Caffeine-Induced Surface Blebbing and Budding in the Acellular Slime Mold *Physarum polycephalum*

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The mechanism of plasma membrane proliferation was studied in the acellular slime mold *Physarum polycephalum* with the aid of light and electron microscopical techniques. Treatment of protoplasmic drops with a Tris-buffered 15 mM caffeine solution causes surface blebbing and budding over periods of 5–90 min. The process of surface blebbing is coupled to a 5–10-fold increase of the surface area in conjunction with characteristic changes in cytoplasmic morphology. Successive constriction of blebs exhibiting different sizes and degree of hyalo-granuloplasmic separation leads to the formation of numerous spherical caffeine droplets. During the process of surface budding and droplet formation the total surface area of the original (genuine) protoplasmic drop is not reduced, but continues to grow.

Freeze-etch studies show that caffeine concomitantly causes characteristic changes in the fine structure of the plasma membrane. During the initial phase of surface blebbing the original density of intramembranous particles (IMP) is reduced from 3676/μm^2 to 1669/μm^2 and the PF: EF ratio (IMP/μm^2 protoplasmic face: exoplasmic face) shifts from 2.4:1 to 2.8:1. When surface budding is completed the IMP-density in the plasma membrane of single caffeine droplets increases again to 2289/μm^2 and the PF:EF ratio changes to 1.5:1. Simultaneously, the isolated caffeine droplets produce numerous small hyaline membrane protrusions, which are pinched off and contain no IMP. Control experiments demonstrate that Tris-buffer without caffeine also shows a weak capacity to induce surface blebbing, to change the IMP-density and the PF:EF ratio (2443/μm^2; 1.5:1); but Tris-buffer fails to cause surface budding. On the other hand, different concentrations of sucrose (25–200 mM) can supress to a certain degree both caffeine- and Tris-buffer-induced surface blebbing, but not caffeine-dependent surface budding.

The caffeine-effect is reversible insofar as protoplasmic drops with blebbing and budding activity recover to normal morphology, fine structure and locomotion when transferred to physiological conditions.

The mechanisms of successive changes in plasma membrane morphology as well as the mode of a participation of the actomyosin system in cell surface dynamics are discussed.

Introduction

Methylxanthines, such as theophylline, theobromine or caffeine, are known to induce changes in cellular behaviour by influencing the relationship between calcium and cyclic nucleotides, which both play an important role in the regulation of cytoplasmic motility [1]. Numerous investigations have dealt with the action of caffeine on smooth and cross-striated muscles (for literature, see [2]). Dependent on the concentration of this drug (1–10 mM), in particular skeletal muscles exhibit a differentiated response ranging from strong, total contraction [3] to wave-like contractions propagated along the fibres [4, 5]. Microinjection-experiments pointed to intracellular sites of action of caffeine [6–8] probably inhibiting the uptake or stimulating the release of Ca^{2+} by the sarcoplasmic reticulum [9, 10]. Although the exact mechanism of caffeine interaction with the calcium-regulating system is still obscure, there is some evidence that the drug causes a transient depolarization of the T-tubules [11, 12], thus initiating a series of steps, such as inhibition of phosphodiesterase, increase of cyclic nucleotides [13, 14], or specific reaction with a "charged molecular complex located at the T-tubule lateral cisternal junction" [2].

In the acellular slime mold *Physarum polycephalum* caffeine was also thoroughly tested and found to cause characteristic effects. Protoplasmic drops [15] treated with 5–10 mM solutions of caffeine reversibly lose their ability to develop a plasmalemma invagination system and to perform locomotion, although the drops can initiate actomyosin fibrillogenesis and normal contraction activ-

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ity [16–18], *i.e.*, caffeine seems to uncouple motive force generation from locomotion [19]. In addition, caffeine stimulates intensive cell surface blebbing and budding processes, which result in the formation of numerous so-called caffeine droplets [20]. The caffeine droplets are Ca^{2+}-sensitive (induction of intracellular movement, see [21–24]) and thus suitable systems for the study of different cell dynamic processes in *Physarum*. In the present investigation changes in the spatial organization of the cytoplasmic actomyosin system and in the ultrastructure of the plasma membrane during the process of caffeine-induced droplet formation were analyzed light and electron microscopically in thin sections and freeze-etch replicas.

Materials and Methods

I. Culture and specimen preparation

Physarum polycephalum (ATCC Nr. 44912) was cultured by combining the methods of Camp [25] and Daniel and Rusch [26]. Axenically grown microplasmodia were transferred to filter paper or agar and transformed to migrating macroplasmodia. Protoplasmic drops were then generated by puncturing plasmoidal strands from the front zone of plasmodia [15]. The drops were always left on the strands for 10 min before isolation and successive treatment with different solutions.

II. Solutions for the investigation of protoplasmic drops in vivo

The following solutions were applied:

A. 15 mM caffeine in 10 mM Tris-maleate-buffer, pH 7.0 [24].
B. 10 mM Tris-maleate-buffer containing 0, 25, 50, 100 and 200 mM sucrose as well as 0 and 15 mM caffeine, pH 7.0.
C. 10 mM Tris-maleate-buffer, pH 7.0.

All solutions were used at room temperature (21 °C). Light microscopical observations and photographic documentation were performed with a Zeiss IM 35–OM2 at different time intervals between 0 and 60 min after application of solutions A–C.

III. Light and electron-microscopical investigation of embedded material

Protoplasmic drops treated for 0, 15 and 60 min in solution A and for 0 and 30 min in solution C were fixed in 1% OsO_4/0.5% $HgCl_2$ [27] for 60 min. Dehydration was carried out in a graded series of ethanol increasing from 30% to 100%, including a staining step in 70% ethanol containing 1% PTA and 0.5% uranyl acetate. Embedding was performed in styrene methacrylate [28]. Semithin and ultrathin sections cut on LKB-ultrotomes were investigated in a Leitz-Ortholux-Orthomat light microscope or in a Philips 301 electron microscope. Profile views of semithin median sections were used to determine morphometrically the surface area of controls, Tris- and caffeine-treated drops.

