Surface Responses in Cultured Fibroblasts Elicited by Ethylenediaminetetraacetic Acid*

By ERNST J. DORNFELD, Ph.D., and ALFRED OWCZARZAK, Ph.D.

(*From the Department of Zoology, Oregon State College, Corvallis, Oregon*)

Plates 127 to 131

(Received for publication, November 6, 1957)

ABSTRACT

Cultures of chick heart fibroblasts were perfused with the chelating agent ethylenediaminetetraacetic acid (EDTA). Cellular responses were observed under phase optics and recorded by time-lapse cinemicrography.

In interphasic fibroblasts, EDTA induces cellular contraction followed by continuous protrusion and retraction of ectoplasmic blebs ("surface bubbling"), formation of motile vermiform processes, and production of rotatory ectoplasmic swellings. The contraction and surface bubbling closely resemble the metaphase contraction and "anaphase bubbling" normally displayed by cultured fibroblasts.

In dividing cells, EDTA does not affect metaphases, but anaphase bubbling appears and persists; telophasic expansion and migration of daughter cells are prevented. Initiation of new mitoses occurs during and after exposure to EDTA.

No cellular responses are induced by calcium, magnesium, or ferrous chelates of EDTA. The EDTA effects are completely reversible on removal of the chelating agent, resulting in the restoration of the normal interphasic cell form and the normal expansion and migration of mitotic products.

The EDTA effects are interpreted to result from the chelation and removal of divalent cations from the cell surface. Possible relations to surface activities observed in normal mitosis are considered, and an hypothesis is presented regarding the role of the developing spindle in cation transfer.

INTRODUCTION

The division of fibroblasts, as observed in tissue culture preparations, is accompanied by retraction of cell processes during prophase, followed by a general rounding of the cell as metaphase approaches. During anaphase and cleavage, the cell surface becomes markedly active in the protrusion and retraction of numerous cytoplasmic blebs ("anaphase bubbling"). The formation of these blebs comes to a halt during telophase, at which time the daughter cells expand, send out pseudopodial extensions, and migrate away from each other. These phenomena have been frequently observed, recorded, and temporally correlated with concomitant events in the cell interior, such as the dissolution of the nuclear membrane, spindle formation, chromosome movements, etc. (3, 4, 12, 19, 25). Little, however, is known of the mechanisms involved in these cell surface activities.

In recent years Lettré and coworkers have reported a series of experiments which relate these phenomena to changes in localized concentrations of adenosine triphosphate (ATP). Specifically, the peripheral ATP level, assumed to provide the energy necessary to maintain cell form during interphase, becomes reduced at mitosis, resulting in a relaxation of the cell surface that leads to cell rounding and intermittent bubbling (15, 18). The principal evidence is derived from the experimental suppression of cell rounding and anaphasic surface motility by addition of ATP to the culture medium, and by the induction of such surface or cortical activity in interphase cells when grown in an anaerobic environment, or when exposed to mitochondrial poisons or agents that inhibit respiration or phosphorylation (16, 17).

* Supported by a grant (C-2178 C3) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

Apart, however, from consideration of the mechanisms that enter into the energetic relations of these phenomena, it may be expected that during such events as prophasic contraction, bleb formation, and telophasic expansion, the cell surface undergoes immediate modifications in its molecular and ionic configuration. Calculations by Webb and Danielli (26) have shown that the cell membrane may be regarded as a reservoir of divalent ions, and direct demonstration by microincineration methods of high calcium and magnesium concentrations in the plasma membranes of tissue cells has been provided by Lansing and Scott (13). The importance of calcium and other cations in cellular properties and activities has been reviewed by Heilbrunn (9). With reference to fibroblasts, it may be supposed that bivalent cations, notably calcium, acting as intermolecular links, contribute to the molecular packing and ridigity of the interphasic cell surface and that alterations in the distribution, or a reduced concentration of these ions, may result in instability of cell form and bring about general or localized changes in the surface such as those observed.

Experimental removal of divalent cations might produce in interphasic fibroblasts effects similar and possibly related to those occurring during cell division. If so, some explanation of the mitosis-associated surface phenomena may be suggested. The treatment of cultured fibroblasts with the chelating agent ethylenediaminetetraacetic acid (EDTA) offers a simple means for the rapid removal of divalent cations, including calcium (5, 20). The effects of such procedure on interphasic and dividing cells are described in the present study.

Procedure

Heart explants of 9 day old chick embryos were employed. Pooled fragments were arranged, 4 to 6 per culture, on coverslips and embedded in plasma clots consisting of equal parts of chicken plasma and embryo extract. The desiccated embryo extract (Difco) was reconstituted in the prescribed manner (7) and diluted with either 8 ml. of Earle's solution (7) or 8 ml. of Simms's Z 16 (7). The latter is calcium-free. The preparations were mounted in perfusion chambers of the type designed by Rose (23) and incubated at 37.5°C. Treatments were carried out at this temperature on cultures ranging from 21 to 76 hours in age. Time-lapse cinemicrographic records under phase optics were made of control, treated, and recovering fields. Experiments were carried out on a total of 150 cultures and 4000 feet of film records were obtained.

One of four isotonic salt solutions (SS), adjusted to a final pH of about 7.85, was used as perfusion agent. Of these, Earle's solution and Parker's 199 (7) contain calcium and magnesium; Simms's Z 16 is calcium-free; and Moscona's solution (21) lacks both calcium and magnesium. All except Earle's served as carriers for disodium ethylenediaminetetraacetate (EDTA; versene) made up in concentrations varying from 0.01 M to 0.001 M, with 0.003 M adopted as standard for most experiments. At the latter concentration, calculations indicated that sufficient unbound EDTA remained after maximum uptake of the external Ca and Mg (from SS and plasma clot) to affect the cells themselves: 0.0005 M for Parker's 199, 0.0017 M for Simms's Z 16, and 0.0026 M for Moscona's solution. Calcium, magnesium, and ferrous chelates of EDTA were also prepared by combination with equimolar concentrations of the corresponding chlorides.

