MORPHOLOGICAL CHANGES IN S-180 TUMOR CELLS INDUCED BY VARIOUS PERTURBING AGENTS AS DETERMINED BY USING TIME-LAPSE CINEPHOTOMICROGRAPHY AND SCANNING ELECTRON MICROSCOPY

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SANDRA CANTWELL McGUIRE

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INDUCED BY VARIOUS PERTURBING AGENTS

AS DETERMINED BY USING TIME-LAPSE

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SCANNING ELECTRON

MICROSCOPY

Thesis Approved:

Norman N. Durham
Thesis Adviser

Kent Carraway

Charlotte L. Dunkley

Roger E. Koeppe

Earl D. Mitchell

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Today many methods are offered to help approach the study of morphological changes in cells. Some of the methods include light and scanning electron microscopy.

Time-lapse photography is particularly useful in permitting a continuous recording of data, although it is not used as frequently as it should be due to the specialized apparatus required.

Light microscopes can be used to observe slides of cells, but the procedures used to mount and prepare specimens such as staining and heat-fixing causes unnecessary alterations in cells. This causes additional problems when one is trying to determine morphological changes in cells due to perturbing agents. This problem can be diminished considerably by using a wet mount or culture growth chamber containing viable cells placed on a microscope recording morphological changes by use of time-lapse photomicrography (Dvorak et al., 1971). An additional advantage is that the fate of the same field of cells can be followed before, during and after the perturbation sequence.

The permanent recording of morphological changes by photomicrography also permits the specimens to be studied at a later date by the observer and others, giving a less biased opinion. Movies permit a greater detailed study of the events occurring with the cells, since there is no time limit for observation of these events as there is in

unphotographed views through the microscope. Since there is little contrast between the cells and background, phase contrast microscopy is utilized, giving much more contrast and detail to the observed cells rather than conventional bright-field microscopy.

Some technique must be used to permit the microculture of the cells to be used in the experiment. The equipment for a controlled culture system must be useable with extant transmitted light such as phase contrast microscopy with high numerical aperture, short working distance objectives and condensers. The chamber must be made of durable construction with biologically inert, non-toxic materials. Sterilizability of the assembled chamber prior to use is essential to avoid contamination from the external environment. The design must have simplicity to permit rapid and easy cleaning and assembly of the chamber. Long-term maintenance of optimum physiological conditions for the cell is necessary to allow for recovery after treatment. One important feature is the rapid exchange or replacement of the culture media to observe and study the effects of varying physiological parameters as well as the instantaneous fixation of cells for subsequent scanning electron microscopy (Dvorak et al., 1971). Quensel (1969) has recently reviewed such methods and their associated technical difficulties. The use of a growth chamber is very helpful for such studies since it allows observation of cells between two coverglasses and has sealed entrance and exit hubs that permit the perfusion of growth medium or perturbing agents.

Time-lapse cinephotomicrography has been a useful tool in recording both subtle and dynamic morphological changes observed in growth

chambers (Rose, 1963). Equipment is commercially available for automatically exposing single frames of film at desired regular intervals of time, thus preserving the data from an experiment which may take many hours. To minimize focus instability and field drift the temperature of the apparatus must be constant and under very reliable control. To aid in this control of constant temperature regulation, an air curtain is connected to a stage plate on the microscope. This constant temperature monitoring helps to keep the cells viable under their optimum growth conditions and allows for cinephotomicrography. In addition many microscopes have positions for 35 mm photographic cameras to be attached. Both photographic methods may be used simultaneously, resulting in time-lapse movies and 35 mm prints from one experiment. Analysis projectors can be used for analyzing the films since these projectors can be run at slow and accelerated speeds. This projector also permits the forward and reverse showing of the film, in addition to being able to stop at individual frames of the film sequence for critical analysis. Most of these projectors have a frame number indicator allowing for the exact time of the experiment for each frame of the film to be calculated. Such instruments greatly facilitate time-motion studies of research films.

An aid in studying the fine surface structural changes due to the perturbing agents is scanning electron microscopy (SEM) (Kessel et al., 1974). The cells are grown on small coverglasses in small disposable Petri dishes until normal spindle morphology is reached. Individual plates are used for each perturbing agent and control. The samples are fixed with glutaraldehyde (Luftig et al., 1977), post-fixed with osmium tetroxide and then dehydrated with ethanol. After dehydration the

cells are critical-point dried before scanning (Kessel et al., 1974). Critical point drying prevents serious surface changes in the cells during drying. The coverglass samples are placed in the dryer which is sealed and filled with liquid carbon dioxide. When the carbon dioxide is heated and reaches its critical temperature and pressure, the carbon dioxide becomes gaseous. After release of the pressure of the gas, the samples are removed and coated with gold-palladium. Attached to the microscope is a Polaroid camera so that pictures and negatives can be obtained within minutes. One disadvantage of SEM is that the same field of cells cannot be observed before, during and after treatment.

Attachment of cells in serum-containing medium depends on the interaction with serum proteins rather than direct attachment to the substratum (Grinnel, 1976a). This attachment of cells can be described in a series of steps: 1) adsorption of serum components onto the substratum, 2) contact between the cells and substratum, 3) initial attachment, 4) and progressive attachments leading to cell spreading, 5) increased strength of cell spreading, and 6) increased strength of cell attachments (Grinnel, 1976b). Electrostatic charges are involved in the attachment process. Glass and most cell surfaces are negatively charged whereas the serum components are positively charged. This results in a negative-positive-negative attraction of the cells, serum components and glass, respectively (Weiss, 1972). Furthermore, a specific component, commonly called the attachment factor, has been isolated from various purified sera (Grinnel, 1976b). It is currently thought by some investigators that cell surface glycoproteins and mucopolysaccharides are involved in adhesion (Grinnel et al., 1973).

One of the purposes of this study was to survey the use of perturbing agents on cell morphology.

Ionophores are molecules that greatly increase the permeability of membranes to specific ions. An influx of ions such as Na^{+} and Ca^{++} might lead to alterations in cell morphology. Ionophores form rings that have a nonpolar periphery and therefore spontaneously insert themselves in membranes, while the interior of the ring closely fits a particular inorganic cation and also provides carbonyl groups that coordinate with it, replacing its normal hydration shell (Davis et al., 1973).

The divalent cation ionophore A23187 changes the permeability of some membranes to calcium. This ionophore causes changes in the membrane potential resulting in an increase of calcium levels in cells (Rasmussen, 1975). Experiments have shown that increasing the uptake of calcium into the cell and/or causing its release from an internal store by treatment of the particular cell with an appropriate concentration of A23187 causes an activation of the smooth muscle cell which results in contraction (Rasmussen, 1975).

Calcium, by being an integral component of the cell membrane, can also effect the overall packing and motional freedom of the surface phospholipids. Displacement of calcium may therefore change the cell morphology and mobility of surface receptors (Vladavsky et al., 1977). Changes in the intracellular levels of calcium by A23187 can form a reversible complex with calcium and by virtue of the lipophylic nature of the ionophore, A23187 can serve as a mobile carrier across biological membranes (Vlodavsky et al., 1977).

Virtually all animal cell membranes are actively involved in ion

transport. These ion transport processes lead to an asymmetric distribution of ions across the membrane (Rasmussen, 1975). Metallic activators are uni- or bivalent metallic cations such as Na^{+}, K^{+}, Mg^{++}, Zn^{++}, Ca^{++}, Fe^{++} and Co^{++}. Since these ions are important in maintaining normal cell morphology, various concentrations of two of them (Ca^{++} and Zn^{++}) were used as perturbants.

The calcium ion distribution in and around the cell is rather different from monovalent cations, in that the bulk of the cell's calcium is concentrated within the mitochondria. Depletion of the cell's calcium by the addition of chelating agents results sequestering of the ion. The calcium ion and cyclic adenosine monophosphate (cyclic AMP) appear to be two of the few general messengers involved in cell activation (Rasmussen, 1975).