IV. Electron microscopical investigation of freeze-fractured material

Freeze-fracture replicas were obtained with a Biotech 2005 (Leybold-Heraeus) from the following specimens:

1. Protoplasmic drops treated for 30–60 min with solution A. This preparation includes isolated caffeine droplets budded off from protoplasmic drops.
2. Protoplasmic drops treated for 30–40 min with solution C.
3. Protoplasmic drops treated for 15 min with solution A until showing intensive formation of caffeine droplets, then washed for 5 × 3 min in solution C and, finally transferred to 1:10 SD-agar (nutritional agar) for a period of 15–20 min until exhibiting a smooth surface. From a total of 30 protoplasmic drops treated in this way, 6 were taken for freeze-fracturing and 24 as controls to test their ability to grow out to normal plasmodia after caffeine treatment (locomotion test).
4. Isolated caffeine droplets derived from protoplasmic drops after treatment in solution A for 40–60 min.

All specimens were prepared for freeze-fracturing in the living state, *i.e.*, without chemical fixation or infiltration with freeze-protecting agents as described recently [29].

To determine the membrane particle density (IMP/μm^2) on EF and PF of protoplasmic drops and caffeine droplets, 7–33 picture areas (size 0.25 μm^2) were chosen randomly from micrographs (100000:1 final magnification) of different probes and replicas. A total of approximately 220 samples were taken for the quantitative evaluation.

Results

I. Light microscopic investigation of protoplasmic drops

For all experiments a caffeine concentration of 15 mM was used because the lower concentrations of 5 mM and 10 mM acted more slowly but in the same manner. Drops showing a regular smooth contour (Fig. 1 g) and an average diameter of 1.5 mm (Fig. 1 a) started to produce single, small blebs at the cell surface within the first 5 min during caffeine treatment. After 15–20 min (Fig. 1 b), the blebbing process expands over the entire periphery producing protrusions of different size and contents (Fig. 1 h). At the same time, morphometric measurements reveal an increase of 270% in the total surface area as compared to the 0 min-state (Fig. 1 a). The blebs now start to constrict from the drop and persist in the sample as so-called caffeine droplets. In spite of a permanent loss of protoplasmic material by budding, the surface area of the original drop continues to grow during the following 30–40 min up to values exceeding the 0 min-stage by 480% (compare Figs. 1 a and c). Simultaneously, the diameter of the drop increases almost two-fold. As the morphometric evaluation of the 60 min-stage does not include the caffeine droplets that are lost during the embedding procedures the actual value for the increase of surface area by caffeine treatment of protoplasmic drops is much higher than can be determined in semithin sections.

Similar results were obtained when Tris-buffer lacking caffeine was used (Fig. 1 f). The diameter of the protoplasmic drop (0 min-stage not shown) also increases, and intensive surface blebbing occurs over the entire periphery (Fig. 1 i). However, the blebs never reach the same size and quantity as in caffeine-treated drops, and budding of droplets was not observed.

The addition of sucrose in varying concentrations (25–200 mM) to Tris- and caffeine-solutions supresses the processes of swelling and surface blebbing of protoplasmic drops (Figs. 1 d and e). Even after a 45 min-treatment with 15 mM caffeine and 10 mM Tris-buffer containing 200 mM sucrose the diameter of the drop is not changed and surface blebs are scarce (compare Figs. 1 c and e). However, the formation of caffeine droplets is not fundamentally blocked.

The changes in cell surface morphology caused by caffeine in protoplasmic drops are reversible. Control experiments, in which protoplasmic drops during intensive surface blebbing and budding were removed from the caffeine-solution, washed in caffeine-free solution and transferred to nutritional agar, revealed a normal morphology (after 20 min) and behaviour (after 20 h) (see below IV).

II. Ultrastructural changes at the surface of drops during surface blebbing and budding

Semithin and ultrathin sections from the periphery of caffeine-treated protoplasmic drops during the phase of intensive surface blebbing and budding exhibit characteristic morphological changes. The cortical filament layer consisting of cytoplasmic actomyosin and normally delineating the surface of 10 min-old protoplasmic drops, locally starts to increase in thickness (Fig. 2 a, c) and to detach from the plasmalemma. The blebs, apparently forming as a result of this detachment (Fig. 2 a, arrow), grow in size by the further influx of cytoplasm extruded through the meshwork of the filament cortex. Depending on the width of mesh in the cortical filamentous network, the growing blebs contain hyaloplasm (Figs. 1 h, 2 a and b, Hp) or additionally membrane limited components (vesicles, mitochondria or nuclei), representing a typical granuloplasm (Figs. 1 h, 2 d, Gp). Consequently, the budding process following surface blebbing results in the generation of two different types of caffeine droplets: (a) hyaline droplets containing exclusively groundplasm (Figs. 2 a and c) and (b) granular droplets, additionally containing membrane-surrounded cell organelles (Figs. 1 k, 2 d).

The constriction of both hyaline and granular blebs from protoplasmic drops is performed by a mechanism resembling cell plate formation during cytokinesis in plants. Numerous vesicles without visible content seem to assemble in a linear, plate-like arrangement at the base of the blebs (Fig. 2 a, arrowheads); these vesicles are flattened in the direction of the perspective border between the growing caffeine droplet and the original drop (Fig. 2 b, double arrow). The successive fusion of the aligned vesicles seems to cause the constriction and thus the final partition of the caffeine droplets.

