyrophosphatase. The control medium did not contain β-glycerophosphate.

Following incubation for both the enzymes, rinsing was carried

out three times for 5 minutes each in 0.05 M veronal - acetate buffer (pH 7.4) containing 7.5% sucrose. Post-fixation was carried out in 1% osmium tetroxide in 0.05 M veronal - acetate buffer (pH 7.4) for 2 hours at 4°C, with one change (Smith and Farquhar, 1966). The embryo pieces were then stained en-block with 0.5% uranyl acetate in 0.05 M veronal - acetate buffer (pH 5.5) for 2 hours at 4°C. Material was dehydrated at 4°C in a graded series of ethanol and embedded in Epon (Luft, 1961). Silver colour sections were studied without any further staining.

RESULTS

1. Fertilized Unfertilized Eggs

The fertilized unfertilized embryo is roughly spherical with an average diameter of 1.5 mm and has three distinct areas as seen through a dissecting microscope. The pigmented upper half is the animal pole, slightly less pigmented and considerably narrower is the gray crescent and the lower, non-pigmented half is the vegetal pole. The cytoplasm contains several structures: yolk platelets, lipid droplets, mitochondria, Golgi body, endoplasmic reticulum, pigment granules, a single nucleus and electron dense particles. The first cleavage is holoblastic, vertical, and starts first at the animal pole, dividing the embryo into two halves. The whole embryo is enclosed in a relatively strong and elastic vitelline membrane, composed of filamentous and granular material (Grey *et al.*, 1974). On the outside the embryo has thick jelly coats (Salthe, 1963; Freeman, 1968).

2. Pigment Granules

Pigment granules had a remarkable uniformity in size (0.5 μ diameter) and showed a characteristic distribution. Prior to the start of the first cleavage the pigment granules aligned into a single row (single stripe stage) and marked the plane of the future furrow. Within 45 seconds two parallel rows (double stripe stage) of pigment granules marked the start of the visible part of the cleavage process. Roughly 15 minutes after the start of the cleavage, many pigment granules formed a dense, circular spot in the animal pole which persisted through

deeper regions the granules were infrequent or absent.

3c. Terminology and Timings

In order to accurately describe specific changes of the first embryonic cleavage, a terminology modified from that of Blumenfeld (1971a) has been used. A clear distinction has been made between the terms "groove" and "furrow" which were previously used synonymously (Blumenfeld, 1971a). The former will refer only to the externally visible cleavage characteristics while the latter will refer to the membrane partition which develops between the blastomeres. These are clearly distinct aspects of the process as demonstrated by the fact that as the cleavage groove has completely encircled the embryos the furrow is only some 300 μ deep in the animal pole (Fig. 1). The proposed terminology is compared with that of Blumenfeld (1971a, b) in Table 1.

The early part of the groove, up to 180° (Fig. 2), was achieved in less than 7 minutes, however, progress of the groove was slow between 180° and 360°. There was a time lag of about 12 minutes between the end of the first and the start of the second cleavage groove (Fig. 2). The first cleavage was not completed until after the appearance of the second cleavage groove (Fig. 2).

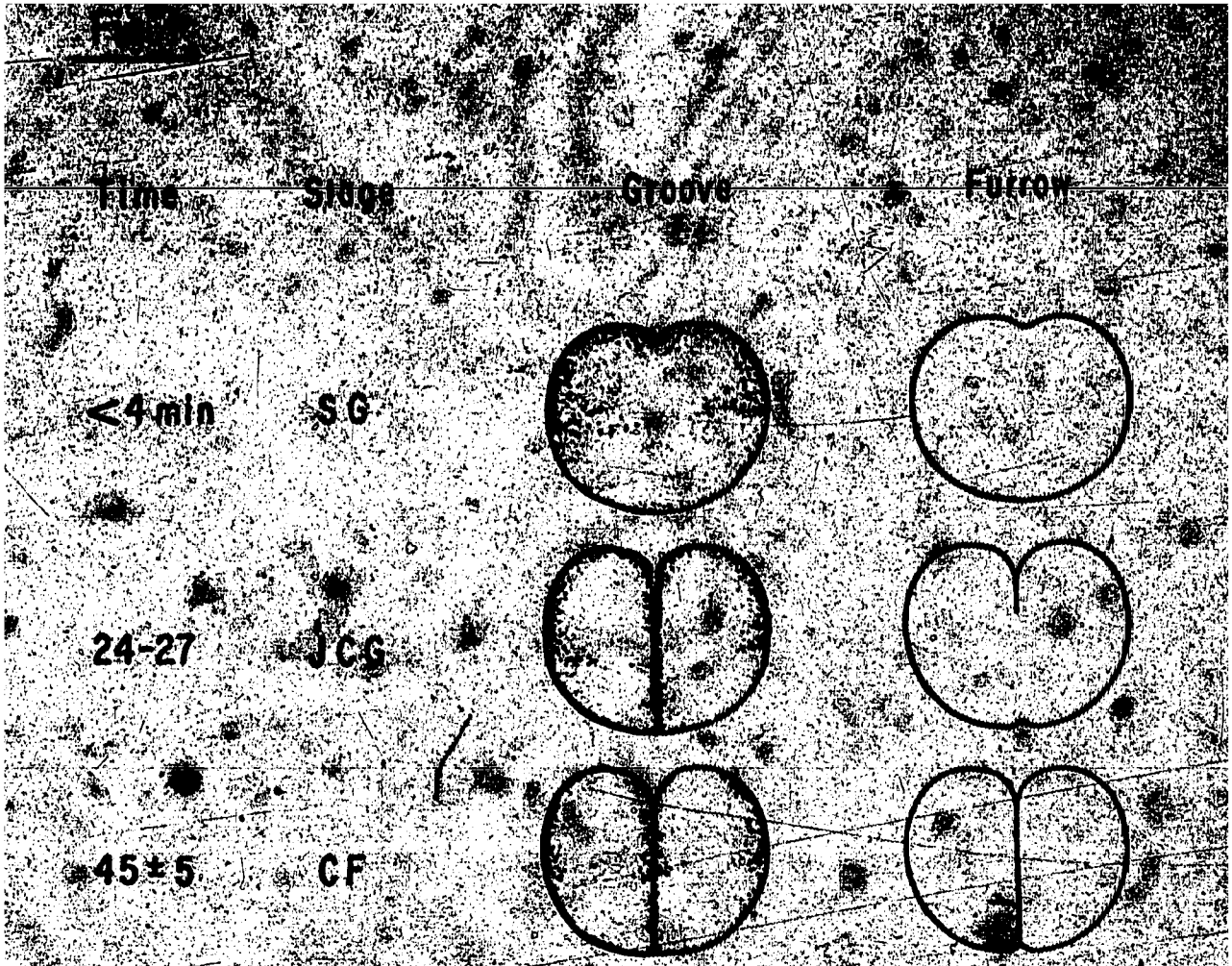


Figure 3. Three different developmental stages of the first cleavage embryo. Figure shows the distinction between the groove and the furrow. At JCG stage (just completed groove) groove is complete all around the embryo while the furrow is only about 300 μ deep. For comparison of groove and furrow in other stages consult Table 1. SG: shallow groove; CF: completed furrow.

TABLE I. TERMINOLOGY

Used in the Present Report. Stage	As Used by Bluemink (1971a, b). Stage	Time**	Spreading of the Groove	Depth of the Animal Pole Furrow
Single Stripe (SS)*	Single Stripe (SS)	0	0°	None
Double Stripe (DS)	Not Reported	<45 secs	45°	25 μ
Shallow Groove (SG)	Shallow Groove (SG)	< 4 mins	90°	100 μ
Half-Advanced Groove (HAG)	Half-Advanced Furrow (HAF)	< 7 mins	180°	145 μ
Far-Advanced Groove (FAG)	Far-Advanced Furrow (FAF)	15-18 mins	270°	170-180 μ
Just-Completed Groove (JCG)	Just-Completed Furrow (JCF)	24-27 mins	360°	300 μ 50 μ (vegetal pole)
Completed Furrow (CF)	Not Reported	45±5 mins	360°	complete through the embryo

* Marks the start of the cleavage

** Time after first appearance of SS stage, at 21±1°C

Fig. 2

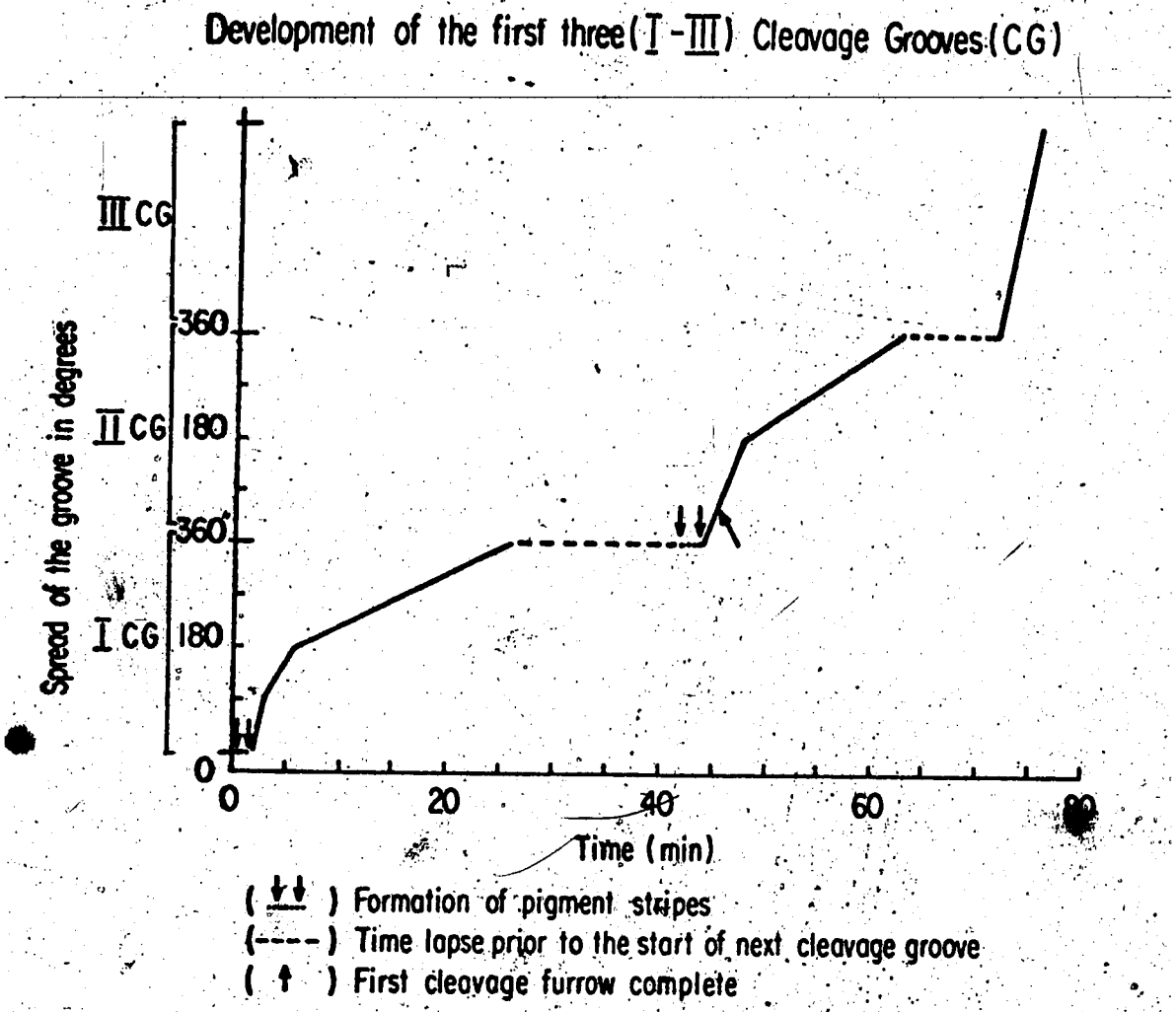


Figure 2. Graph showing developmental time for the first three cleavage grooves (CG). In first and second CG the upper half (up to 180 degrees) of the groove appears in a relatively short time (>7 mins). The first cleavage furrow is not complete until after the II CG has traversed more than 90 degrees on the embryo surface. Third CG starts simultaneously at four different points and is thus not represented in degrees.

4. Ultrastructural Features Associated with the Cleavage

a. Scanning Electron Microscope Observations

Fixed and air-dried embryos were more brittle in comparison with frozen-dried specimens. In the fixed embryos, where the vitelline membrane was removed during fixation, the outer surface remote from the cleavage groove revealed a relatively smooth surface with few undulations (Fig. 3). In the immediate vicinity of the groove, however, the surface was thrown into folds extending 150 - 200 μ (Figs. 3, 4). These folds clearly were not artefacts since they occurred in precisely the region of stress or tension lines which can be seen in living embryos. The folds appeared immediately after the DS stage and became increasingly prominent with the growth of the furrow up to FAG stage, after which they declined in number and size. After the JCG stage where the furrow has grown roughly 300 μ into the embryo the stress folds were restricted to a length of 25 μ on either side of the groove. In the vegetal pole groove the folds were relatively less prominent. Observations at higher magnification showed that the surface of the stress-fold region was rough, owing to the presence of blunt protuberances (Figs. 4, 5) and was clearly distinguishable from the smooth surface remote from the groove. Examination of thin sections through the stress-fold region also revealed these protuberances (Fig. 6) and in addition this region also showed an underlying filamentous network (Fig. 6).

In order to examine the lateral surfaces of the blastomeres, embryos at the CF stage were manually separated along the cleavage plane. When not previously removed, the dried vitelline membrane, stretched across the furrow, was easily cracked by applying pressure

Figs. 3 and 4. Scanning electron micrographs.

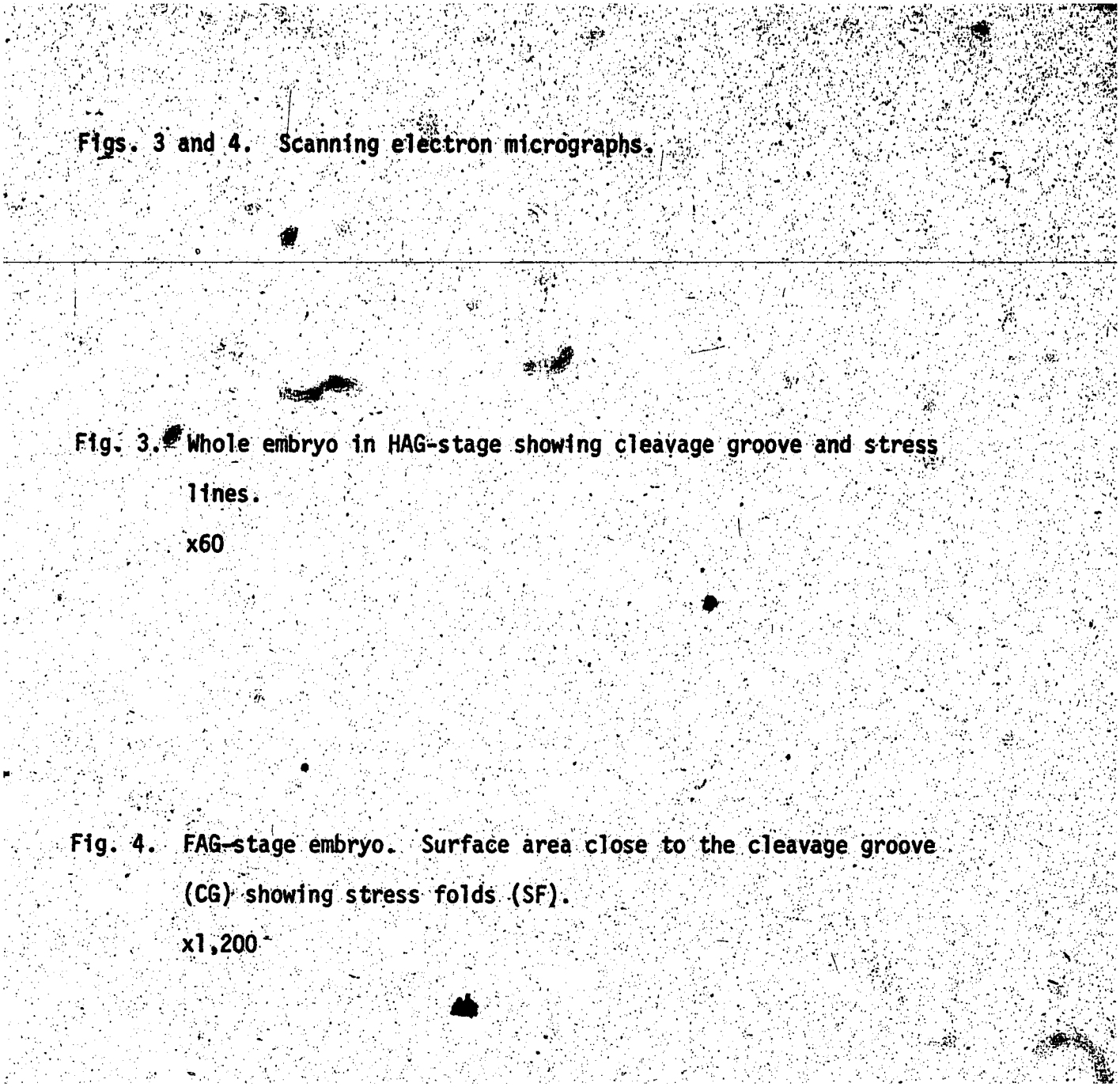
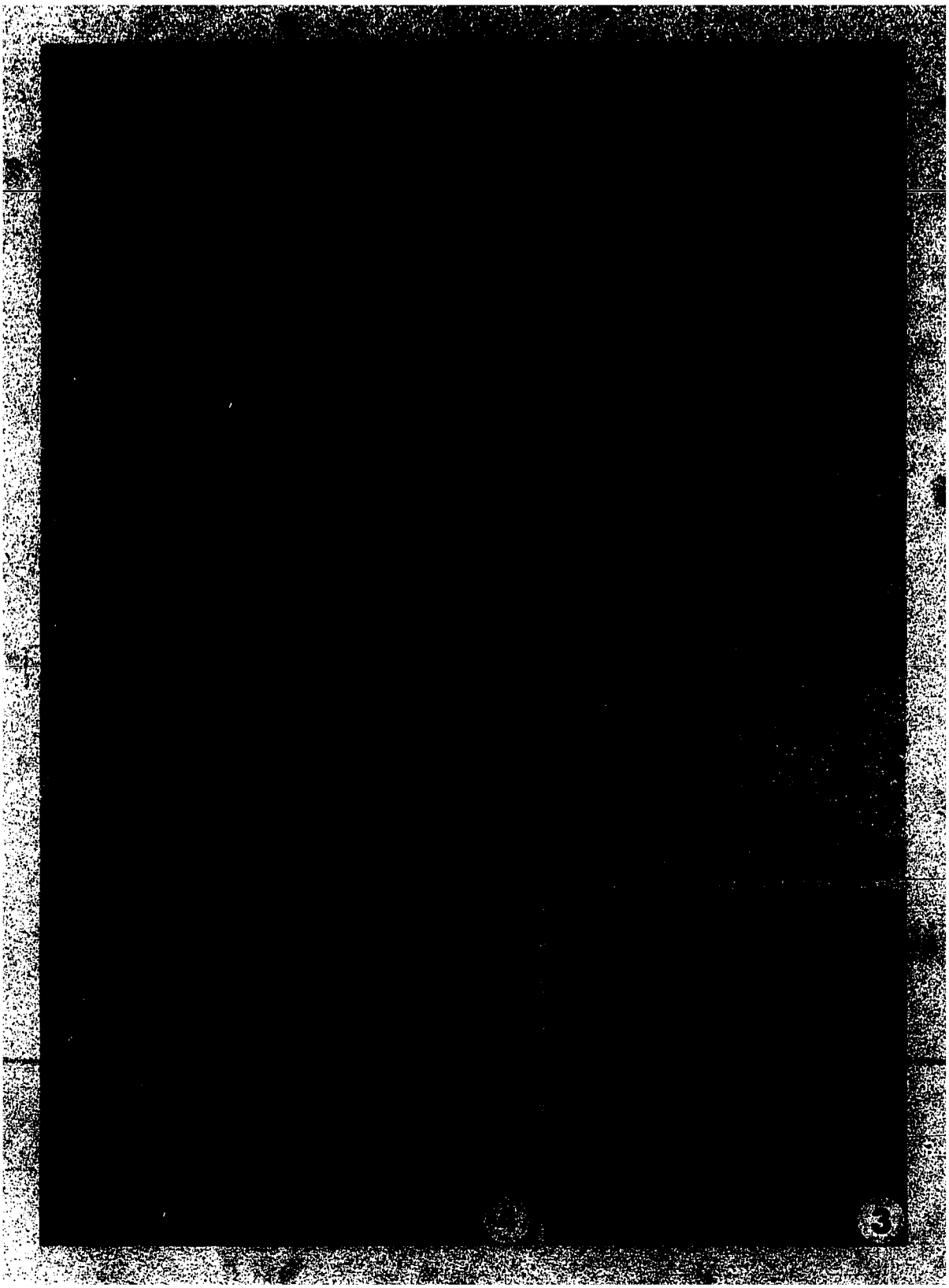


Fig. 3. Whole embryo in HAG-stage showing cleavage groove and stress lines.
x60

Fig. 4. FAG-stage embryo. Surface area close to the cleavage groove (CG) showing stress folds (SF).
x1,200



