CYTOPLASMIC PROTRUSIONS IN INSECT CELLS DURING MITOSIS IN VITRO

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The formation of transient cytoplasmic protrusions, so called "bubbling," has frequently been observed in a number of living vertebrate cells since the early description of it in salamander epithelium by Peremeschko (1879). In cultured vertebrate cells, this phenomenon has been observed occasionally during mitosis (Strangeways, 1922; Boss, 1955). Hughes (1950) reported increased bubbling during artificially delayed anaphase. In vertebrates, Novikoff (1939) reported bubbling of *Sabellaria* eggs, and Baumgartner (1933) observed it in grasshopper and cricket spermatocytes. Chambers (1938) studied the induction of blebs in relation to cell injury. In the past, the reports have described protrusions of the cell surface mainly during late anaphase and telophase or at the time of cytokinesis. Surface bubbling of invertebrate cells in continuous culture in vitro has not been reported up to now. The present paper describes observations on dividing leafhopper cells in vitro.

MATERIALS AND METHODS

Cultured cells of the six-spotted leafhopper, *Macrosteles fascifrons* Stål (Homoptera: Cicadellidae) were studied. For the in vitro cultivation of the insect tissues, embryos were used at blastokinetic movement stage (Hirumi and Maramorosch, 1964). The culture techniques employed were the same as those described previously (Mitsuhashi and Maramorosch, 1964), except that trypsinization was omitted. The culture medium contained the following constituents (in mg per 100 ml): 650 NaCl, 20 $NaH_2PO_4 \cdot H_2O$, 60 KCl, 20 $NaHCO_3$, 60 $CaCl_2 \cdot 2H_2O$, 60 $MgCl_2 \cdot 6H_2O$, 500 D-glucose, 500 lactoalbumin hydrolyzate, 500 Bacto-peptone; it also contained 1.00 ml L-glutamine (200 mM), 0.4 ml phenol red (0.5%), and 100 units/ml Kanamycin. The vitamins were the same as those in Grace's medium (Grace, 1962), but Ca-pantothenate was omitted. The organic acids also were the same as those in Grace's medium, but the concentration was only one-tenth. Water was added to make 80 ml, and 20 ml of fetal bovine serum was added after sterilization through a Seitz filter. The pH of the medium was adjusted to 6.5 with potassium hydroxide before the addition of fetal bovine serum. The cultures were incubated at 25°C, and the media were changed once a week.

OBSERVATIONS

Cell Divisions

The observations of mitosis were made on epithelial-type cells. The beginning of prophase was recognized under a phase contrast microscope by condensation of the chromosomes (Fig. 16). At prophase, the chromosomes were situated so that they could be observed in polar view under the microscope, while the poles of the forthcoming division already had been established and were always parallel to the glass surface of the culture vessel. The equatorial plates were always seen in side view. No polar view of the metaphase has been seen in these insect tissue cultures. Although the chromosomes were observed in exact side view at early metaphase, the equatorial plates, seen clearly at first, sometimes became obscure (Fig. 6); they appeared like oval rings or were dark. In these cases, the equatorial plates were not at right angles to the glass surface of the culture vessels (Figs.

11–15). The beginning of anaphase was recognized by chromosome separation, and the beginning of telophase was defined by cleavage furrow formation instead of chromosome movement. Often chromosome movements could not be followed clearly at late anaphase.

Cytoplasmic Protrusions (Bubbling)

During metaphase, anaphase, and telophase, the surface of the cultured cells showed small cytoplasmic processes, 2–8 μ in diameter, which protruded and retracted. An individual process usually retracted within 10 min after it appeared. The time at which the first protrusions appeared varied from early metaphase to telophase. The results of the observations are illustrated diagrammatically (Fig. 27).

Mitosis was observed in 71 cells: 59 cells were at partial metaphase and later stages, and 12 cells were at prophase. The first bubbling was manifest at metaphase in 20 cells, at anaphase in 16 cells (Figs. 6–8, Fig. 24), and at early telophase in 12 cells (Figs. 13 and 25). In the group of cells observed throughout the whole metaphase, those that produced no protrusions required 22–36 min for the completion of metaphase; the one exception was a cell that required 85 min for metaphase and showed cytokinesis, but no nuclear division (Table I). In bubbling cells, metaphase required 45–75 min. Additional data were obtained on bubbling cells observed for more than 20 min, but not throughout the whole period of metaphase; these cells also required much longer periods to complete metaphase than cells without protrusions. These observations are summarized in the right part of Table I. Anaphase lasted for 3 min, irrespective of bubbling.