It has been found that when cells are disaggregated with chelating agents (substances which bind divalent cations such as Ca^{++} and Mg^{++}), macromolecules of a mucoid nature appear in the medium. These macromolecules are suggested to comprise the surface coat on cells (Ambrose et al., 1970). Adhesive bonds can be disrupted by chelating agents such as EGTA and EDTA. De-adhesion caused by EDTA appears to be a result of a +2 metal requirement in the formation and/or the stabilization of the adhesive bond (Grinnel et al., 1971).

Colchicine, an alkaloid used in studying chromosomes, specifically inhibits the formation of spindle fibers. Dividing cells treated with colchicine are arrested at metaphase. The repulsive forces still operate between the centromeres, but spindle fibers do not become attached and the cell cannot move into anaphase. Colchicine prevents the formation of the mitotic spindle which is composed of microtubules

(Ambrose et al., 1970). Since colchicine inhibits the formation of microtubules and microtubules might be involved in cell surface modification, the effect of colchicine on cell morphology was studied.

Digestive enzymes, such as trypsin, catalyse the hydrolysis of certain peptide bonds. Trypsin acts on the peptide linkages involving the carboxyl groups of arginine and lysine (Ambrose et al., 1970). Trypsin has been shown to break the cell adhesive forces leading to cell rounding (Rees et al., 1977). This loss of cell adhesiveness is reversible and recovery is a time-dependent process (Grinnel et al., 1973). Cultured Sarcoma-180 tumor cells become rounded, but do not detach from the substratum when subjected to mild trypsinization. Removal of trypsin permits the cells to go back to their original spindle shape over an 8-20 hour period (Huggins et al., 1976). The above studies were all at the light microscopic level and the purpose of using trypsin in this investigation was to use cinephotomicrography and SEM to observe the changes.

CHAPTER II

MATERIALS AND METHODS

Test Organism

Sarcoma 180 (CCRF-S180 II), purchased from the American Type Culture Collection Cell Repository was used for the experiments. S-180 is a tumor derived from mice and was established by Foley et al. in 1959. Stock cultures were grown in milk dilution bottles on McCoy's 5a modified medium with 10% calf serum. The cells were transferred twice a week to keep subconfluent cells available for experiments.

Chemicals Used for Cinephotomicrography Experiments

See Table I (p. 21).

Growth Medium

1 X McCoy's 5a modified medium plus 10% calf serum.

Hepes Buffered Saline (HBS)

Hepes = N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid

NaCl	8.0 g
KCl	0.4 g

Na_2HPO_4	0.1 g
Dextrose	1.0 g
Hepes	2.3 g

Reagents were added to a one liter volumetric flask and filled with deionized distilled water. The pH was adjusted to 7.4 with NaOH. It was then filtered to sterilize.

Chemicals Used for Scanning Electron Microscopy

3% Glutaraldehyde	(Ted Pella, Inc.)
2% Osmium tetroxide	(Polysciences, Inc.)
Absolute ethanol	(U. S. I. Chem. Co.)

Maintenance of Cell Lines

Cells were grown to subconfluency in milk dilution bottles on McCoy's 5a modified medium plus ten percent calf serum. The old medium in the bottles was aseptically removed and then ten mls of fresh growth medium was added to each bottle. The cells were harvested by scraping with a rubber policeman (Huggins et al., 1975). To insure single cells, rather than aggregates of cells, the cell-medium suspension was pipetted at least twenty times. Aliquots of the cell suspension, ranging from 0.1 ml to 0.5 ml were transferred to individual sterile milk dilution bottles. To each bottle 10 ml of fresh medium was added. The pH was adjusted with CO_2 prior to sealing with rubber stoppers. The bottles were stored flat in a 37°C walk-in incubator. All procedures were done in a Bioflow Germ-Free hood located in a transfer room of the tissue culture laboratory to maintain

aseptic conditions. The cells were transferred twice a week (Penso et al., 1963).

Preparation of Coverglasses for Chamber

Coverglasses of No. 1½ thickness were selected. The coverglasses were placed in a beaker containing concentrated nitric acid-water (1:1) solution. The solution was heated to 90°C in a fume hood. The heat was turned off and the coverglasses remained in the acid solution overnight. The coverglasses were then thoroughly rinsed with tap water followed with five rinses in distilled-deionized water. They were rinsed with absolute ethanol and placed in a beaker filled with absolute ethanol for at least one-half hour. The individual coverglasses were removed with clean, plastic tipped forceps and dried with lens paper, being cautious to prevent skin oils from contacting the ethanol or coverglasses. The dried polished coverglasses were placed on filter paper in a clean Petri dish until assembly of the growth chamber (Dvorak, 1975b).

Preparation for Time-Lapse Experiments

In preparation for an experiment the cells were grown in milk dilution bottles as described in maintenance of cell lines. To set up an experiment, a bottle of cells was obtained from the walk-in incubator. Working in the germ-free hood, the cells were harvested using a rubber policeman, while leaving the old medium in the bottle. The old medium was left in the bottle so that after a dilution for correct cell density was made some of the medium was pre-conditioned by the cells to minimize the transitory shock to the cells. The cell

suspension was pipetted at least twenty times to insure single cells. The cells were then counted by use of a Bright Line hemacytometer made by American Optical Company and then diluted to 2×10^5 cells/ml using fresh medium.

A sterile Dvorak-Stotler growth chamber, made by Carl Zeiss, Inc., was prepared for loading. Bardic I. V. extension tubing was fitted on to the outlet port hub of the chamber and on one position of a three-way valve which had already been attached to the inlet port hub of the chamber. The free-ends of tubing were placed into sterile 250 ml Erlenmeyer flasks with sterile aluminum foil covering the mouths of the flask. A one milliliter sterile tuberculin syringe was filled with the diluted cell suspension. The syringe was attached to the three-way valve with the open valve pointing to the inlet hub and syringe only. The suspension was slowly injected into the chamber, being careful to eliminate all air bubbles by tilting the outlet port hub up. Leaving the syringe in place the chamber was inverted and placed on a Lucite holder. The inverted position allows the cells to settle to the upper coverglass and attach to the serum coated on the coverglass. The chamber was incubated in this position at 37°C in a CO_2-O_2 incubator. At the end of the settling period the tuberculin syringe was replaced with another extension tubing. A 10-20 ml glass syringe was filled with 37°C fresh medium and was attached to the end of the extension tubing. The three-way valve was then opened to the tubing in the bypass Erlenmeyer flask. Fresh medium was pumped through the tubing in the syringe until it passed through the three-way valve to the by-pass tubing. This procedure eliminated any excess air bubbles from entering the chamber. The valve was then turned to the chamber and

fresh medium was perfused through the chamber. This flushing of the chamber removed any nonadhering cells and cell debris. During the process the outlet hub of the chamber was tilted upward to expel all air bubbles.

The chamber was then placed on a mechanical stage plate on the microscope for observation. A Sage Instrument Model 279 air curtain maintained the 37°C physiological temperature via a thermister touching the chamber.

The cells in the chamber remained on the microscope stage at a constant 37°C until they reached their normal flattened spindle-shaped morphology. At this time the cells were ready for treatment and time-lapse cinephotomicrography experiments.