Treatments of the cultures followed one of the following sequences:

1. SS→EDTA→SS
2. SS→EDTA→X-EDTA
3. SS→X-EDTA→EDTA→SS
4. SS→X-EDTA→EDTA→X-EDTA

Solutions were perfused through the culture chamber by gravity flow. The fluid volume of the chamber proper was approximately 2 ml., and that of the entire system, with perfusion tubes attached, 7 ml. To assure complete replacement of fluids, 12 ml. of solution were perfused through the chamber; the flow of this volume took, on the average, 2½ minutes.

The procedure followed for each treatment can be summarized as follows: Culture chambers were filled with SS and incubated at 37.5°C. until used (30 minutes to 6 hours). EDTA treatment was limited to 30 minutes, with a few exceptions. Pretreatment with X-EDTA, when used, ranged from 30 to 70 minutes, and the recovery period (in SS or X-EDTA) from 27 to 150 minutes. The length of this final sequence was governed by speed of recovery and number of fields recorded within the same culture.

Cinematography began within 15 seconds of the beginning of perfusion in all cases, and terminated 1 minute before the end of each step of the experiment. This interval permitted marking the film with blank frames to signify a change of solution and allowed for the necessary preparations for the coming perfusion. Attempts were made to include one or more mitotic cells for each control, pretreatment, and treatment sequence. Whenever possible, the same field was recorded throughout the experiment.

OBSERVATIONS

Cultures of fibroblasts respond to treatment with EDTA in a marked and characteristic manner. The pattern of cellular reaction begins almost immediately, reaches a maximum effect usually

within 20 minutes, and can be completely reversed by removal of the chelating agent. The general appearance of a culture before treatment, at full response, and after recovery is shown in Figs. 1 to 3.

Effect of EDTA on Non-Dividing Cells.—The initial response of interphasic fibroblasts almost invariably becomes evident within 5 minutes from the beginning of perfusion. A withdrawal of cell processes and progressive cellular contraction take place, which may or may not end in complete rounding of the cell (Figs. 4, 7, and 11). In the same photographic field and throughout an entire culture, varying degrees of contraction may be found. The rounding up usually precedes, but may accompany, other forms of surface activity. Frequently, cells in early stages of contraction may be observed to display a pulsating motion, a slight periodic swelling, and contraction. EDTA concentrations below 0.0025 M generally elicit contractile responses, but are less effective in inducing further types of surface motility.

During the contraction phase of the response, most of the cells display a surface bubbling (Figs. 4 *c* to *i*, 9 *c*) very similar to that which accompanies normal anaphase (Fig. 6). This is characteristic for EDTA concentrations of 0.003 M or above. Sometimes it occurs early in contraction, but more commonly near its conclusion. Ectoplasmic blebs are projected and withdrawn from any region of the cell surface, and this activity persists throughout the exposure period. With the passage of time the numbers of cells affected and the intensity of the reaction increase. As in anaphase bubbling, the blebs are mostly devoid of cytoplasmic inclusions and closely resemble the former in size, numbers, and manner of formation and retraction. In the viewing of cinemicrographs, taken at 2- or 4-second frame intervals, the blebs appear to be protruded somewhat explosively and to be more gradually withdrawn. Measurements show that their formation actually requires about 10 seconds, and withdrawal is effected in a period of 20 to 30 seconds.

Two variants of this type of surface activity occur. One variant may appear in fully rounded cells and is characterized by the formation of a single large ectoplasmic swelling involving a quarter to a half of the cell's surface. This bleb progresses around the cell is a circular movement and may at any point reverse direction or be withdrawn (Fig. 13). It is generally rather persistent, its rotation passing through several cycles or repeated reversals. Except for its broader base and manner of movement the bleb appears structurally similar to the smaller kind of protuberance.

The second variant consists in the protrusion of elongated ectoplasmic processes which may appear tubular, finger-shaped, or vermiform (Figs. 4 *e* to *i*, 11 *c*). These may originate from partially contracted or fully rounded cells, one or several per cell, and once formed generally persist. The connective stalks of these processes can be long and tenuous and the tips large and bulbous. They are usually mobile, describing waving or winding movements and frequently display on their surface peristaltic contractions which move distoproximally. Occasionally, the vermiform processes become detached from the cell body and migrate about the culture (Figs. 12 *a* and *b*). Rotating blebs may occasionally transform into vermiform processes.

Both variants as well as surface bubbling may occur in the same field, the activity continuing throughout the duration of treatment, providing the EDTA concentration does not exceed 0.003 M. Lethal effects may appear at higher concentrations, damage being marked by slight swelling of the cells and abrupt cessation of all surface and interior movement.

Effect of EDTA on Dividing Cells.—In the presence of EDTA, the course of mitosis is rarely modified in timing or mechanics until late anaphase or telophase (Figs. 4, 7, and 9). During metaphase the rounded cell surface remains relatively quiescent, differing in this respect from EDTA-affected interphase cells whose contracted surface shows vigorous bubbling. Spindle formation, chromosomal movements, and cleavage proceed normally. Anaphase bubbling, however, is more intense than usual, and once begun persists in the daughter cells as long as the culture is exposed to EDTA. The division products fail to expand and to migrate away from each other.

A very few early metaphases were observed to begin premature bubbling, but this activity ceased abruptly before onset of anaphase movement and was resumed again sometime during cleavage. Rare instances of prolonged metaphases were noted in which the onset of anaphase was greatly retarded, sometimes never appearing.

New mitoses may be initiated during EDTA treatment and were observed to appear through the entire range of concentrations and durations.

These divisions are completed normally and manifest surface phenomena identical to those of cells already in division at the beginning of treatment. One of the more outstanding examples occurred in a 0.01 M EDTA solution: seven mitoses were initiated between the 5th and 20th minute of treatment; five of the seven cleaved by the end of treatment at 33 minutes. At the beginning of treatment, these cells were indistinguishable from other interphasic fibroblasts, being fully expanded and showing characteristic interkinetic nuclei with prominent nucleoli.

Treatment of Cultures with Chelated EDTA.—Perfusion of cultures with Ca-EDTA, Mg-EDTA, or ferrous-EDTA induces no cellular responses (Figs. 14 to 16). Interphasic fibroblasts remain fully expanded and display normal migratory movements. The mitotic process is not affected; surface bubbling occurs during anaphase, and cleavage is followed by telophasic expansion and cellular separation. New mitoses are initiated during treatment.

