PHASE CONTRAST OBSERVATIONS OF THE ENDOPLASMIC RETICULUM IN LIVING TISSUE CULTURES

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ABSTRACT

Cells from three human sources (two malignant and one fetal) were observed through phase contrast microscopy to contain unusual cytoplasmic images. These were photographed and are discussed as representing the endoplasmic reticulum in the living cell.

INTRODUCTION

The endoplasmic reticulum (ER) was first observed by Porter *et al.* (1) in thinly spread tissue cultured cells viewed with the electron microscope (EM). With the improvement of EM preparation procedures, numerous reports have followed and have indicated the potentially universal occurrence of this cytoplasmic membranous system in animal cells. Since these reports have resulted only from EM analyses, the endoplasmic reticulum, generally, has been considered a component of submicroscopic size. However, a recent observation by Fawcett and Ito (2) demonstrated that phase contrast images observed in freshly isolated testicular cells were equivalent to EM profiles of the endoplasmic reticulum, and thus removed this organelle from the exclusive realm of submicroscopic analysis.

By the use of an innovation of the tissue culture technique (3), we have observed, in cells of one fetal-lung and two malignant-tissue cultures, structures which appear in every respect to be equivalent to the endoplasmic reticulum as reported by Fawcett and Ito in their phase contrast photographs. Although these structures were not proved by EM techniques to be the endoplasmic reticulum, their relationship to the form and size described by Fawcett and Ito and their location in the endoplasm, indicate that there can be little doubt as to their identity.

MATERIALS AND METHODS

Tissue Cultures: Fresh human specimens from a papillary adenocarcinoma (metastatic to the omentum), a melanoma, and an 18-week fetal lung were cut with scalpels into pieces of approximately 1 mm. in

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diameter, placed on coverslips of multipurpose culture chambers (3), and then covered with full sheets (2 × 3 inches) or narrower strips (1 × 3 inches) of unperforated and nutrient-moistened cellophane of the type used for dialysis. After this the chambers were assembled and filled with a fluid nutrient composed of medium No. 1066 (75 per cent), calf serum (20 per cent), and whole egg ultrafiltrate (5 per cent). This arrangement provided two environmental conditions for the cultures: (1) full sheet of cellophane—a nutrient dialysate environment, and (2) strip of cellophane—a complete nutrient environment. The full sheets isolated the explants from the main nutrient-containing compartment of the chamber although they permitted an access to it through their minute pores. The strips, being narrower than the diameter of the gasket hole, provided a condition in which there was direct access to the main nutrient vault around their edges as well as an access by dialysis.

Phase Contrast Studies: Cells emigrating from these tissue explants were observed with a Bausch and Lomb phase contrast microscope fitted with a long working distance condenser. Photographs were made with a Hasselblad 120 roll film camera using Ansco IFF 13 film and a Bausch and Lomb 100 watt research lamp light source.

RESULTS

The endoplasmic reticulum (ER) could be observed only in cells cultivated under the full sheets of cellophane and not found in similar cells cultivated under the cellophane strips.

After 8 days of cultivation, emigrations from the papillary adenocarcinoma contained a small percentage of cells which appeared to have a cytoplasm sharply delineated into an endoplasm and ectoplasm. With the high power, phase contrast objective (97×), the endoplasm was observed to be full of tiny forms of complex structure. Two of these cells were photographed and are shown in Figs. 1 through 4. The endoplasmic area in both of these cells contained a multiplicity of forms with erratic bends or curves, of varying lengths, and often in equally spaced parallel array. Besides these structures, the larger and denser elongated forms of mitochondria were easily identified. The ratio between the diameters

Explanation of Figures

Abbreviations Used in Figures

N, nucleus
ER, endoplasmic reticulum
M, mitochondria
Go, Golgi complex, juxtanuclear apparatus
En, endoplasm
Ec, ectoplasm
P, process of endoplasm
a, artifact, lens dust

FIGURES 1 through 4

High power, phase contrast photographs of two cells of emigration from a human cystic adenocarcinoma cultivated under sheets of cellophane in the multipurpose culture chamber for 8 days. Fig. 4 has a magnification line for all of the illustrations. × 1400.

FIGURES 1 and 2

One cell photographed at a 3-minute interval and at two levels of focus. Two distinct patterns of the endoplasmic reticulum (*ER*) in the upper portion of the cell are shown. In Fig. 2 the phase darker ectoplasm (*Ec*) and the phase lighter endoplasm (*En*) are more pronounced. The mitochondria (*M*) which are phase darker than the endoplasmic reticulum are shown in both the ectoplasm and endoplasm. A juxtanuclear area with dark and light granules presumably is the Golgi apparatus (*Go*).