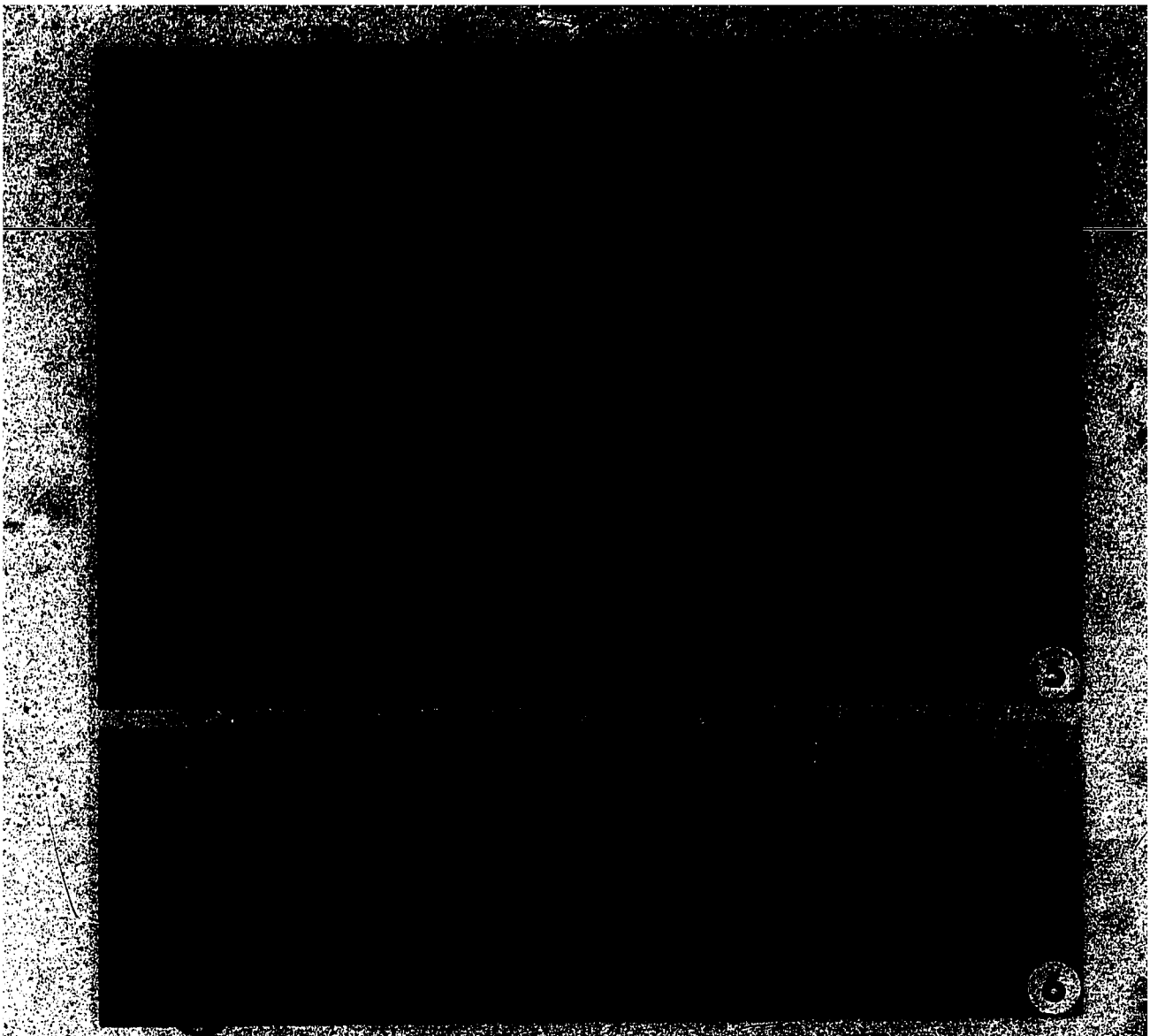


Figure 5. Same as in Fig. 4. Surface of a stress fold (SF) showing membrane protuberances. x4,600

Figure 6. Transmission electron micrograph of an area corresponding to the one shown in Fig. 5, illustrating the protuberances and subsurface microfilaments. x34,000

with an eyelash over the furrow area. Different methods employing an eyelash, finely drawn glass needles or razor blade were used to break the fixed or frozen-dried embryos along the cleavage plane. In the fixed specimen it was almost impossible to obtain intact interblastomeric surface. Tarin (1971), using similar methods for fracturing gastrulating and neurulating chemically fixed frog embryos, was unsuccessful in getting good results, but he still got some interesting fractures by chance. However, in the first cleavage CF stage embryos, the interblastomeric area is very large because of the comparatively bigger cells, and not a single fractured embryo showed an entirely intact interblastomeric surface. Accordingly this procedure proved good only for viewing the naturally exposed surface. The criterion used in the present study to judge the intactness of the surface was the appearance of yolk platelets when the specimen was viewed at high magnification. The presence of these easily recognizable organelles was a clear indication that cytoplasm had been exposed. In comparison with the fixed specimens, it was very easy to break frozen-dried embryos along the cleavage plane. However, in frozen-dried embryos it was not possibly to study the outer surface of the cells due to the difficulty of removing the vitelline membrane during or after freezing.

In a separated half, progressing toward the furrow, the stress folds and protuberances became less prominent and merged into an area 5 - 10 μ wide where the surface displayed a villous transformation (Figs. 7a, b). This area, which appeared as a band, completely encircled the embryo, and its presence was confirmed by transmission electron microscopy (Figs. 13a, 14, 17). Branched microvilli were also noticed

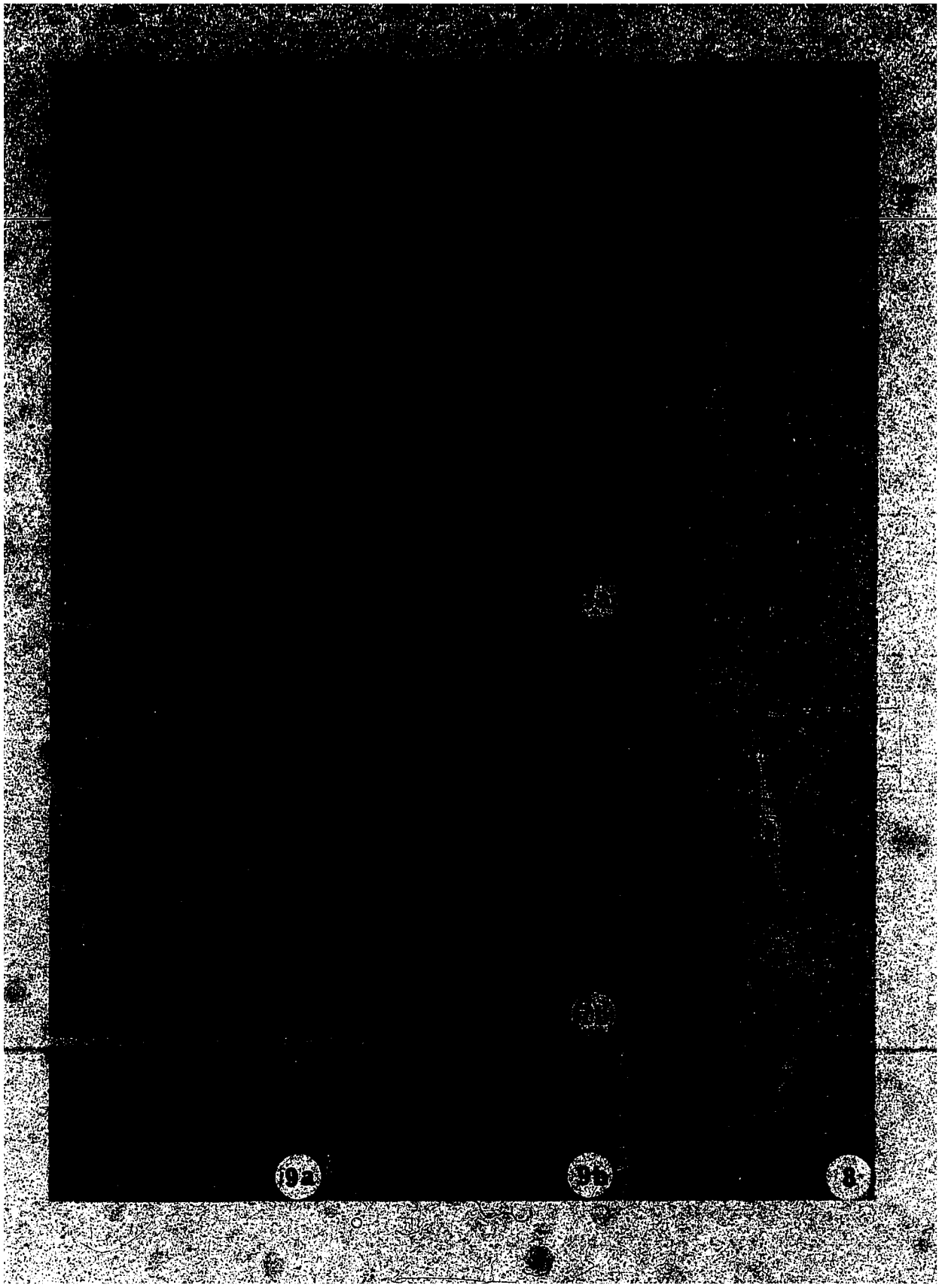
Figs. 7a and b. Lateral surface of a blastomere from a CF-stage embryo.

a. Microvilli are present in a band: RS, rough surface due to membrane protuberances; SS, smoother surface. x3,300

b. Enlargement showing branching of microvilli. x10,400

Fig. 8. Low power transmission electron micrograph showing cleavage furrow at JCG-stage. Outer region of the membranes (between arrows) shows close apposition; FT I, furrow tip I. x650

Figs. 9a and b. Interblastomeric contacts where the membranes approach within 30 Å. x12,600



(Figs. 7b, 13a, 7d). More deeply, beyond the band of microvilli, the lateral surface became relatively smooth (Fig. 7a), and protuberances of the type seen in the stress-fold region were entirely absent.

In the present study chemically fixed, air-dried and frozen-dried specimens provided information regarding the outer blastomeric surface and interblastomeric surface respectively. Combined they gave a picture of the entire cell surface with its regional variations in appearance. The method of examination would seem to be well suited to the study of a number of cell types in which the intercellular surfaces can be exposed, thereby giving information regarding the spatial arrangement of surface projections in cell-to-cell attachment.

b. Transmission Electron Microscope Observations

Study of many thin sections through cleaving embryos approaching the CF stage lead to the diagrammatic representation of animal pole furrow morphology shown in Fig. 10. At the JCG stage the furrow had progressed approximately 300 μ to the level of the dashed line in Fig. 10, and terminated at "furrow tip I" (FT I). Subsequent growth of the furrow is indicated below the dashed line with its "furrow tip II" (FT II). Regardless of the subsequent growth, furrow tip I remained stationary and recognizable at the position indicated.

JCG Stage

All results refer to the animal pole furrow unless otherwise stated. At this stage several characteristic regions could be distinguished in the furrow (Fig. 8). Starting from the animal pole, the

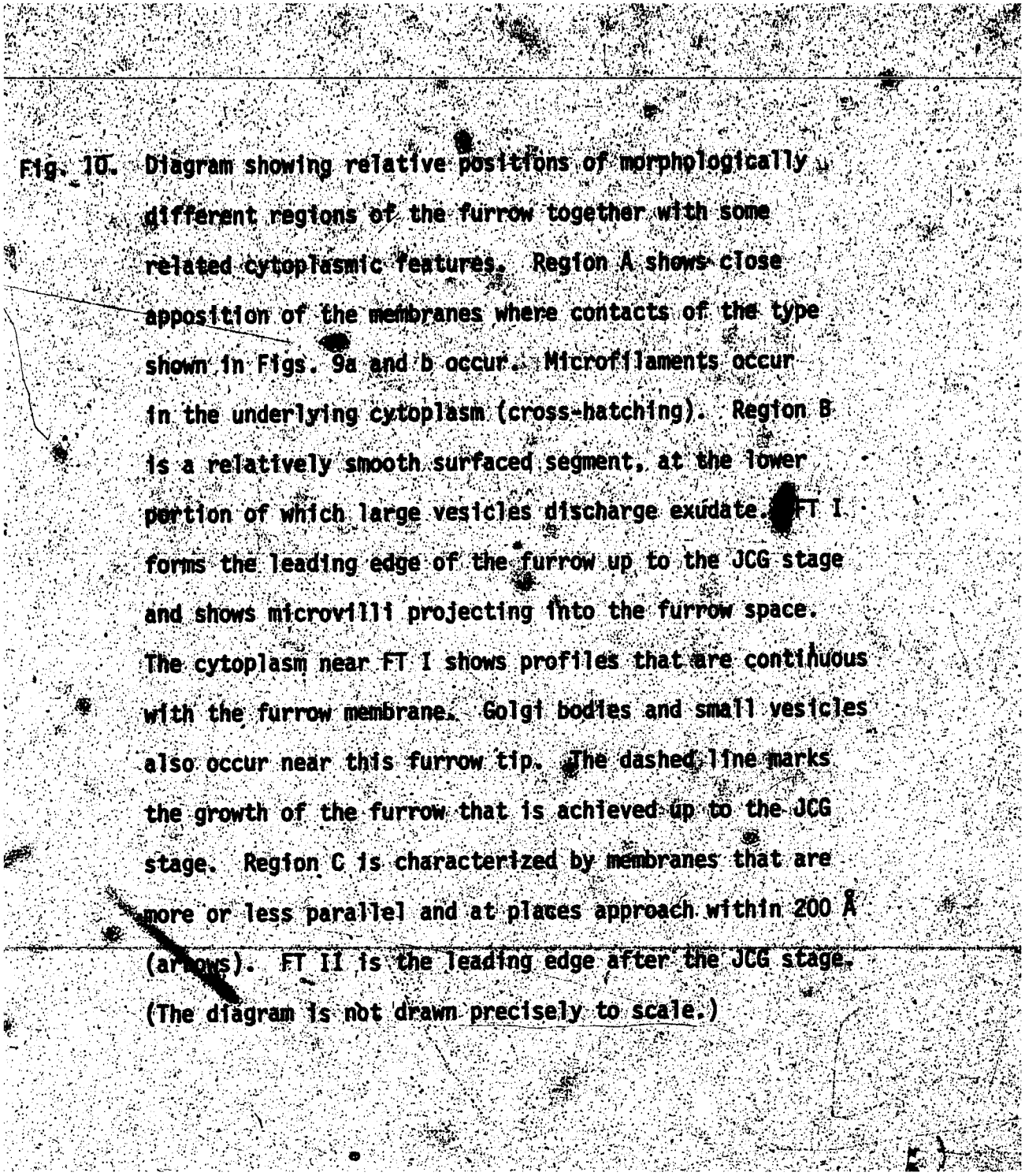


Fig. 10. Diagram showing relative positions of morphologically different regions of the furrow together with some related cytoplasmic features. Region A shows close apposition of the membranes where contacts of the type shown in Figs. 9a and b occur. Microfilaments occur in the underlying cytoplasm (cross-hatching). Region B is a relatively smooth surfaced segment, at the lower portion of which large vesicles discharge exudate. FT I forms the leading edge of the furrow up to the JCG stage and shows microvilli projecting into the furrow space. The cytoplasm near FT I shows profiles that are continuous with the furrow membrane. Golgi bodies and small vesicles also occur near this furrow tip. The dashed line marks the growth of the furrow that is achieved up to the JCG stage. Region C is characterized by membranes that are more or less parallel and at places approach within 200 Å (arrows). FT II is the leading edge after the JCG stage. (The diagram is not drawn precisely to scale.)

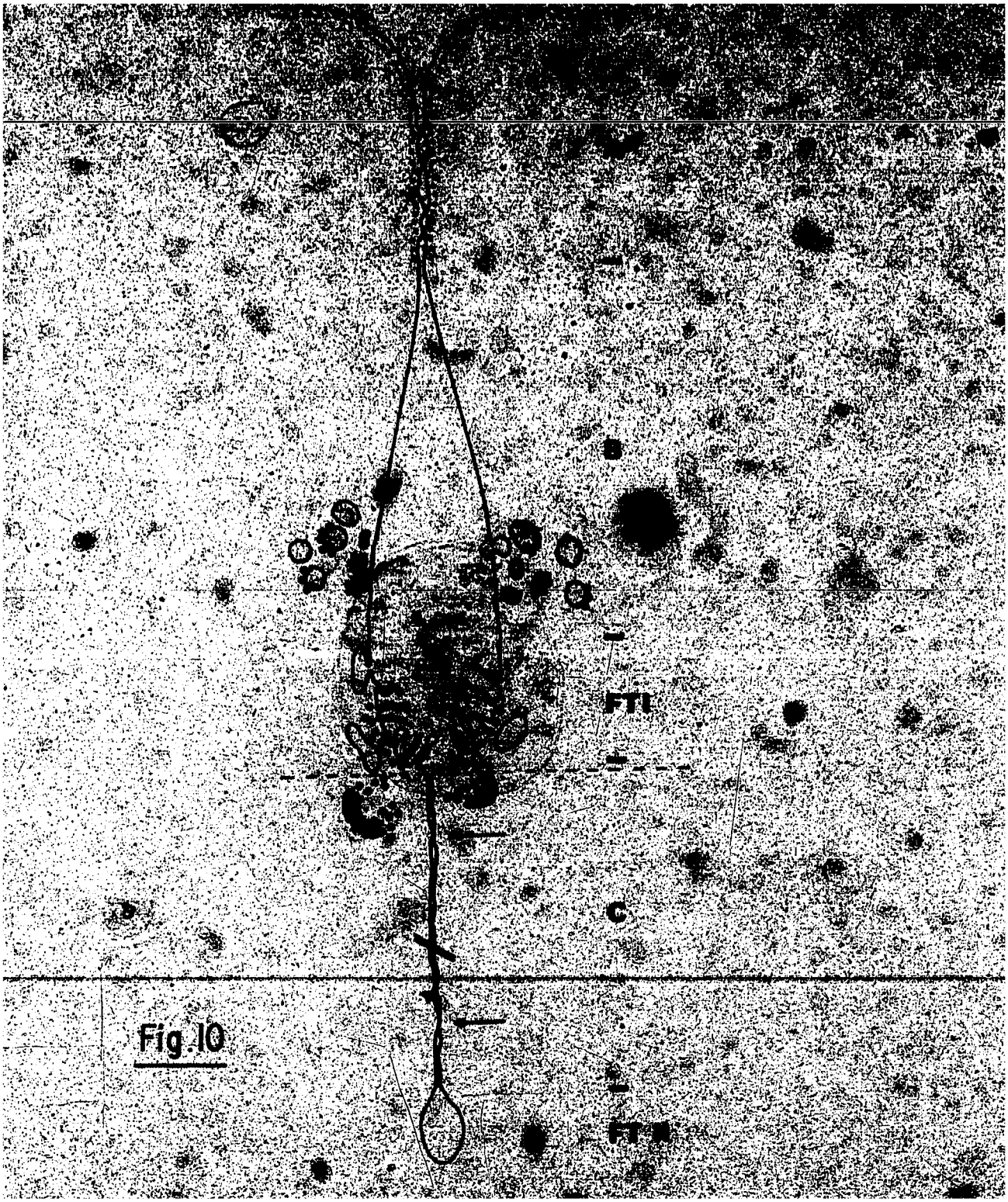


Fig.10

outermost region showed [illegible]

40 - 50 Å. The width [illegible]

in the widest [illegible] terminated at the furrow floor or [illegible]. The apical furrow was 40 - 50 µm deep.

The outermost region (A in Fig. 10) was characterized by the presence of randomly arranged cytoplasmic projections, as reported by Kalt (1971b). Apposing projections seldom approached one another closer than about 200 Å, but the intercellular space between these projections, in a restricted area near the polar axis, was occasionally reduced to a width of less than 30 Å, forming a gap junction (Figs. 9a, b). It could not be established with certainty whether focal tight junctions (Treherne *et al.*, 1967) were present, although a number of profiles suggested their probable existence (Fig. 21).

The following area of membrane apposition (B in Fig. 10) extended up to 200 µm in length and was characterized by the presence of many relatively large (0.07 - 0.5 µm diameter) vesicles in the cytoplasm on each side of the furrow (Figs. 11, 12), particularly in the deeper region of this zone. The presence of these vesicles in the process of fusing with the furrow membrane imparted undulations to the furrow surface here. Under the conditions of fixation and staining used in the present work, the vesicles were frequently observed while apparently releasing a moderately electron-dense fibrous exudate onto the furrow surface (Fig. 11), and adjacent membrane areas showed a material of similar appearance attached to its surface (Fig. 11). In some of the embryos the furrow space was virtually filled with this

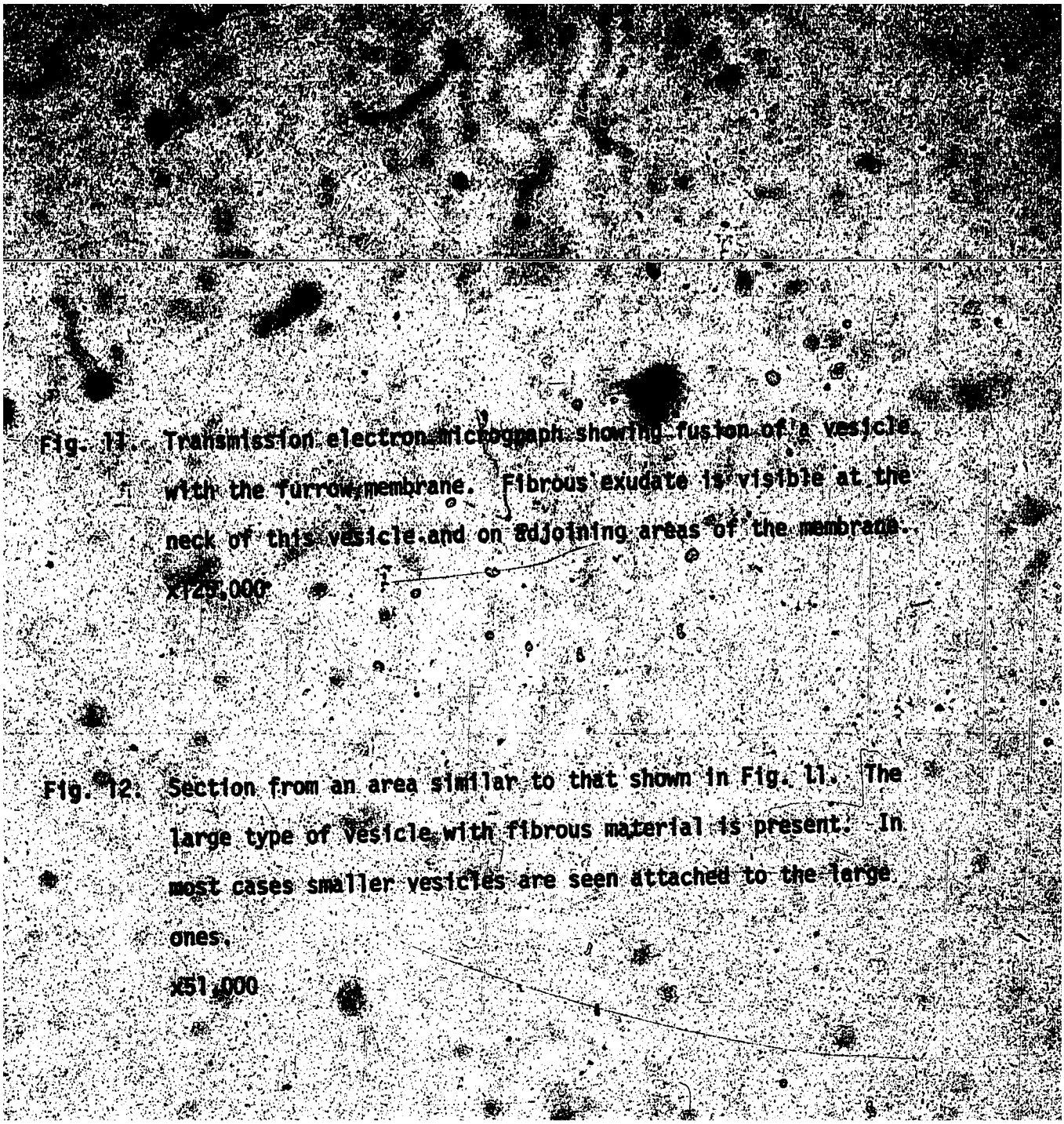


Fig. 11. Transmission electron micrograph showing fusion of a vesicle with the furrow membrane. Fibrous exudate is visible at the neck of this vesicle and on adjoining areas of the membrane. ×129,000

Fig. 12. Section from an area similar to that shown in Fig. 11. The large type of vesicle with fibrous material is present. In most cases smaller vesicles are seen attached to the large ones. ×51,000

11

12

material. Smaller vesicles were observed attached to the larger vesicles, often while the latter were apparently discharging their contents (Fig. 12). Vesicles close to the furrow showed a trilaminar limiting membrane similar in appearance to the plasma membrane (Fig. 11). In contrast, the vesicles farther away from the furrow often lacked the trilaminar pattern.