Cell Activity

In most instances, protrusions appeared before the middle of metaphase. Two cells, observed during the whole metaphase, showed bubbling very early; one of them continued to bubble during the whole metaphase, while the other bubbled for 44 min during a 45-min metaphase (Fig. 27). In cells that bubbled at metaphase, this process of retraction and protrusion of the cell surface usually continued into anaphase and telophase (Figs. 16–23). One cell had only two blebs at metaphase and none at anaphase and telophase. In a few cells, bubbling started 10 min after the cleavage furrow formed, but in most of the cells the bubbling appeared at the time of cytokinesis.

Size of Blebs

Individual blebs were relatively small (2–6 μ in diameter) during metaphase and somewhat larger (6–8 μ in diameter) at anaphase and telophase, as shown in Figs. 16–23. Also, the frequency of their formation and their total number on the cell surface were comparatively low at metaphase. The frequency of bleb formation was greatest at late anaphase or early telophase, and it steadily de-

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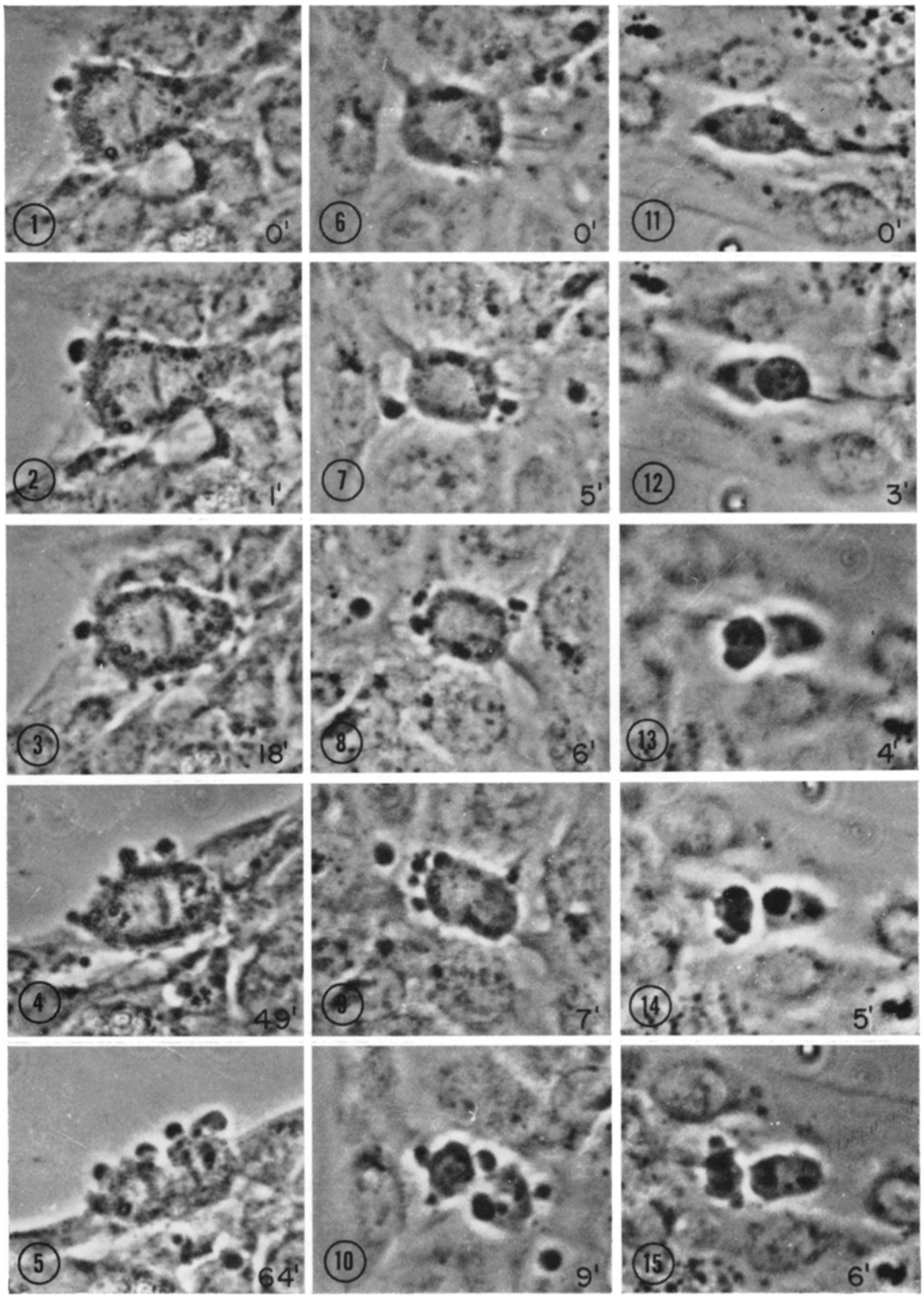
FIGURES 1–5 While chromosomes were in the equatorial plate, a projection (bleb) was observed at the surface of the cell about 71 min before beginning of anaphase (Figs. 1, 2). The cell rounded up and the blebs increased in number during late metaphase (Figs. 3, 4); bubbling became more active at early telophase (Fig. 5). × 800.