FIGURES 3 and 4

These photographs were taken at a 26-minute interval and indicate the variation in the endoplasmic reticulum (*ER*) pattern after a prolonged period of time. The darker staining mitochondria (*M*), endoplasm (*En*), ectoplasm (*Ec*), and Golgi apparatus (*Go*) are indicated.

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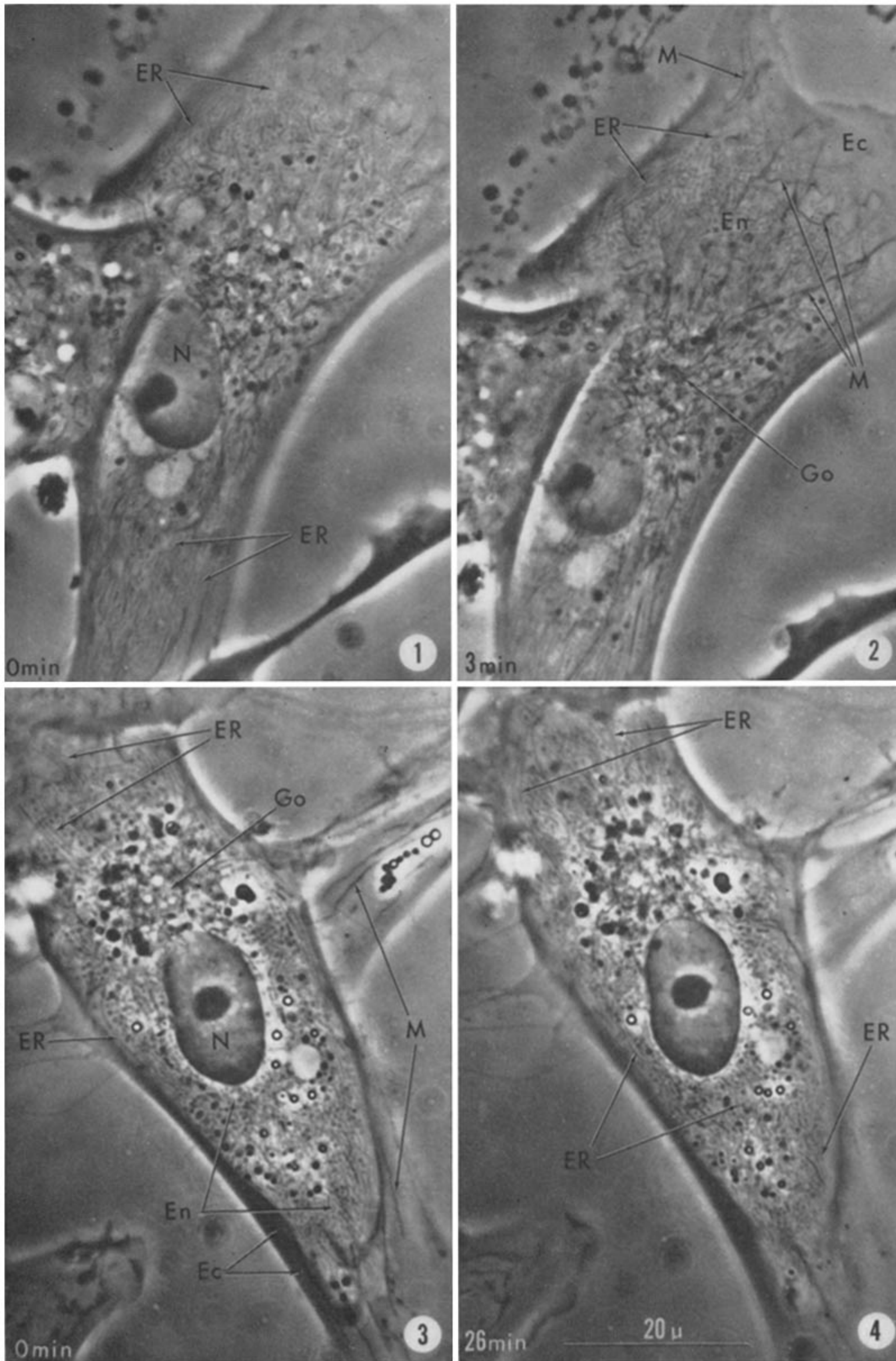

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of mitochondria and ER elements in these photomicrographs is similar to that evaluated from the phase contrast images published by Fawcett and Ito. The Golgi areas in both of these cells were observed and appeared to contain secretory droplets. Figs. 1 and 2 taken at a 3 minute interval, and Figs. 3 and 4 at a 26 minute interval, show the relative changes which occurred.