III. Ultrastructural changes in caffeine droplets

During and after separation from the genuine drop the caffeine droplets undergo further charac-

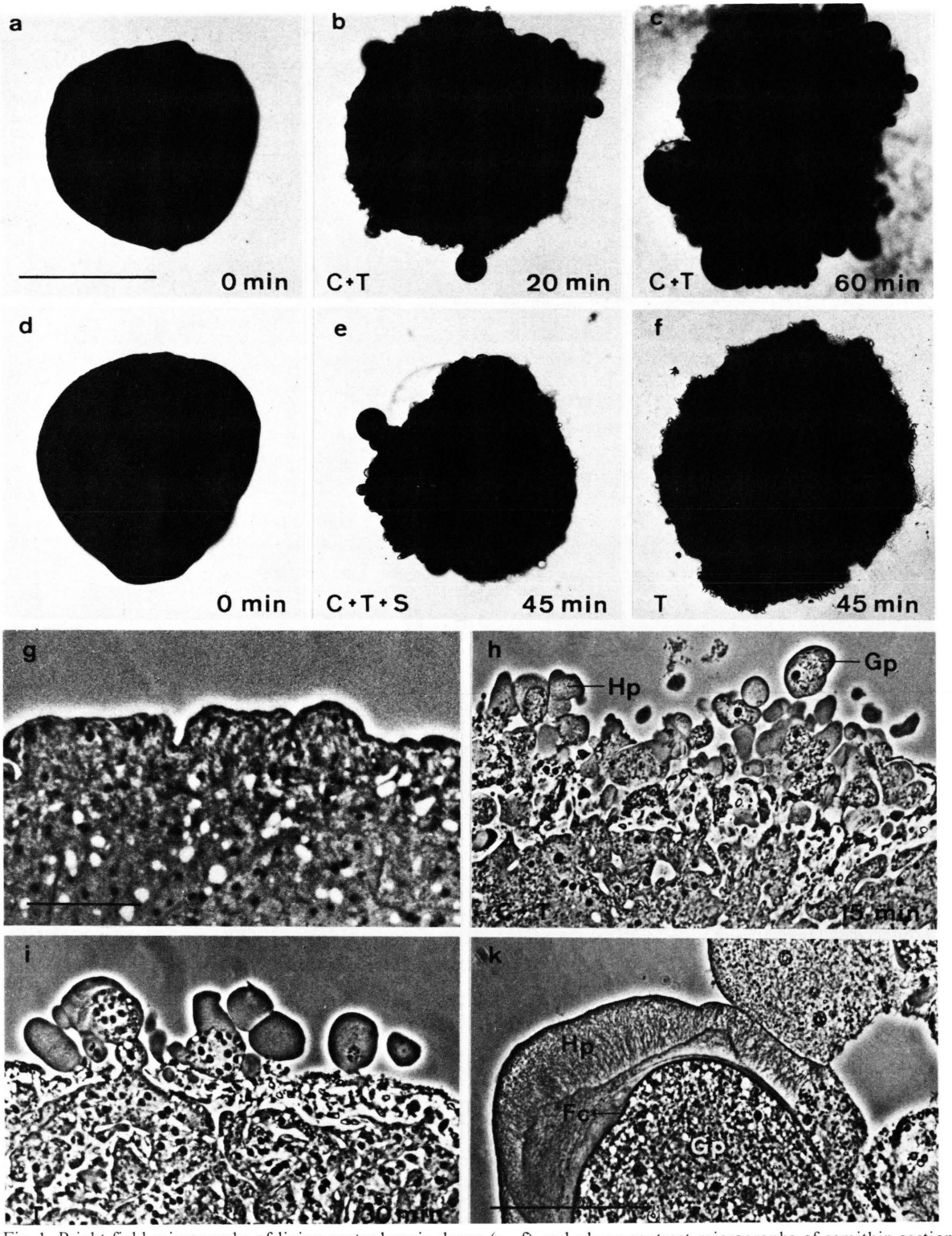

Fig. 1. Bright-field micrographs of living protoplasmic drops (a–f) and phase-contrast micrographs of semithin sections of the periphery of protoplasmic drops (g–k) after treatment with different solutions. a–c: 15 mM caffeine (C) and 10 mM Tris-buffer (T); the same drop after a = 0 min (untreated control), b = 20 min and c = 60 min treatment. d and e: 15 mM caffeine (C), 10 mM Tris-buffer (T) and 200 mM sucrose (S); the same drop after d = 0 min (untreated control) and e = 45 min treatment. f: 10 mM Tris-buffer (T) after 45 min treatment. g: Periphery of an untreated protoplasmic drop at 10 min after its generation (control). h: Periphery of a protoplasmic drop treated for 15 min with 15 mM caffeine (C) and 10 mM Tris-buffer (T). Blebs contain hyaloplasm (Hp) or granuloplasm (Gp). i: Periphery of a protoplasmic drop treated for 30 min with 10 mM Tris-buffer (T). k: Caffeine droplet containing a central granuloplasm (Gp) separated from a peripheral hyaloplasm (Hp) by a filament cortex (Fc). Scale: a–f = 1 mm, g–i = 50 μm, k = 50 μm.