Recovery from EDTA Effects.—The characteristic responses of interphasic and dividing fibroblasts to non-lethal concentrations of EDTA are completely reversible on removal of the chelating agent. This may be effected by flushing the culture chamber with unfortified carrier solution: Parker's 199, Earle's solution, or Simms's Z 16; also by addition of $CaCl_2$ in an amount sufficient to bind the versene, or by perfusion with Ca- or Mg-EDTA. Flushing with Moscona's solution, however, does not produce recovery, and the characteristic EDTA responses continue to be displayed.

In recovery, most, if not all, of the cells of a field begin to show cessation of surface activity within 5 minutes. The sequence of events is the reverse of that occurring on EDTA treatment: reduction of bubbling, vermiform process formation, and ectoplasmic circular movements precede cell expansion (Figs. 8 and 10). The formation of blebs gradually declines, and finally stops. Attached vermiform processes, though they may be greatly attenuated and mobile, become completely retracted into the parent cell (Fig. 17); the migration of freed processes slows down, their peristaltic movements cease, and rounded masses of aparticulate ectoplasm remain. Cells with circular movements generally are the last to assume normal shape. Eventually, all fibroblasts become fully expanded and take on a completely normal appearance (Fig. 12 *c*). Full recovery of a whole culture is attained in 1 to 2 hours.

Cells in late telophase follow a similar course of behavior, eventually spreading and migrating away from each other in a normal fashion (Figs. 5 and 8 *c*). Inhibited metaphases usually continue into anaphase and cleavage, leaving no indication of their previous mitotic arrest. New mitoses appear in fair numbers shortly after the cultures have been flushed and proceed to completion (Fig. 8 *c*). Cultures observed for several hours after the flushing operation remain mitotically active and appear fully normal.

Extended Exposure to EDTA.—Several cultures were subjected to successive half-hour renewals of 0.003 M EDTA for periods up to 4½ hours. Surface bubbling diminished or largely disappeared after 2½ hours, but the cells remained in an intact, though contracted condition. When finally returned to Parker's 199 for 2 hours, bubbling appeared and was followed by normal expansion.

Other cultures were subjected to four or five successive treatment-and-recovery cycles (30 minutes of 0.003 M EDTA, followed by 60 minutes of Parker's 199). In these cultures the characteristic responses regularly recurred.

Extended Exposure to Ionically Deficient Salt Solutions.—EDTA-like effects, cellular contraction, and continuous surface bubbling, were observed to arise in culture chambers that were repeatedly perfused simply with Simms's Z 16. After cumulative exposure of 6 to 8 hours to this calcium-free medium, the characteristic surface effects became evident. Similar treatment with Parker's 199 or Earle's solution produced no cellular reaction.

However, in Moscona's solution (lacking both calcium and magnesium) cellular contraction and bubbling was induced rapidly, a vigorous reaction appearing within 30 minutes. With continuous perfusion the surface bubbling ceased within 2 hours, but without being followed by cell expansion. Parker's 199, introduced after 6 hours, restored the expanded state.

DISCUSSION

Borei and Björklund (2), treating unfertilized, anisodiametric eggs of sea urchins with EDTA, found that these cells responded by rapid rounding. This is similar to the initial response of fibroblasts. Borei and Björklund further showed that loss of surface rigidity occurred upon EDTA

treatment, for centrifuging produced greater elongation in treated than in untreated eggs. In view of the chelating properties of EDTA, this may be related to the observation of Shapiro (24) that centrifuged sea urchin eggs elongate more rapidly and also recover their sphericity in shorter time in calcium-free than in normal sea water. Likewise in the case of fibroblasts, EDTA-like effects are displayed sooner or later upon exposure to calcium-free salt solutions (Moscona's or Simms's Z 16). Since fibroblasts are responsive to EDTA, but not to EDTA chelates of calcium and magnesium, it can be assumed that the effects are due to the chelating activity of EDTA alone, that changes in the disposition of divalent cations are produced, and that these changes are responsible for the surface activities observed. Nishimura, DiPaolo, and Hill (22) have noted that EDTA diminishes the viscosity of the peripheral cytoplasm in mouse ascites cells, as determined by the displacement of peripheral lipide granules under centrifugation; addition of calcium prevents this reduction in viscosity, whereas the effect of magnesium is so slight that much higher concentrations are required. An actual decrease of calcium and magnesium levels in EDTA-extracted *Chlamydomonas* has been measured by Eversole and Tatum (8).

The surface motility induced by EDTA—bubbling, circular movement of ectoplasmic blebs, and vermiform process formation—is strikingly similar to that observed by Holtfreter (10, 11) in isolated cells of amphibian gastrulae. In the latter, the appearance of such surface activity was promoted by "agents which weaken or liquefy the cell membrane," *e.g.*, isotonic saline solutions lacking calcium or having a pH above 9. It is conceivable that these formations are related to changes in permeability consequent to localized and fluctuating alterations in the molecular organization of the cell membrane. The rotation of ectoplasmic bulges and the progression of peristaltic contraction waves suggest that a directional propagation of such alterations may occur. It is noteworthy that on removal of the chelating agent cessation of surface activity is the first phase of recovery.

The speed with which reversal of EDTA effects takes place, as well as the ability of cells to withstand successive cycles of treatment and recovery, suggests that the action of the agent is limited to the surface. This would likewise explain its lack of interference with the mitotic spindle. On the assumption that EDTA removes divalent cations from the lipoprotein structure of the cell membrane, their replacement must be rather rapidly effected from the exterior or interior environment. This may be expected, in accordance with Webb and Danielli's (26) study on ion interactions with palmitate monolayers.

Of particular interest is the close similarity between the responses of interphasic fibroblasts to EDTA and the normal surface activities that accompany their division, *viz.*, retraction of cytoplasmic processes, assumption of spherical form, and the elevation and retraction of ectoplasmic blebs ("anaphase bubbling"). In order of appearance as well as in cytological characteristics, the resemblance is striking. Exception must be made to the EDTA-induced rotatory movements and vermiform processes, which may, however, be regarded as extreme effects not reached under normal conditions. The sequence of events following removal of the chelating agent likewise compares favorably with the restoration of interphase form that begins in telophase. It is conceivable that a mechanism similar to EDTA action is operative during normal cell division.