Equipment Used for Time-Lapse Cinephotomicrography

The chamber used to conduct the cinematography experiments was a Dvorak-Stotler culture system developed by J. A. Dvorak and W. F. Stotler of the National Institutes of Health, Bethesda, Maryland. The chamber is commercially available through Carl Zeiss, Inc. The metal parts of the chamber were made of Type 303 stainless steel. The culture chamber consists of seven parts. The holder had a 19.0 mm lower aperture, a 30.5 mm upper aperture and a total thickness of 8.9 mm. The platform on which the lower coverglass rests was coated with an 80 μm layer of self-adherent Teflon. This teflon layer was required at all steel-glass interfaces to prevent the sharp steel edge from acting as a die and cutting the coverglass under the influence of high perfusion pressures. Two coverglasses of 25 mm

diameter and No. 1½ thickness are used. These have been acid-washed and polished with lens paper (Dvorak, 1975b). The spacer has a 19.0 mm aperture and a thickness of 635 microns. Critical control of the thickness of the finished spacer to a tolerance of 25 microns is essential for proper performance of the assembled chamber. Perfusion ports consist of 27 gauge stainless steel tubing fitted with stainless steel luer-taper hubs. The needles pass through the body of the spacer and are permanently bonded. Both surfaces of the spacer which contact the coverglasses are coated with an 80 μm layer of self-adherent teflon. The pressure plate, with a 19.8 mm aperture and a total thickness of 1.7 mm, has a 2.6 mm wide by 785 μm thick stepped surface which contacts the upper coverglass. This step, which is coated with an 80 μm layer of self-adherent teflon, applies uniform pressure on the coverglass-spacer assembly. A silicon O-ring has its outer diameter ground flat to fit the recessed well of the holder. Keeping all parts intact was achieved by the stainless steel snap ring which fit into a groove of the holder. The assembled chamber was then wrapped with a small piece of aluminum foil covering each hub and a large sheet of aluminum foil wrapping the entire chamber. The wrapped chamber then was placed into a large glass Petri dish and sterilized for use (Dvorak et al., 1971).

The temperature of the chamber on the microscope was maintained with a Sage Instruments Air Curtain, Model 279. The relative large mass of the steel holder acts as a thermal buffer, thus restricting temperature fluctuations to approximately 0.2°C.

A Sage Syringe pump Model 341 was used to perfuse medium or perturbants through the chamber to the cells. The pump was equipped to

perfuse over a broad range of perfusion rates.

The apparatus for conducting time-lapse cinephotomicrography was a Wild-Heerbrugg M20 phase contrast microscope, marketed by Heerbrugg, Switzerland and Farmingdale, New York. The microscope was used in conjunction with a Sage Instrument Control Module Series 500 Cinephotomicrographic apparatus. To this was attached a Bolex H-16 M 16 mm movie camera equipped with a motor for time-lapse studies. The control module had a variety of choices of rates of filming and exposure times.

The experiments were run at an exposure time of 0.4 second and a light setting of 0.06 μA. Most experiments were filmed at 30 frames/minute.

The basic M20 research microscope was illuminated by a tungsten-halogen lamp. The long focal length phase contract condenser was used. A 10 X phase contrast objective lens was also used. The body tube was equipped with a 1.25 X magnifier and beam splitter which partitioned the image between a horizontal eyepiece, a vertical monocular tube containing a 10 X photoeyepiece, and a diagonal monocular tube containing a 10 X photoeyepiece. A Nikon AFM automatic exposure shutter was attached to the diagonal tube and to the shutter was connected a Nikon M-35S film camera back for 35 mm photographs. Above the vertical tube was a Bolex H-16 movie camera equipped with a 50 mm f/1.8 lens. The low available light required the use at full aperture for cinephotomicrography, so the lens was set at infinity.

Attached to the movie camera was an auxiliary motor to power the single frame exposures and film advance. This motor was actuated by a pulse transmitter unit which offered a wide variety of filming rates. For recovery experiments, the microscope was fitted with an

electromagnetic shutter which shielded the chamber and cells for illumination except during the exposure or focus checks.

Eastman Kodak Tri-X Reversal black and white 16 mm movie film (No. 7278) was used throughout the investigation. The exposure index of the film with a tungsten lamp is ASA 160. To obtain proper exposure, the light output of the microscope lamp was adjusted via a potentiometer on its power supply. A photocell in the cinefocusing tube wired to an external microammeter indicated the intensity of the illumination.

Eastman Kodak Tri-X black and white 35 mm single frame film was used in the Nikon camera. By using Diafine developer the exposure index was increased to ASA 1600 and developed in the darkroom of the Biochemistry Department.

The exposed rolls of 16 mm film were reversal processed by a commercial laboratory (Arthur Sweeney, Inc., Tulsa, Oklahoma).

Preparation for Scanning Electron Microscopy

Round coverslips of No. 1 thickness glass (Corning) were cut to a diameter of approximately eight millimeters using a diamond tipped pencil. To maintain sterile conditions, subsequent preparation was done in a germ-free hood. The individual coverglasses were immersed in absolute ethanol and then flamed. When all the ethanol had burned off and the coverglass was allowed to cool for a few seconds, it was placed in sterile disposable thirty-five millimeter Petri dishes. At least one duplicate coverglass was added to each plate. The washing with absolute ethanol was necessary to remove oils from the glass, in addition to preventing water spots on the coverglasses which might have

inhibited proper attachment and flattening of the cells. Approximately 3×10^4 cells were pipetted onto the coverglass. To each plate, 2 ml of fresh medium was carefully pipetted at the side of the dish to minimize convection currents. These dishes were then placed into a CO_2-O_2 incubator. Periodically, the plates were removed from the incubator to monitor the cells for their normal spindle-shaped morphology by use of an inverted microscope. This process may take several days, so the coverglasses were seeded at a low density to obtain mostly single cells for experimentation.

When the cells were flat the growth medium was carefully aspirated off and the perturbant or control reagent was quickly added to prevent any air drying of the cells. At the same time the reagent-cell contact time was being monitored with a stop watch. To stop the experiment in each plate a three percent glutaraldehyde solution buffered with 0.05 M sodium cacodylate (pH 7.2) was added. The plates were then stored at 4°C. After the glutaraldehyde fixation was complete, the cells were washed two times with 0.1 M sodium cacodylate buffer. All preparative steps were done in the Petri dishes. This buffer was carefully aspirated off at the side of the dishes to prevent aspirating any cells off of the coverglasses. The cells were then post-fixed with two percent osmium tetroxide in 0.05 M sodium cacodylate buffer. This incubation was done in a fume hood since it is very toxic. The osmication process was complete in thirty minutes since monolayer of cells are being used. The cells were then washed two times with 0.1 M sodium cacodylate buffer and were ready for dehydration.

The cells were then dehydrated with increasing concentrations of ethanol. Absolute ethanol was used because of its purity. The

sequential dehydration allows for the water to be replaced with ethanol. Initially 30% ethanol-water (v/v) was added to the plate and then aspirated off. It was added again and allowed to incubate for fifteen minutes. Rushing or incomplete dehydration of samples will cause morphological artifacts, so care must be taken to allow complete dehydration of the samples at each increasing concentration of ethanol. After the fifteen minutes the ethanol was aspirated and quickly replenished with 50% ethanol to prevent air drying of samples. The fifteen minute incubation was repeated and the process was repeated with 70%, 80%, 90% and 95% ethanol. The cells could then be stored at 4°C or can be dehydrated three times with absolute ethanol and critical point dried. It is very important to remove all water before critical point drying (Kessel et al., 1974; Boyde et al., 1972).

The boat or specimen holder for the critical point dryer was filled with absolute ethanol before inserting the samples. A loosely coiled spring works well for separating the coverglasses and keeping them from getting mixed up. The boat or holder was inserted into the critical point dryer which had been cooled to 20°C prior to the insertion. The drying apparatus was sealed and filled with liquid CO_2. The ethanol formed a layer below the carbon dioxide so that the ethanol could be drained. More liquid carbon dioxide was gently added while draining the ethanol. This step took approximately fifteen minutes but the objective was to remove all ethanol and substitute it with liquid carbon dioxide. All valves and drains were closed to allow for impregnation of carbon dioxide into the cells for thirty minutes. After this time fresh liquid carbon dioxide was added while the carbon dioxide used for impregnation was drained out. This step helped insure

that any residual ethanol in the cells was removed. The level of carbon dioxide must never get below the samples or the samples would be exposed to air. The carbon dioxide was slowly heated to 37°C and a pressure of 1200-1500 psi. When the temperature approached 30°C the meniscus became flattened and soon disappeared. The temperature and pressure continued to increase to assure that all carbon dioxide was in the form of a gas. The temperature was kept at 37°C so that as the gas was vented, the carbon dioxide would not change back to the liquid state. The gas must be vented slowly to prevent turbulence and condensation which would ruin the samples. When the pressure reached zero the dryer was opened and the samples were removed. Each coverglass was placed on sample identification coded stubs. The edge of the coverglass was then painted on three places with silver conductivity paint (Ladd Industries).