One cell which emigrated from a melanoma explant was photographed after 11 days of cultivation in a time-lapse sequence to illustrate mitochondrial activity, but after photographic development, the cytoplasm was observed to contain lesser-sized forms which appeared to be the endoplasmic reticulum. This is illustrated in Figs. 5 and 6, which show only the lower tip of a rather large cell. The delineation between the endoplasm and ectoplasm is not sharp in this photograph, but the rapid change in form of the ER which occurred after 2 minutes may be observed.

The cellular emigration from the human fetal lung was generally in two forms: (1) an epithelium, and (2) an unusually large and isolated fibroblastic element which moved about very slowly. Both of these cell forms displayed networks similar to those in Figs. 1 through 6, and a portion of one of the larger fibroblastic cells is shown in Figs. 7 through 11. Once again, the relationship to the larger and denser filamentous mitochondria is easily made. Figs. 7 through 10 were taken at 1-minute intervals and show the progressive change occurring in the ER and mitochondria. The ER changed form more rapidly than the mitochondria and appeared to be in a solated region which was phase lighter than the phase darker periphery (ectoplasm). The ER was continuously influenced by brownian bombardment whereas the mitochondria were not.

Another fibroblastic cell from the fetal lung is shown in Figs. 12 through 17. Fig. 17 is a montage of most of the cell, and Figs. 12 through 16 were taken at 3-minute intervals and illustrate only the lower pole of the cell. Here the ectoplasm and endoplasm delineation was very sharp and its labile nature depicted. The long mitochondria were not observed in this cell.

DISCUSSION

Because the cytoplasmic structures we have found are very similar to those described in reference 2 and because the latter were identified as ER elements by parallel electron microscopical observations, we postulate that these structures represent the endoplasmic reticulum *in vivo*.

FIGURES 5 and 6

High power, phase contrast photographs of a portion of one cell which emigrated from a human melanoma cultivated under a sheet of cellophane in the multipurpose culture chamber for 11 days. The pictures were taken at a 2-minute interval and indicate the relative change of the mitochondria versus that of the endoplasmic reticulum. The form of the endoplasmic reticulum has shifted considerably whereas that of the mitochondrial array has remained relatively stable. × 1300.

FIGURES 7 through 11

High power phase contrast observations of the polar portion of a fibroblastic cell which emigrated from the human fetal lung cultivated for 23 days under a full sheet of cellophane in the multipurpose culture chamber. The magnification line for Figs. 7 through 10 is shown in Fig. 7, and *a* represents lens dust artifacts.

FIGURES 7 through 10

These photographs were taken at a 1-minute interval and show the relative change taking place in the endoplasmic reticulum and mitochondrial array. × 950.

FIGURE 11

A high power enlargement of Fig. 10 showing greater detail. The phase displays of the endoplasmic reticulum and ectoplasm (*Ec*) are essentially equivalent whereas the endoplasm (*En*) is phase lighter and, thus, renders the *ER* visible. × 2133.