In constructing such an hypothesis, an observation of special moment is that EDTA excites no immediate reaction in rounded metaphase cells, *i.e.*, in the presence of a newly formed spindle the induction of surface bubbling is considerably delayed. It does not, in fact, appear until anaphase (as normally), but then persists until the chelating agent is removed. This is quite unlike the reaction of interphasic cells, in which surface bubbling follows cell rounding immediately. Also, where interphasic fibroblasts in the presence of EDTA enter mitosis, the rounded and bubbling surface becomes quiescent with formation of the metaphase spindle, and remains so until anaphase.

The apparently diverse responses of interphasic and dividing cells to EDTA can be reconciled in a concept that also attempts to account for the surface phenomena accompanying normal fibroblast division. It is suggested that prophasic retraction of cell processes and assumption of spherical form results from removal of divalent cations from the cell surface. This is accomplished by EDTA in the present experiments. In normal mitosis the developing spindle may be assumed to incorporate divalent ions into its micellar structure, drawing these from the cell surface and the extracellular medium until interior requirements

are fully satisfied. During this period of ionic transfer, the cell surface becomes and remains relaxed (rounded) and of increased permeability to the aqueous environment. In this connection it may be noted that Barer and Joseph (1) have determined by immersion refractometry that the solid concentration of the cytoplasm in dividing insect spermatocytes drops to a minimum at prometaphase, rising again at telophase.

In non-dividing cells exposed to EDTA, surface bubbling follows immediately after rounding, this being assumedly related to the rapid redistribution of cations from the cell interior to the surface. This redistribution may be pictured as proceeding unevently, leaving unreconstructed patches which become stretched or "blown out" by interior hydrostatic pressure. With incorporation of cations continuing into the surface of the formed blebs, contraction and recession of the blebs is effected. Permanent fixation of ions, however, is not achieved, since they continue to be drawn off by the chelating agent. Escape of cations also continues when EDTA is replaced by the calcium- and magnesium-free Moscona's solution. In both instances normal membrane reconstruction is prevented and bubbling continues. Since the supply of available intracellular cations will in time become depleted, the surface bubbling may be expected to cease after prolonged exposure to EDTA or Moscona's solution, and the cells to remain in a contracted state. This is, indeed, observed. By replacing either EDTA or Moscona's solution with a medium containing divalent ions, normal restoration of the cell membrane is promoted. Though Simms's Z 16 lacks calcium, its magnesium content appears to be sufficiently effective in this respect, except over an extended period.

With regard to dividing cells, the divalent cations are assumed to become firmly bound to the growing spindle, do not move to the surface, are inaccessible to EDTA, and the cell remains smoothly rounded. With degeneration of the spindle beyond anaphase, release of cations takes place and these accumulate in the surface. Bubbling is initiated, and in the presence of EDTA will continue for an extended period, since fixation of these ions in the cell surface cannot be achieved. Under normal conditions, ionic reconstruction of the membrane may commence as soon as the spindle is functionally developed, the cations being initially contributed by the extracellular medium. Bubbling appears, but ceases when uniform distribution and fixation is attained. Since quiescence of the surface is not reestablished until late telophase, cations released from the degenerating spindle may also contribute to reconstruction of the surface.

The above interpretation of the experimental observations is admittedly speculative and stresses the possible role of structural modifications in the cell surface. It may be that by high resolution electron microscopy some evidence of pertinent membrane changes can be detected. Coman (6), in an electron microscope investigation on liver cells whose adhesiveness was destroyed by EDTA, has, in fact, reported a partial disintegration of the cell membrane. A study on the ultrastructure of EDTA-treated and dividing fibroblasts is in progress.

The possibility that EDTA in the present experiments affects ATP-energized processes cannot be excluded. Since divalent cations are indispensable as activators of various metabolic processes involving ATP, including oxidative phosphorylation (14), their removal might be expected to decrease the availability of energy derived from breakdown of ATP. In the view of Lettré (15, 18) the drop in available energy would account for loss of membrane rigidity and produce rounding of the cell. If the limiting conditions for energy production are conceived as localized to or near the cell surface, the redistribution of the activating ions from the cell interior or from a proper exterior medium would reverse the effects. A mechanism of this nature does not necessarily exclude the alternative hypothesis of a direct effect on membrane structure; both processes could be operative.

Literature Cited

1. Barer, R., and Joseph, S., Phase-contrast and interference microscopy in the study of cell structure, *Symp. Soc. Exp. Biol.*, 1957, **10**, 160.
2. Borei, G. G., and Björklund, U., Effect of versene on sea urchin eggs, *Exp. Cell Research*, 1953, **5**, 216.
3. Boss, J., Mitosis in cultures of newt tissues. iv. The cell surface in late anaphase and the movements of ribonucleoprotein, *Exp. Cell Research*, 1955, **8**, 181.
4. Chambers, R., Structural and kinetic aspects of cell division, *J. Cell. and Comp. Physiol.*, 1938, **12**, 149.
5. Chenoweth, M. B., Chelation as a mechanism of

pharmacological action, *Pharmacol. Rev.*, 1956, **8**, 57.