The stubs were placed on a Hummer II coater. The vacuum was pulled to 110 millitorr. The high voltage switch was turned on to 9 volts. Simultaneously the timer was set to automatic for a coating time of 1.75 minutes which resulted in a coating thickness of 175Å. During this coating process the run must be adjusted to 10 DC milliamperes which was at a vacuum between 90-110 millitorr. After the coating, the vacuum was released and the stubs were put into specimen holder boxes. The boxes were then stored in a glass dessicator filled with moisture indicator dessicant until the samples were observed on the scanning electron microscope.

Equipment Used for Scanning Electron Microscopy

Most of the equipment used was provided by the Physiological Sciences Department of the Veterinary Medicine School at Oklahoma State University.

Two apparatuses were available for critical point drying. The easiest and most suitable for samples on coverglasses was the Polaron Critical Point Dryer of Hartford, England. Inserted in the Polaron boat was a loosely coiled spring so that each coverglass was placed vertically into the individual coils of the spring. The boat was filled with absolute ethanol prior to loading of samples to prevent any air drying. The other dryer was a Tousimis Samdri PVT-3 from Rockville, Maryland. This holder had to be placed in a dish of ethanol before loading of samples. The samples were very difficult to load since they had a tendency to fall out of the holder.

After drying the samples were placed on metal stubs made by Ted Pella, Inc. The coverglass edge was then painted with silver conductivity paint before coating.

The samples were then placed on a Hummer II by Technics, Inc. to be coated with gold-palladium. The samples were coated for 1.75 minutes (175 Å) at nine volts using 10 DC milliamperes.

The samples were then labeled and stored in specimen holder boxes made by Ted Pella, Inc. and placed in a dessicator to prevent rehydration.

A JEOL JSM-35 scanning electron microscope was used to observe the samples. A Polaroid camera was mounted onto the scope for

recording the image on either Type 52 (positive only) or Type 55 (positive/negative) film.

Maintenance of Records

A log book was kept for all time-lapse cinephotomicrography experiments. Records were made when the chamber was loaded with cells and the cell density. All information regarding exposure time, rate of filming, name and concentration of perturbant including total time and rate of perfusion. Careful recording was necessary so that the films could be analyzed properly.

A log book was also kept recording all 35 mm pictures taken during the experiment. The event time of the perturbant was noted for each picture.

When the processed films were received, the films were observed using a Lafayette Film Analyzer Projector. The film could be observed at various frame rates and individual frames could be studied.

TABLE I

CHEMICALS USED FOR CINEPHOTOMICROGRAPHY EXPERIMENTS

Chemical	Diluent
1 mM EDTA	HBS
1 mM EGTA	HBS
1 mM $CaCl_2$	HBS
5 mM $CaCl_2$	HBS
10 mM NaN_3	HBS
3 µg/ml Ionophore A23187 (Eli Lilly Co.)	HBS + 0.06% DMSO
3 µg/ml A23187 + 1 mM $CaCl_2$	HBS + 0.06% DMSO
3 µg/ml A23187 + 1 mM EGTA	HBS + 0.06% DMSO
HBS	-
McCoy's + 10% Calf Serum	-
1 mM $ZnCl_2$	H_2O
0.75 mM $ZnCl_2$	HBS
0.50 mM $ZnCl_2$	HBS
40 mM Tris Buffer	-
1 mM Colchicine	HBS + 0.01% EtOH
10 mM Colchicine	HBS + 0.10% EtOH
Butyric Acid	McCoy's + Calf
Dibutyril cyclic AMP	McCoy's + Calf
Thrombin	-
80 µg/ml Concanavalin A	HBS
SA-180-Zn Membrane Antiserum	-
Lyophilized Normal Mouse Serum Absorbed to Antiserum	McCoy's + Calf

CHAPTER III

RESULTS AND DISCUSSION OF CINEPHOTOMICROGRAPHY EXPERIMENTS

EDTA

The use of 1 mM EDTA (in HBS) caused a definite effect upon the morphology of S-180 cells. At time zero, nearly all cells were flat. After perfusion of the effector for five minutes approximately 60% of the cell population were rounded or in the process of rounding. By ten minutes of contact with EDTA, 100% of the field were rounded. The cells were then flushed with fresh medium to remove the perturbant. Five hours later the cells were beginning to reflatten. By twenty-three hours after treatment, the reflattening process was complete (Figures 1, 2, 3, 4, in Appendix).

Trypsin

The effect of trypsin (5 µg/ml) on the tumor cells was a relatively slow process. After five minutes only a few cells were rounded. After fifteen minutes of perfusion approximately 50% of the cells were round. The perfusion was stopped and the cells were allowed to remain in the trypsin solution for one hour. After one hour 94% of the cells were round. The cells were then flushed with fresh medium. Twenty-four hours later the cells had regained their normal flattened

morphology (Figures 5, 6, 7, 8, in Appendix).

Hepes Buffered Saline Control

The perfusion of hepes buffered saline (pH 7.4) had little or no apparent effect on the morphology of the cells. After six minutes of perfusion 100% of the cell population was flat. Even after fifteen minutes or one hour of treatment of the cells greater than 99% remained flattened. Because of the minimal effect on the morphology hepes buffered saline was used as the diluent in the majority of the experiments (Figures 9, 10, 11, 12, in Appendix).

Colchicine

The treatment of cells with 1 µg/ml colchicine (in HBS + 0.01% EtOH) for 6 minutes resulted in rounding of 30% of the cell population. After 11 minutes of treatment approximately 55% of the population were round. At the end of fifteen minutes of perfusion approximately 80% were rounded. The cells were then flushed with fresh medium and allowed to recover (Figures 13, 14, 15, 16, in Appendix), but 24 hours later the cells appeared dead.

EGTA

Treating the cells with 1 mM EGTA for one minute resulted in rounding of 30% of the population. By the end of eight minutes approximately 70% were round. At the end of the fifteen minute period of treatment 100% of the population were round. The cells were flushed with fresh medium and allowed to recover (Figures 17, 18, 19, 20, in Appendix).

Sodium Azide

During the first two - three minutes of sodium azide (10 mM) treatment ruffling of the peripheries of the cells was observed. After perfusion for fifteen minutes, the cells were then flushed with fresh medium. Approximately twelve hours after the treatment the cells appeared dead.

$CaCl_2$ and Ionophore A23187

Treatment of the cells with 1 mM $CaCl_2$ (in HBS) for fifteen minutes caused approximately 50% of the cell population to round.

Using 5 mM $CaCl_2$ (in HBS) caused the cells to round up in a manner that was different than most other treatments. Most of the cells rounded from their leading end (head) to the tail portion, rather than the usual tail-to-head rounding. Approximately 80% of the cells had rounded after seven minutes. The cells were flushed with fresh medium and allowed to recover. Twenty-four hours later the cells were treated with 3 µg/ml A23187 (in 5 mM $CaCl_2$). At three minutes some of the cells were beginning to round. After thirty minutes of treatment about 50% of the cells had rounded. The cells were then flushed with fresh medium and allowed to reflatten. About 24 hours later the cells were treated for thirty minutes with 3 µg/ml A23187 (in HBS). There was no apparent effect on the cells.

A fresh set of cells was used to perfuse 3 µg/ml A23187 + 1 mM $CaCl_2$ (in HBS). After fifteen minutes approximately 50% had rounded or were just beginning to round.