FIGURES 6–10 The equatorial plate was not clearly discernible and appeared like an oval ring at the equatorial region (Fig. 6). During anaphase, bubbling started on the surface of the cell (Figs. 7, 8). Since it was difficult to recognize the end of anaphase, the beginning of telophase was identified as the time of cleavage furrow formation (Fig. 9). The greatest bubbling activity was observed at early telophase (Fig. 10). × 870.

FIGURES 11–15 The chromosomes seemed to be scattered in the equatorial plate at late metaphase (Fig. 11); the spindle body was not parallel to the glass surface of the culture vessel (Fig. 12). The bubbling started at telophase (Fig. 13) and became more active at late telophase (Figs. 14, 15). × 870.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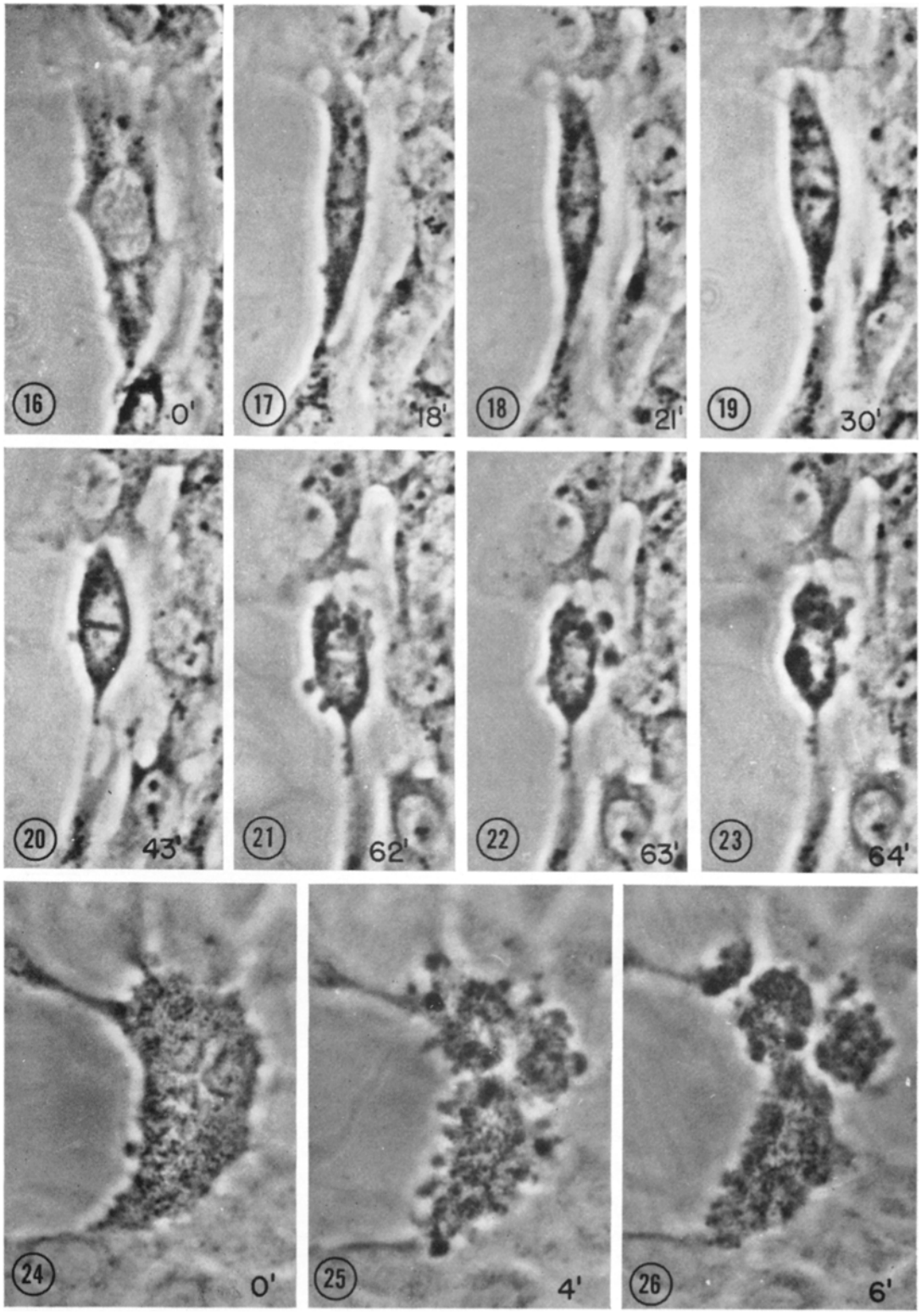