6. Coman, D. R., Cellular adhesiveness in relation to the invasiveness of cancer: electron microscopy of liver perfused with a chelating agent, *Cancer Research*, 1954, **14**, 519.
7. Difco Labs., Reagents and media for tissue culture and virus propagation, Bull. 140, Detroit, 1955.
8. Eversole, R. A., and Tatum, E. L., Chemical alteration of crossing over frequency in *Chlamydomonas*, *Proc. Nat. Acad. Sc.*, 1956, **42**, 68.
9. Heilbrunn, L. V., An Outline of General Physiology, 3rd edition, Philadelphia, Saunders, 1952.
10. Holtfreter, J., Structure, motility and locomotion in isolated embryonic amphibian cells, *J. Morphol.*, 1946, **79**, 27.
11. Holtfreter, J., Significance of the cell membrane in embryonic processes, *Ann. New York Acad. Sc.*, 1948, **49**, 709.
12. Hughes, A. F., and Swann, M. M., Anaphase movements in the living cell. A study with phase contrast and polarized light on chick tissue cultures, *J. Exp. Biol.*, 1948, **25**, 45.
13. Lansing, A. I., and Scott, G. H., The effect oJ perfusion with sodium citrate on the content and distribution of the minerals in various cells of the cat as shown by electron microscopy and microincineration, *Anat. Rec.*, 1942, **84**, 91.
14. Lardy, H. A., The influence of inorganic ions on phosphorylation reactions, A Symposium on Phosphorus Metabolism, (W. D. McElroy and B. Glass, editors), Baltimore, Johns Hopkins Press, 1951, 477.
15. Lettré, H., Zellstoffwechsel und Zellteilung, *Z. Krebsforsch.*, 1952, **58**, 621.
16. Lettré, H., Albrecht, M., and Lettré, R., Verhalten von Fibroblasten unter aeroben und anaeroben Bedingungen, *Naturwissenschaften*, 1951, **38**, 504.
17. Lettré, H., Albrecht, M., and Lettré, R., Zur Auslösung von Plasmabewegungen in Ruhezellen, *Naturwissenschaften*, 1951, **38**, 505.
18. Lettré, H., and Schleich, A., Zur Bedeutung der Adenosintriphosphorsäure für Formkonstanz und Formänderungen von Zellen, *Protoplasma*, 1954, **44**, 314.
19. Lewis, W. H., The relation of the viscosity changes of protoplasm to ameboid locomotion and cell division, A Symposium on the Structure of Protoplasm, (W. Seifriz, editor), Ames, Iowa, Iowa State College Press, 1942, 163.
20. Martell, A. E., and Calvin, M., Chemistry of the Metal Chelate Compounds, New York, Prentice-Hall, 1952.
21. Moscona, A., Cell suspensions from organ rudiments of chick embryos, *Exp. Cell Research*, 1952, **3**, 535.
22. Nishimura, E. T., DiPaolo, J., and Hill, W. T., Studies on the viscosity of the cytoplasm of mouse Ehrlich ascites tumor cells, *Arch. Path.*, 1955, **59**, 487.
23. Rose, G., A separable and multipurpose tissue culture chamber, *Texas Rep. Biol. and Med.*, 1954, **12**, 1074.
24. Shapiro, H., Centrifugal elongation of cells, and some conditions governing the return to sphericity, and cleavage time, *J. Cell. and Comp. Physiol.*, 1941, **18**, 61.
25. Strangeways, T. S. P., Observations on the changes seen in living cells during growth and division, *Proc. Roy. Soc. London, Series B*, 1922, **94**, 137.
26. Webb, D. A., and Danielli, J. F., Concentration of cations at negatively charged surfaces, *Nature*, 1940, **146**, 197.

Explanation of Plates

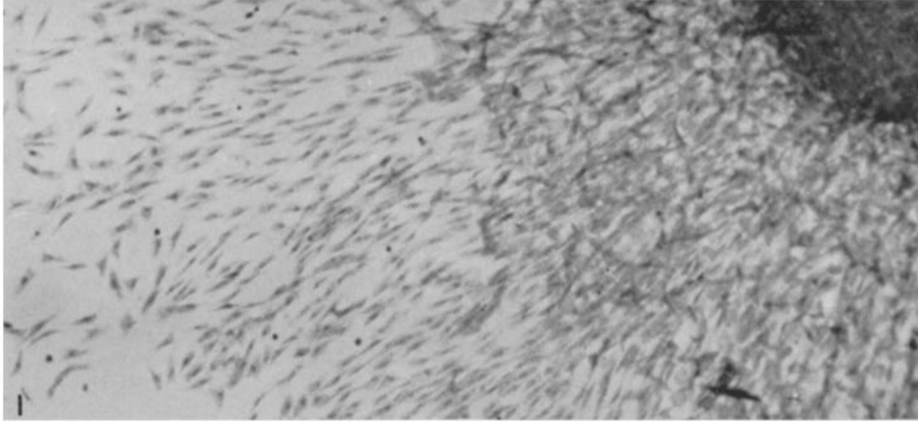
Plate 127

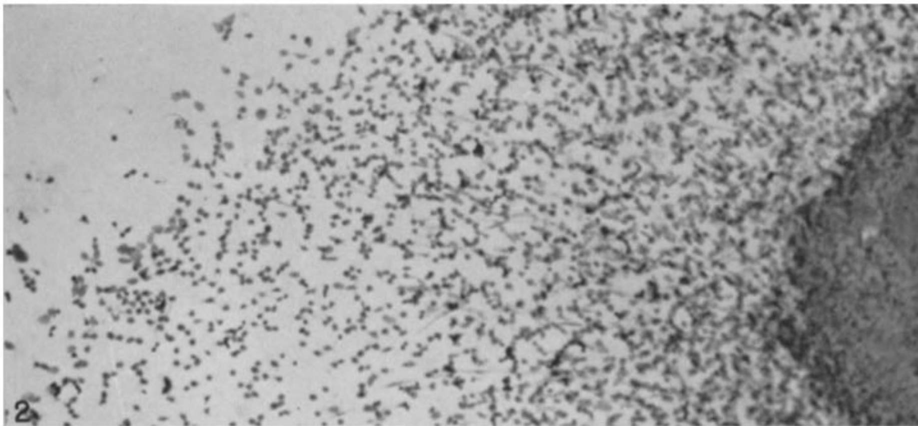
Fig. 1. 46-hour culture, ½ hour in Simms's Z 16. × 76.

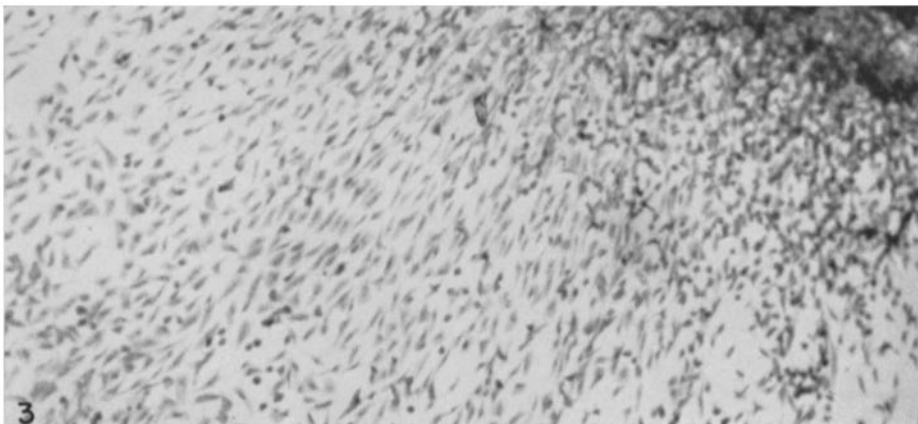
Fig. 2. 46-hour culture, ½ hour in 0.003 M EDTA/Simms's Z 16. × 76.