Subsequent experiments, months later, were done using A23187 +

Ca and A23187 + EGTA. Little morphological change occurred.

$ZnCl_2$-Tris Buffer

This set of experiments was done to see if pretreatment with $ZnCl_2$, which stabilizes membranes, would prevent swelling of cells after immediate treatment with 40 mM Tris buffer.

A set of cells were treated with 1 mM $ZnCl_2$ (in H_2O). Immediately the cells became rounded and swollen. Subsequent addition of 40 mM Tris caused vacuoles in the cells to form. The cells were flushed with fresh medium, but never recovered.

Another set of cells were treated with 0.75 mM $ZnCl_2$ (in HBS). After eight minutes of treatment a few cells had rounded but no swelling occurred. The cells were then treated for three minutes with 40 mM Tris which caused some swelling and protrusion from the flat cell. The cells were flushed with fresh medium and allowed to recover. The following day the cells were treated with 0.50 mM $ZnCl_2$ (in HBS). At three minutes, blebs formed on the flattened cells. The cells were then treated with 40 mM Tris for three minutes which caused additional blebs and swelling of the flattened cells. Fresh medium was flushed through the chamber to allow for cell recovery.

Additional Chemicals Used

Various other chemicals were used in surveying morphological changes in cells caused by perturbing agents. Some agents caused no apparent change while other agents effects were not consistently reproducible to be valid. Some of these agents were 2 mM Butyric acid, 1 mM Dibutyril cyclic AMP, Thrombin and Concanavalin A. Cold growth medium

was also used, but due to the slow perfusion rates and heat from the light source the temperature leaving the chamber (measured by thermister inserted in outlet hub of chamber) was not low enough to cause morphological changes.

CHAPTER IV

RESULTS AND DISCUSSION OF SEM EXPERIMENTS

Growth Medium Controls

Controls were obtained as follows: the growth medium containing non-adhering cells was removed from the dishes and the samples were immediately flooded with glutaraldehyde fixation.

The sample population of cells had a normal spindle-shape morphology (Figure 21, in Appendix). At higher magnification the cells had numerous microvilli, retractile fibrils and other attachment processes (Figure 22, in Appendix).

Hepes Buffered Saline Controls

Hepes buffered saline was the diluent in the SEM experiments, therefore cells were treated with HBS for a control to make sure morphological changes were not due to the diluent itself.

At ten and fifteen minutes of incubation in HBS the cells retained their normal morphology. Most cells were flat with microvilli, retractile fibrils, thick filopodia and some lamellipodia (Figure 23, in Appendix).

Ionophore A23187 and Related Experiments

These three sets of experiments consisted of treating cells with

3 µg/ml Ionophore A23187 (in HBS + 0.06% DMSO), 3 µg/ml A23187 + 1 mM $CaCl_2$ (in HBS + 0.06% DMSO), and 3 µg/ml A23187 + 1 mM EGTA (in HBS + 0.06% DMSO).

When cells were treated with only A23187, most of the cells in the population had partially rounded after contact with the effector at both five and fifteen minutes. After five minutes, thick filopodia and lamellipodia were present, in addition to ridges on the cell surface, rather than distinct microvilli. Upon completion of fifteen minutes of incubation in the effector, most of the thick filopodia were gone, but lamellipodia and ridges remained on the surface (Figure 24, in Appendix).

After treating cells with A23187 + $CaCl_2$ distinct differences in morphology can be seen at the different incubation times. At five minutes of treatment over half of the cells present were in various stages of rounding. Few attachment fibrils are present and ruffling at the cell margin was evident. After fifteen minutes of incubation in A23187 + $CaCl_2$ few cells were left in the population. Most of the remaining cells had short retractile fibrils, thick filopodia, and lamellipodia surrounding the cells. Other cells, (Figures 25, 26, in Appendix) appeared quite different with many large ballooning, zeiotic blebs protruding from their otherwise featureless surface. The background of these samples is covered with some type of cell vesicles or debris, which could account for the low cell density after treatment.

No differences in cell morphology were seen after A23187 + EGTA at the various incubation times (Figures 27, 28, in Appendix) except that nearly all the cells in the population were rounded and had lamellipodia at their cell margins. Extending outward from the

lamellipodia were many retractile fibrils and thick filopodia. Therefore the ionophore alone appeared to cause a "ruffling" of the cell surface, but when an increase in Ca^{++} concentration accompanies the ionophore, large blebs from the surface were observed. If calcium was removed from the medium with EDTA, neither of the above effects was seen. Thus the ruffling and blebbing seem to be somewhat dependent on the presence of Ca^{++}.

1 mM EDTA

EDTA had a more drastic effect on cell morphology than other treatments. At four minutes (Figure 29, in Appendix) about fifty percent were flat and there were many cells in the population. At a higher magnification numerous retractile fibrils and microvilli could be seen (Figure 30, in Appendix).

After fifteen minutes in EDTA, most of the cells had detached from the coverglass leaving cell residue behind (Figure 31, in Appendix). At higher magnification retractile fibrils, thick filopodia and microvilli were present (Figure 32, in Appendix) as were lamellipodia.

1 mM EGTA

The effect of EGTA was similar to that of EDTA except, after fifteen minutes of exposure to the effector many cells were still present (Figure 33, in Appendix). At higher magnification (Figure 34, in Appendix) many thick and long filopodia and some unattached filopodia were present. Retractile fibrils and microvilli were also observed.

$CaCl_2$

After treatment with 1 mM and 5 mM $CaCl_2$, the majority of the cells were flat (Figure 35, in Appendix). The elongated cells had undergone some partial rounding. The treated cells varied from round, bipolar and multipolar, with many retractile fibrils present. 5 mM $CaCl_2$ caused more rounding than 1 mM $CaCl_2$ and left a larger area of lamellipodia at the cell margins (Figure 36, in Appendix). Extending from the cells treated at both concentrations of calcium are thick filopodia, long retractile fibrils and microvilli.

Trypsin

After fifteen minutes of treatment, the majority of the cell population had undergone various stages of rounding (Figure 37, in Appendix). Many of the cells were spherical and bipolar rounded.

At higher magnification (Figure 38, in Appendix), numerous microvilli and thick filopodia were still present after fifteen minutes, but there were few retractile fibrils.

Sodium Azide

Treatment of cells for fifteen minutes produced little rounding of the cells in the population (Figure 39, in Appendix). It appeared that the cells initially began to round up leaving an uneven lamellipodia at the cell periphery (Figure 40, in Appendix). Few retractile fibrils were present, but microvilli were pronounced and there were some formation of blebs.

CHAPTER V

SUMMARY AND CONCLUSIONS

Cinephotomicrography is a useful tool for observing morphological changes in cells. The extent and impact of the movies cannot be reasonably expressed through photographs and writings. Observations of the films is the best way to get a clear picture of morphological changes occurring. Much data can be overlooked from micrographs and descriptions. Unfortunately, at the present time, correlative and descriptive nomenclature has not been firmly established as to the various forms and activities of cells during perturbation sequences in writings.

Results obtained show that most of the agents used perturbed the cells in both cinephotomicrography and scanning electron microscopy (SEM) experiments. With SEM samples many subtle and inapparent changes in morphology observed by light microscopy were very dramatic when observed by scanning electron microscopy. Although much detailed information can be obtained from electron micrographs, the fate of the same set of cells or individual cell cannot be followed.

A major problem which occurred in this study was the instability of the cell line. At the beginning of the study, aliquots of cells should have been stored for future use to help insure the stability, validity, and comparative capabilities of the experiments.

Future work will use the techniques of both time-lapse cinephoto-

micrography and scanning electron microscopy to explain and understand the biology of cells and their interaction with their environment.

LITERATURE CITED

Ambrose, E. J., and D. M. Easty. 1970. Cell biology. Thomas Nelson and Sons, LTD., Great Britain.

Boyde, A., R. A. Weiss, and P. Vesely. 1972. Scanning electron microscopy of cells in culture. Exp. Cell Res. 71:313.

Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. Berry Wood, Jr. 1973. Microbiology, p. 127-128. Harper and Row Publishers, Inc., New York.

Dvorak, J. A. Suggested protocol for the preparation and use of the Dvorak-Stotler controlled environment culture system. Nicholson Precision Instruments, Inc., Bethesda, Maryland.

Dvorak, J. A., and W. F. Stottler. 1971. A controlled-environment culture system for high resolution light microscopy. Exp. Cell Res. 68:144-148.

Grinnel, F. and P. A. Srere. 1971. Inhibition of cellular adhesiveness by sulfhydryl blocking agents. J. of Cell Phys. 78:153-158.

Grinnel, F., M. Milan, and P. A. Srere. 1973. Studies on cell adhesion: IV Adhesion of baby hamster kidney cells. J. Cell Biol. 56:659-665.

Grinnel, F. 1976a. The serum dependence of baby hamster kidney cell attachment to a substratum. Exp. Cell Res. 97:265-275.

Grinnel, F. 1976b. Cell spreading factor: Occurrence and specificity of action. Exp. Cell Res. 102:51-62.

Huggins, J. W., R. W. Chesnut, N. N. Durham, and K. L. Carraway. 1975. Glycoprotein differences between the ascites and cultured forms of the Sarcoma 180 tumor. Bioch. and Biophys. Res. Comm. 65:497-502.

Huggins, J. W., R. W. Chesnut, N. N. Durham, and K. L. Carraway. 1976. Molecular changes in cell surface membranes resulting from trypsinization of Sarcoma 180 tumor cells. Biochim. et Biophys. Acta 426:630-637.

Kessel, R. G., and C. Y. Shih. 1974. Scanning electron microscopy in biology. Springer-Verlag Publishers, Inc., New York.

Luftig, R. B., E. Wehrli, and P. N. McMillan. 1977. Life Sciences, 21:285-300.

Penso, G., and D. Balducci. 1963. Tissue cultures in biological research. Elsevier Publishing Co., New York.

Quesnel, L. B. 1969. Methods in microculture, p. 365-425. In J. R. Norris and D. W. Robbins (eds.). Methods in microbiology, Vol. 1, Academic Press, Inc., New York.

Rasmussen, H. 1975. Ions as 'second messengers', p. 203-212. In G. Weissman, and R. Clairborne (eds.), Cell membranes: biochemistry, cell biology and pathology. H. P. Publishing Co., Inc., New York.

Rees, D. A., C. W. Lloyd, and D. Thom. 1977. Control of grip and stick in cell adhesion through lateral relationships of membrane glycoproteins. Nature 267:124-128.

Rose, G. G. (ed.). 1963. Cinemicrography in cell biology. Academic Press, New York.

Vlodavsky, I. and Le Sachs. 1977. Difference in the calcium regulation of concanavalin A agllutinability and surface microvilli in normal and transformed cells. Exp. Cell Res. 105:179-189.

Weiss, L. 1972. Studies on cellular adhesion in tissue culture: XI Some effects of ouabain. Exp. Cell Res. 71:281-284.

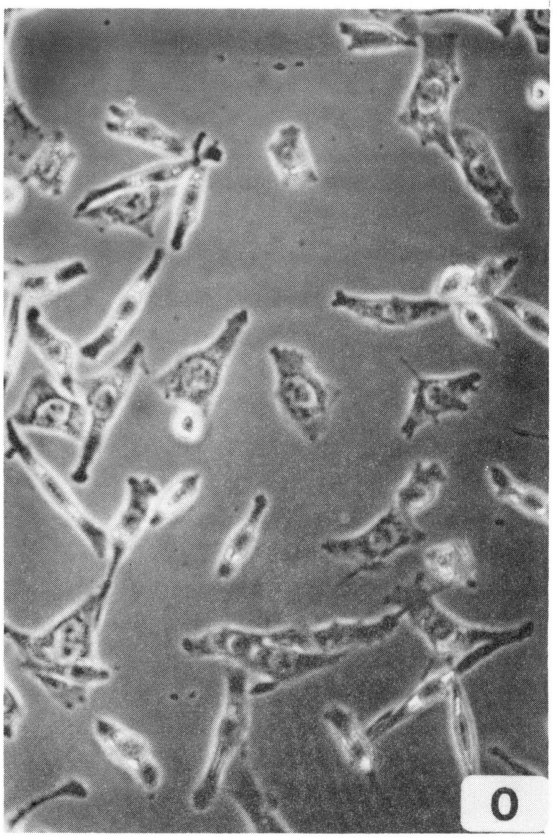
APPENDIXES

Figure 1. S-180 Cells Before Treatment

Figure 2. 1 mM EDTA - 5 Minutes of Treatment

Figure 3. 1 mM EDTA - 10 Minutes of Treatment

Figure 4. 1 mM EDTA - 23 Hours After Treatment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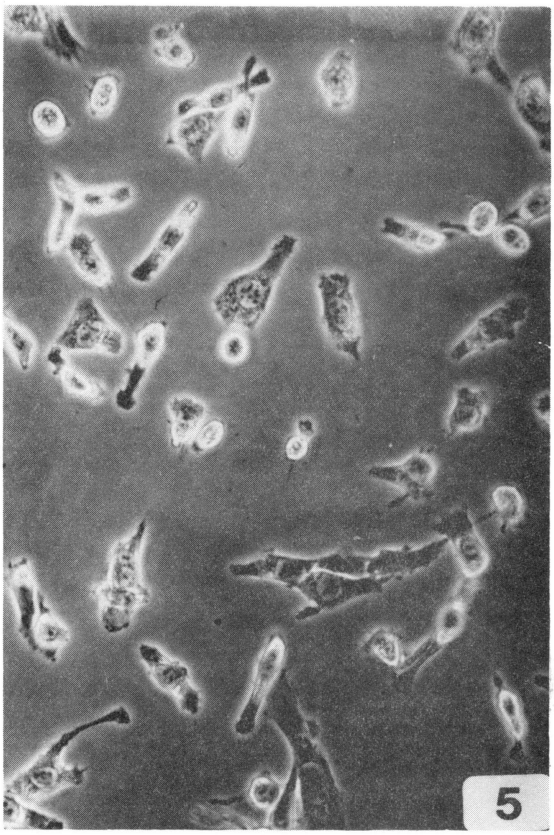
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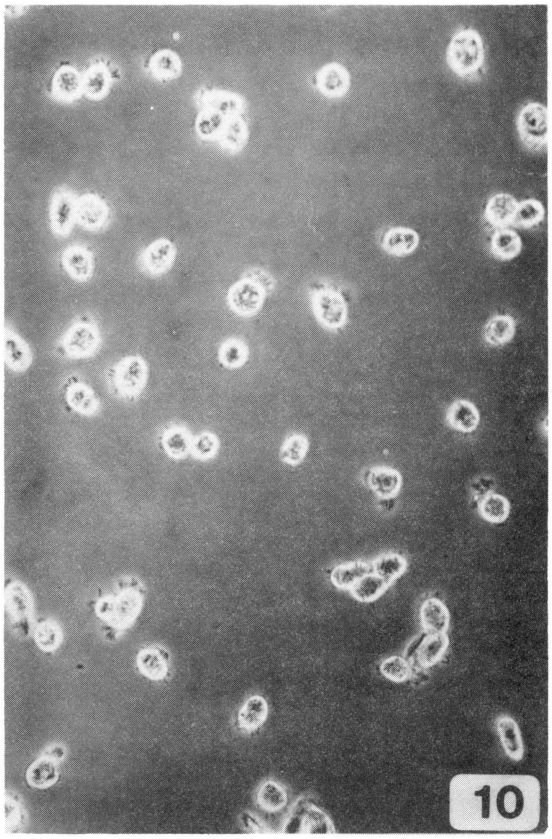
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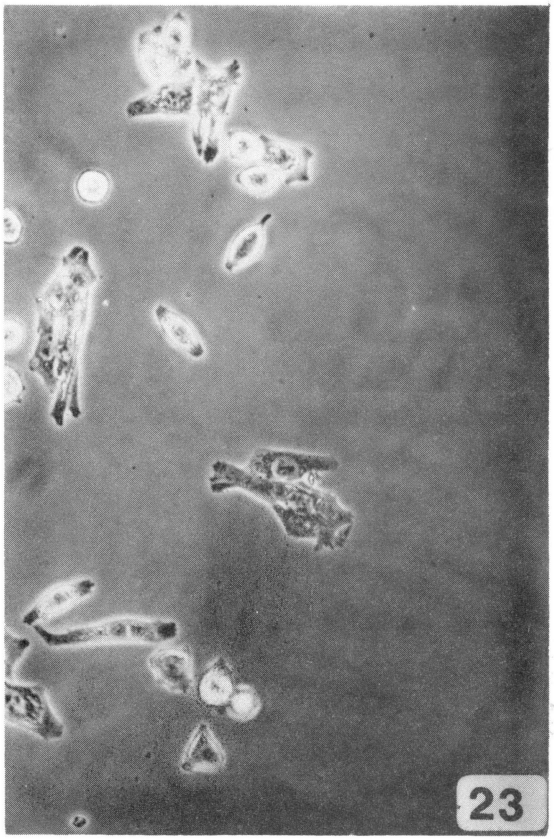
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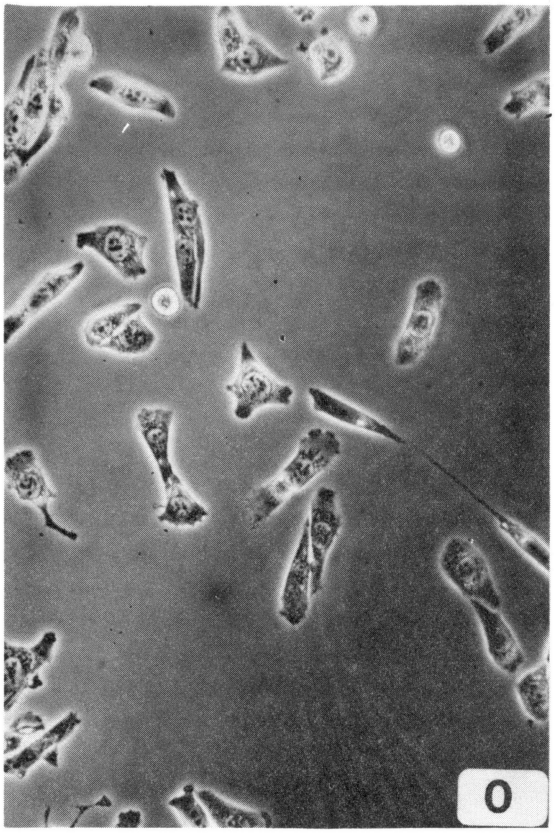
Figure 5. S-180 Cells Prior to Treatment