The furrow tip (FT I in Fig. 10) showed a villous transformation (Figs. 13a, 14, 17) noted earlier by scanning electron microscopy (Figs. 7a, b). These microvilli remained in the position occupied at the JCG stage despite further growth of the furrow (Figs. 69, 83), and were subsequently oriented as the band shown in Figs. 7a and b (CF stage). Up until the JCG stage the microvilli advanced with the furrow tip to form the leading edge. The band of microvilli thus demarcated the part of the furrow that had grown between the SG and JCG stages from that which had subsequently progressed to the CF stage.

The presence of microfilaments immediately subjacent to the furrow membrane has been described by Bluemink (1971b) up to the JCG stage. As the furrow advanced beyond FT I the filaments underlying the lateral furrow surfaces and FT II were much less clearly defined than before.

In the region of FT I, examination of sections often gave the impression of large empty vacuoles in close association with the furrow tip (Fig. 15). Study of serial sections, however, revealed that these were actually continuous with the furrow space and, by their lack of fibrous contents and their location, could be clearly distinguished

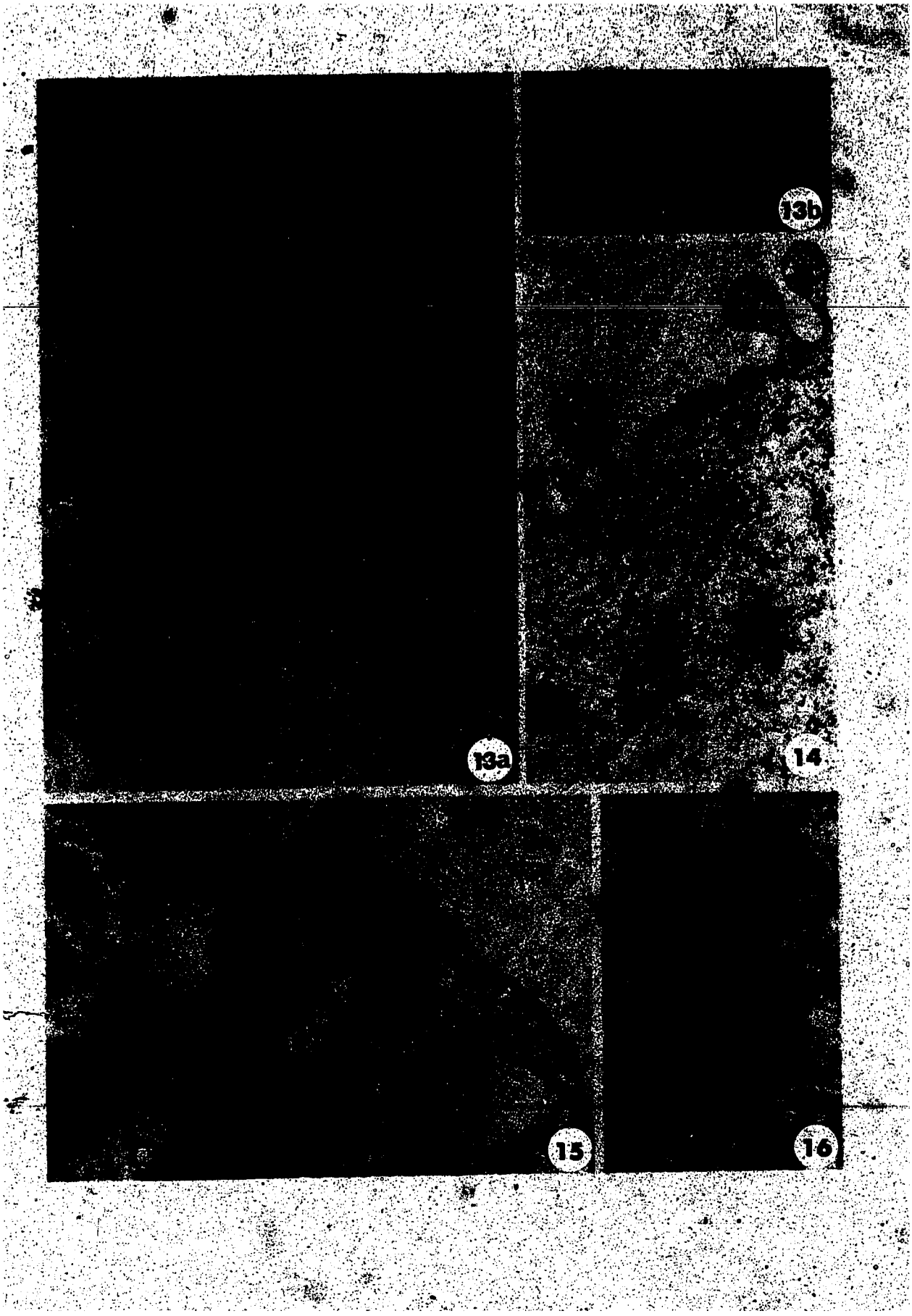
Fig. 13. a. Furrow tip I. Microvilli are seen projecting into the furrow space. Arrows point to the scattered portions of the mid-body.
x20,500

b. Enlargement of the mid-body showing tubules cut transversely as well as obliquely.
x73,000

Fig. 14. Furrow tip I with microvilli; small vesicles are seen associated with a Golgi-like body (arrow).
x22,000

Fig. 15. Furrow tip I shows vacuoles that are actually continuous with the furrow membrane. A population of small vesicles can be seen scattered close to the limiting membrane of the furrow and vacuoles (arrows).
x39,000

Fig. 16. Golgi body and small vesicles close to the furrow tip I.
x43,000


13b
13a
14
15
16

from the vesicles described earlier.

Small (400 - 600 Å diameter), round or oval vesicles with moderately electron dense contents were present in the cytoplasm near FT I (Figs. 14, 15). These were frequently seen in close association with the furrow tip and also with the Golgi elements located close to the tip area (Figs. 14, 16). These small vesicles were clearly distinguishable from the larger vesicles by their contents, size and location.

A mid-body was located below the furrow tip of the JCG stage with portions scattered through the cytoplasm of this region (Fig. 13a). The mid-body contained a small number of tubules (Fig. 13b), approximately 200 Å outer diameter, similar to those observed in the spindle of other mitotic cells (Krishan and Buck, 1965). In the present case the tubules were not observed in pairs but were dispersed singly throughout the diffuse electron dense material. Despite thorough searching, the mid-body was never seen after the JCG stage and its dissolution was presumed. From the fact that the furrow is established in the animal pole, it would be expected that the mitotic apparatus and hence the mid-body would be located excentrically towards this pole, in conformity with the situation which exists in other cleaving eggs (Rappaport, 1971). However, this observation is at variance with that previously reported (Kalt, 1971b), where the mid-body was described in a location well into the vegetal pole.

Unique V-shape or circular membrane profiles were observed in the cytoplasm subjacent to FT I membrane (Figs. 17, 18). The profiles had a pentalaminar (3 dark + 2 light) pattern of a tight

Fig. 17. Furrow tip I. Microvilli are seen projecting into the furrow space. Arrow points to the V-shape pentalaminar membrane profile.
x85,000

Fig. 18. Furrow tip I. Arrows point to the circular profile showing pentalaminar arrangement of membranes. F = furrow.
x70,000

Fig. 19. Portion of the furrow of a CF-stage embryo. Small vesicles are seen scattered in the furrow space. Vesicles with double membrane are also visible (arrow). P = pigment granule which has been lost from the section.
x43,000

junction. The material present in the concavity of the V-shape or circular profiles was quite comparable with the surrounding cytoplasm. These "intracytoplasmic tight junctions" were observed only near the furrow tip I.

The vegetal pole furrow showed only a few small microvilli at its tip and the microfilaments subjacent to the membrane were less distinct than in the corresponding region of the animal pole furrow. Vesicles were seen in close proximity to the furrow membrane (Fig. 63). Interblastomeric contacts closer than 200 Å were not observed, although a few cytoplasmic projections into the furrow space were present. The vegetal pole furrow progressed to a depth of about 250 μ before fusing with the animal pole furrow which had advanced some 1,200 μ through the embryo.

CF Stage

During most of the cleavage process the furrow showed either fibrous electron opaque contents or was electron optically empty, depending on the fixation. However, the embryos fixed after the CF stage had a significant number of small (200 - 600 Å diameter) vesicles in the furrow (Figs. 19 - 23), some of which showed a well defined double membrane (Fig. 19). The contents of the vesicles were diffuse and electron lucent. A large number of vesicles of similar size, shape and appearance were also present in the cytoplasm adjacent to the furrow membrane (Fig. 22). In most of the furrow the limiting membrane was apparent except for a few cytoplasmic projections where the membrane definition was lost (Fig. 22). These profiles gave the impression of

Figs. 20-23. Different areas of the furrow in CF-stage embryos.

Fig. 20. Vesicles are seen in the furrow. At [redacted]een arrows) the interblastomeric space is reduce[redacted] Å.
x43,000

Fig. 21. Vesicles are present in the furrow. At places (arrows) the trilaminar pattern of the limiting membrane is apparent. A close membrane contact is also visible.
x65,000

Fig. 22. Vesicles are present in the furrow as well as in the adjoining cytoplasm. Arrow points to what might be the plane of section grazing through a membrane projection.
x54,000

Fig. 23. Embryo fixed with osmium tetroxide - zinc iodide (OZI) fluid. Here, also, the furrow shows small vesicles and granules. Dense deposits of OZI are seen aligned on either side of the furrow. LD, stellate shape lipid droplet; YP, yolk platelet.
x15,000

cytoplasm open to the furrow space. Interestingly, the contents of such cytoplasmic projections were composed of vesicles or membranes. At places even the trilaminar pattern of the limiting membranes was discernible (Fig. 21). The presence of vesicles in the furrow as well as in the adjoining cytoplasm was also confirmed in OsO_4 - ZnI_2 fixed embryos (Fig. 23).

5. Incorporation of Fucose-^{3}H into Trialdehyde Fixed Embryos

a. Counting Studies

In a preliminary set of experiments embryos were incubated in fucose solution (50 µC/ml) for different lengths of time as described in Fig. 24. The purpose of these experiments was to obtain maximum incorporation of the tracer without affecting the normal development of the embryos. The amount of fucose incorporated into the fixed embryos increased with the length of incubation time (Table 2; Fig. 24). However, 15 minutes incubation proved a lethal dose for the embryos.

TABLE 2. FUCOSE-^{3}H UPTAKE INTO THE EMBRYOS: RESPONSE TO THE LENGTH OF INCUBATION IN FUCOSE-^{3}H SOLUTION (50 µC/ml)

Incubation time	2 mins	4 mins	6 mins	9 mins	12 mins	15 mins
DPM per embryo	24 27 (27) 30	31 32 (33) 36	37 39 (39) 42	43 45 (45) 47	63 65 (66) 69	73 74 (76) 81

Values in brackets represent mean of the three experiments.

Fig. 24. Uncleaved embryos were grown at room temperature in SWS containing fucose-H^3 (40 uC/ml). Embryos were left in the solution for 2, 3, 6, 9, 12 and 15 minutes. Immediately after their removal the material was thoroughly washed and fixed in the trialdehyde mixture. Counts were made on the fixed embryos. The results represent the mean of three experiments. The amount of fucose incorporated into the embryos increased with the length of incubation time in the fucose-H^3 solution.

Fig. 25. Uncleaved embryos were grown in fucose-H^3 solution for 12 minutes as described above. Thereafter the material was thoroughly washed and only the uncleaved embryos were post-grown in SPSS for 0, 5, 20, 40 and 80 minutes. Counting was carried out on fixed embryos as described above. Activity increased slightly for up to 20 minutes; thereafter it declined and was more or less comparable with zero minute group.

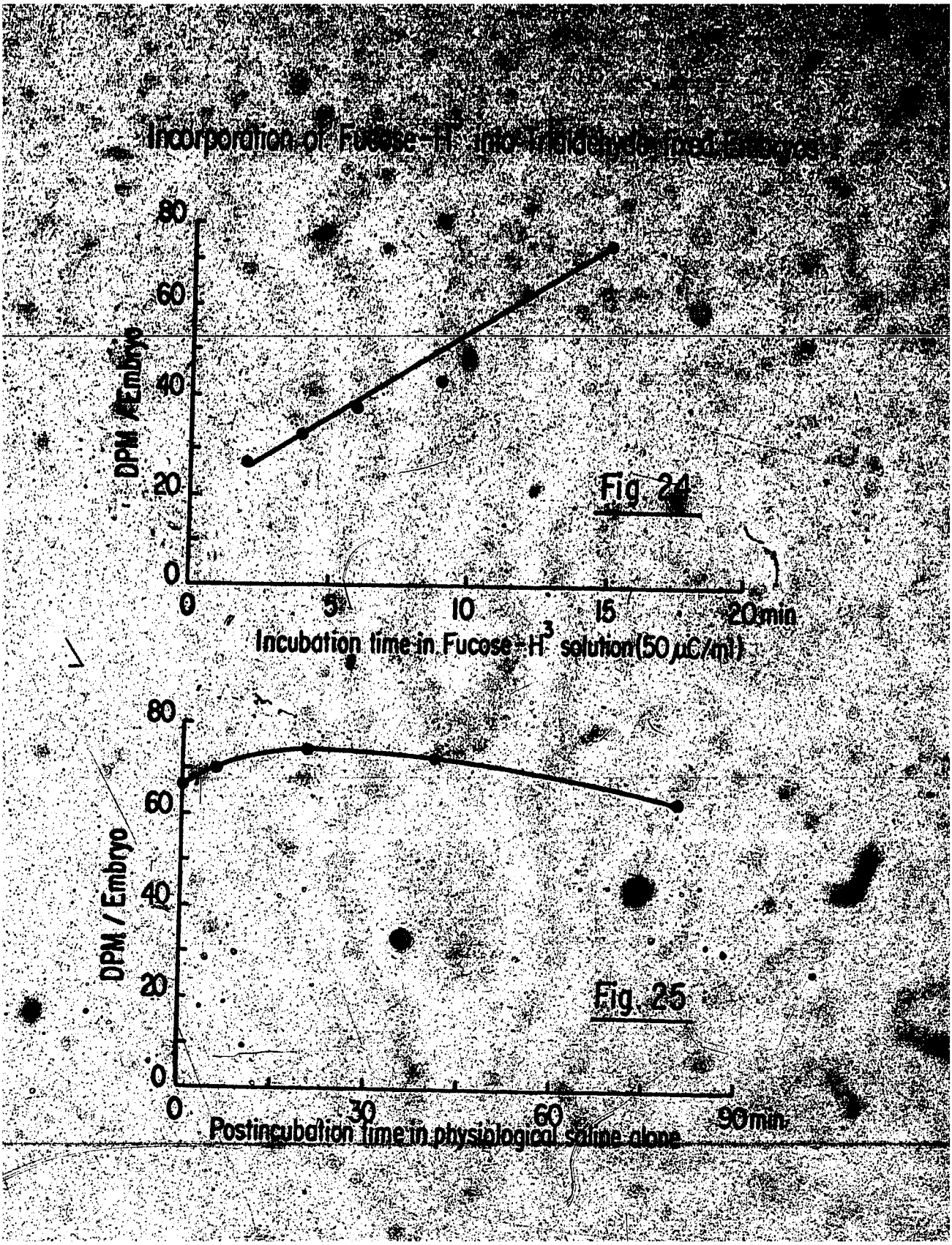

Incorporation of Fucose-H3 into trichloracetic acid precipitate
80
60
40
20
0
DPM / Embryo
0
5
10
15
20min
Incubation time in Fucose-H3 solution (50 µC/ml)
80
60
40
20
0
DPM / Embryo
0
30
60
90min
Postincubation time in physiological saline alone

Fig. 24

Fig. 25

The latter showed white spots in the normally pigmented area and did not develop. Embryos grown for 12 minutes developed normally up to the formation of the blastopore (maximum time observed). Vitelline membranes from 10 embryos (grown in fucose-^{3}H solution and fixed in trialdehyde mixture) were surgically removed and pooled together to check for any radioactivity. The membranes did not incorporate any fucose and embryos burnt (in a Packard oxidizer) with or without the vitelline membrane showed no difference in their counts.

After 12 minutes incubation in fucose-^{3}H solution the embryos were washed and grown in fresh SPSS solution for 0, 5, 20, 40 and 80 minutes, followed by fixation in the trialdehyde mixture. The activity was then checked in the fixed embryos and the results shown in Table 3 (Fig. 25). Until 20 minutes each embryo on the average showed a slight increase in dpm (66 at 0 minutes to 74 at 20 minutes); thereafter the level of activity decreased gradually to 63 at 80 minutes (Table 3).

TABLE 3. FUCOSE-^{3}H UPTAKE INTO THE EMBRYOS: EFFECT OF POST-INCUBATION FOLLOWING 12 MINUTES TREATMENT IN FUCOSE-^{3}H SOLUTION (50 µC/ml)

Post-incubation time	0 mins	5 mins	20 mins	40 mins	80 mins
DPM per embryo	63	72	73	69	58
	65 (66)	73 (70)	72 (74)	70 (72)	62 (63)
	69	66	77	75	68

Values in brackets represent mean of the three experiments

b. Autoradiography

Thick sections from the fucose-treated embryos, grown subsequently for 0, 5, 20, 40 and 80 minutes and trialdehyde fixed were exposed to Ilford L4 emulsion for several weeks. Distribution of the silver grains was judged by visual inspection. It was difficult to tell the presence of silver grains in a area containing a lot of pigment granules, even if the latter was bleached prior to emulsion exposure. Two criteria were adopted to differentiate between these two dense structures:

1. Silver grains of Ilford L4 emulsion were smaller (0.12 μ, Caro and Van Tubergen, 1962) in size than the pigment granules (0.5 μ diameter)
2. Pigment granules showed a hollow center (optical effect) when they were slightly out of focus and could be differentiated from silver grains which did not show such an effect. This occurred when the silver grains on top of a thick section were in focus and the pigment granules at a slightly lower level were not in focus (Figs. 26 - 29).

In all the embryos studied the vitelline membrane (VM) did not show any grains, thus supporting the liquid scintillation counting studies where the isolated VM did not show any counts. Grains were scattered in the cytoplasm with no special distribution except that they were particularly present on or around the yolk platelets. Due to the lack of resolution it could not be ascertained whether the grains were associated with the superficial material of the yolk plate-

Figs. 26-29. Autoradiographs of the embryos that were grown in fucose-H^3 solution for 12 minutes and were post-grown in SPSS alone for different lengths of time.

Fig. 26. Post-incubation time: 0 minute. Furrow formation has not yet started. Silver grains are mostly present in the cytoplasm. A non-specific distribution of grains can be seen near the outer surface (OS).
x900

Fig. 27. Post-incubation time: 5 minutes. Furrow formation has not yet started. Silver grains are seen (arrows) only in the cytoplasm and no grains are present near the outer surface (OS).
x900

Fig. 28. Post-incubation time: 20 minutes. Furrow formation has begun and silver grains are present in the furrow (F). In the cytoplasm grains can also be seen near or on the yolk platelets (arrow).
x900

Fig. 29. Post-incubation time: 40 minutes. Furrow has progressed beyond FT I. Large number of grains are present in the ampullaceous leading edge of the furrow (F).
x900

lets or were present in the cytoplasm around it. However, certain profiles did support the former possibility (Fig. 28).

In the embryos fixed immediately after (0 minutes) the fucose treatment, most of the activity was seen in the cytoplasm. A few grains were also observed along the cell surface (Fig. 26). After 5 minutes no activity was observable along the outer surface (Fig. 27) and the grains were restricted mainly to the cytoplasm. After 20 minutes the cleavage furrow began to appear and also silver grains in the furrow area (Fig. 28). Within 40 minutes a large amount of activity was observed in the ampullaceous furrow tip II (Fig. 29) and the grains were relatively less frequent in the cytoplasm. After 80 minutes the grains were observed mainly in the completed furrow and the outer surface in the immediate vicinity of the cleavage groove also showed some activity.

6. Morphology of Cell Inclusions

a. Lipid Droplets

Much of the cytoplasm is filled with electron dense yolk platelets and with comparatively less dense inclusion bodies (Figs. 30, 35). A detailed account of the former in the *Xenopus* embryo and oocyte is already available (Balinsky and Devis, 1963; Armstrong, 1972; Leonard *et al.*, 1972). In light microscope study of the *Rana pipiens* oocyte the inclusion bodies have been called "lipochondria" (Holtfreter, 1946), a term used by Ries (1935) to describe osmiophilic granules in pancreatic cells and to indicate their lipid nature. Subsequently these inclusions have been described in a variety of other oocytes (Kemp,

1956; Balinsky and Devis, 1963; Karasaki, 1963b; Wischnitzer, 1966) and have been referred to by such names as fat droplets, lipid droplets, lipid bodies, liposomes, lipo-protein bodies and lipochondria. All of these terms indicate that the inclusions contain mainly lipids. For the present purpose the term "lipid droplets" will be used.

The lipid droplets were round, oval or multilobed in profile (Figs. 30, 31, 35) and varied in size from 0.3 μ to 8 μ although most of them were in a size range from 0.8 μ to 2.0 μ. Occasionally the droplets were seen clumped together. At places the lipid droplets were seen in close association with the yolk platelets, often having a concave face fitting onto the convex surface of the platelet (Fig. 30). In certain cases, especially towards the vegetal pole, the lipid droplet spheres formed a complete ring around a yolk platelet (Fig. 35).

Sections from the embryos fixed only in trialdehyde with no post-osmication, stained for 15 minutes each in 5% uranyl acetate and lead citrate, did not show any lipid droplets. Empty spaces of a similar size and distribution were observed (Fig. 34). On the other hand, in embryos fixed with osmium tetroxide alone the lipid droplet showed angular or stellate contours (Fig. 33). A similar appearance of lipid droplets was apparent in OsO_4 - ZnI_2 fixed embryos (Figs. 23, 74).