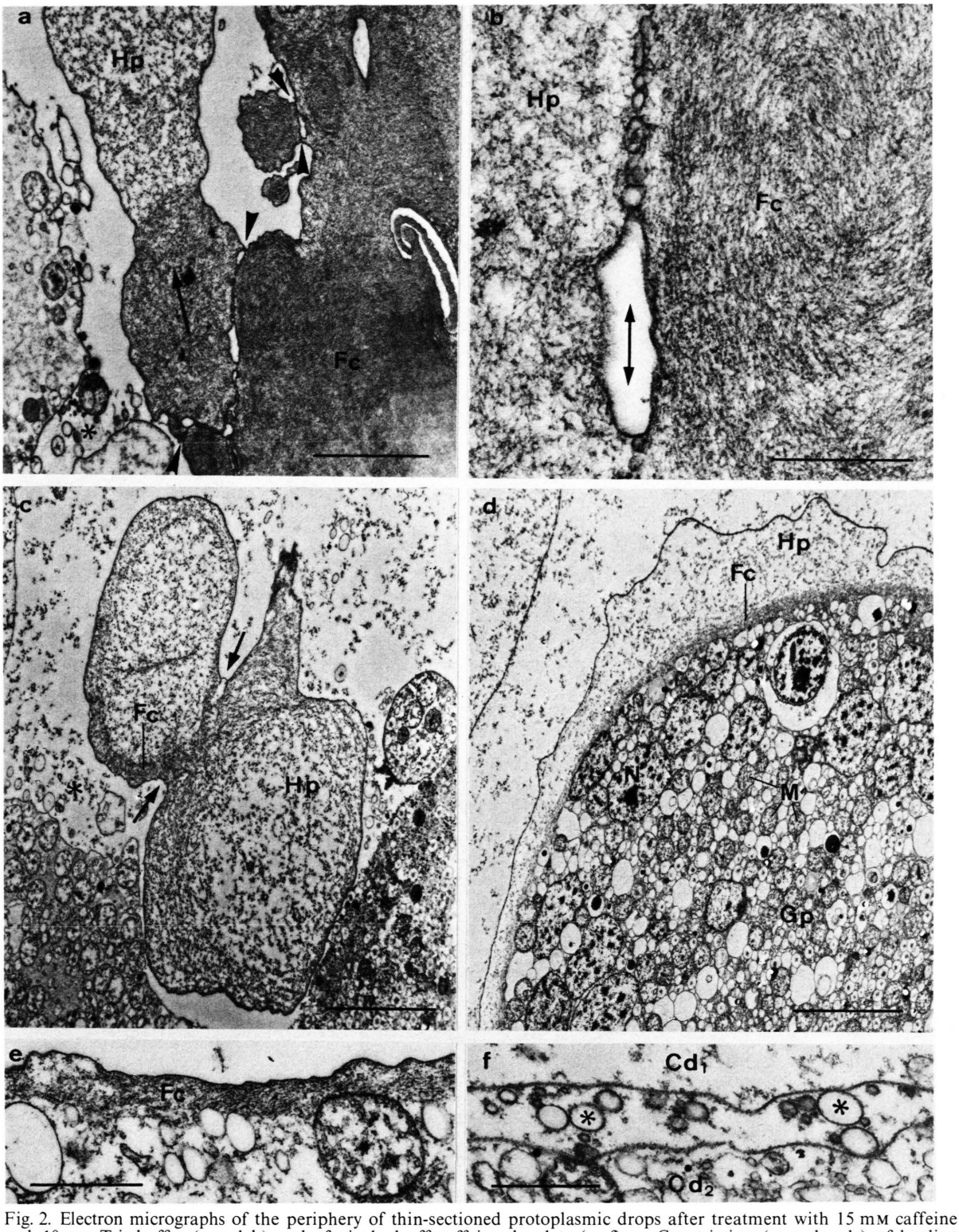

Fig. 2. Electron micrographs of the periphery of thin-sectioned protoplasmic drops after treatment with 15 mM caffeine and 10 mM Tris-buffer (a and b) and of pinched off caffeine droplets (c–f). a: Constriction (arrowheads) of hyaline caffeine droplets (Hp) from the periphery of a protoplasmic drop with a thick filament cortex (Fc); the arrow indicates direction of budding. b: Detail from Fig. 2a showing the aligned vesicles in the plane of constriction (double-arrow) at higher magnification (for labels see a). c: Hyaline (Hp) caffeine droplet undergoing division into smaller components (arrows) by the local contractile activity of a filament cortex (Fc). d: Granular (Gp) caffeine droplet showing sequestration of peripheral hyaloplasm (Hp) by contraction of the entire filament cortex (Fc); N = nucleus, M = mitochondria. e: Detail from the periphery of a granular caffeine droplet with a well-developed filament cortex (Fc) in close contact to the plasmalemma. f: Details from the peripheries of a hyaline (Cd 1) and granular caffeine droplet (Cd 2) with numerous small vesicles in the extracellular space between these droplets (stars). Corresponding vesicles are also indicated by stars in a) and c). Scale: a = 3 μm, b = 0.5 μm, c and d = 5 μm, e and f = 1 μm.