Figure 6. 5 µg/ml Trypsin (in HBS) - 5 Minutes of Treatment

Figure 7. 5 µg/ml Trypsin (in HBS) - 15 Minutes of Treatment

Figure 8. 5 µg/ml Trypsin (in HBS) - 60 Minutes of Treatment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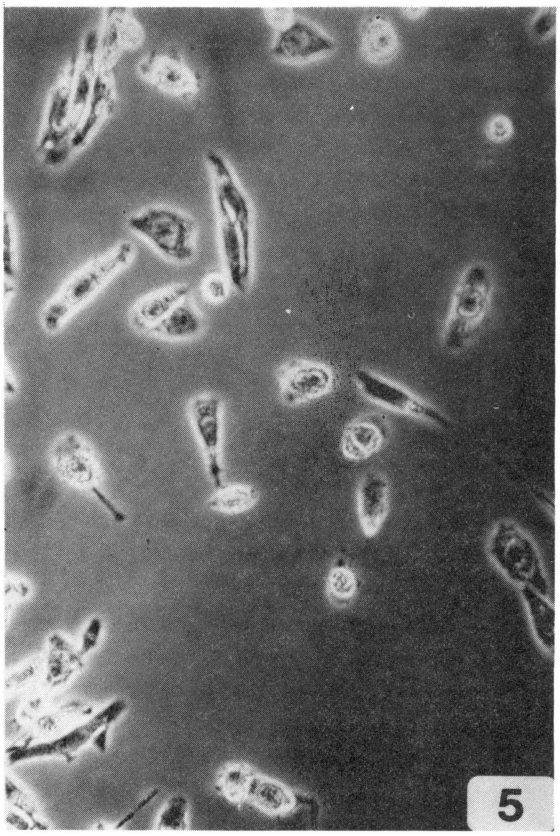
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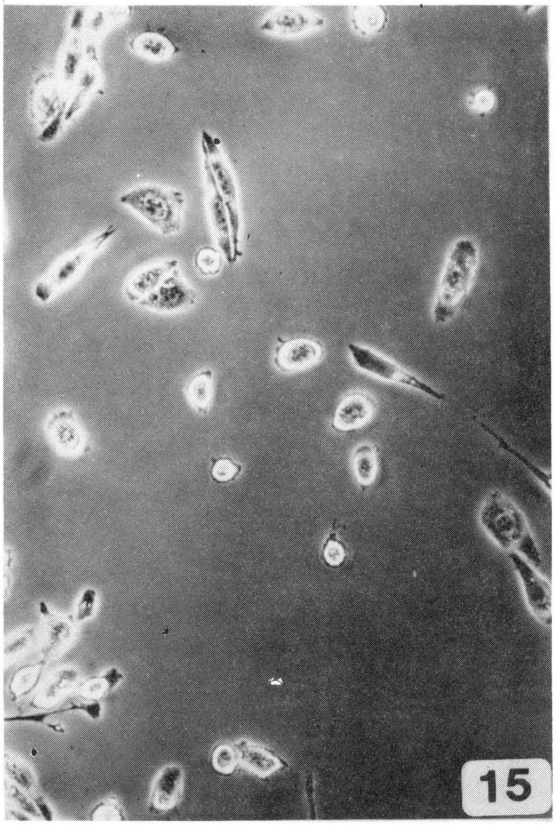
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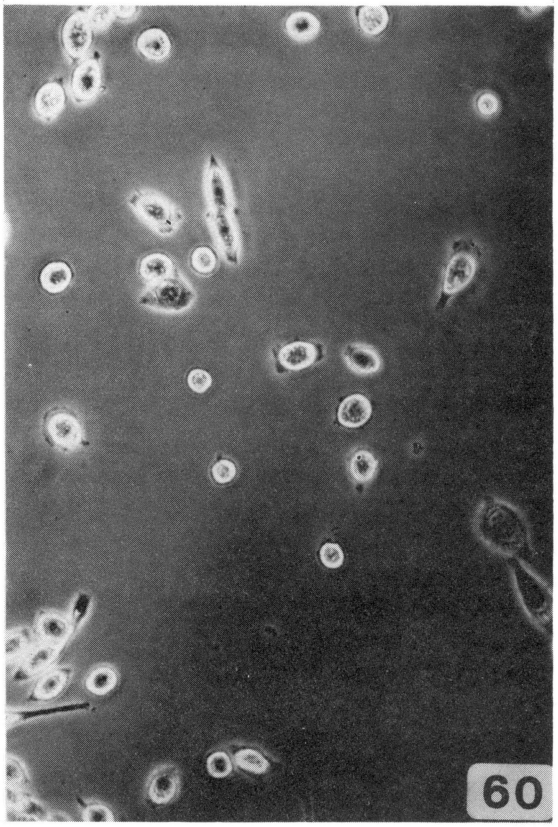
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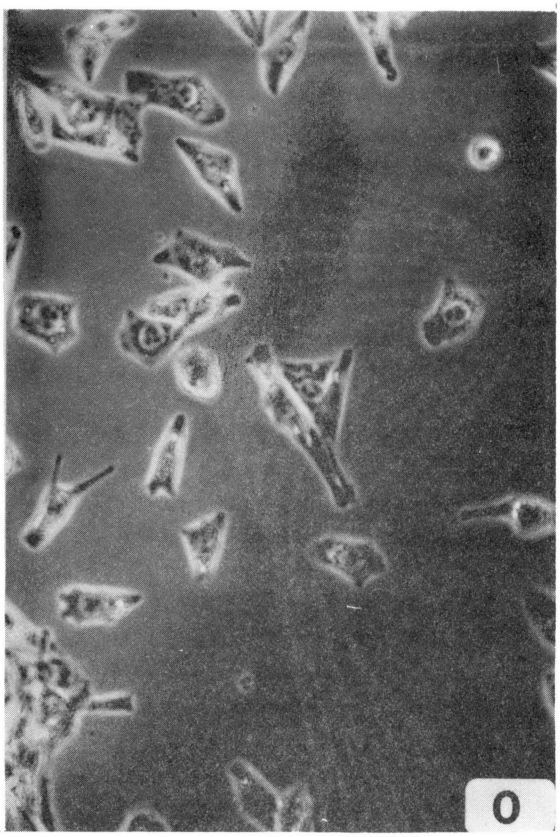
Figure 9. S-180 Cells Prior to Treatment