As with yolk platelets, the lipid droplets showed a gradient in their distribution, being more frequent towards the vegetal pole. Despite their similar size and distribution the two structures were completely different in their internal structure and could readily be

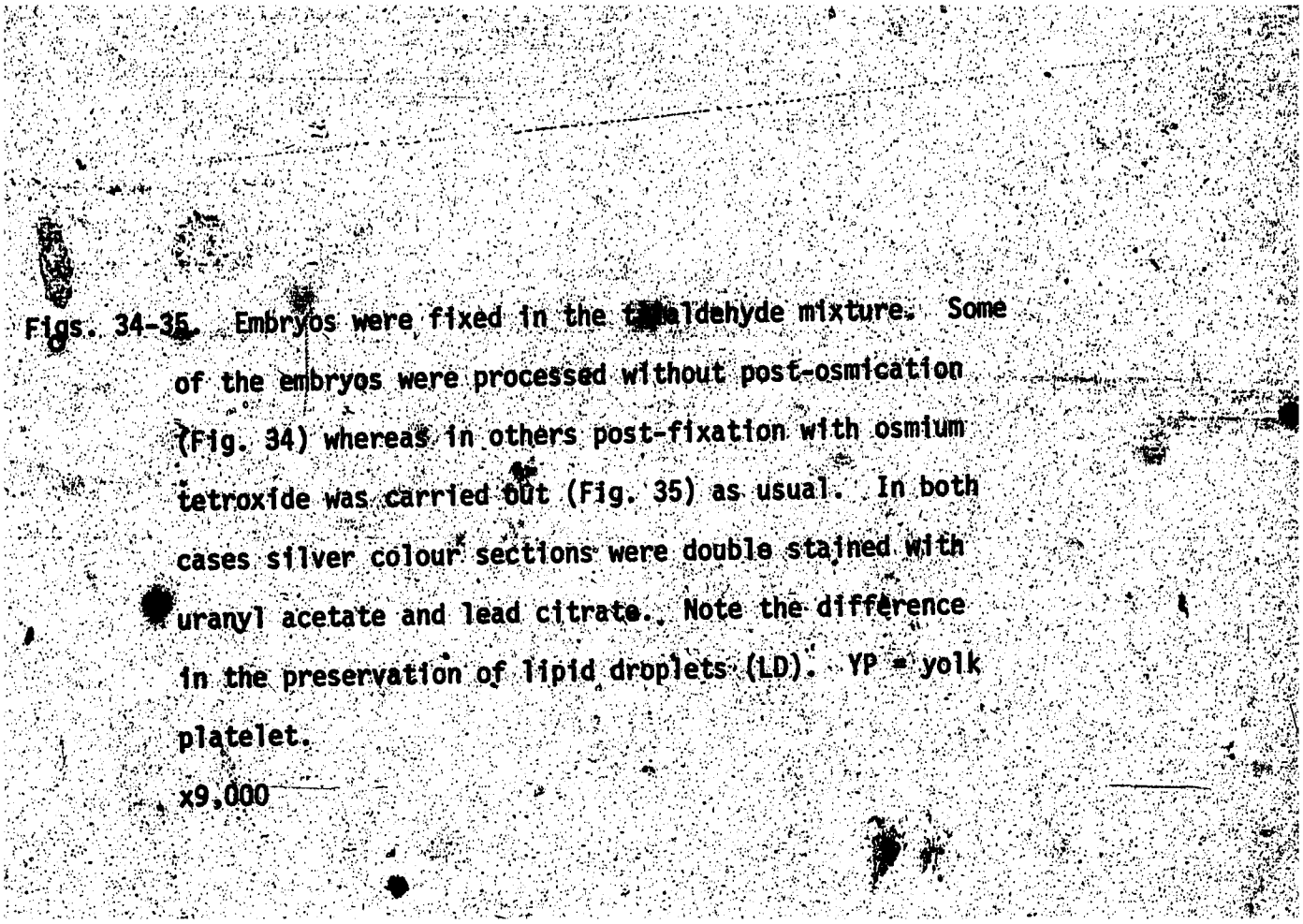
Fig. 30. A portion of the animal pole cytoplasm. Lipid droplets (arrow) and yolk platelets (YP) show a comparable distribution. A yolk platelet is seen in a lipid droplet concavity (double arrow). x2,200

Fig. 31. Multilobed lipid droplet with a relatively dense margin. Glutaraldehyde-osmium tetroxide. x23,500

Fig. 32. An electron dense growing or receding partition in a lipid droplet. Glutaraldehyde-osmium tetroxide. x70,000

Fig. 33. Angular or stellate lipid droplets after 1% osmium tetroxide fixation. x13,000

30
31
32
33



Figs. 34-35. Embryos were fixed in the [illegible]aldehyde mixture. Some of the embryos were processed without post-osmication (Fig. 34) whereas in others post-fixation with osmium tetroxide was carried out (Fig. 35) as usual. In both cases silver colour sections were double stained with uranyl acetate and lead citrate. Note the difference in the preservation of lipid droplets (LD). YP = yolk platelet.
x9,000

distinguished from each other. Yolk platelets showed a crystalline core (Fig. 36), partially surrounded by a diffuse granular substance (Figs. 34, 42, 43) and had a well defined limiting membrane (Figs. 42, 43) as has been shown by others (Balinsky and Devis, 1963; Karasaki, 1963a, b; Armstrong, 1972; Leonard *et al.*, 1972). By contrast, lipid droplets were uniformly grey in appearance and lacked any crystalline structure. With few exceptions there was no distinct peripheral or inner zone, and a limiting membrane could not be resolved in any of them, although in certain cases the boundary was relatively dark in appearance (Fig. 31). Certain lipid droplets showed what may have been the process of fission or fusion and an apparently growing or receding partition was observed (Fig. 32).

Joint configurations involving a lipid droplet and endoplasmic reticulum and also a lipid droplet and Golgi body (Figs. 37, 38) were observed. Such configurations were infrequent. Although lipid droplets themselves did not show any limiting membrane, membrane-bound tubular cisternae were occasionally seen projecting from them, and such cisternae had ribosomes attached to the cytoplasmic side giving the appearance of rough endoplasmic reticulum (RER, Fig. 37). The clear lumen of the RER cisterna was continuous with a cavity in the lipid droplet, which was not membrane-bound (Fig. 37). In joint configurations of a lipid droplet and Golgi body, the outermost membrane at the convex face of the latter was continuous with the droplet. As in the case of the lipid droplet - RER complex, the lumen of this Golgi cisterna was common with a cavity in the lipid droplet (Fig. 38). The cavity again lacked a limiting membrane. The

Fig. 36. A portion of a yolk platelet showing the crystalline area and granular superficial layer. A boundary is apparent between the crystalline and peripheral regions (arrows).
x180,000

Fig. 37. Endoplasmic reticulum membrane projecting from a lipid droplet showing ribosomes attached to the outer surface. Lumen of the ER is continuous with a cavity in the lipid droplet.
x43,000

Fig. 38. Joint configuration of a Golgi body and a lipid droplet. The membrane of the outermost Golgi cisterna loses definition in the lipid droplet and the cisternal space is continuous with a cavity in the lipid droplet. Small vesicles and electron dense particles are apparent at the concave face of the Golgi body.
x36,000

Golgi body showed small vesicles and electron dense particles scattered in its concavity. They were also seen in close proximity with the lipid droplets (Fig. 90). The RER and the Golgi body were never present on a common lipid droplet.

b. Endoplasmic Reticulum

The endoplasmic reticulum was predominantly comprised of tubular cisternae, up to 2 μ long and 0.2 μ in diameter, and vesicles (0.15 - 0.5 μ in diameter) (Figs. 39-43). The former were usually seen in pairs (Figs. 39-43). Vesicular as well as cisternal endoplasmic reticulum showed short filamentous projections embedded in the luminal surface of the membrane (Figs. 39-43), giving a characteristic fringed appearance and henceforth this type will be referred to as "Fringed Endoplasmic Reticulum". The fringed ER membrane showed ribosomes attached to the cytoplasmic surface which frequently showed a spacing of approximately 150 Å (Figs. 39, 40). Paired cisternae had ribosomes on their interfaces, and in some such pairs membrane continuity between cisternae was also noticed (Fig. 40). Partners of a pair were not necessarily equidistant and often had dissimilar lengths (Fig. 39).

Another feature shown by the fringed ER cisternae was their close apposition with yolk platelets (Figs. 42, 43). The cisterna nearer to the yolk platelet was asymmetric inasmuch as the portion adjoining the platelet's limiting membrane did not show any ribosomes, although the latter were apparent on the membrane away from such contacts (Figs. 42, 43). The fringed ER - yolk platelet contacts were of

Figs. 38-41. Paired cisternae of endoplasmic reticulum. The fringed interior is due to the presence of filamentous projections on the luminal surface. Ribosomes are present on the outer surface.

Fig. 39. Paired ER cisternae of unequal lengths. Ribosomes are evenly spaced on the membrane surface.
x44,000

Fig. 40. Cisternae showing a membrane continuity (arrow).
x43,000

Fig. 41. A large number of fringed vesicles present close to a pair of cisternae.
x36,000

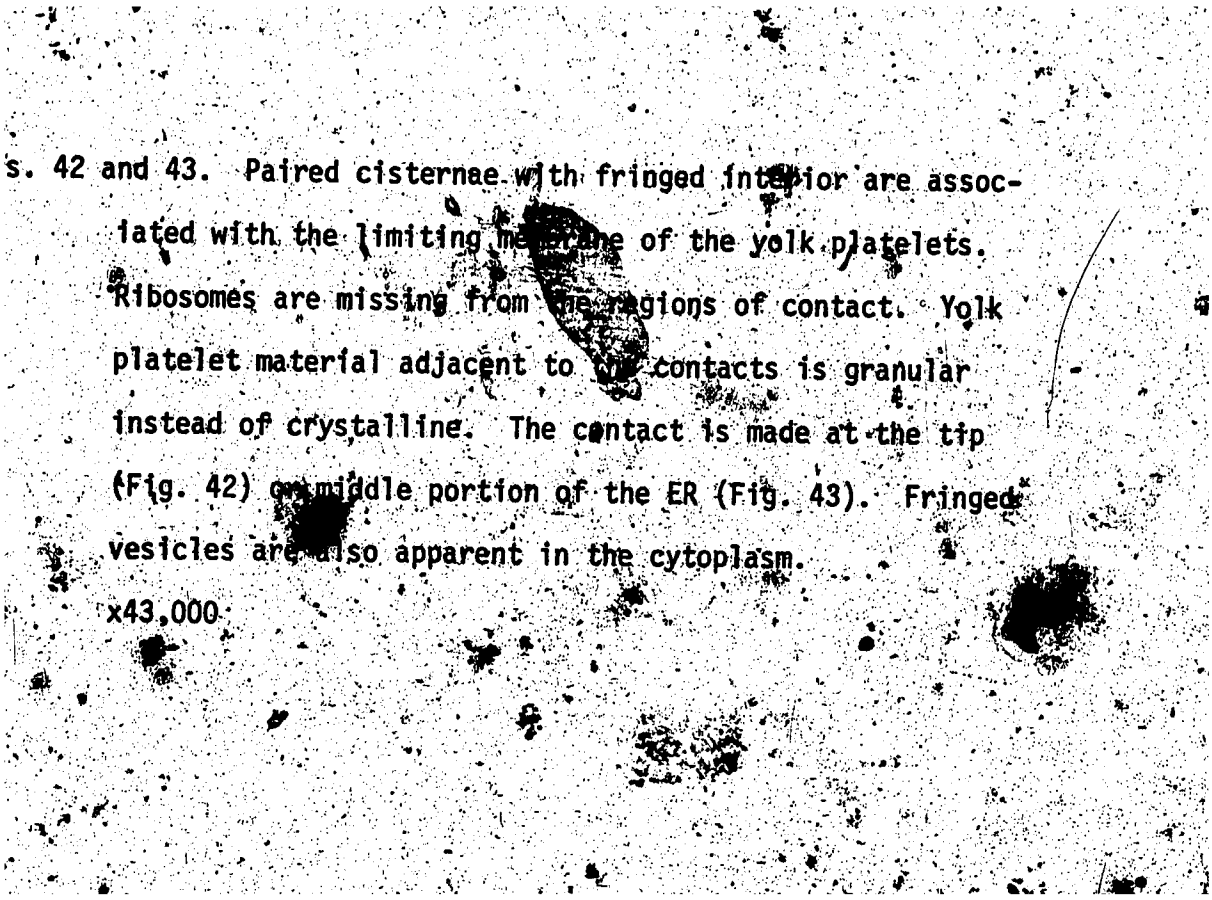
39
40
41

varying lengths, and different portions of a cisterna, such as the tip, mid-section or terminal section (Figs. 42, 43), were involved in such contacts. Yolk platelets, showing attached fringed ER membrane, had granular peripheral material adjacent to such contacts (Figs. 42, 43). In certain cases the ends of the fringed ER cisternae were dilated (Fig. 43).

Some of the paired tubular cisternae were within 0.2 μ of a Golgi body (Figs. 44, 45, 51). However, in this case only one of the cisternae had a fringed interior (Fig. 45), while the other was narrow in diameter and contained a moderately electron dense substance comparable with that of the nearby Golgi cisternae. Such pairs showed none, or very few, attached ribosomes (Figs. 44, 45, 51) and the cytoplasm between these ER cisternae and the Golgi body showed small vesicles (Fig. 51). Fringed ER cisternae were also seen as an integral part of the Golgi body (Fig. 54) and is discussed later.

Fringed ER vesicles ranged from 0.1 μ to 0.5 μ in diameter, were always seen associated with fringed ER cisternae (Figs. 41, 42), and possessed ribosomes on their outer surface. The fringed ER vesicles were also seen scattered elsewhere in the cytoplasm, particularly near the convex face of the Golgi bodies (Figs. 45, 46, 55). In certain cases the membrane of the Golgi cisterna was seen projecting into the fringed ER vesicle, the membrane showing close apposition (Fig. 55). Neither fringed ER cisternae nor vesicles showed any morphologic relationship with the furrow membrane.

In addition to the ER types described above, lone ER cisternae were observed that showed a diffuse electron lucent contents instead of



s. 42 and 43. Paired cisternae with fringed interior are associated with the limiting membrane of the yolk platelets. Ribosomes are missing from the regions of contact. Yolk platelet material adjacent to the contacts is granular instead of crystalline. The contact is made at the tip (Fig. 42) or middle portion of the ER (Fig. 43). Fringed vesicles are also apparent in the cytoplasm.

x43,000

A
B

Fig. 44. Paired cisternae (C) located near a Golgi body (GB). The cisterna towards the Golgi body is narrow and its appearance is similar to the Golgi cisternae. The dilated ER cisterna still has ribosomes on the side away from the Golgi body. x43,000

Fig. 45. Paired cisternae located near the Golgi body as in Fig. 44 but the dilated partner of the pair still shows some fringes in the lumen. The Golgi body has an attached cisterna which is dilated and is similar to fringed ER. Coated vesicles are also present (arrows). x43,000

Fig. 46. Fringed ER cisterna has evenly spaced ribosomes attached to the outer surface. A fringed vesicle is associated with the Golgi body. x42,000

Fig. 47. Small vesicles are scattered between the Golgi body and an ER cisterna that has uniformly electron-lucent interior (arrow). Compare the internal appearance of this ER cisterna with that of the fringed ER (FER). x42,000

Figs. 48 and 49. Golgi body with a single fenestrated cisterna and its associated small vesicles. ER vesicles are also present (arrows). x36,000

44
46
47
48
49

the fringed interior (Figs. 47, 51). These irregularly shaped ER cisternae also had ribosomes attached to the surface, but they were less frequent than on the fringed ER and were randomly distributed. The cisternae were scattered in the cytoplasm without any preferential distribution. Whenever seen close to the Golgi body such ER cisternae did not show ribosomes on the side facing the Golgi body, and small vesicles were seen scattered between the two organelles (Fig. 47). Unlike fringed ER, these membranes were never seen associated with the yolk platelets.

c. Golgi Bodies

The term Golgi body has been used here in a broader sense and includes a range of structures from a single fenestrated cisterna to a stack of cisternae with its adjoining vesicles. Although the Golgi bodies were distributed throughout the cytoplasm they were more frequent within a distance of 5 μ from the growing furrow. Some of the Golgi bodies were just 2 μ away from the growing furrow. The simplest form of the Golgi body seen in these embryos was a single fenestrated cisterna accompanied by ER membranes (Figs. 48, 49). The interior of such cisternae was moderately and uniformly electron opaque in appearance. Small (200 - 400 Å diameter), round or oval vesicles of a similar density were seen associated with these cisternae (Fig. 49). Study of serial sections excluded the possibility that these cisternae were a portion of more developed Golgi bodies. They could not have been a part of annulate lamellae as the latter were quite infrequent and could not account for the relatively frequent

occurrence of such cisternae.

In further developed Golgi bodies two or three cisternae were observed. Although the cisternae were arranged in a stack the organelle was without morphological polarity and did not show any curvature (Fig. 50). Vesicles associated with either of the faces were similar in appearance. Depending on the plane of the section, cisternae either appeared as an array of circular profiles or as well defined parallel membranes, and were usually narrow in the middle and slightly dilated towards the edges (Fig. 50). Coated vesicles were also noticed in association with such Golgi bodies (Fig. 50).

Golgi bodies showing convex and concave faces were also observed (Figs. 51, 53), and fringed vesicles were usually seen associated with the convex side (Fig. 51). Study of a large number of thin sections through such Golgi bodies revealed that the cisternae were more or less cup-shaped, and a section through the rim of such a Golgi body showed three to four cisternae [illegible] concentric rings (Fig. 52). The innermost ring had a diame[illegible] most spanned about 0.5 μ. The cup-shaped [illegible] plane was about 1 μ in height. The cytoplasm [illegible] the convex side of some of the Golgi bodies had a cisterna (Fig. 53) that was fenestrated in appearance. Small, round or oval vesicles were seen scattered between the Golgi body and this fenestrated cisterna, and both the small vesicles and the cisterna had a similar internal appearance. Coated vesicles were noticed among the vesicles associated with the Golgi body and in certain cases they appeared to have been fixed in the process of pinching off from the Golgi cisternae as well as from the fenestrated cisterna (Fig.

Fig. 50. Golgi body with flat cisternae which have a central plate-like region and are dilated at their ends. Vesicles present at both the faces are similar in appearance. Arrows indicate membrane protrusions similar to the formation or fusion of coated vesicles. x35,500

Fig. 51. Golgi body with cup-shaped cisternae. Fringed vesicles are scattered mostly in the cytoplasm towards the convex face of the Golgi body. Paired cisternae (PC) of the kind shown in Figs. 44 and 45 are apparent and also show a possible formation of vesicles. Arrow indicates a coated vesicle. x23,000

Fig. 52. Golgi body with cup-shaped cisternae. The plane of the section has passed through the rim and the cisternae appear as concentric rings. LD, lipid droplet. x2[illegible],500

Fig. 53. Cup-shaped Golgi body. Small vesicles are present in the cytoplasm between a fenestrated cisterna (FC) and the Golgi body. The innermost cisterna (GC) of the Golgi body is also fenestrated. Arrows indicate coated vesicles. x42,000

Fig. 54. Golgi body with seven cisternae. The outermost cisterna has a fringed interior and has ribosomes only on the surface away from the organelle. The third cisterna (arrow) is also dilated and is similar to the fringed ER cisternae but lacks ribosomes. The innermost cisterna of the Golgi body is fenestrated. Arrow head indicates coated vesicle. x36,000

53). This type of Golgi body was more frequently observed than the other types reported here.

The most highly developed Golgi bodies observed here had a total of seven well defined flat or curved cisternae with a clear morphological polarity. The outermost cisterna on the convex side had a bifacial symmetry, inasmuch as only the side away from the Golgi body showed ribosomes (Fig. 54). This cisterna had a fringed appearance. The third dilated cisterna was often similar to the one described above, but totally lacked ribosomes (Fig. 54). The rest of the cisternae were relatively narrow and had a moderately electron dense interior. A study of serial sections confirmed that this electron opacity was not due to the plane of the section grazing the cisternal membrane. The cisternae on the concave side were frequently fenestrated and showed associated vesicles (Fig. 54). Coated vesicles were either continuous with the Golgi cisternae (Fig. 54) or were seen lo[illegible]ng other Golgi-associated vesicles.

Golgi bodies were also noticed in pairs (Fig. 55). The two partners seldom had a similar number of cisternae and most frequently the number was three in one and four in the other. A special type of morphological relationship between the fringed ER vesicles and the Golgi cisternae has been discussed above.

d. Lamellar Bodies

Based on the arrangement and relative thickness of the lamellae, three types of lamellar bodies were observed. In the first type, the body showed dense, radially arranged lines interspersed by less dense lines (Figs. 56a-c). The appearance was similar to a

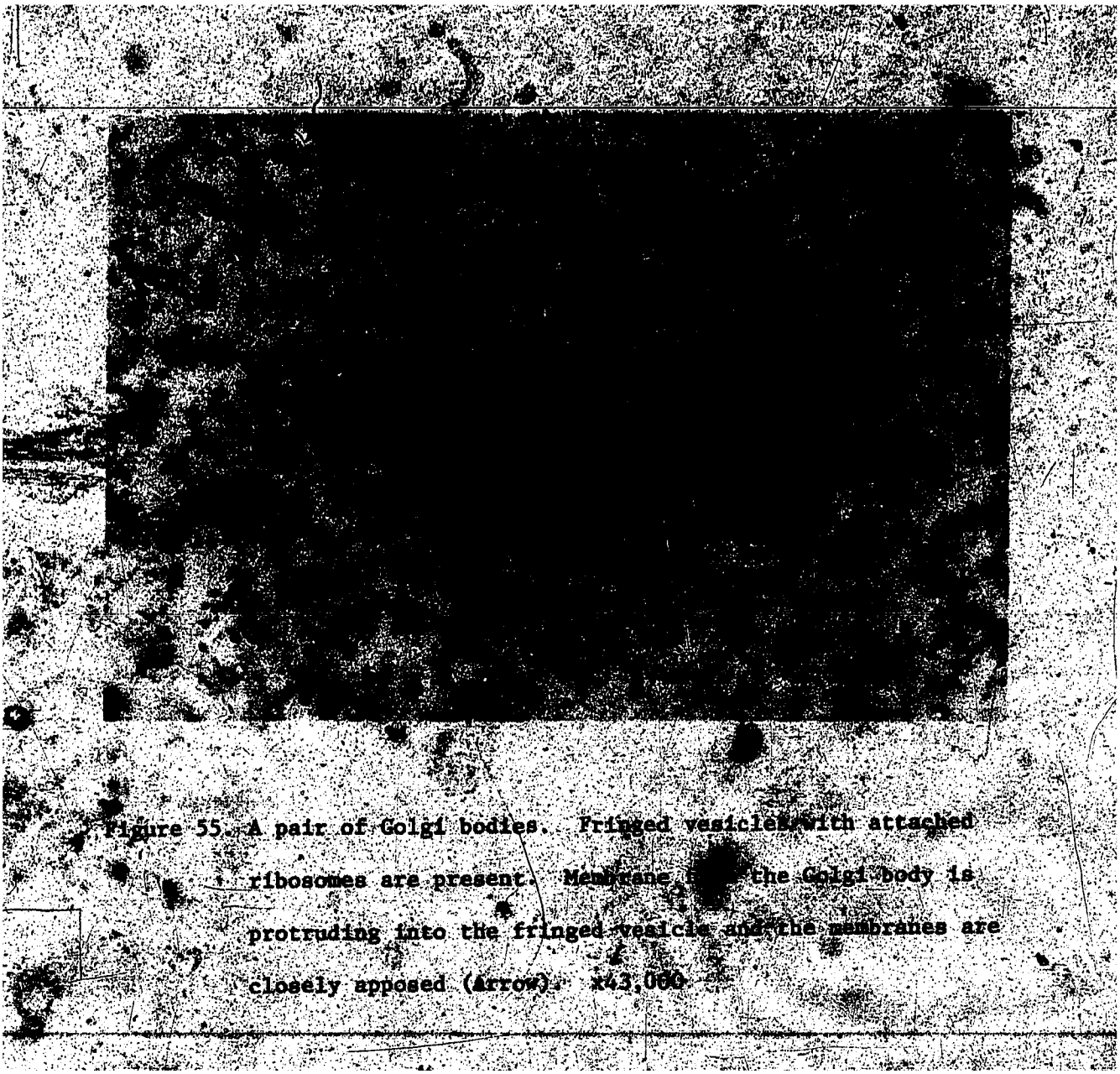


Figure 55. A pair of Golgi bodies. Fringed vesicles with attached ribosomes are present. Membrane [illegible] the Golgi body is protruding into the fringed vesicle and the membranes are closely apposed (Arrow). x43,000

typical myelin figure (Fig. 56b) and had a repeat distance of 100 Å as recorded from micrographs. Such membrane profiles were observed only near the furrow tip 1 (Fig. 56a).