Fig. 3. 47½-hour culture, 1 hour recovery in Simms's Z 16 after ½ hour in 0.003 M EDTA/Simms's Z 16. × 76.

Above specimens were fixed in acetic alcohol and stained in May-Gruenwald Giemsa.







(Dornfeld and Owczarzak: Effects of EDTA on fibroblasts)

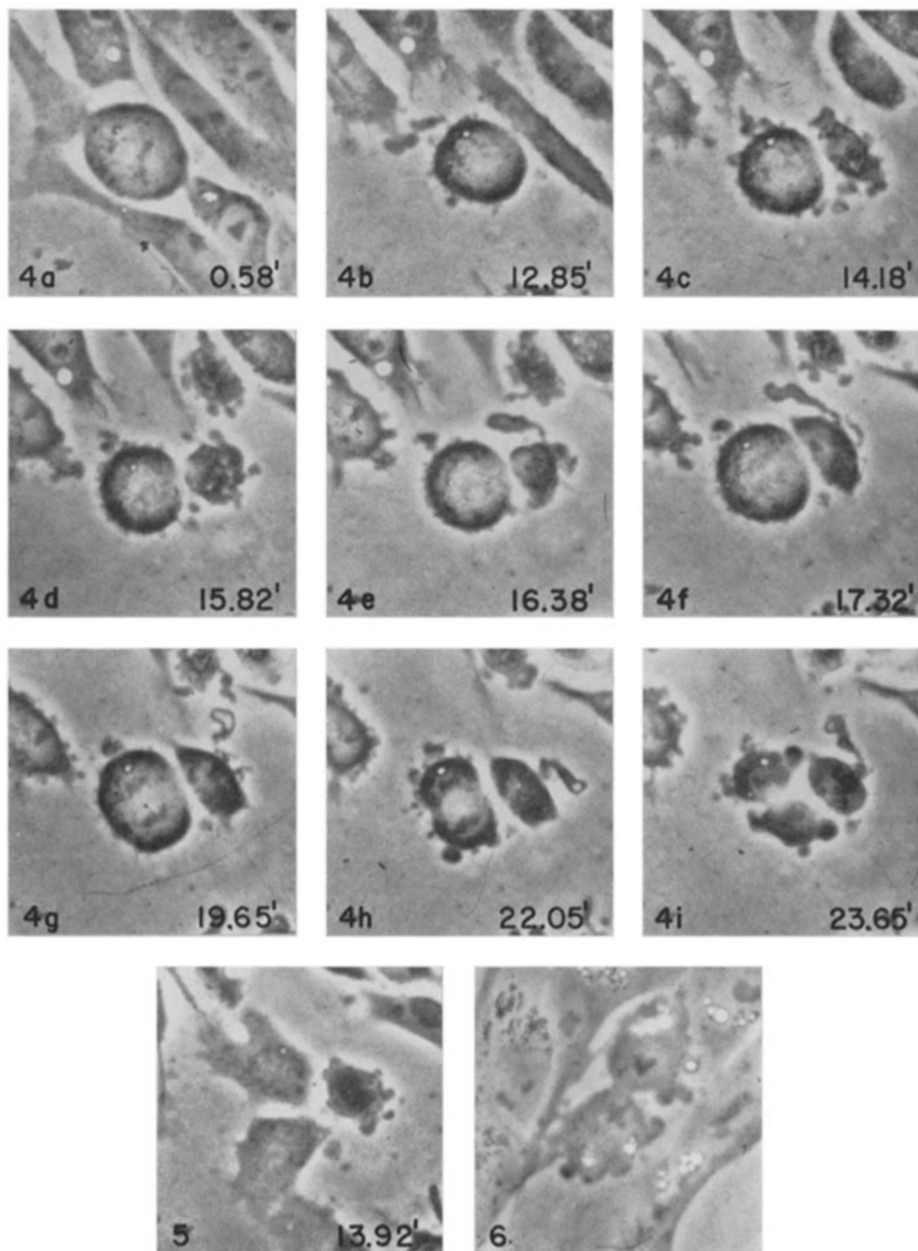
Plate 128

All figures are taken from 16 mm. cinematographic films recorded with phase contrast optics. Numerals in the right-hand corners indicate minutes and hundredths of minutes elapsed after initiation of the specified treatment.

Figs. 4 *a* to *i*. Response to treatment with 0.003 M EDTA/Parker's 199. Note cellular contraction, surface bubbling, and vermiform processes; mitosis going to completion. × 560.

Fig. 5. Recovery of above cells in 0.003 M Ca-EDTA/Parker's 199. Note expansion of cells. × 560.

Fig. 6. Normal anaphase bubbling. Culture in Parker's 199. × 560.



(Dornfeld and Owczarzak: Effects of EDTA on fibroblasts)

Plate 129

All figures are taken from 16 mm. cinematographic films recorded with phase contrast optics. Numerals in the right-hand corners indicate minutes and hundredths of minutes elapsed after initiation of the specified treatment.

Figs. 7 *a* to *c*. Response to treatment with 0.003 m EDTA/Parker's 199. Note contraction and bubbling of interphase cells; lack of surface activity in mitotic cells until anaphase. × 560.