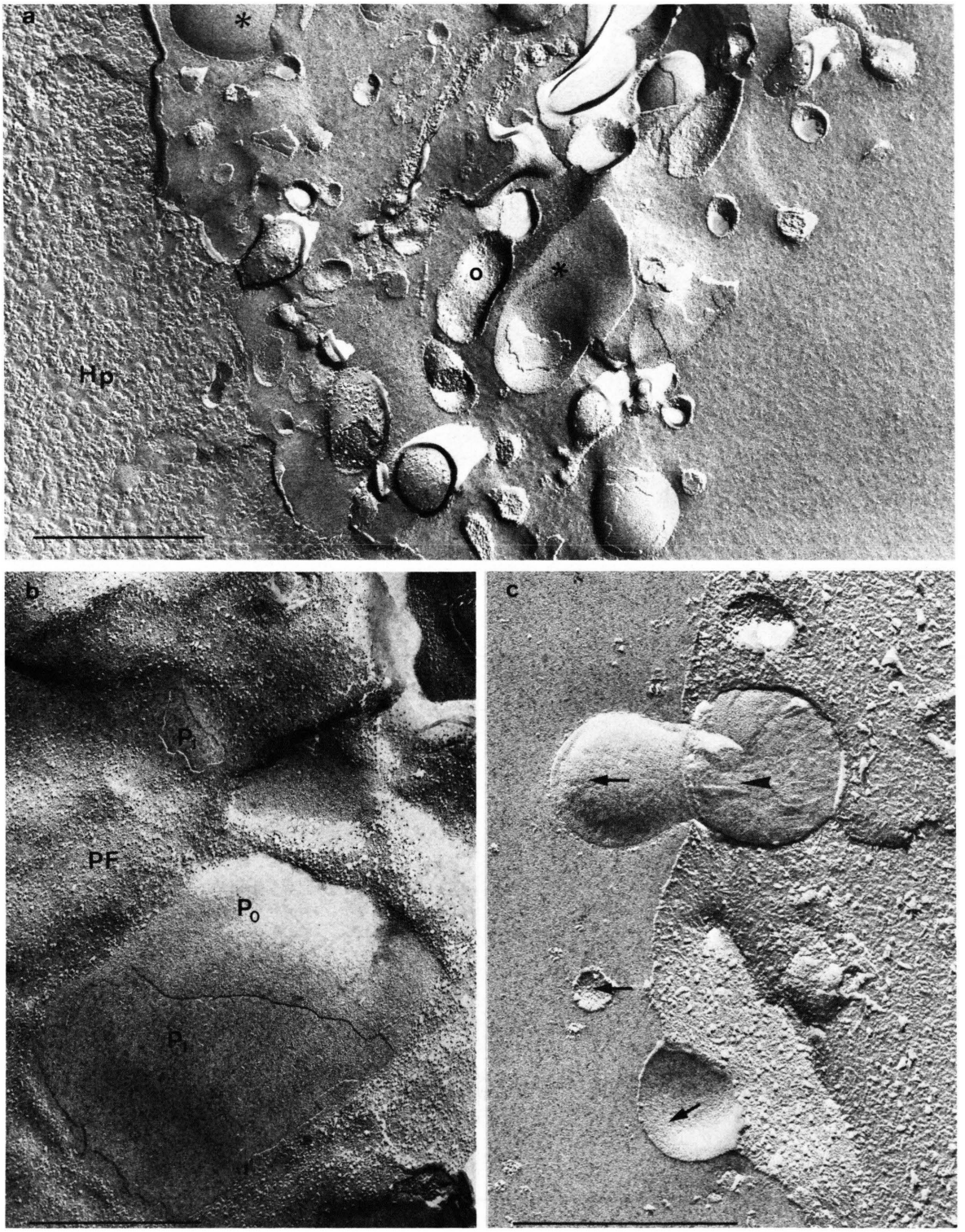

Fig. 3. Electron microscopy of freeze-fractured caffeine droplets derived from protoplasmic drops treated with 15 mM caffeine and 10 mM Tris-buffer. a: Cross-fractured periphery of a hyaline caffeine droplet (Hp) showing intense proliferation of small vesicles. Most vesicles exhibit smooth membrane fracture-faces without IMP (stars); only occasionally a few IMP are present (circle). b: IMP-rich PF of a caffeine droplet showing circumscribed smooth, double-layered areas (po, p1). c: Cross-fractured periphery of a caffeine droplet showing intracellular fusion of a lipid droplet with the plasmalemma (arrowhead) and segregation of vesicular lipid material into the surrounding medium (arrows). Scale: a–c = 0.5 μm.