In the second type of lamellar body (Figs. 57a-c) tetrads of lamellae were observed in which two dense lines sandwiched between the two less dense lines, a uniform clear [illegible]ating all the four (a septalaminar arrangement). Each tetrad of lamellae spanned a thickness of approximately 160 Å. In any single lamellar body two to four such tetrads were present in a circular fashion enclosing a diffuse electron dense substance (Fig. 57a). The space between any two tetrads was of uneven dimensions (Figs. 57b-c). This type of lamellar body was frequently observed near lipid droplets (Fig. 57a).

The third type of lamellar body (0.15 to 1 µ in diameter) a band [illegible]ten lamellae was interrupted [illegible]random (Fig. 58b). All the lamel[illegible]the band were uniformly electron dense and were evenly spaced (spacing, 35 Å, Figs. 58b, c). In some of the lamellar bodies the lamellar portion was separated from its limiting membrane by a large space (Fig. 58a) while in others a membrane continuity between the lamellar part and the limiting membrane was apparent (Fig. 58b). This apparent difference may have been due to the plane of the sections passing through different regions of the body. Such membrane profiles were more frequent near the furrow membrane (Fig. 58b), and were seen associated with the concave face of the [illegible]shaped Golgi bodies described earlier (Fig. 58a). Such Golgi bodies were also seen close to the growing furrow.

Figs. 56-58. Three types of lamellar bodies. Figs. 56c, 57c and 58c show simplified version of the arrangement of laminae and their relative thickness in the lamellar bodies.

Fig. 56. a. Furrow tip I (FT I) with microvilli. The lamellar body (arrow) is seen near the tip.
x17,000

b. Enlargement showing myelin-arrangement of laminae in the body.
x110,000

Fig. 57. a. Membrane whorls close to a lipid droplet (LD).
x52,000

b. Enlargement showing two heavy-dark laminae sandwiched between two less dark laminae (arrows).
x290,000

Fig. 58. a. Lamellar body (arrow) closely associated with the cup-shaped Golgi body.
x50,000

b. Band of laminae intertwined at random. F, furrow.
x40,000

7. Cytochemical Studies

a. Lanthanum Treatment

The presence of lanthanum nitrate during fixation resulted in the deposition of an electron dense layer external to the cell membrane which obliterated the outermost dark lamina of the trilaminar membrane, although the inner dark lamina was still discernible (Figs. 60, 62, 64). The presence of this stained layer facilitated the examination of surface topography. The thickness of the stainable layer, measured where the membrane was cut transversely, showed variations according to the region of the furrow. These variations were consistently obtained.

The outermost region of the furrow displayed a uniformly thick (100 - 150 Å) lanthanum stained layer similar to that present on the surface remote from the furrow (Fig. 59). At a depth of approximately 70 μ the thickness gradually began to increase, reaching a maximum of about 0.25 μ (Fig. 60) where the large vesicles and exudate were associated with the furrow membrane (Figs. 11, 12); more deeply the stained layer showed a gradual decrease in thickness (Fig. 61); in the furrow tip area (FT I) the thickness of the layer was at a decreased level but was still greater than that on the outer surface, and was uniform, or occasionally patchy (Fig. 62). Stained material was also present loose within the furrow space. The vegetal pole furrow (Fig. 63) showed a fairly uniform layer throughout, about 100 - 150 Å thick, and similar to that seen in the outermost region of the animal pole furrow.

After the furrow had passed the JCG stage and had traversed approximately two-thirds of the embryo, the opposing membranes ran almost parallel to one another (C in Fig. 10). Occasionally the membranes

Figs. 59-62. Embryos fixed in the presence of lanthanum ions.

Fig. 59. Outer surface showing uniformly stained layer.
x44,000

Fig. 60. Furrow surface corresponding to the lower half of the segment 8 in Fig. 10. Note the increase in thickness of uniformly stained surface.
x45,000

Fig. 61. Furrow surface close to region FT I in Fig. 10, showing gradual decrease in thickness of the stained layer.
x43,000

Fig. 62. Furrow tip I showing patchily stained surface.
x44,000

[redacted]

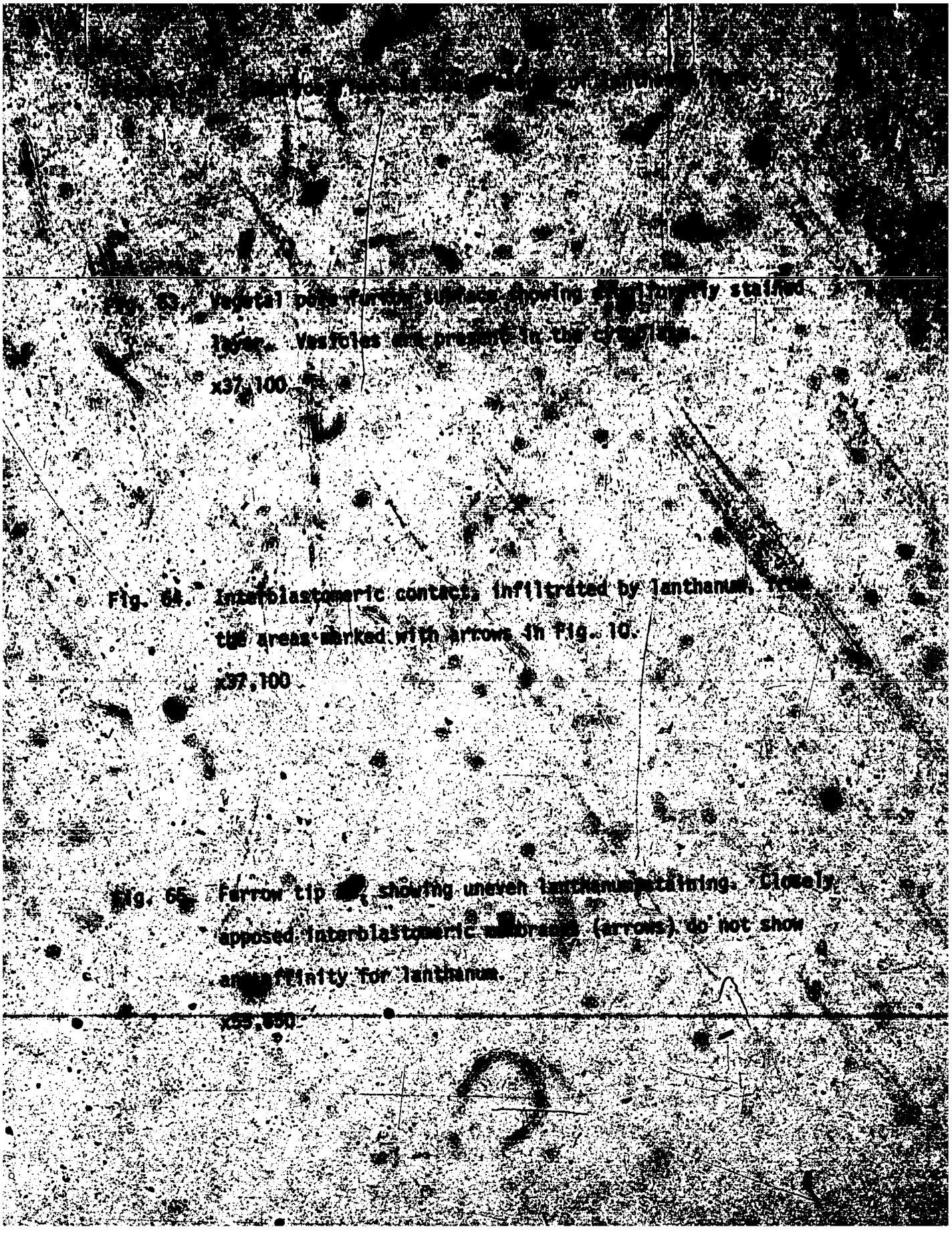
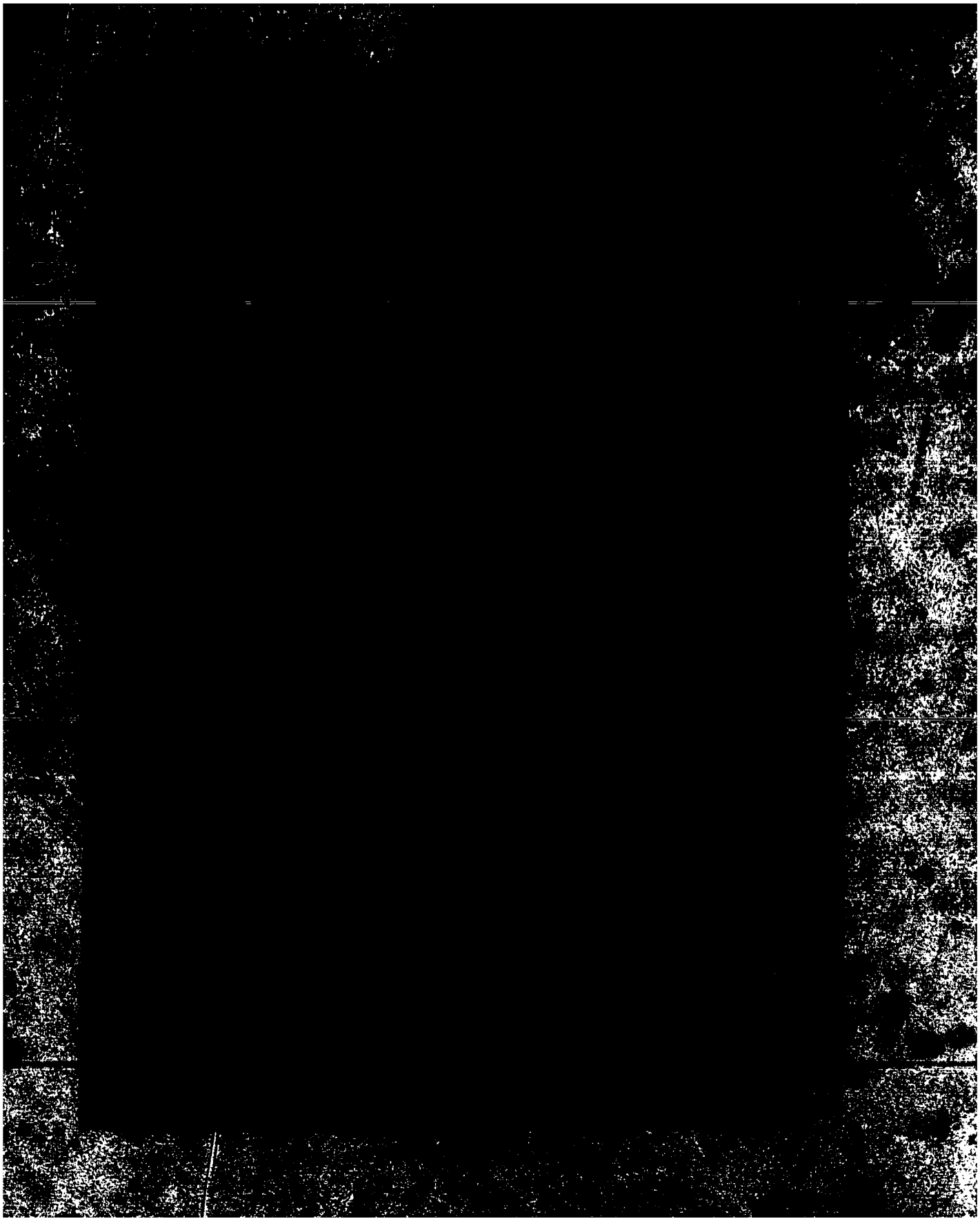


Fig. 63. Vegetal pole furrow surface showing slightly stained lower. Vesicles are present in the cytoplasm. x37,100

Fig. 64. Interblastomeric contacts infiltrated by lanthanum, from the areas marked with arrows in Fig. 10. x37,100

Fig. 65. Furrow tip showing uneven lanthanum staining. Closely apposed interblastomeric membranes (arrows) do not show an affinity for lanthanum. x35,000



[illegible] (Fig. 64)

[illegible] (Fig. 65) was

[illegible] manner (Figs. 66, [illegible]) quently appeared as isolated clumps and showed a different [illegible] than that found on the surface of other furrow regions.

b. Osmium Tetroxide - Zinc Iodide (OZI) Fixation

The trilaminar pattern of the membrane observed after lanthanum treatment (Figs. 60, [illegible] 64) was never seen after OZI fixation. The electron dense metal (OZI) deposits obliterated the limiting membrane of the embryo. The deposit on the membrane surface was present as small globules. The globular appearance was more apparent in tangentially cut surfaces (Fig. 67). The distribution of the OZI positive material in the furrow varied according to the stage of development.

In the FAG stage the interdigitatory stage in the growing furrow was not as pronounced as in the UCF stage (Fig. 10) and the microvilli at the FT I were seen closely packed (Fig. 66). The interdigitations were similar to those described for blastula stages (Fig. 6 in Sanders and Zalik, 1972a) of *Xenopus* embryos. The boundaries of these interdigitating microvilli were outlined by electron dense deposits (Figs. 66, 68). The tip of the sectioned furrow at this stage of development was virtually filled with microvilli (Fig. 66). A small number of empty spaces lined with dense deposits were noticed among the microvilli and in the adjoining cytoplasm. These spaces, in a series

Figs. 66-72. Embryos fixed with ruthenium tetroxide - cell surface (CS) flocculent staining deposits (OZI) over the furrow.

Fig. 66. Furrow tip in a PAG-stage embryo. Microvilli are most closely packed and their boundaries are outlined by electron dense deposits. The clear areas are continuities of the furrow space. x11,000

Fig. 67. Electron dense deposits on a transversely cut furrow surface are seen as globules. F, furrow; C, cytoplasm. x68,000

Fig. 68. Furrow tip after PAG-stage. Microvilli (MV) are still closely packed and on their surface show OZI deposits. F, furrow. x60,000

Fig. 69. A portion of the furrow during JCG-stage. Microvilli at the furrow tip I (FT I) lack deposits. The furrow beyond FT I shows the deposits (arrows). x10,000

Fig. 70. A portion of the furrow during JCG-stage. Microvilli at FT I lack deposits. OZI positive material is present in the subjacent cytoplasm and is membrane bound. x15,000

Fig. 71. FT I area of the furrow during JCG-stage showing transitional zone. Membranes beyond FT I are obliterated by the deposits and the furrow is represented by a thick dark band. x39,000

Fig. 72. A portion of the furrow at CF-stage. Deeper areas of the furrow (about 400 μ from FT I) do not show deposits. Note the abrupt ending of the electron dense layer. An electron dense profile is visible in the subjacent cytoplasm. x28,000

granules and OZI positive [illegible] adjacent cytoplasm and in the cut ends of the [illegible]. The rest of the furrow membrane at the [illegible] did not show any deposits.

As the furrow progresses [illegible] during the J66 stage the microvilli at FT I, which [illegible] previous [illegible] did not show any deposits although some material was present there in the furrow (Fig. 69). OZI positive granules and vesicles, however, were present in the subjacent cytoplasm (Fig. 70). It has been pointed out above that the microvilli remained stationary during the post-J66 stage development while the furrow was lead by FT II. The lamellostomeric membranes between FT I and FT II showed an OZI positive material which almost filled the intercellular space (Figs. 69, 71).

At the CF stage the whole of the furrow, except for a small segment, showed metal (OZI) deposits. This non-reactive portion was about 200 µ long and occurred at a depth of approximately 400 µ from the FT I. The segment usually enclosed a spherical cavity (Fig. 72); however, in some of the embryos the membranes were also [illegible] but again lacked OZI deposits. Another interesting feature was that electron dense material ended abruptly near the [illegible] zone (Fig. 72).

The vitelline membrane and the outer [illegible] show deposits at any of the developmental [illegible] (Fig. 73). Small granules and vesicles containing metal (OZI) deposits were [illegible] cytoplasm (Fig. 73). In the cytoplasm the metal [illegible] were present, were always membrane bound. The Golgi bodies did not [illegible]

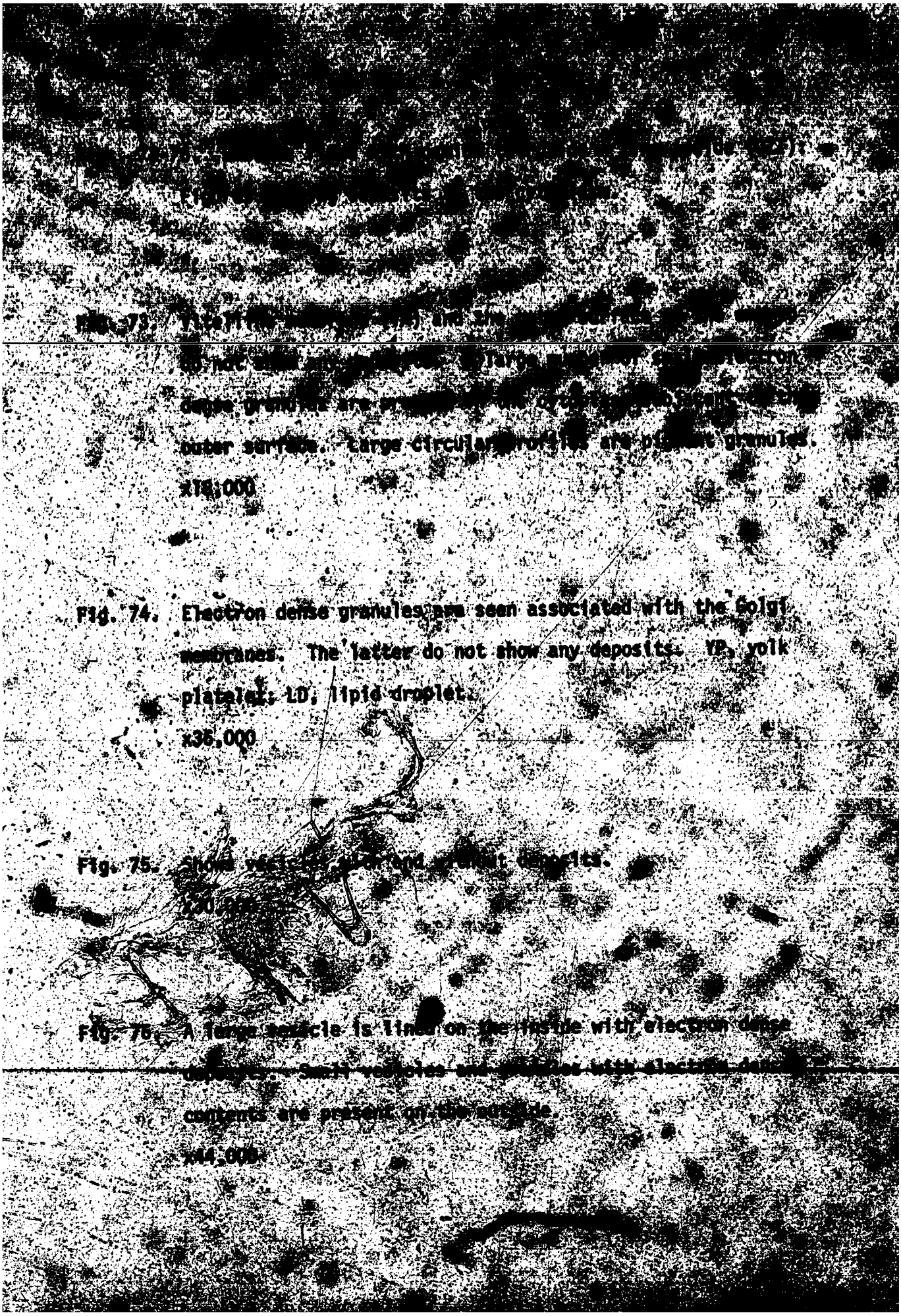
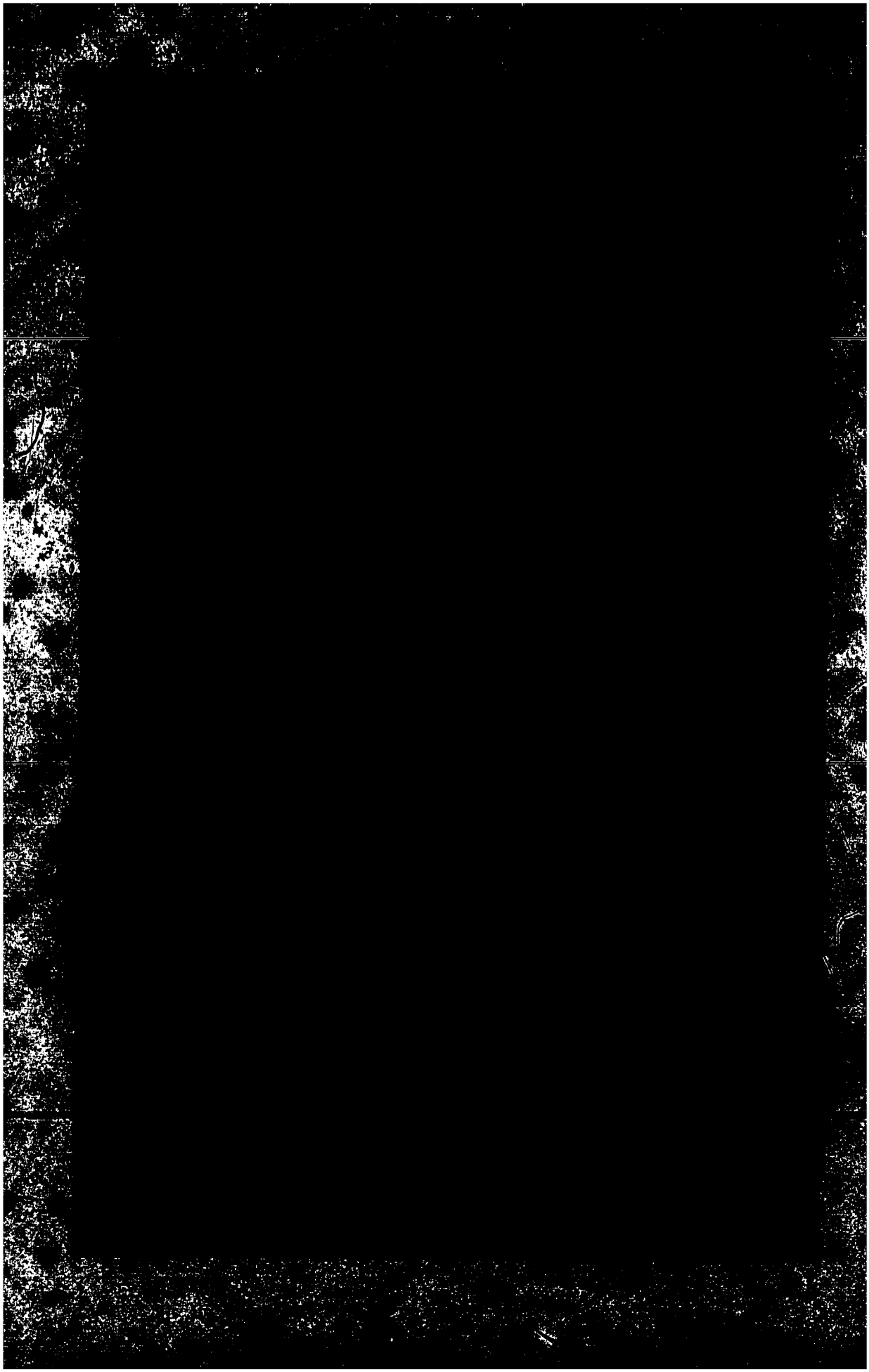