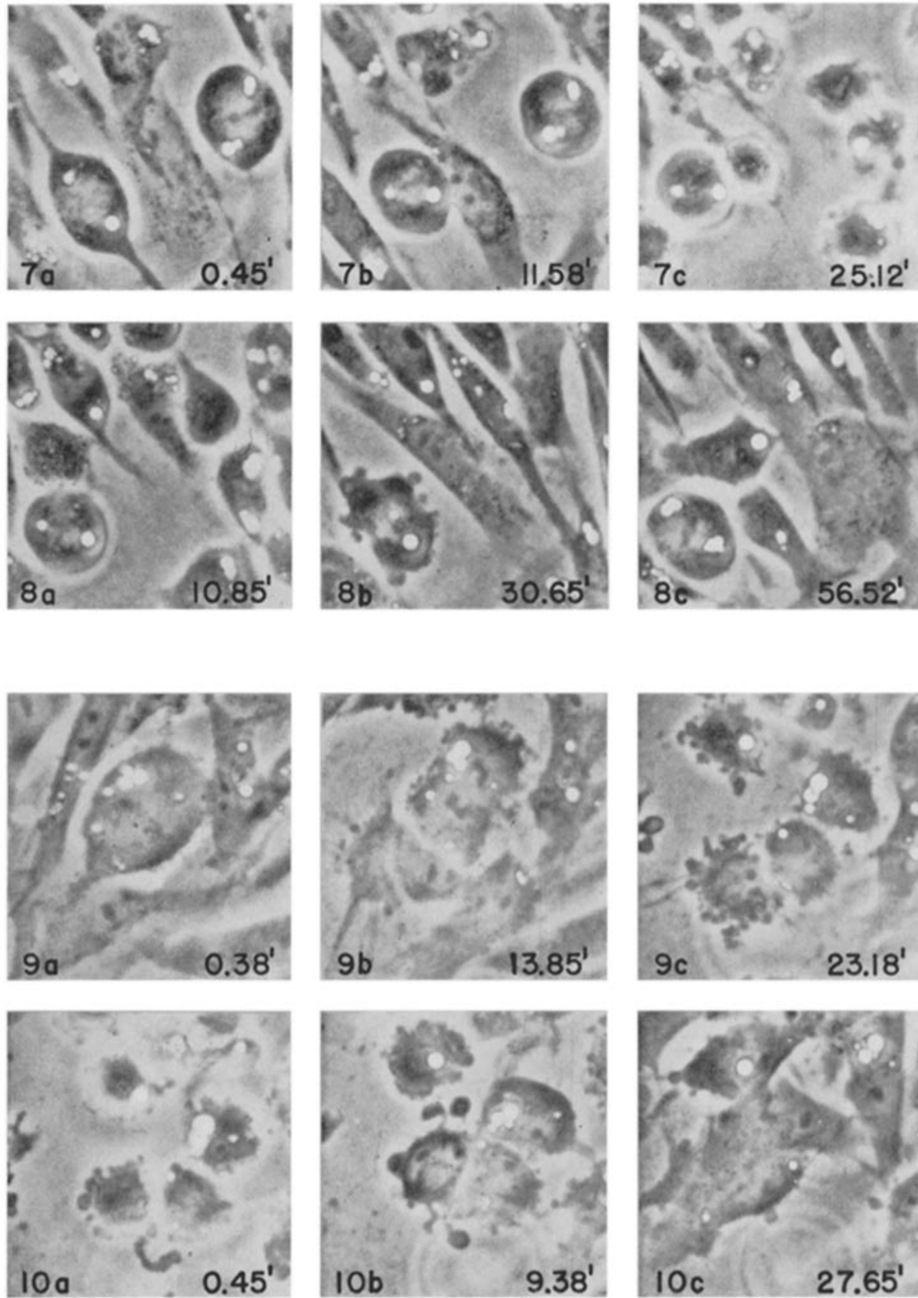
Figs. 8 *a* to *c*. Same field. Recovery in Parker's 199. Note expansion of cells, completion of division, and initiation of new mitosis. × 560.

Figs. 9 *a* to *c*. Response to treatment with 0.003 m EDTA/Parker's 199. Note cellular contraction and bubbling; mitosis in progress. × 560.

Figs. 10 *a* to *c*. Same field. Recovery in 0.003 m Ca-EDTA/Parker's 199. Note retraction of vermiform process and cell expansion. × 560.

THE JOURNAL OF
BIOPHYSICAL AND BIOCHEMICAL
CYTOLOGY

PLATE 129
VOL. 4



(Dornfeld and Owczarzak: Effects of EDTA on fibroblasts)

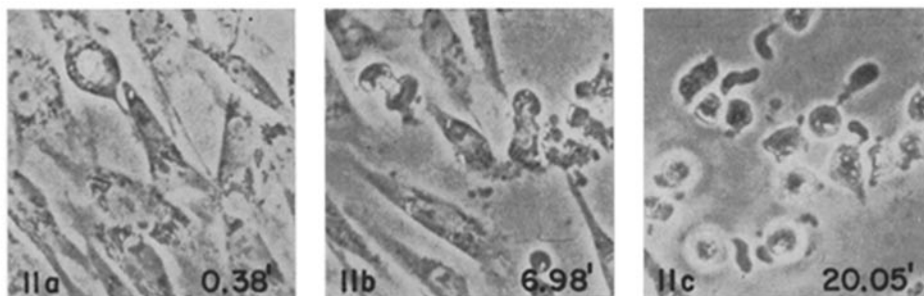
PLATE 130

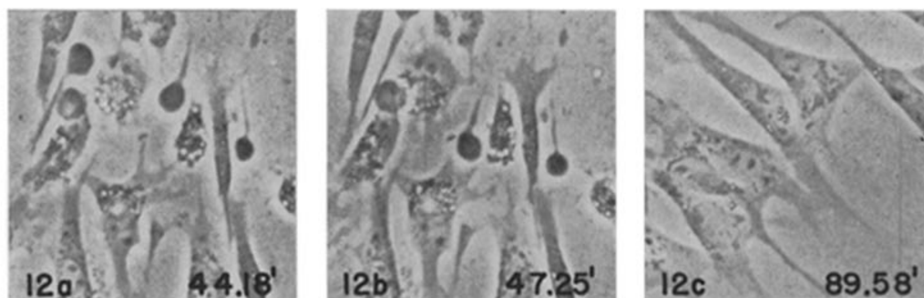
All figures are taken from 16 mm. cinematographic films recorded with phase contrast optics. Numerals in the right-hand corners indicate minutes and hundredths of minutes elapsed after initiation of the specified treatment.