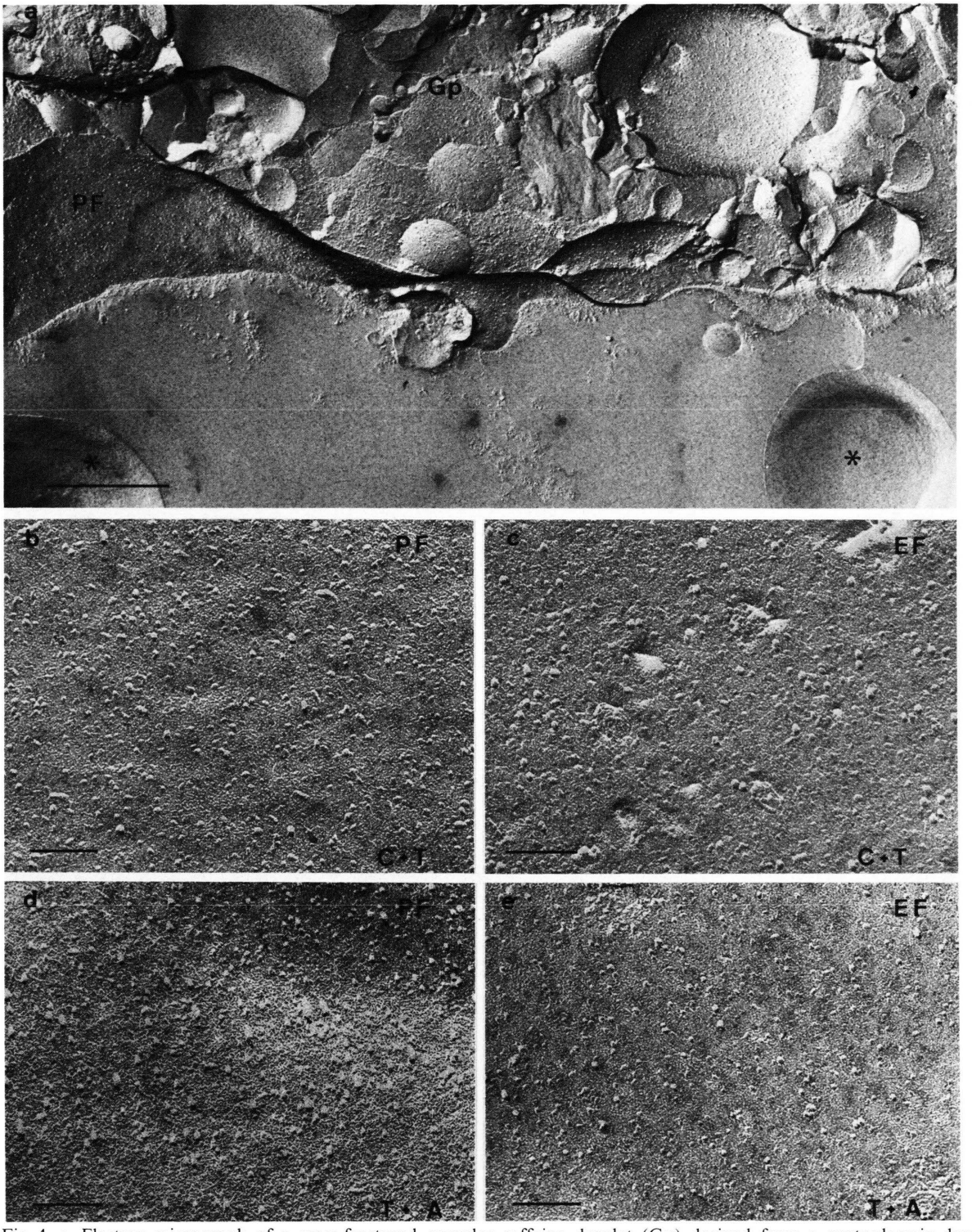

Fig. 4. a. Electron micrograph of a cross-fractured granular caffeine droplet (Gp) derived from a protoplasmic drop treated with 15 mM caffeine and 10 mM Tris-buffer. Stars mark segregated vesicular lipid material. b–e. Representative micrographs of PF (b and d) and EF (c and e) of protoplasmic drops treated with 15 mM caffeine (C) and 10 mM Tris-buffer (T) (b and c), or washed in Tris-buffer (T) and recovered on nutritional agar (A) (d and e). Note the abundance of large IMP ($\varnothing \geqq 10$ nm) in c. Scale: a = 0.5 μm, b–e = 0.1 μm.

teristic alterations in morphology. Both hyaline (Fig. 2c) and granular droplets (Fig. 2d, e) start to display a new filament cortex beneath the plasma membrane. The contractile activity of this filament cortex seems to initiate additional morphogenetic processes, such as constriction of smaller portions from the caffeine droplets (Fig. 2c, arrows) or, in conjunction with a loosening contact to the plasma membrane, a separation of peripheral hyaloplasm from central granuloplasm (Figs. 1k, 2d). Simultaneously, numerous small hyaline membrane protrusions are pinched off at the surface of caffeine droplets and released into the surrounding medium. Under favourable situations they are arrested in narrow spaces between neighbouring caffeine droplets and thus not lost during embedding (Figs. 2a, c and f, stars).

The analysis of freeze-fractured material contributes further details on the formation and nature of the protrusions at the cell surface of hyaline (Fig. 3a) and granular caffeine droplets (Fig. 4a). Intracellular fusion of lipid droplets with the plasmalemma (Fig. 3c, arrowhead) and segregation of IMP-free membrane material from the plasma membrane can be observed at many sites (Fig. 3c, arrows). This is especially evident in fracture-faces from surface regions where segregation of lipid protrusions has just occurred (Fig. 3b): This replica exhibits different IMP-free layers (po, p1) probably above a lipid droplet which continue laterally into a normal IMP-rich PF-face. Although smooth fracture-faces of segregated protrusions prevail (Figs. 3a, 4a, stars), areas with a few IMP are also present (Fig. 3a, circle).

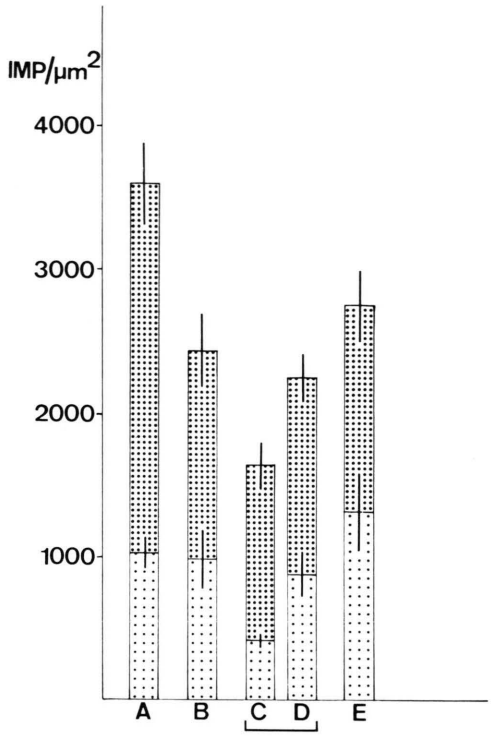

Fig. 5. Diagram summarizing the quantitative evaluation of IMP/µm² in protoplasmic drops and caffeine droplets after treatment with different solutions. A = 10 min-old protoplasmic drops without any pretreatment (see [29]). B = 10 min-old protoplasmic drops after 30–40 min in 10 mM Tris-buffer. C = 10 min-old protoplasmic drops after 30–60 min in 15 mM caffeine and 10 mM Tris-buffer. D = Isolated caffeine droplets derived from protoplasmic drops 60 min treated in 15 mM caffeine and 10 mM Tris-buffer. E = 10 min-old protoplasmic drops after 15 min in 15 mM caffeine and 10 mM Tris-buffer, followed by 5 × 3 min in 10 mM Tris-buffer and a stay of 10–17 min on nutritional agar. Densely-dotted portion of the columns = PF. Sparsely-dotted portion of the columns = EF. Vertical bars = standard deviation.