Fig. 73. [illegible] and the [illegible] do not show any [illegible] dense granules are [illegible] outer surface. Large circular profiles are pigment granules. ×18,000

Fig. 74. Electron dense granules are seen associated with the Golgi membranes. The latter do not show any deposits. YP, yolk platelet; LD, lipid droplet. ×36,000

Fig. 75. Shows yolk [illegible] and granular deposits. ×30,000

Fig. 76. A large vesicle is lined on the inside with electron dense [illegible]. Small vesicles and [illegible] with electron dense contents are present on the outside. ×44,000



[illegible] large vesicles (0.[illegible]-3.9 [illegible] diameter) [illegible] zone (OZ), [illegible] two horizons (Fig. [illegible]). [illegible] the peripheral [illegible] surface [illegible] were usually seen close to the furrow as well as lipid [illegible]

a. Thiamine Pyrophosphatase

In the embryos incubated for TPPase activity the reaction product, lead phosphate, was seen in the cytoplasm and in a limited area of the [illegible]

The deposits in the cytoplasm were always membrane bound and were never seen loose in the matrix. The appearance of the reaction product was [illegible]. For example, some of the Golgi cisternae showed dense deposits filling the lumen while in others the reaction product was restricted to a portion of the cisternae (Figs. 77, 78). In others the Golgi cisternae showed a diffuse type of reaction product (Figs. 79, 80). In any single Golgi body one to three cisternae showed the activity. Wherever the morphological polarity of the Golgi body was observable the cisterna at the convex face showed metal deposits (Figs. 77, 79). Some of the vesicles associated with the Golgi body and the growing furrow showed a variable degree of deposits (Figs. 80-84). A diffused reaction product present at the periphery of some of the lipid droplets and pigment granules was also seen in the controls.

In the furrow at the 3CB stage only the microvilli at FF I showed sporadic deposits (Fig. 82). Golgi bodies and some of the

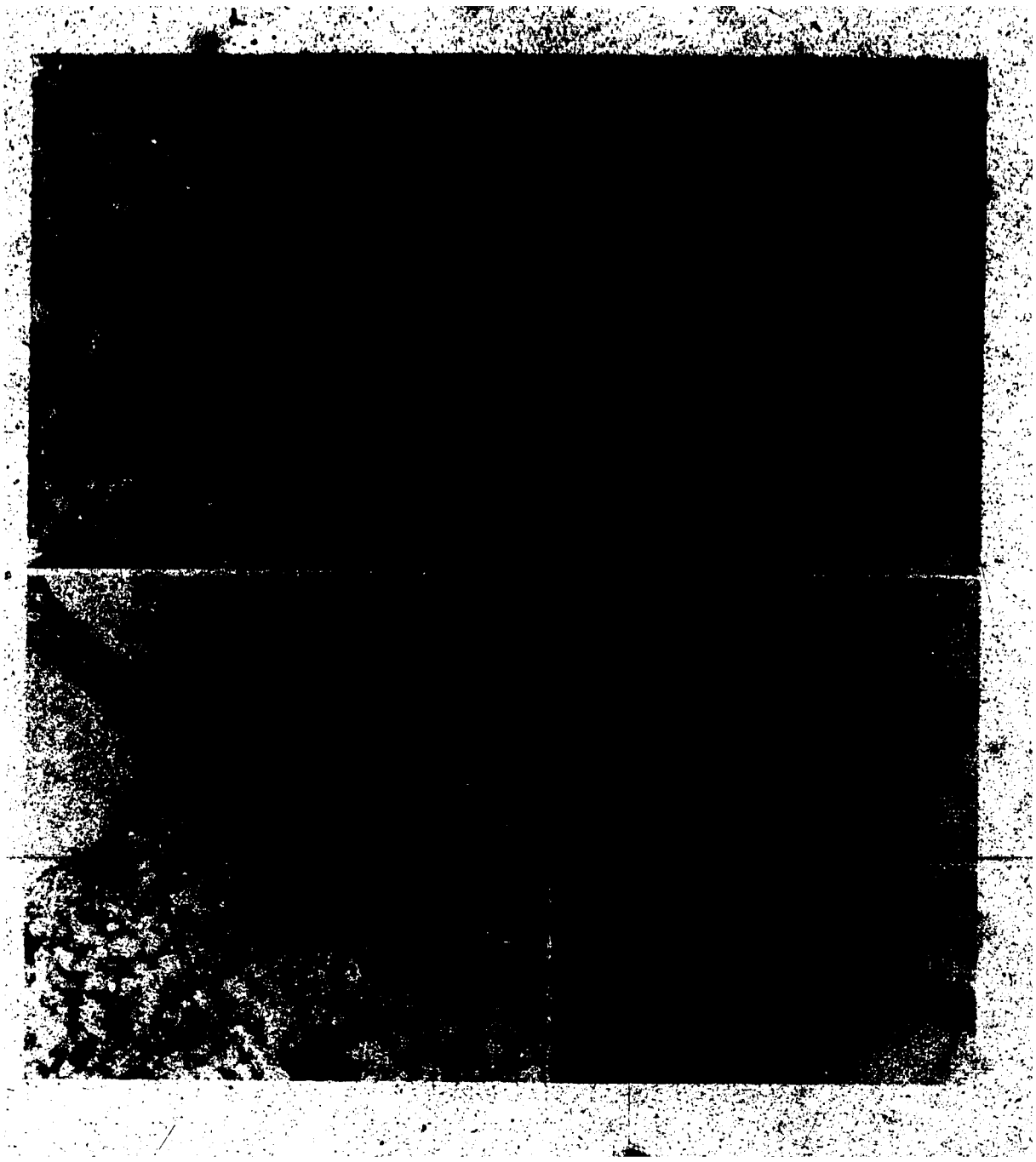
Figs. 77-80. Embryos incubated for the demonstration of thiamine pyrophosphatase: Showing the appearance of reaction product in the Golgi bodies.

Fig. 77. Uniformly dense reaction product is seen in a single cisterna at the convex face of the Golgi body. x27,500

Fig. 78. Reaction product is visible in three of the Golgi cisternae and in some of the associated vesicles.
x27,500

Fig. 79. A Golgi body (G) near the furrow. Cisterna at the convex face shows a diffused reaction product.
x27,500

Fig. 80. A diffused type of reaction product is seen in the Golgi body and in most of the associated vesicles.
x35,000



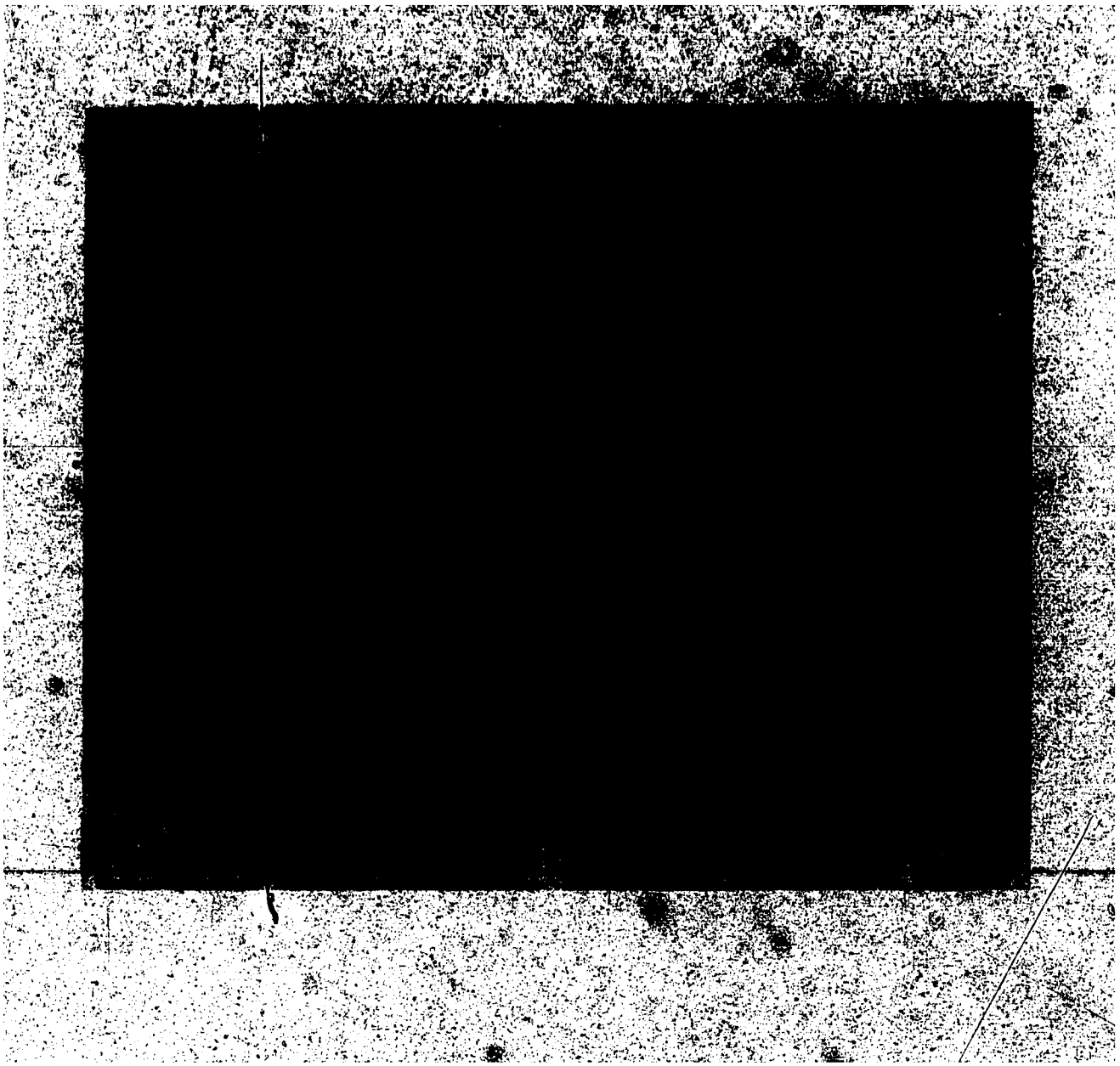
Figs. 81-84. Embryos incubated for the demonstration of thiamine pyrophosphatase. Reaction product is seen in and near the furrow.

Fig. 81. Electron dense deposits are seen in the vesicles as well as in the membrane cisternae close to the furrow (F). Surface of the furrow membrane shows little or no deposit.
x22,400

Fig. 82. A portion of the furrow of a JCG-stage embryo. Microvilli and the furrow membrane show scattered deposits similar in appearance to the one shown by Golgi cisternae in Fig. 79.
x37,000

Fig. 83. A portion of the furrow after JCG-stage. Microvilli at furrow tip I (FT I) are negative and the furrow area immediately ahead of FT I shows uniformly dense deposits.
x6,000

Fig. 84. A portion of the furrow after JCG-stage. Area immediately ahead of FT I showing the reaction product. Membrane cisternae and vesicles in the subjacent cytoplasm also show the deposits.
x37,000



vesicles present close to the [illegible]

diffused reaction product (Figs. 79, 80). [illegible]

development heavy deposits were observed on the [illegible]

below FT I (Fig. 83). Some of the smooth reticular cisternae and vesicles in the subjacent cytoplasm also showed dense deposits in their lumen (Fig. 84). The rest of the furrow and the outer surface were negative. It is pertinent to record that prior to the 2CC stage there was no reaction observed in the furrow, though some of the vesicles and cisternae present in the subjacent cytoplasm showed ATPase activity (Fig. 81).

d. Acid Phosphatase

Two attempts were made for the demonstration of acid phosphatase in the *Xenopus* embryos. Reaction product was observed only in a limited area of the furrow. The deposits were present as spaced aggregates in the FT I area on the surface of the microvilli (Fig. 88). Towards the outer surface the distance between the aggregates increased (Figs. 86, 87) and no deposits were observed on the outer surface itself (Fig. 85). Deeper areas of the furrow beyond FT I were also negative in terms of showing any reaction product.

In the cytoplasm the Golgi bodies (Fig. 90) and paired ER cisternae (Fig. 85) described earlier did not show any deposits. However, more experimentation is required to establish the absence of acid phosphatase activity in the Golgi bodies. Acid phosphatase has been demonstrated in the Golgi bodies of unfertilized [illegible] from *Rana pipiens* (Kessel and Decker, 1972).

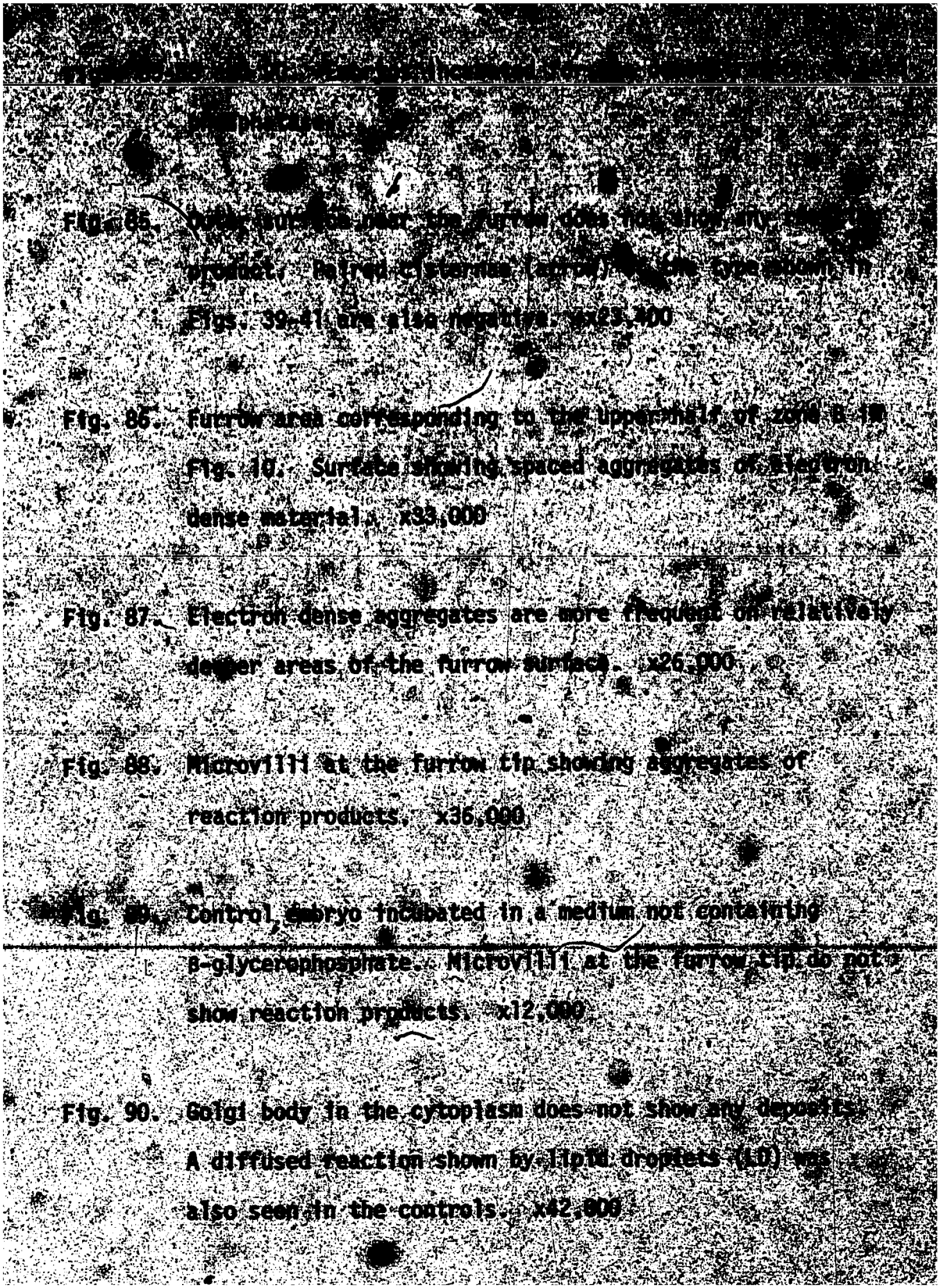


Fig. 85. Outer surface near the furrow does not show any deposit of reaction products. Retrovitellinae (arrow) and the cytosome in Figs. 39-41 are also negative. x23,400

Fig. 86. Furrow area corresponding to the upper half of zone 6 in Fig. 10. Surface showing spaced aggregates of electron dense material. x33,000

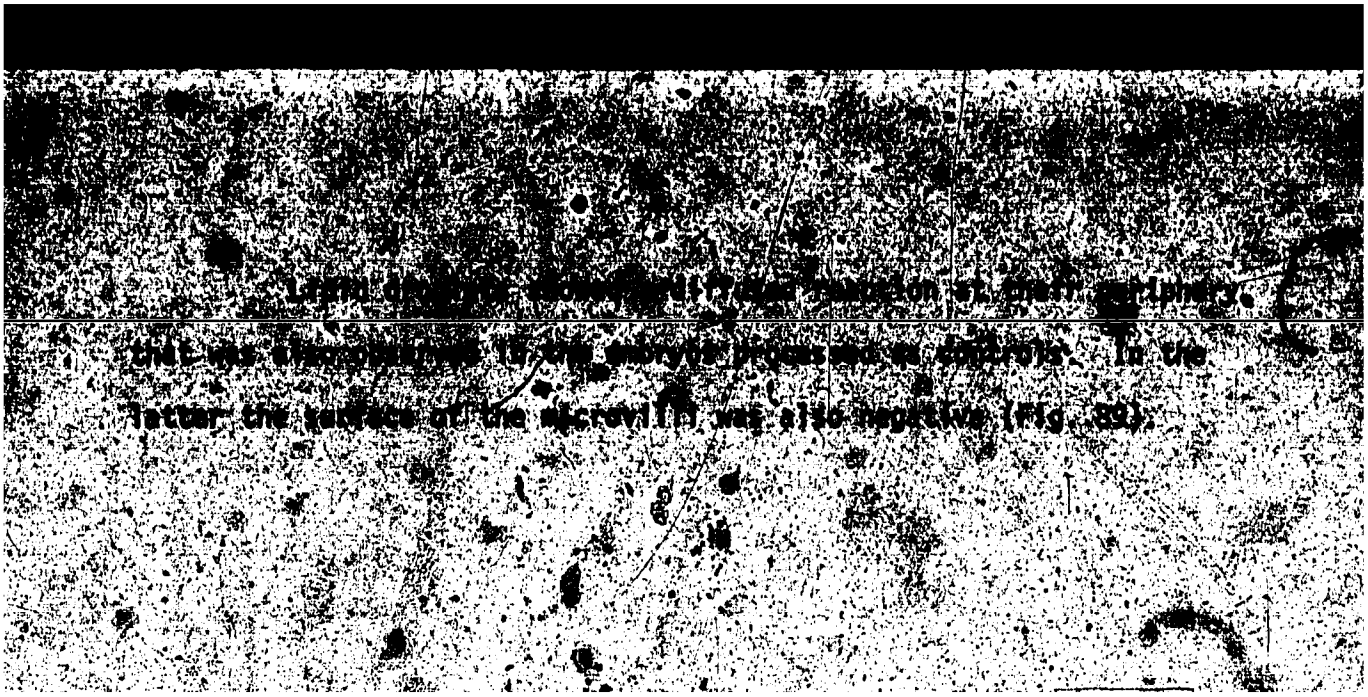
Fig. 87. Electron dense aggregates are more frequent on relatively deeper areas of the furrow surface. x26,000

Fig. 88. Microvilli at the furrow tip showing aggregates of reaction products. x36,000

Fig. 89. Control embryo incubated in a medium not containing β-glycerophosphate. Microvilli at the furrow tip do not show reaction products. x12,000

Fig. 90. Golgi body in the cytoplasm does not show any deposits. A diffused reaction shown by lipid droplets (LD) was also seen in the controls. x42,800

[redacted]



Legend to Figure 8

Light micrographs showing glial cells on a fresh capillary, that was thoroughly rinsed in the enzyme process and as controls. In the latter the surface of the microvilli was also negative (Fig. 8B).