FIGURES 16–23 When a cell was observed from prophase (Fig. 16), the first bleb (Fig. 17) appeared soon after the chromosomes became arranged at the equatorial plate. The places at which the blebs appeared at early metaphase were not predictable (Figs. 18–20). The cell usually produced only two blebs simultaneously at metaphase. During anaphase and telophase, the bubbling became more active and the number of blebs increased (Figs. 21–23). × 830.

FIGURES 24–26 Anaphase bubbling that started at early anaphase (Fig. 24) became most intensive at early telophase (Fig. 25) and subsided during telophase (Fig. 26). × 830.

TABLE I

Comparison of Durations of Metaphase of Cells with and without Bubbling

Occurrence of bubbling	Duration of metaphase in cells observed during whole metaphase (*min*)					Duration of metaphase in cells observed more than 20 min during metaphase (*min*)						
Bubbling at metaphase	57 (50)	61 (43)	75 (75)	45 (44)		72 (72)	34 (20)	36 (32)	35 (35)	61 (61)	29 (29)	
Bubbling at anaphase or telophase	22	31	26			20	28	38	27	24	37	32
No bubbling	26	28	36	85	27	35	37	63				

Numerals in parentheses represent the duration of bubbling at metaphase.

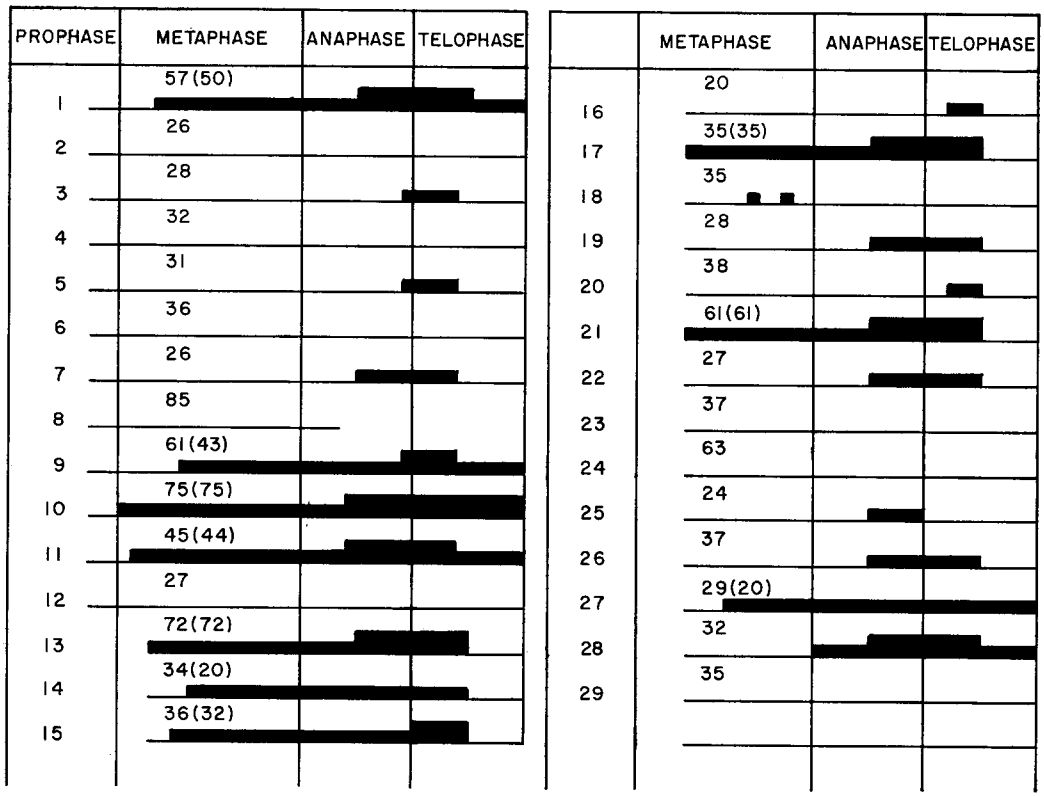

FIGURE 27 Diagram presenting the length of time at which bubbling was observed on the cell surface. The summary is based on 29 cells observed continuously during the whole metaphase, or for more than 20 min during metaphase. Each horizontal bar represents the period during which a cell was observed; the double height of the bar represents periods of increased size and frequency of the blebs. Numerals in metaphase column represent the metaphase duration in minutes and the time observed during metaphase; numerals in parentheses represent the period of bubbling.

creased during the later part of the telophase. At metaphase, bubbling was usually observed before a cell assumed its round shape (Figs. 1–5, 16–24). The size of the blebs after anaphase was not related to the starting time of bubbling.

Site of Blebs

At metaphase, the first blebs appeared at random sites, as shown in Figs. 1–4 and 17–20. In a few cells, the first blebs were noticed near the chromosomes; these cells bubbled afterwards at various places. In other cells, the first blebs appeared at the poles. Apparently, in leafhopper cells the places at which the first blebs appeared at metaphase were not limited to the vicinity of chromosomes, as was found for bubbling in newt fibroblasts (Boss, 1955).

DISCUSSION