IV. Quantitative analysis of IMP during surface blebbing and budding

The density and vertical distribution of IMP was evaluated in EF and PF planes of caffeine and Tris-buffer-treated drops during different stages of plasma membrane proliferation (Fig. 5). The original IMP-density on EF and PF of the plasmalemma, which amounts to 3676/µm² in 10 min-old protoplasmic drops (Fig. 5, column A; see [29]) decreases during a 30–60 min combined treatment in 15 mM caffeine and 10 mM Tris-buffer to 1669/µm² (Fig. 5, column C). At the same time, the PF:EF ratio shifts slightly from 2.4:1 to 2.8:1. Later, when surface budding is completed, the IMP-density in isolated caffeine droplets increases again to 2289/µm² (Fig. 5, column D), thus attaining a value similar to that of protoplasmic drops incubated in 10 mM Tris-buffer without caffeine (2443/µm²; Fig. 5, column B). A corresponding coincidence is observed for the PF:EF ratio, which amounts 1.5:1 in both samples. Protoplasmic drops first treated with caffeine and then washed in Tris-buffer recover to more normal IMP-values when transferred to nutritional agar (2788/µm²; Fig. 5, column E), with the exception that the PF:EF ratio shifts to 1.1:1.

Distinguishing a separate evaluation of two classes of IMP with respect to their diameter

($\varnothing < 10$ nm; $\varnothing \geqq 10$ nm) reveals that in untreated protoplasmic drops most IMP are of small size (1–2% $\varnothing \geqq 10$ nm on both PF and EF), whereas caffeine remarkably increases the number of IMP with large size (5% $\varnothing \geqq 10$ nm on PF and 43% $\varnothing \geqq 10$ nm on EF, see also Figs. 4b and c). In protoplasmic drops treated with Tris-buffer (Fig. 5, column B) or transferred to nutritional agar (Fig. 5, column E) the number of large IMP is again similar to controls (1–2.5% $\varnothing \geqq 10$ nm; Figs. 4d and e).

Discussion

Dynamic changes in cell surface morphology and rapid proliferation of plasma membrane material, initiated by caffeine in protoplasmic drops of *Physarum polycephalum* [20], are common cell biological phenomena of general importance [30]. It is well known that the treatment of living cells with strongly hypotonic or toxic solutions causes swelling and sometimes blebbing of the protoplast by extension of short membrane extrusions [31]. In a large variety of cell types the blebs originating under these conditions show a hyaline appearance, *e.g.*, in embryonic isolated amphibian cells during circus movement [32, 33], in cultured cells during mitosis and spreading [34, 35], or in amebas during treatment with osmotically inbalanced solutions [36].

A conclusive interpretation for the mechanism of bleb-formation is still lacking. Several authors supposed an increase of intracellular hydrostatic pressure as a necessary precondition for the generation of surface blebs at special weakened sites of the plasma membrane [37–39]. An enhanced hydrostatic pressure is normally achieved by the influx of water and solutes into the cytoplasm as postulated in the "respiratory model" [30]. This is supported by experiments in which hypertonic solutions were able to prevent swelling and blebbing [37, 38]. Disturbances in the osmotic balance must also be involved in the effects caused by caffeine, and to a lesser extent, by Tris-buffer, because the degree of swelling and blebbing is suppressed by higher concentrations of sucrose. However, investigations on *Amoeba proteus* using actin-specific drugs, such as phalloidin [40] or DNAase I [41], demonstrate that the process of bleb-formation is not merely induced by osmotic changes in the cell, but also by the activation of its contractile differentiations. The actomyosin system of amebas, which forms a filament cortex beneath the cell surface and generates the motive force for cytoplasmic streaming and changes in surface morphology [42–44], detaches at single sites from the plasma membrane under normal and experimental conditions. Hyaline or granular cytoplasmic material is then squeezed out through the meshwork of the filament cortex and causes a prolongation of the cell extrusions. Similar observations were made in *Physarum polycephalum* to explain the formation of blebs at the surface of protoplasmic drops after treatment with caffeine and Tris-buffer (Fig. 6). Regions where the cortical filament layer is in close contact with the plasma membrane alternate with discrete areas where this contact has been lost (Fig. 6B, arrowheads). The increase of internal hydrostatic pressure by both the influx of water, as a consequence of enhanced membrane permeability, and the active contraction of the filament layer can produce the numerous surface blebs and thereby the distinct enlargement of cell surface area. Caffeine is known to influence the contractile activity of the actomyosin system in acellular slime molds and muscle cells by interfering with calcium regulating systems (for literature, see Introduction of this paper), present also in *Physarum polycephalum* [45].

The external, but not the internal application of caffeine induces a strong relaxing effect on the contractile activities of *Physarum* as measured tensiometrically [17]. In protoplasmic drops, the drug does not inhibit actomyosin fibrillogenesis and an uptake of contractile activities, but reversibly impedes the regeneration of plasmalemma invaginations [18]. The transmission of forces generated by the contractile system and thus an uptake of locomotory activity is bound to the presence of plasmalemma invaginations [19]. From these experiences, the above-cited authors suggested that caffeine directly or indirectly interferes with the interaction of actin filaments and membranes. Disturbed filament-membrane interconnections may also be involved in blebbing and budding phenomena at the cell surface of protoplasmic drops.