DISCUSSION

Electron microscopy has made a very great contribution to our understanding of cell architecture and function. Although it is a very useful technique, it only provides a static picture of a dynamic system, often making interpretation hazardous. Moreover, the picture obtained with a conventional type of electron microscope is essentially two-dimensional. However, comparative studies using more than one fixation procedure and serial sections of important cellular areas help in minimizing some of these shortcomings. The technique also becomes more informative when coupled with cytochemical investigations. Although electron microscopy is an indispensible technique for cellular studies, great care is essential when drawing correlations between structure and function.

1. Ultrastructural Analysis of the Cleavage Process and Furrow Growth

The demonstration of membrane junctions possessing an intercellular gap of less than 30 Å is the first clear description of these contacts in this material. It has been suggested (Kalt, 1971b) that the presence of interblastomeric contacts in the animal pole furrow probably acts as a barrier or seal between the furrow space and the perivitelline space. In order for this to be the case, two requirements have to be met: first, the presence of the contacts all along the groove functioning as a terminal bar, and second, an intercellular gap narrow enough to check the passage of small molecules. The first requirement is shown here to be morpholo-

gically implausible, and the second, which would contradict the presence of tight junctions, is unlikely to be fulfilled since the intercellular space varied from 30-200 Å; although the presence of focal tight junctions (Trelstad *et al.*, 1967) could not be ruled out entirely. It should be pointed out here that CF-stage embryos fixed with OZI solution lacked the electron dense deposits in a restricted area (about 400 μ deep from FT 1) of the furrow. The abrupt ending of the deposits at either end of this OZI negative zone (Fig. 72) suggests that this portion of the furrow might present a barrier to this fixing fluid through interblastomeric contacts. These contacts in the outermost region have been suggested to be adhesive in nature (Bluemink, 1971a, b). The presence of lanthanum binding material in close (200 Å) contacts (Fig. 64) and the fact that they are randomly distributed, supports the contention that these contacts are also adhesive rather than sealing, and may play an integral role in the formation of the furrow. Lanthanum binding material in other cell types (Khan and Overton, 1969; Overton, 1969) has also been assigned an adhesive role.

Ultrastructural features often used to identify a "gap" or a "low resistance" junction (see review by McNutt and Weinstein, 1973) are:

1. a septalaminar arrangement, i.e. two trilaminar membranes separated by a 20-30 Å electron lucent zone (Revel and Karnovsky, 1967).
2. its sub-structure shows subunits packed in a

hexagonal array with a [illegible] center-to-center spacing. This arrangement of subunits in gap junctions has been confirmed by tantalum tracer penetration studies (e.g. Revel and Karnovsky, 1967) and by freeze-cleave replication studies (e.g. McNutt and Weinstein, 1970).

The presence of a gap less than 30 Å in width contacts observed here satisfies part of the requirement of low resistance electrical junctions (Loewenstein, 1966) and may provide a morphological basis for the electrical coupling demonstrated between blastomeres of cleaving *Xenopus* embryos (Palmer and Slack, 1970; De Laat *et al.*, 1973; DiCaprio *et al.*, 1974). There have been several speculations concerning the role of low resistance electrical junctions in embryonic systems. Material passing through these junctions may, for example, influence ionic balance, growth, development and differentiation of the cells (Potter *et al.*, 1966; Loewenstein, 1966, 1968; Sheridan, 1971).

Despite some views to the contrary (Rappaport, 1971) the role of vesicle fusion with the plasma membrane during furrow formation has often been speculated upon (Selman and Perry, 1970; Kalt, 1971b) but the localization of such membrane insertion, if any, has been uncertain. The present results indicate the probable existance of at least two such sites, each of which is associated with a morphologically distinct type of vesicle. The large vesicles, containing fibrous material, were present some distance behind the tip (FT 1), and some were apparently continuous with the furrow membrane.

[illegible] of the [illegible] of the [illegible] of the cytoplasm of the [illegible] of the membrane undergoing rapid growth, together with [illegible] pools of membrane and cell surface material.

In view of the fact that the opposing membranes of the furrow appear to be held in position relative to one another at the outermost region (A in Fig. 10) by intercellular contacts, it is possible that the addition of new membrane behind the leading edge of the furrow (B in Fig. 10) provides at least some of the motive force required for cleavage advancement.

Random fusion of vesicles has been reported in *Xenopus* embryos (Bluemink, 1971b), but the present observation suggests that, in addition, fusion in a localized area also occurs. In certain mammalian cells the fusion of vesicles, one with the other, forms the apposing surfaces of the dividing cells (Buck and Zimmerman, 1962), in contrast to vesicle fusion with the existing membrane as reported here. The possibility of direct insertion of membrane precursor molecules during *Xenopus* cleavage has also been speculated upon (Bluemink, 1971b; Bluemink and de Laat, 1973). This speculation does not seem to be tenable if the plasma membrane of *Xenopus* embryo conforms to the generally assumed structure of cell membranes, i.e. with a hydrophobic core, owing to fatty acid chains and hydrophobic proteins covered on either side by hydrophilic lipid polar heads, proteins and carbohydrates (see review by Singer and Nicolson, 1972). Moreover, the limiting membrane of the embryo has extracellular surface material as observed in the present study. Nevertheless, some

[illegible]

[illegible] would [illegible] passage of hydrophilic molecules through the hydrophobic centre of the membrane — a thermodynamically unfavourable situation.

The presence of small vesicles (200-600 Å in diameter) in the furrow of CF-stage embryos (Figs. 19-23) raises two questions, namely, what is their function and how do they reach the furrow space? Regarding the purpose of the vesicles in the furrow there can be two possibilities: (a) secretory material, once synthesized, is insulated from the cytoplasm by the small vesicles, which are transferred intact into the furrow space where they rupture to supply their contents. In other words it is a mode of secretion with the vesicles acting as carriers. (b) Vesicles are needed to supply materials to specific parts of the furrow by releasing their contents only at certain sites. However, the latter possibility seems to be improbable as the vesicles were seen scattered at random in the furrow. Thus the first possibility is favoured.

The answer to the second question, how these vesicles enter the furrow, is not clear. Kurosumi (1961), while giving an ultrastructural analysis of secretion in a variety of cells, has reviewed six different ways by which cells extrude materials. Briefly they are:

Type I: *Holocrine secretion*: dissolution of the whole cell which releases the secretion, as represented by the cells of the sebaceous gland.

Type II: *Merocrine secretion*: the luminal surface of the gland cells sends out membrane projections which are pinched off and is found in the apocrine sweat gland, the mammary gland and the thyroid gland.

Type III: *Microapocrine secretion*: expanded tips of microvilli are pinched off as occurs in the choroid plexus and in the intrahepatic bile duct.

Type IV: *Macrocrine secretion*: the secretory vesicle approaches the plasma membrane, a small opening occurs through plasma and vesicular membrane and the contents are poured out to the lumen of the gland, e.g. pancreatic gland.

Type V: In this type the product may diffuse through the intact plasma membrane. There is no morphological evidence for this type, however, although it may occur in gastric body chief cells.

Type VI: When two or more types of extrusion occur in the same cell. Parietal cells of stomach display two types (III and IV) of secretion release.

Rhodin (1971) working on adrenal cortex cells, has described still another mode of excretion that has some resemblance to the "Extrusion Type III" of human apocrine sweat glands (Kurosumi, 1961). The present case does not resemble any of the seven types described inasmuch as vesicles, even after extrusion, are seen intact. Vesicular extrusion in *Xenopus* embryos has been speculated upon by Kalt (1971b) as one of the modes by which

glycogen might be transferred from the cytoplasm to the outside. According to him, the outer membrane of a double-membrane vesicle fuses with the plasma membrane and the material, still delimited by the inner membrane, is released into the furrow. However, the speculation lacks structural evidence. Although intact vesicles in the furrow were observed during the present study, Kalt's (1971b) suggestion cannot be adopted to explain vesicle transfer because some of the vesicles in the furrow still showed a double membrane (Fig. 19). Moreover, not all the vesicles in the nearby cytoplasm had a double membrane.

There is no general agreement concerning the origin of the vesicles which supply membrane to the growing furrow. In *Xenopus* it has been suggested that the vesicles could be a product of the rough endoplasmic reticulum (Bluemink, 1971b; Bluemink and De Laat, 1973) or the Golgi complex (Kalt, 1971b). Present observations support the latter view inasmuch as the small vesicles were closely associated with the Golgi complex and scattered between it and the furrow tip. The Golgi origin of the larger vesicles is also very likely, since similar vesicles are observed in juxtaposition with this organelle at later stages of development (Sanders, 1973) and in other cell types (Wise and Flickinger, 1970a). Vesicles seen continuous with the furrow membrane and subjacent to it do not seem to be an example of pinocytosis as is indicated by the use of ferritin.

Ferritin is an iron-containing protein and has been used in the past to demonstrate pinocytosis (e.g. Farquhar and Palade, 1960). It has the advantage of being an electron dense molecule

large enough (about 100 Å diameter) to be seen individually (Farrant, 1984; Massover *et al.*, 1973). Surgically denuded SG-stage embryos were grown in different concentrations of ferritin (25, 50 and 100 mg %) for 20 minutes. In thin sections of these embryos, vesicles of the type described above were present in the cytoplasm near the furrow, but they did not show any ferritin in them, indicating that the vesicles continuous with the furrow most likely were merging. Although very little ferritin was bound to the outer or to the furrow surface, the result showed that the ferritin was available in the furrow. Moreover, the flow of materials from the cytoplasm to the furrow is indicated by the following:

1. observation of profiles where vesicles were seen apparently discharging their exudate (Fig. 11) into the furrow.
2. appearance of thiamine pyrophosphatase activity in the furrow during development, while earlier the activity was restricted to the Golgi bodies and vesicles in the cytoplasm.
3. presence of a large number of intact vesicles in the furrow.

The weight of previous evidence (for example Bennett and Leblond, 1970; Wise and Flickinger, 1970a) also favours the passage of vesicles from the Golgi complex to the cell surface over the reverse direction.

A microvillous transformation in the cleavage furrow was

observed both with the scanning and transmission electron microscopes. Prior to the JCG-stage this was present at the advancing tip and thereafter it remained stationary as an encircling band. The reason for its presence at the advancing tip is unclear, although the association of such structures with areas involved in rapid membrane production (Buck and Tisdale, 1962; Selman and Perry, 1970; Bluemink, 1972) or utilization (Sanders, 1970) suggests that they accompany sites of increased membrane turnover. Some observations (Fig. 15) suggest that they may be the result of vacuole fusion and the subsequent excess of membrane at the surface. The absence of microvillous transformation from furrow tip II correlates with the absence of localized vacuole fusion.

The presence of microfilaments immediately below the membrane during cleavage has been described (Zotin, 1964; Selman and Perry, 1970; Bluemink, 1971a, b) together with the speculation that they might constitute a contractile system. For example, while studying the effect of cytochalasin B on *Xenopus* embryos Bluemink (1971b) concluded that filaments are attached to the plasma membrane, and in an earlier study on axolotl eggs it has been suggested that the filaments are anchored in the cytoplasm through 65 Å filaments which are randomly oriented (Bluemink, 1970). Such an arrangement of filaments gives support to the view that during contraction, filaments pull in the surface membrane (Bluemink, 1971b) and may be responsible for the presence of the stress folds and blunt protuberances observed in the present study. It is significant in this regard that the region of subcortical cytoplasm

which displays furrow inducing capacity (Sawai, 1972) corresponds very closely to the region of surface in which these stress folds and blunt protuberances were observed.

In a transmission electron microscope study of *Bufo regularis* and *Rhynobatrachus natalensis* eggs, a honeycomb-like structure, comparable to membrane protuberances shown here by scanning electron microscopy (Fig. 5), has been reported as appearing in the animal pole surface, 10 minutes after fertilization (Balinsky, 1966). The membrane roughings are temporary in existence and disappear in the next 30 minutes. According to Balinsky (1966) if the movements of the egg cortex, that lead to the formation of the grey crescent, "on the dorsal side are more far-reaching than those on the ventral side, there would be created an excess of cortex on the animal hemisphere, and this might cause the plasmalemma to be thrown into folds and create a puckered honeycomb-like surface". The reappearance of these protuberances near the groove at the beginning of first cleavage might be an indication of some displacement of cortical cytoplasm during the initial phase of cleavage.

The term *glycocalyx* was coined by Bennett (1963) to designate the glycoprotein and polysaccharide macromolecular coat present on the external surface of animal cells. The study of cell surface material is of great interest because of its suspected role in cell-cell recognition, cell adhesion and association, contact phenomena and growth control and a number of other cellular activities (see reviews by Winzler, 1970; Rambourg, 1971; Hughes,

1973). The presence and chemistry of cell surface material can be analyzed by the application of different histochemical techniques such as PA-Schiff to detect glycoprotein and cationic dyes to detect acidic residues. These and many other techniques used in the study of cell surface material have been reviewed in great detail by Rambourg (1971). In first cleavage *Xenopus* embryos surface material in the furrow is rendered electron dense with ruthenium red (Bluemink and De Laat, 1973). In mid-blastula stages the extracellular material is stained with ruthenium red and colloidal iron (Sanders and Zalik, 1972a). It is known that these cationic agents, colloidal iron (Mowry, 1963) and ruthenium red (Luft, 1971), indicate the presence of acidic residues in the cell surface material. The precise specificities of the lanthanum deposition are unclear; however it has been established by a number of studies that this electron dense deposit is a fairly accurate indicator of the presence of extracellular material and its relative thickness (Doggenweiler and Frenk, 1965; Lesseps, 1967; Overton, 1968; Martinez-Palomo, 1970).

The animal pole furrow surface showed characteristic variations in affinity for lanthanum throughout its length. The region showing the greatest deposit corresponded with the region where the larger vesicles with fibrous contents were observed fusing with the surface and discharging. This correlation suggests that not only is membrane inserted at this region, but also a considerable amount of extracellular surface material is added here. On either side of this heavily staining region, there was a

gradual decrease in the thickness of the lanthanum layer, thus indicating that the exuded material is diffusing away from the site of initial appearance on the surface. Such translatory movements of the components of the cell surface in the plane of the membrane have been demonstrated in several cell types (Frye and Edidin, 1970; Edidin, 1972), and the possibility that these components may become redistributed under certain conditions in other embryonic tissue has been suggested (Sanders and Zalik, 1972b). While the present results cannot indicate that this area of fusion of the larger vesicles is the only site of exudation of surface material, it appears to be a major one.

Another region of the furrow that showed varied lanthanum deposits was the furrow tip I. In some embryos the deposit was observed in fairly evenly spaced patches, while in others it was uniform in appearance. A similar distribution of the surface material at FT I was noticed after testing for acid phosphatase and thiamine pyrophosphatase. The patchy distribution could have been due to a selective removal of the deposit from certain areas, although the presence of a uniform layer of material in adjacent areas renders this possibility unlikely. It could also reflect the fact that the lanthanum binding surface material is released from vesicles at spaced sites in this region, and this may correlate with the presence of the small, moderately dense, Golgi-associated vesicles in the adjacent cytoplasm. The question arises why this patchy appearance, observed after three different techniques (lanthanum, acid phosphatase,

[redacted] was not completely contained. One possible explanation could be that the material was able to diffuse away from its site of initial extrusion and thus even out the non-uniformity.

A major part of the closely apposed, parallel inter-blastomeric surfaces in the deeper areas of the furrow between FT I and FT II did not show any lanthanum deposits. An interpretation of this observation could be that it is due to lack of penetration of the lanthanum, but this is unlikely since deposits were present in some closely apposed surfaces (fig. 64). It has been reported (Martinez-Palomo, 1970) that loss of lanthanum deposit during preparation is minimal and it is therefore tentatively concluded that lanthanum binding surface material is extremely sparse in the furrow between FT I and FT II.

At furrow tip II the lanthanum was deposited in large clumps rather than uniformly, and although a similar result has been obtained with other embryonic tissue (Sanders and Zalik, 1972b), its significance is unclear.

The specificity of the osmium tetroxide - zinc iodide (OZI) reaction is not known; however, some speculation has been made as to the nature of the material involved in the reaction. According to Niebauer *et al.* (1969), extraction of the tissue with lipid solvents abolishes OZI staining, indicating that the reaction might be due to lipids. More recently, Elias *et al.* (1972) reported that pretreatment of living *Hydra* with hyaluronidase prevents OZI deposition, suggesting that the reaction could

be due to mucopolysaccharides. Reportedly, OZI has a marked tendency to stain the cell surface and the Golgi apparatus (e.g. Trabucchi et. [illegible]69; Elias *et al.*, 1972; Dauwalder and Whaley, 1973) and this [illegible] rationale to survey the distribution of OZI positive [illegible] in *Xenopus* embryos in order to help in elucidating the [illegible] between the Golgi bodies and the cell surface.

The [illegible] native-stain (OZI) gave reproducible results. The [illegible] of some but not all vesicles in the present investigation is [illegible] with the findings of Elias *et al.* (1972) in *Hydra* and Dauwalder and Whaley (1973) in maize root tips. Lack of staining of some of the vesicles could result from absence, masking or loss of stainable materials during preparation of the embryo. However, the frequent occurrence of stained and unstained vesicles very near to each other (Fig. 75) and the general quality of fixation excludes the possibility that this diversity in staining of the vesicles could be artifactual. The staining variations might reflect the difference in nature of the material present in such vesicles. The OZI staining of some of the Golgi associated vesicles and not the Golgi body itself, irrespective of whether vesicles are coming from, or merging into, the Golgi body, indicates an abrupt change in the nature of the vesicular contents.

The presence of OZI positive material in the furrow, and in some of the Golgi associated vesicles suggests a link between the furrow and the Golgi bodies. Such a link is probably provided through OZI positive vesicles. The question arises as

to which way the flow is proceeding. Considering the fact that the outer surface, perivitelline space and vitelline membrane did not possess any OZI positive material, which could have migrated to the furrow, the only option left is that OZI staining material is supplied from the cytoplasm. If OZI was staining the Golgi derived material then the variability of staining shown by the Golgi associated vesicles is in line with an earlier suggestion that different types of vesicles can be produced simultaneously by the same Golgi body (Stockem, 1969; Wise and Flickinger, 1970b).

2. Incorporation of Fucose-H^3 into the Embryos

The fact that the vitelline membrane did not show any counts and lacked autoradiographic activity showed convincingly that the membrane did not incorporate fucose-H^3. The presence of activity in the fixed embryos conclusively showed that the vitelline membrane as well as plasma membrane was permeable to the sugar. In addition, it indicated that fucose-H^3 was incorporated into macromolecules that were preserved by the fixative mixture, although the possibility of non-specific adherence of the label cannot be entirely ruled out. Reportedly, fucose-H^3 is a stable sugar, selectively taken up into glycoprotein (Coffey *et al.*, 1964; Bekesi and Winzler, 1967) and is located at the end of the carbohydrate side chain (Spiro, 1969). The probability of incorporation of fucose into macromolecules is also substantiated by the fact that no appreciable loss of activity (Fig. 25) was observed between 0 to 80 minutes.

One explanation for the slight increase in the counts observed from 0 to 20 minutes (Table 3) could be that when the embryos were taken out of the fucose solution and thoroughly washed, there still remained some fucose in the perivitelline space that was not incorporated and thus lost during fixation at 0 minute. However, if the embryos were allowed to grow (5 minutes and 20 minutes) before fixation the fucose in the perivitelline space was incorporated into the embryo and resulted in an increased count.

In light microscopic autoradiography the exact path followed by fucose at a subcellular level is not clear due to the lack of resolution. However, the study reveals that fucose was incorporated, and that during cleavage the activity moved to the furrow area (Figs. 28, 29). This could mean one of two things: first, the macromolecule containing the fucose residue is released into the furrow. Second, the fucose molecule is cleaved and released into the furrow. The latter possibility is improbable because unincorporated fucose molecules would most likely be lost during fixation and would not show up in the furrow as observed here. The other possible ways of fucose-H^3 loss, such as breakdown to metabolites or conversion to other sugars followed by their breakdown, have found little support in the literature (e.g. Bennett *et al.*, 1974). Fucose is usually incorporated into newly synthesized glycoproteins (Bekesi and Winzler, 1967; Kaufman and Ginsberg, 1968; Herscov[illegible]970). Incorporation of fucose into glycolipids can also take [illegible] (Bosmann *et al.*, 1968, 1969). In

the present study the label was initially observed in the cytoplasm and later it migrated to the furrow space. Irrespective of the nature of the label-carrier molecule, whether it be glycoprotein or glycolipid or any other type of macromolecule, the study clearly shows that fucose is part of the molecule which is supplied to the furrow from within the cell.

3. Interrelationships of Cytomembranes in the Embryo

This study provides the first detailed morphological description of the lipid droplets, the endoplasmic reticulum and the Golgi bodies in first cleavage *Xenopus* embryos. A possible interrelationship between these organelles based on the present observations is outlined in Fig. 91.

a. Lipid droplets

The first ultrastructural description of lipochondria was provided by Kemp (1956) using the *Rana pipiens* oocyte. Subsequently similar inclusions have been reported in a variety of oocytes (Wischnitzer, 1958, 1966; Ward, 1962; Balinsky and Devis, 1963; Karasaki, 1963) and their morphology and distribution is very similar to the lipid droplets of the present study. Although certain lipid droplets described here possessed a dense margin and partition, they generally lacked the structural characteristics of a true membrane, as is true for lipochondria (Wischnitzer, 1966). The stellate appearance of lipid droplets, after osmium fixation alone, is similar to the lipochondria of Balinsky and Devis (1963).