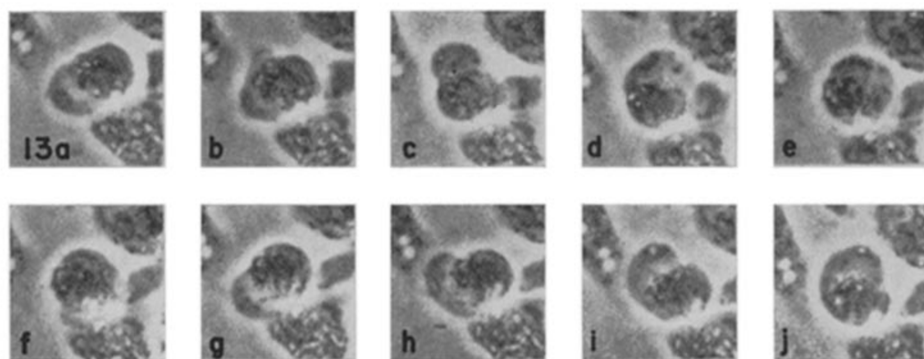
FIGS. 11 *a* to *c*. Response to treatment with 0.003 M EDTA/Simms's Z 16. Note contraction, surface bubbling, and vermiform processes; mitosis going to completion. × 336.

FIGS. 12 *a* to *c*. Recovery in Simms's Z 16 after EDTA treatment. Note migration of three detached vermiform processes in *a* and *b*; *c* shows normal appearance of cells after approximately 1½ hours. × 336.

FIGS. 13 *a* to *j*. Circular movement of an ectoplasmic bleb induced by treatment with 0.003 M EDTA/Simms's Z 16. Consecutive frames represent four second intervals. × 720.







(Dornfeld and Owczarzak: Effects of EDTA on fibroblasts)

Plate 131

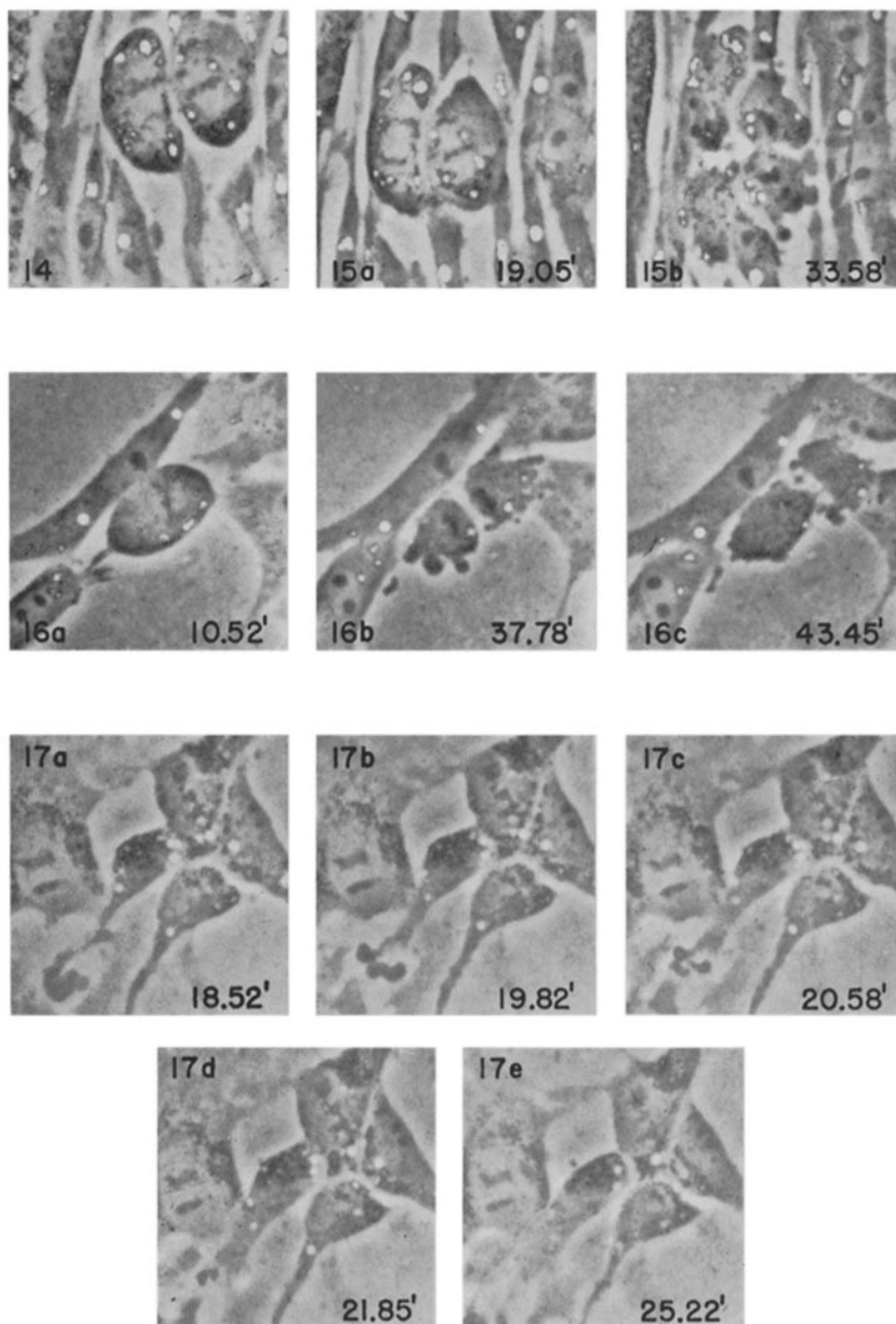
All figures are taken from 16 mm. cinematographic films recorded with phase contrast optics. Numerals in the right-hand corners indicate minutes and hundredths of minutes elapsed after initiation of the specified treatment.

Fig. 14. Control field in Parker's 199. × 560.

Figs. 15 *a* and *b*. Same field treated with 0.003 M Ca-EDTA/Parker's 199. Note normal appearance of the entire field (*a*); bubbling occurring only in the early division products as normally (*b*). × 560.

Figs. 16 *a* to *c*. Treatment with 0.003 M Ca-EDTA/Parker's 199. Note the absence of bubbling in interphasic cells, the normal appearance and bubbling in the mitotic cell, and migration of the expanded daughter cells. × 560.

Figs. 17 *a* to *e*. Retraction of vermiform process in Earle's solution following treatment with 0.005 M EDTA/Simms's Z 16. × 560.



(Dornfeld and Owczarzak: Effects of EDTA on fibroblasts)