The authors first recognized bubbling in cultured cells of the six-spotted leafhopper during a study of the effect on cell growth of different concentrations of mineral salts and glucose in the culture medium. Bubbling seemed to be a regular phenomenon in cultured cicadellid cells, and there seemed to be a definite correlation between the degree of bubbling and the length of the mitotic intervals. The bubbling of leafhopper cells in vitro differed in certain aspects from the bubbling described for vertebrate cells. In the latter, active cell protuberances appeared mainly during late anaphase and telophase (Strangeways, 1922; Boss, 1955). Boss studies the association between bubbling and the behavior of the mitotic apparatus by observing the ribonucleoprotein (RNP) in cultured newt fibroblasts. According to his interpretation, the RNP, which the chromosome acquired at prophase, bubbles as it is shed from the chromosomes at anaphase. Swann and Mitchison (1958), observing sea urchin eggs during cleavage, suggested that bubbling may be caused by the expansion of the cell surface resulting from the release of certain substances from chromosomes during anaphase. Ethylenediaminetetraacetic acid (EDTA) and victoria blue elicit similar bubbling in fibroblasts (Dornfeld and Owczarzak, 1958; Lettré and Schleich, 1955). In leafhopper cells, the formation of protuberances was recognized at a very early stage in metaphase. The prolonged metaphase of bubbling insect cells seems to suggest a functional relationship between the formation of cell protuberances and the duration of the metaphase. Protrusions similar to those described in this paper were illustrated in photographs of beetle cells by Roberts and Johnson (1966), but no mention of the phenomenon of bubbling was made by those authors.

Robbins and Micali (1965) studied the responses of the mammalian HeLa cell to osmotic shock in hypotonic solution. They postulated that the Ca^{2+}-mediated gelation differential which exists between cortex and endoplasm during interphase and disappears in the mitotic cell is responsible for the bubbling response in hypotonic solution during interphase. It would be interesting to compare the mammalian cell responses to osmotic shock and the bubbling observed in leafhopper cells, but it is not certain whether these phenomena are related. In leafhopper cells, the high incidence of blebbing during metaphase is of particular interest. This blebbing is more intense than that usually observed in vertebrate cells. Also of interest is the tendency for blebbing in metaphase to prolong this period of mitosis in cicadellid cells.

Carlson (1946) described changes in viscosity during mitosis of grasshopper neuroblasts. The viscosity was found to be relatively high in all parts of the cytosome during interphase and prophase, and to decline in late prophase. It was observed to be at its lowest at anaphase and then gradually to rise to its original high level during telophase. In leafhopper cells, the bubbling was less pronounced at metaphase than at anaphase and telophase. Perhaps a changed viscosity may account for the formation of larger cytoplasmic protrusions at anaphase and early telophase in leafhopper cells.