Increase of cell volume, surface blebbing and proliferation by budding are coupled to a more or less rapid growth and *"de-novo"* formation of plasma membrane material. Investigations on dividing amphibian eggs [46–48], *Drosophila* embryos during blastoderm formation [49], cultured cells during mitosis [50] or on nerve cells during axonal

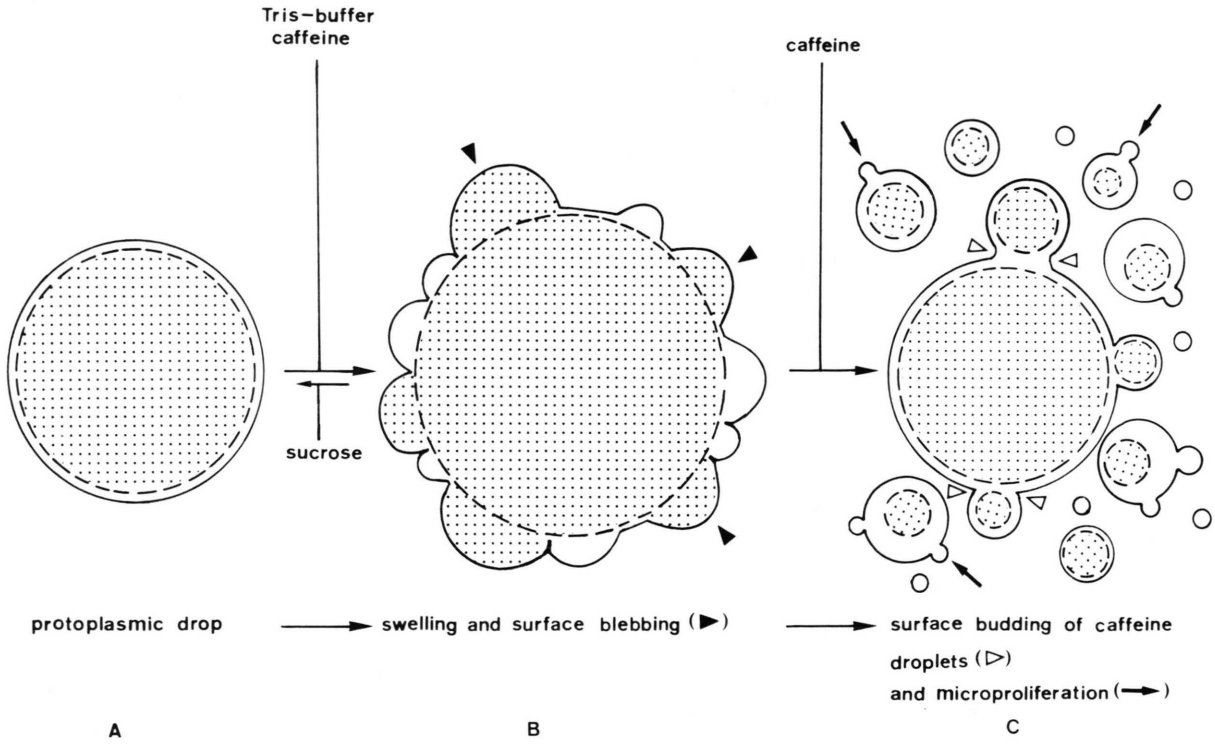

Fig. 6. Schematic drawing summarizing the caffeine- and Tris-buffer-induced changes in protoplasmic drops and caffeine droplets. Dotted areas = granuloplasm; white areas = hyaloplasm; broken lines = filament cortex; continuous lines = plasma membrane (for details see Discussion).

elongation [51, 52] gave evidence for the incorporation of material rich in phospholipids and poor in IMP into rapidly growing plasma membranes (for literature, see [47]). The morphologically visible fusion of lipid droplets with the plasmalemma (Figs. 3b and c) and the distinct changes in IMP-density during intensive cell surface proliferation (Fig. 5) also point to an intussusception of lipid-like membrane components in *Physarum polycephalum*. On the other hand, one cannot exclude that the decrease in IMP-density during surface blebbing by more than 100% may also be caused by an aggregation of rather small IMP ($\varnothing < 10$ nm) to large ones ($\varnothing \geqq 10$ nm). The abundant appearance of large IMP, especially on EF during caffeine treatment (Fig. 4c), supports this assumption. Characteristic changes in both the size and number of IMP in conjunction with surface alterations were also shown by Niedermeyer *et al.* [53] in a freeze-etch study on osmotically swelling and shrinking yeast cell tonoplasts.

Cytotic processes, as described during cell plate formation in plant cells [54], also participate in cellular budding during the generation of isolated caffeine droplets (Fig. 6, C, open arrowheads, and Figs. 2a and b). Comparable to the situation when plasmodial strands of *Physarum* are punctured and produce protoplasmic drops which regenerate a new plasmalemma [55] or during the subsequent *de-novo* formation of the plasmalemma invagination system [19], caffeine-treated drops can also provide rapid membrane growth by fusion and exocytosis of pre-existing, vesicular membrane material. Therefore, both possibilities of membrane growth, *i.e.*, the intussusception of lipid material and cytotic influx of pre-existing, complete membranes must be taken into account to explain the fluctuation in cell surface area and plasma membrane morphology during caffeine-induced blebbing and budding.

In this connection it seems to be of interest that not only the incorporation of membrane precursors can rapidly increase the cell surface area but that the rapid release of plasma membrane constituents into the external environment can also decrease the surface area again and, in this way, change the ultrastructure of the membrane. This is evident

from the release of numerous vesicles exhibiting a smooth surface completely devoid of or poor in IMP from isolated caffeine droplets (Fig. 6, C, arrows). This striking output of large amounts of lipid material may explain the increase in IMP-density in the isolated caffeine droplets (see Fig. 5, column D).

Caffeine droplets derived from protoplasmic drops of *Physarum polycephalum* cannot only be used as a model to study generation and control of contractile activity and cytoplasmic streaming; they are obviously also suitable objects for the analysis of physiological and morphological changes of cell membranes, *e.g.*, for a structural analysis of the effects of Ca^{2+} and temperature changes.

Acknowledgements

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