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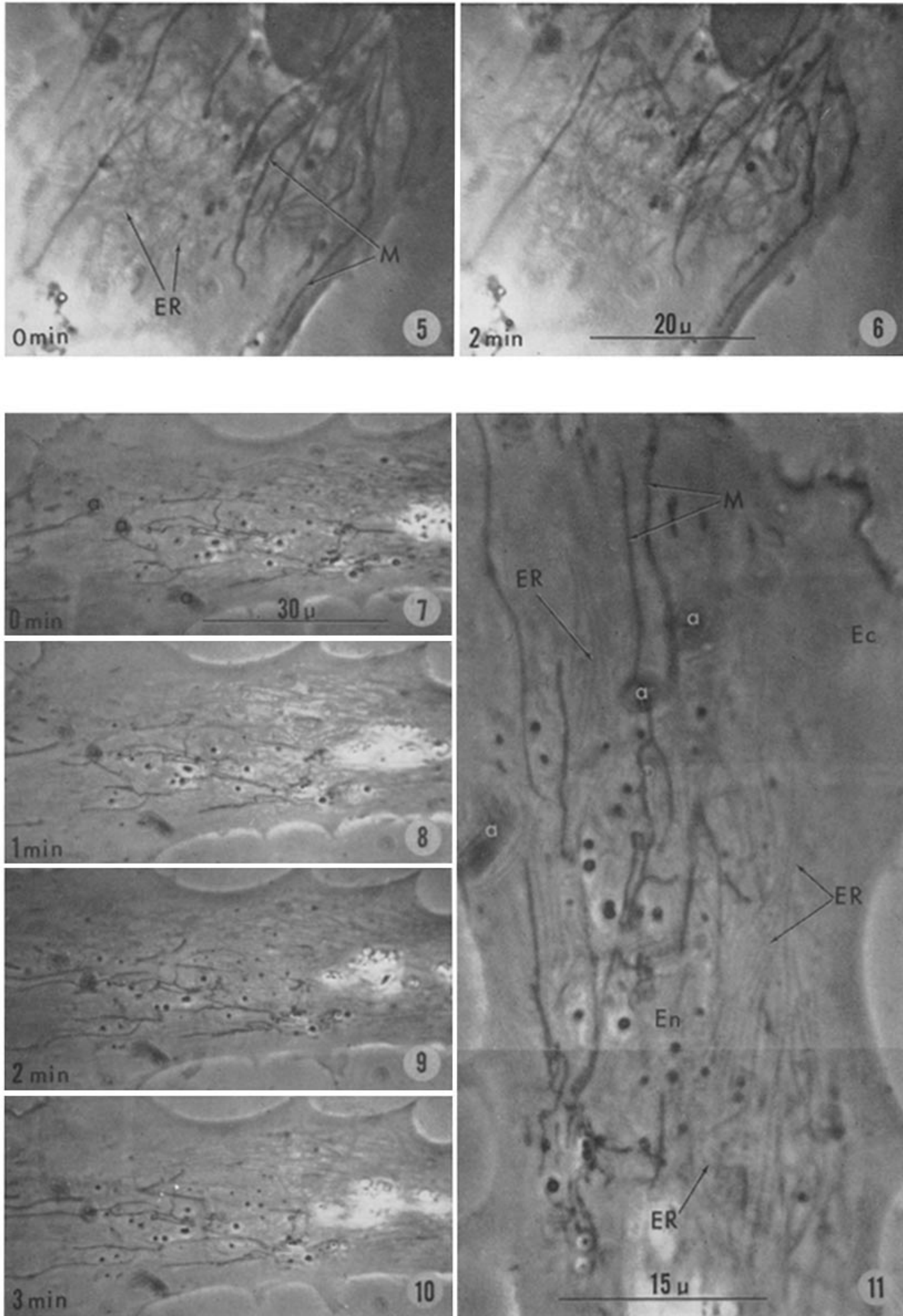

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Phase contrast microscopy was introduced in the mid-1940's and has become an increasingly useful tool for those engaged in tissue culture. It seems unusual, then, that after this length of time and with as many people making tissue culture observations as there are, that the endoplasmic reticulum as shown in these living cells has not heretofore been observed and reported. Even the refined techniques of Frederic (6) with the anoptral optical system, and the relevant phase contrast searches of Chèvremont (7) have not revealed equivalent cytoplasmic forms; although the descriptions by Thiéry (8–10) of plasmocytes and by Palay and Wissig (11) of fresh optic neurons suspended in hypertonic sucrose bear definite similarities. Since the indicated cultures were established in a novel way, which has been shown to favor cellular differentiation (3–5), it seems quite likely that this dialysis cellophane system may have been conducive to a greater activity of the endoplasmic reticulum and, thus, permitted it to be more visible.

Fawcett (12) has suggested that the lowering of the refractive index of the cytoplasmic matrix associated with solation results in enough differential contrast to make the ER visible under phase contrast optics. In most situations the endoplasm is gelated and has the same index of refraction as the ER, so the two are phase equivalent and the ER invisible. Although this is quite possibly the reason for seeing the ER, the solation of the endoplasm to produce a visible ER may be due to the dialysate environment produced by the cellophane procedures.

The question may arise whether the images reported as the ER were not really "surface patterns" of the type frequently encountered in certain epithelial cells (oral epithelia, for instance). The phenomena, of course, were not limited to epithelial cells, and these ER structures were focused in the midzone of the cell as may be determined by the sharp focus of the nuclear wall and/or cell wall. However, these structures were located in a fluid zone and were observed as a shimmering mass due to brownian bombardment, and this makes a relationship with "surface patterns" unlikely.

The very active appearance of the Golgi zones illustrated in the cells of Figs. 1 through 4, partially supports the relationship between the endoplasmic reticulum and the Golgi zone postulated by Palade (13, 14) and Palay (15). With time-lapse motion picture techniques, it should now be possible to follow the living morphological activity and responses of the endoplasmic reticulum.

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Figures 12 through 17

These are phase contrast photographs of a single fibroblastic cell which emigrated from a human fetal-lung explant cultivated under a sheet of cellophane in the multipurpose culture chamber for 22 days. A magnification line for Figs. 12 through 16 is in Fig. 12.