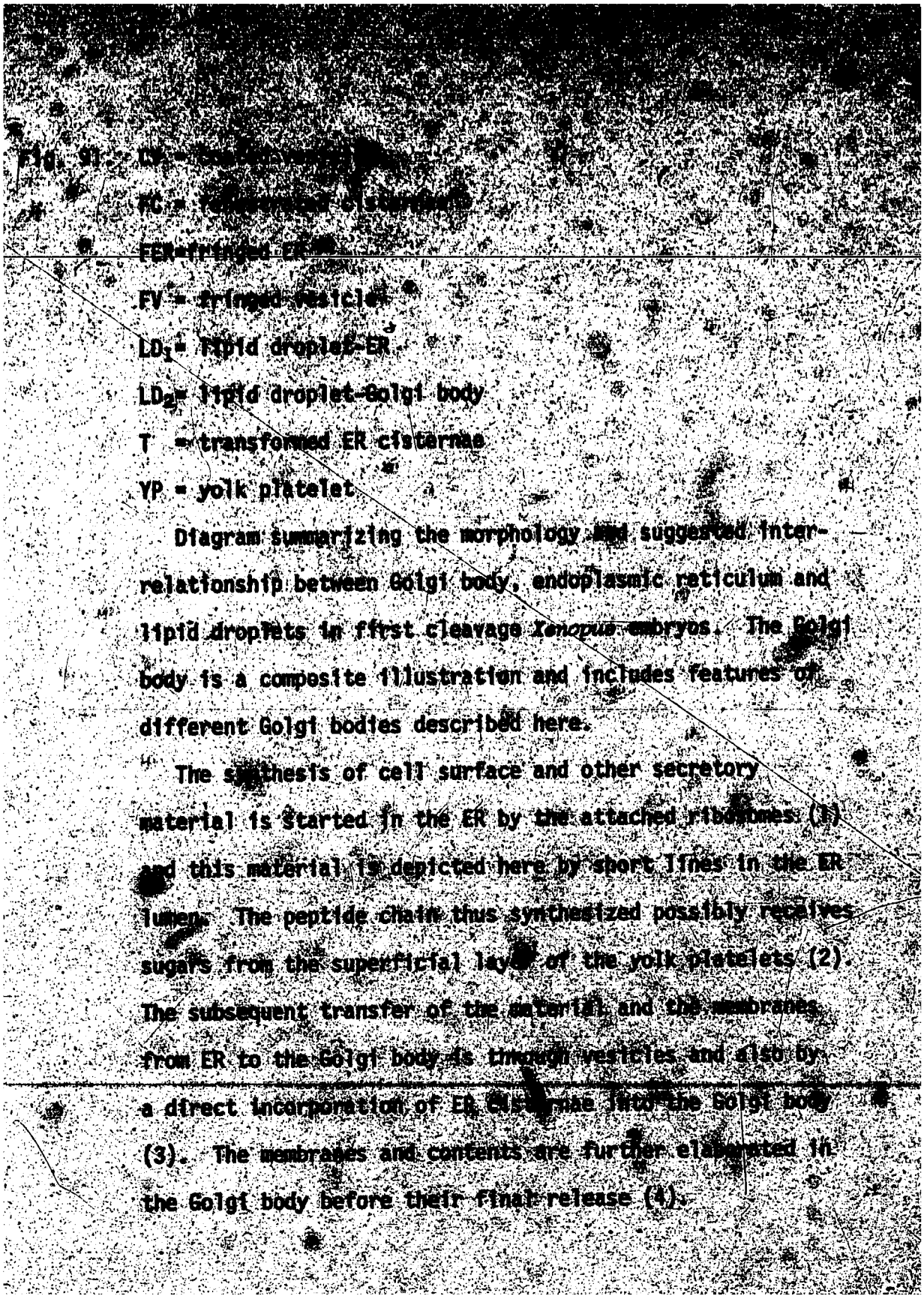


Fig. 91. CV = [illegible]

FC = [illegible]

FER = fringed ER

FV = fringed vesicle

LD_1 = lipid droplet-ER

LD_2 = lipid droplet-Golgi body

T = transformed ER cisternae

YP = yolk platelet

Diagram summarizing the morphology and suggested interrelationship between Golgi body, endoplasmic reticulum and lipid droplets in first cleavage *Xenopus* embryos. The Golgi body is a composite illustration and includes features of different Golgi bodies described here.

The synthesis of cell surface and other secretory material is started in the ER by the attached ribosomes (1) and this material is depicted here by short lines in the ER lumen. The peptide chain thus synthesized possibly receives sugars from the superficial layer of the yolk platelets (2). The subsequent transfer of the material and the membranes from ER to the Golgi body is through vesicles and also by a direct incorporation of ER cisternae into the Golgi body (3). The membranes and contents are further elaborated in the Golgi body before their final release (4).

After fixation with trialdehyde alone the lipid droplets were represented by rounded clear spaces. This is either due to their lack of preservation by trialdehyde alone or else they are preserved in such a way that the lipid droplets fail to pick up any stains. However, if the trialdehyde fixation was followed by post-osmication the lipid droplets appeared as grey rounded bodies. It seems that osmium tetroxide is essential for the preservation of lipid droplets and reflects their lipid nature. Certain morphological features of lipid droplets such as the lack of a limiting membrane, occasional multilobed appearance and the presence of a growing or receding partition suggest that these inclusions behave like droplets and may be fragile in nature.

The association of lipid droplets with the rough endoplasmic reticulum (RER) and the Golgi body showed two pertinent features. First, although the RER and the Golgi body showed a well defined membrane, this was not true for the lipid droplets. Second, at the junction of these membranes with lipid droplets a clear space or cavity was observed in the latter that was continuous with the lumen of the RER cisterna or of the Golgi body cisterna. Such profiles indicate that the membranes might be a product of precursor molecules from lipid droplets and the cavity in the latter might represent the space left by the used material. *In vitro* experiments have demonstrated that phospholipids can form a lamellar phase (Stoeckenius, 1962a), and it has also been shown that lipids and proteins can give rise to membrane profiles

without the presence of preassembled membranes (Maul, 1967). The present case might be an *in vivo* example of this phenomenon. This possibility also finds support from the suggestion that lipochondria, in addition to fatty acids and phosphatides, may contain proteins (Holtfreter, 1946). This would mean that the basic membrane constituents are available in the lipid droplets. The membrane profiles or "blisters" on the surface of lipid droplets in the *Priapulus* oocyte have been interpreted as a product of the droplets (Nørrevang, 1968). A similar formation of structured membrane systems from phospholipid inclusions during differentiation of embryonic cells has been documented by Mercer (1962). According to this worker "many membranous organelles of cells could be the result of the spontaneous dissolution of a mass of phospholipid accumulated in a vacuole (comparable to lipid droplets of the present study) at some early stage in the cell's history".

Lipid droplets may therefore represent a membrane reserve from which genesis of cytomembranes can take place. If they are serving the metabolic role of supply of cytomembranes then they are more than merely storage granules and should be considered as cell organelles.

b. Endoplasmic reticulum

The fringed appearance of the ER was due to the presence of filamentous projections. From an *in vitro* study of microsomes, Redman and Sabatini (1966) have suggested that from the onset of protein synthesis the growing peptide chain is directed towards

the cisternal space. It is possible that the projections observed here might be a synthetic product of the attached ribosomes. The question arises why the ER cisternae continuous with lipid droplets did not show a fringed interior despite the fact that they had ribosomes attached to it. One possible explanation could be that they represent nascent membranes that have just acquired ribosomes.

Fringed ER showed three morphological forms, namely: paired cisternae, single cisternae and vesicles. They all appear related to one another inasmuch as the single cisternae (Fig. 46) could have been one of a pair of cisternae, cut along the longer axis with the plane of the section passing through only one cisterna of the pair. All the embryos studied, and the micrographs presented here (Figs. 39-43) strongly suggest that the fringed ER vesicles are derived from fringed ER cisternae.

The fringed ER vesicles described here are different in several ways from similar fringed vesicles reported in amoebae (Sanders, 1970; Wise and Flickinger, 1970a). The former have ribosomes and are derived from endoplasmic reticulum whereas in amoebae fringed vesicles are devoid of ribosomes (Sanders, 1970; Wise and Flickinger, 1970a) and find their origin in Golgi bodies (Wise and Flickinger, 1970a). In the present case the fringed ER vesicles were seen associated with the convex face of the Golgi body, but in amoebae association of fringed vesicles with Golgi bodies has been shown at the concave face (Wise and Flickinger, 1970a).

Regarding the origin of fringed endoplasmic reticulum

in *Xenopus* embryos, there are two possibilities: it either remains from the oocyte or it is elaborated after fertilization. The former possibility seems to be improbable since in oocytes, including those of *Xenopus*, fringed ER has not been reported (Balinsky and Devis, 1963); even the typical type of RER is poorly developed (Nørrevang, 1968). Different sources, such as nuclear membrane (Beams, 1964) or Golgi bodies (Sakai and Shigenaga, 1967) have been suggested as possible sites of origin of the RER. Cyclical transformations between nuclear envelope, endoplasmic reticulum and annulate lamellae (Palade, 1956; Longo, 1972) can also contribute to the ER system at different developmental stages. However, none of the present observations support this concept for *Xenopus* embryos. Dallner and his co-workers (1966) have postulated that membrane phospholipids and proteins are first assembled in RER, and it seems that these constituents might be made available from lipid droplets as has been discussed above.

Relationship of fringed ER with yolk platelets

Paired ER cisternae exhibited a varying degree of association with yolk platelets. The frequent occurrence and morphological details of such contacts indicate that it is probably more than a chance association and has some functional significance in terms of material exchange. This idea gains support from the fact that the ER regions involved in such contacts were modified by not having any ribosomes, possibly to permit close association of the membranes. Ribosomes were apparent on the ER membranes away from

such regions of apposition. The yolk material immediately adjacent to the contact was granular instead of crystalline. In amphibian oocytes it has been shown that the superficial layer of yolk platelets contains acid polysaccharides (Karasaki, 1963a, b; Ohno *et al.*, 1964; Tandler and LaTorre, 1967; Favard and Favard-Séréno, 1969). It is possible that the fringed ER cisternae after the synthesis of transportable proteins, which reportedly grow into the cisternal space of the ER (Caro and Palade, 1964; Redman and Sabatini, 1966; Jamieson and Palade, 1967), receive sugar moieties from the peripheral portions of the yolk platelets.

Continuity of ER with Golgi body

Functional continuity between ER and the Golgi bodies has been demonstrated by various cytochemical and biochemical techniques (see reviews by Beams and Kessel, 1968; Morre *et al.*, 1971; Northcote, 1971). The present electron microscope observations provide morphological evidence for such a continuity.

Depending upon its morphology, either cisternal or vesicular, the fringed ER showed two types of relationships with the Golgi body. Large segments of ER cisternae contributed directly to the Golgi body by apparently losing their ribosomes and transforming into cisternae that became incorporated into the Golgi body. The suggestion finds support from the observations of ER cisternae, closely apposed to the forming face of the Golgi body, and with bifacial symmetry due to the presence of ribosomes only on the surface away from the Golgi body. It seems that such cisternae

may have been fixed in the process of transformation. A similar kind of ER-Golgi body relationship has also been proposed by Friend (1965). If the third dilated cisterna (Fig. 54) is a transformed fringed ER cisterna, as is suggested by its appearance, then the unfringed cisterna sandwiched between the two fringed cisternae may be either a product of the fringed ER itself or is assembled under its influence. The former possibility is supported by the existence of paired cisternae in which one did not have a fringed interior and thus resembled a Golgi body cisterna (Figs. 44, 45, 51). In addition, such pairs, occurring within 0.2 μ of the Golgi body, indicate that the probable transformation of the ER cisternae into Golgi cisternae can take place even outside the Golgi body.

The functional significance of close association between fringed ER vesicles and the outermost cisterna of the Golgi body (Fig. 55) is not immediately apparent. However, such a close approach of two types of membrane provides a situation for exchange of materials. The fate of these vesicles, probably derived from fringed ER cisternae, remains unknown. Ovtracht and colleagues (1973) have suggested that ER-derived material can directly combine with Golgi-derived vesicles through "boulevard peripherique" and bypass the central plate-like portions of the Golgi cisternae. In the present case no such tubular connections were seen between the ER and the Golgi body, however, the fringed ER vesicles may be providing a transitory functional continuity between the two organelles. It is possible that the fringed ER

vesicles are modified following their association with the Golgi body and no longer remain fringed, thus becoming indistinguishable from other Golgi-derived vesicles by their morphological appearance alone.

Small vesicles with a diffuse electron dense interior, seen scattered between the ER cisternae and the forming face of the Golgi body (Fig. 47) could be a product of the blebbing activity of the former. Such blebbing activity of ER cisternae has been reported in various other cell types (Beams and Kessel, 1968; Thiery, 1969; Franke *et al.*, 1971; Morre *et al.*, 1971; Ovtracht *et al.*, 1973). These vesicles are probably contributing their contents, as well as their membranes, to the Golgi bodies.

The various types of ER associations with Golgi bodies described here may play a major role in the maintenance of the latter in *Xenopus* embryos.

c. The Golgi body

A large amount of information is available regarding the structure of the Golgi body in a wide variety of cells, but its basic architecture is similar throughout (Beams and Kessel, 1968). Golgi cisternae in the present case were either flat or cup-shaped and usually fenestrated at the edges. According to Sakai and Shigenaga (1967), during the course of spermatogenesis in the grasshopper the profile of the Golgi body is transformed from a flat shape to a U-shape or a system of concentric rings. The description matches with the Golgi profiles reported here inasmuch

as the flat and cup-shaped Golgi bodies could be two developmental stages of the organelle. The appearance of concentric rings in the present case, however, is due to the plane of the section passing through the rim of the cup-shaped Golgi body.

The vesicles associated with the concave face are probably budded off from the edges and/or innermost cisterna resulting in a morphological polarity of the Golgi body. In *Tetrahymena*, pinching away of vesicles from an ER cisterna has been related to its fenestrated appearance (Franke *et al.*, 1971). The observation is comparable with the present case as cisternal edges and the innermost cisternae were usually fenestrated. The increased electron density of the cisternal material towards the concave side may be indicative of functional polarity as has been suggested in other Golgi types (Mollenhauer and Morre, 1966; Thiery, 1969). Another conclusive piece of evidence for a cytochemical polarity of the Golgi body in the present material is provided by the thiamine pyrophosphatase localization (Fig. 77) and is discussed below. In certain cases the polarity of the Golgi body could not be ascertained, since the cisternae were flat and vesicles were seen associated at all levels. In such Golgi bodies it is possible that material exchange through vesicles is taking place throughout the organelle. The observation is consistent with the suggestion that potential sites of membrane input into the Golgi body are not restricted to the forming face (Mollenhauer, 1965; Manton, 1967; Novikoff, 1967; Flickinger, 1969; Maul, 1969).

Among the small vesicles seen associated with the Golgi body, some had very fine bristles on their outer surface giving them the appearance of coated vesicles. Such coated vesicles have been reported in a variety of cellular forms, e.g. erythroblasts (Fawcett, 1964), vas deferens cells (Friend, 1965) and oocytes (Kessel and Beams, 1963; Anderson, 1964; Roth and Porter, 1964). In the present case the coated vesicles were not only loose near the Golgi body but were also seen in continuity with the Golgi cisternae and were present at almost all levels of the Golgi body. Usually, coated vesicles have been associated with specific protein uptake (Bowers, 1966), although according to Jamieson (1966, cited by Beams and Kessel, 1968) the material present on the outer surface of these vesicles help in pinching off of vesicles by a contraction device.

The number of Golgi cisternae in different cell types may vary from three to twelve (Beams and Kessel, 1968). In vertebrates the number is at the lower end of the range and in the present case the maximum number of cisternae in any single stack was seven, the usual number being two to four. The number is comparable to that of many other vertebrate cells: two to five in mouse pancreatic cells (Sjöstrand and Hanzon, 1954), two to four in chick liver cells (Karrer and Cox, 1960) and four or fewer in mid-blastula *Xenopus* embryos (Sanders, 1973).

Certain cells show a remarkable localization of the Golgi body. In many secretory cells it is located between the nucleus and the apical surface (Palade *et al.*, 1962; Neutra and

Leblond (1966), while juxtanuclear localization has also been reported (Hicks, 1966; Novikoff, 1967; Beams and Kessel, 1968; Whaley, 1968). In mid-blastula *Xenopus* embryos the organelle usually occurs near the plasma membrane and does not show any juxtanuclear localization (Sanders, 1973). Although in the present study the Golgi bodies were seen associated with the furrow tip and were frequently observed within 5 μ of the growing furrow, they were also apparent in other areas of the cytoplasm. Thus the question of any preferred distribution of the Golgi body in the first cleavage *Xenopus* embryos remains unsettled.

The origin of the Golgi body could either be *de novo* or from other cell organelles (Whaley, 1966). The origin from other organelles such as the nuclear envelope (Morre and McAlear, 1963; Bouck, 1965; Scharrer and Wurzelmann, 1969; Stang-voss, 1970; Kessel, 1971), lamellar bodies (Ruby and Webster, 1972), and endoplasmic reticulum (Friend, 1965; Franke *et al.*, 1971; Morre *et al.*, 1971) is well documented. Although some of the single fenestrated Golgi cisternae in the present study were seen surrounded by RER membranes (Figs. 48, 49), there is not sufficient evidence to postulate an ER origin of the Golgi body. The present study does, however, provide some evidence in favour of a new source from lipid droplets as discussed above. In spite of a similar structure of the Golgi body in a variety of cells it apparently finds origin from different sources.

d. Lamellar bodies

Lamellar bodies have been described previously in *Xenopus* oocytes (Spornitz, 1973) and embryos (Bluemink and De Laat, 1973). However, the present study indicates the occurrence of three different types of lamellar body in *Xenopus* embryos. The periodicity recorded (100 Å) in the first type, the myelin-like figures, is comparable to that reported in true myelin figures (100-130 Å) (Robertson, 1964). The total thickness of the repeating laminar arrangement (4 dark + 3 light) observed in the second type (160 Å) is about double the approximate thickness of a plasma membrane (80 Å). The third type of figure, composed of a band of two to ten lamellae intertwined at random, is similar to the ones reported in *Xenopus* embryos by Bluemink and De Laat (1973). The frequent occurrence of the third type of lamellar bodies near the maturing or concave face of the Golgi body noticed here suggests that these structures or their precursors might be originating from the Golgi body. This type of lamellar body was also seen close to the furrow membrane. The purpose of this closeness is not immediately apparent. One possibility could be that the structures represent another mode of transport of materials from the Golgi body to the growing furrow. Lamellar figures in the cytoplasm near the furrow of cleaving *Xenopus* embryo have been suggested to represent membrane precursors (Bluemink, 1971b).

Some workers (Curgy, 1968; Spornitz, 1973) have expressed the view that laminated figures are artifactual, while

others have reported the involvement of these structures in the formation of mitochondria (Pannese, 1966; Beaulton, 1968) and dictyosomes (Mercer, 1962; Ruby and Webster, 1972). It has been shown that phospholipids when hydrated will form lamellar figures (Stoeckenius, 1962b; Bangham and Horne, 1964; Glauert and Lucy, 1968). If the lamellar bodies observed here are an example of such hydration of phospholipid pools it would be hard to ascertain whether this hydration occurred *in vivo* and the structures are genuine, or whether it occurred during fixation resulting in artificial formation of these structures. In light of the above, any interpretation of these structures should be considered with caution. In the present case, even if the structures are artifacts of fixation, the occurrence of three different types may indicate the existence of a similar number of different pools of precursor materials which have their own relative distribution and association with other organelles in the embryo.

Thiamine pyrophosphatase (TPPase)

Two objections are commonly raised for the localization of TPPase: first, non-enzymatic hydrolysis of the substrate by the lead ion (Moses and Rosenthal, 1968). Second, non-specific binding of the lead ion (Gilles and Page, 1967). In the present study both these possibilities are excluded by appropriate controls in which Golgi bodies, vesicles and the furrow failed to show any deposits. Non-specific binding was shown, however, by lipid droplets and pigment granules. The deposits of lead

phosphate in the Golgi bodies, vesicles and the forming cell membrane. Embryos were varied, ranging from diffused or dense. This may not necessarily reflect the nature of the enzyme activity as the possibility of the diffusion of reaction products after incubation cannot be ruled out (Shnitka and Seligman, 1971). For the present purpose, the presence of deposit will be taken to mean only the presence of enzyme activity and no consideration will be given to the intensity or amount of the deposit.

The presence of enzyme activity in the Golgi body and the furrow indicates a possible functional link between the two. Such a link may be provided by the vesicles that are enzyme-positive. The appearance of enzyme activity in the furrow only after the latter has grown some distance into the embryo gives an indication that the enzyme may be transported there in vesicles originating in Golgi bodies. Formation of vesicles containing TPPase activity by the Golgi body has been reported in other cell types (Griffith and Bondareff, 1973). Localization of enzyme activity within the outermost cisternae of the Golgi body provides strong evidence in favour of a cytochemical polarity of the organelle.

f. Acid Phosphatase

Presence of acid phosphatase activity on the surface of microvilli was not complemented by its localization in the cytoplasm. The lack of activity in the Golgi apparatus provides an interesting contrast to the usually accepted localization of the enzyme in the organelle of various cells including amphibian oocytes (e.g. Kessel and Decker, 1972). However, the observation coincides with that of

Martin and Spitzer (1973). According to these workers the Golgi body in the syncytiotrophoblast of human term placenta is consistently unreactive for acid phosphatase. In the present case the means by which the activity appears in the furrow remains a question. The possibility of artifacts should not be ruled out entirely.

4. Route for the Assembly of Surface Material - A Hypothesis

A proposed assembly line has been summarized in Fig. 91. After release from the lipid droplets the rough endoplasmic reticulum cisternae acquire filamentous projections on the luminal side which are probably a synthetic product of the ribosomes. The projections act as a marker and help in assigning the direction of flow of the membranes. If the peripheral material of the yolk platelets is acid polysaccharide in nature, then the subsequent association of the fringed ER cisternae with the yolk platelets could be for the purpose of adding sugar moieties to the cisternal contents. Thereafter the ER membranes and their contents either become incorporated into the Golgi body or approach it through fringed ER vesicles for further differentiation. Finally, the Golgi-derived vesicles can contribute to the plasma membrane and surface material.

Observations in support of the proposed hypothesis:

1. Nascent endoplasmic reticulum, continuous with lipid droplets, does not show a fringed interior though ribosomes are attached to its surface.
2. The appearance of fringes in the lumen of the endoplasmic reticulum after it has been released from lipid droplets.

3. The association of fringed ER cisternae with yolk platelets and the lack of ribosomes in the region of contact.

4. The yolk material immediately adjacent to such regions of contact is granular instead of crystalline.

5. The granular peripheral material of the yolk platelets contains acid polysaccharides (Ohno *et al.*, 1964; Tandler and LaTorre, 1967; Favard and Favard-Séréno, 1969).

6. The association of fringed ER membranes with the Golgi body either by direct apposition or through fringed vesicles.

7. Vesicles probably derived from the Golgi body are seen in direct continuation with the plasma membrane.

8. Relationship of the Golgi body with the furrow membrane, through vesicles, has also been shown by TPPase and OZI studies.

The cell surface in first cleavage *Xenopus* embryos does not show any filamentous projections similar to those seen in the fringed endoplasmic reticulum. Therefore, a direct transfer of surface material from fringed ER to plasma membrane (Bluemink, 1971b; Bluemink and De Laat, 1973) or vice versa is unlikely to occur in these embryos.

Similar assembly lines, involving synthesis of proteins by ribosomes associated with the ER membrane, sequential addition of sugars at various stages, followed by further modification of this material in the Golgi body and in its derived vesicles, have been proposed by several workers (Northcote, 1971; Whaley *et al.*, 1972; Ovtracht *et al.*, 1973).

If the details of the cleavage process are to be

thoroughly elucidated then it is of prime importance that biochemical correlates be obtained for some of the events proposed here.

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