Hughes (1950) reported that more extensive bubbling occurred in cultured chick cells when anaphase was delayed by treatment with fluoride. Bubbling at the surface of cells in metaphase was also observed, but it was often no greater than the usual bubbling during cleavage. When urethane was applied to the cells, small bubbles were observed at the cell surface of two cells which were arrested in metaphase. Other cells proceeded to anaphase and telophase. In a cell which underwent cleavage, the bubbling was so extensive as to obscure the division of the cell until the two daughter cells had separated.

Substances that inhibit cell division probably operate by changing particular enzyme systems. It is to be expected that treatment with sublethal doses of toxic substances would affect the enzyme systems of cells. However, in the present study the delays in the mitotic intervals in cultured cicadellid cells occurred in ordinary culture media. It would seem worthwhile to study the effect of inhibitory substances or narcotics on cleavage and bubbling in cicadellid cells, since these cells appear amenable to the same methods of observation and treatments as cells of higher animals. Future experiments might attempt to relate the delayed cleavage and more extensive bubbling to biochemical events within the cells.

SUMMARY

Transient protuberances or blebs at the cell surface were observed in epithelial-type cells in insect tissue cultures of the six-spotted leafhopper (*Macrosteles fascifrons*). The protuberances appeared not only during anaphase and telophase, but often also at metaphase and even at very early metaphase. When they occurred at metaphase, the duration of the metaphase was prolonged. The protuberances were most prevalent at anaphase or early telophase, which corresponds with the timing of the occurrence of similar protuberances reported for vertebrate cells by other workers.

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BIBLIOGRAPHY

BAUMGARTNER, W. J. 1933. Pseudopodia and flowing cytoplasmic movements in living germ cells. *Trans. Kansas Acad. Sci.* **36**:209.

BOSS, J. 1955. Mitosis in cultures of newt tissues. IV. The cell surface in late anaphase and the movements of ribonucleoprotein. *Exptl. Cell Res.* **8**:181.

CARLSON, J. G. 1946. Protoplasmic viscosity changes in different regions of the grasshopper neuroblast during mitosis. *Biol. Bull.* **90**:109.

CHAMBERS, R. 1938. Structural and kinetic aspects of cell division. *J. Cell Comp. Physiol.* **12**:149.

DORNFELD, E. J., and A. OWCZARZAK. 1958. Surface responses in cultured fibroblasts elicited by ethylenediaminetetraacetic acid. *J. Biophys. Biochem. Cytol.* **4**:243.

GRACE, T. D. C. 1962. Establishment of four strains of cells from insect tissue grown *in vitro*. *Nature.* **195**:788.

HIRUMI, H., and K. MARAMOROSCH. 1964. Insect tissue culture: use of blastokinetic stage of leafhopper embryo. *Science.* **144**:1465.

HUGHES, A. F. W. 1950. The effect of inhibitory substances on cell division. A study on living cells in tissue cultures. *Quart. J. Microscop. Sci.* **91**:251.

LETTRÉ, H., and A. SCHLEICH. 1955. Zur Bedeutung der Adenosintriphosphorsäure für Formkonstanz und Formänderungen von Zellen. *Protoplasma.* **44**:314.

MITSUHASHI, J., and L. MARAMOROSCH. 1964. Leafhopper tissue culture: embryonic, nymphal, and imaginal tissues from aseptic insects. *Contrib. Boyce Thompson Inst.* **22**:435.

NOVIKOFF, A. B. 1939. Surface changes in unfertilized and fertilized eggs of *Sabellaria vulgaris*. *J. Exptl. Zool.* **82**:217–235.

PEREMESCHKO. 1879. Über die Theilung der thierischen Zellen. *Arch. Mikroscop. Anat.* **16**:437.

ROBBINS, E., and A. MICALI. 1965. The use of osmotic shock in the study of the mammalian HeLa cell surface changes during mitosis with special reference to calcium-containing solutions. *Exptl. Cell Res.* **39**:81.

ROBERTS, H. S., and N. S. JOHNSON. 1956. Cytokinesis of multi-spindle cells. *Biol. Bull.* **110**:334.

STRANGEWAYS, T. S. P. 1922. Observations on the changes seen in living cells during growth and division. *Proc. Roy. Soc. (London), Ser. B.* **94**:137.

SWANN, M. M., and J. M. MITCHISON 1958. The mechanism of cleavage in animal cells. *Biol. Rev. Cambridge Phil. Soc.* **33**:103.