Figure 17

This is a photographic montage which shows most of the fibroblastic cell. At its upper pole there is an endoplasmic (*En*) and ectoplasmic (*Ec*) delineation and the endoplasmic reticulum (*ER*) in this area is somewhat broader than that observed in the lower pole. Filamentous dark mitochondria were not observed in this cell. × 700.

Figures 12 through 16

These are high power enlargements of the lower pole of the cell in Fig. 17 and were taken at 3-minute intervals to illustrate the rapidly changing form of the endoplasmic reticulum (*ER*) as well as the limits of the solated endoplasm. In Fig. 13 the finger-like process (*P*) may be seen to contain three striated forms of the endoplasmic reticulum, and in Fig. 16 four forms are detailed. In all of these figures this process may be observed to have undergone a rapid change. × 1470.

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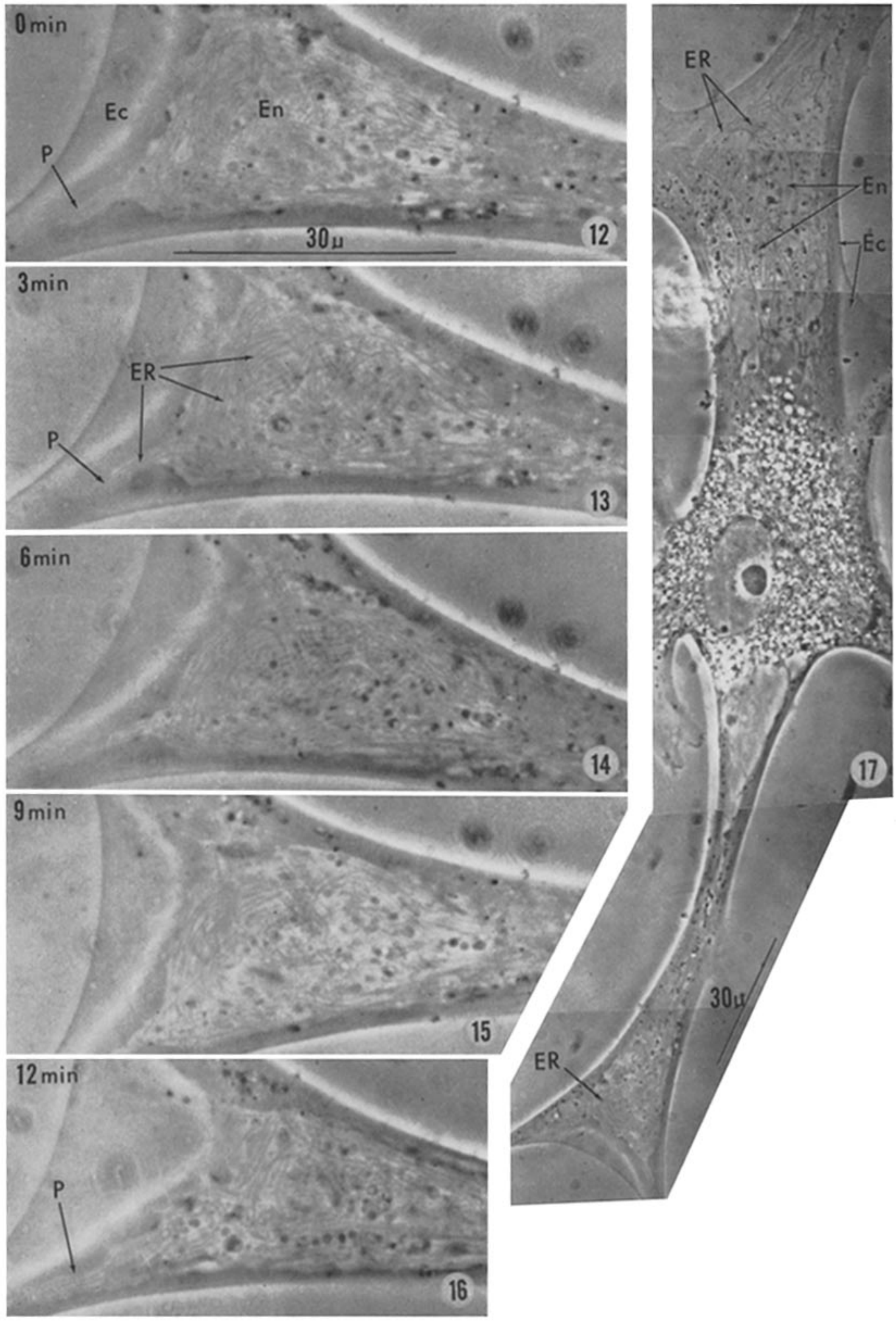

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