Figure 10. HBS Control - 6 Minutes of Treatment

Figure 11. HBS Control - 15 Minutes of Treatment

Figure 12. HBS Control - 30 Minutes of Treatment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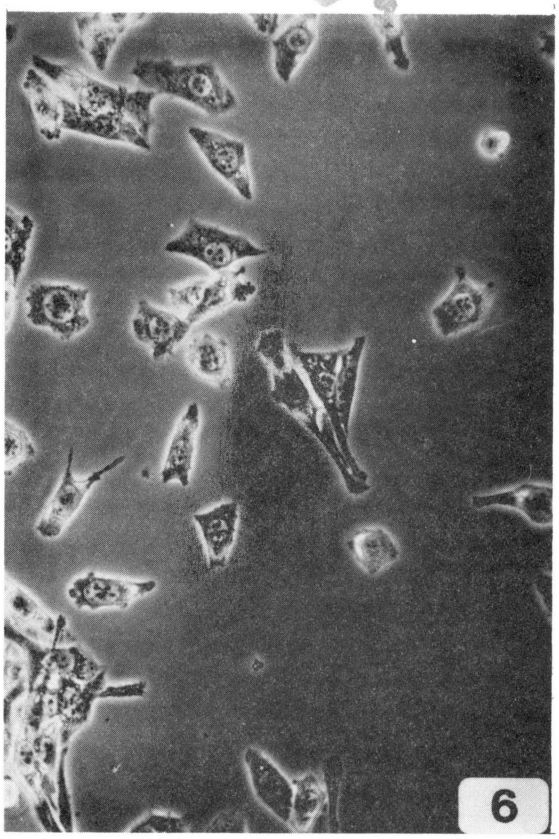
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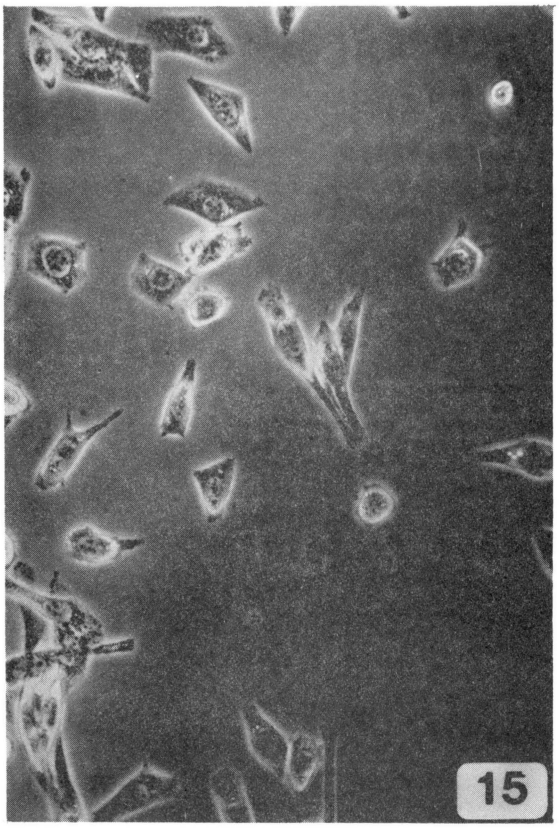
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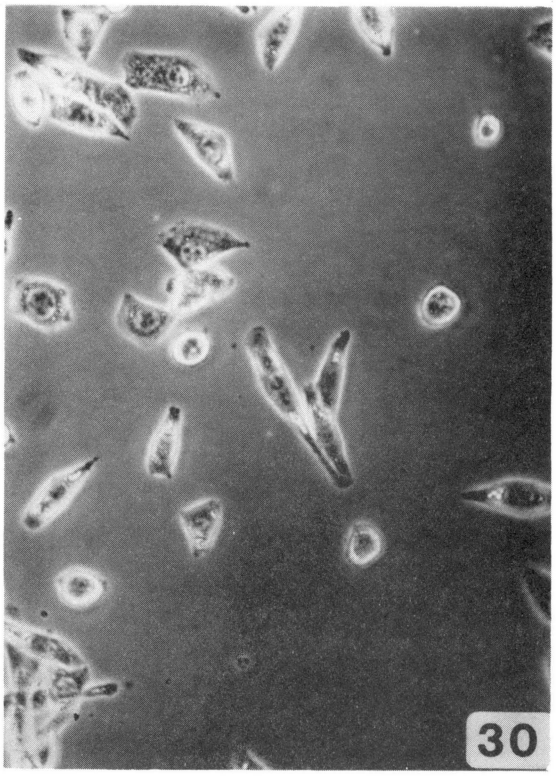
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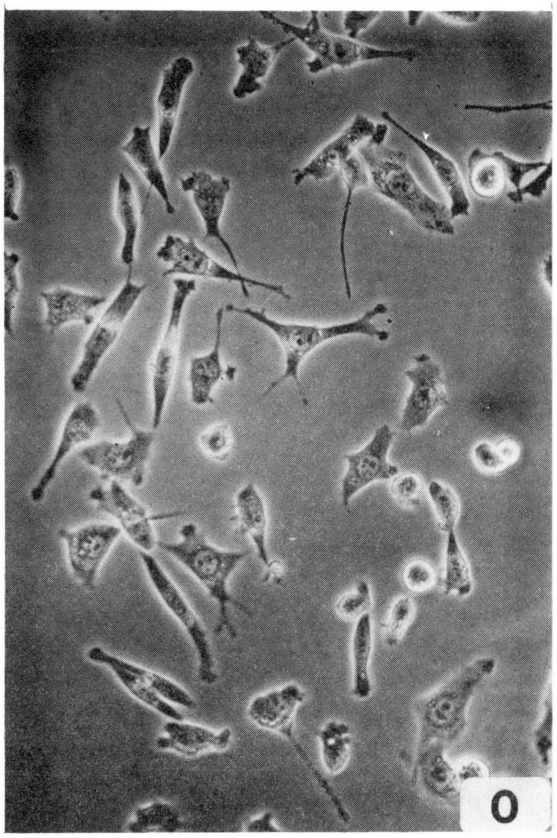
Figure 13. S-180 Cells Prior to Treatment

Figure 14. 1 µg/ml Colchicine (in HBS) - 6 Minutes of Treatment

Figure 15. 1 µg/ml Colchicine (in HBS) - 11 Minutes of Treatment

Figure 16. 1 µg/ml Colchicine (in HBS) - 15 Minutes of Treatment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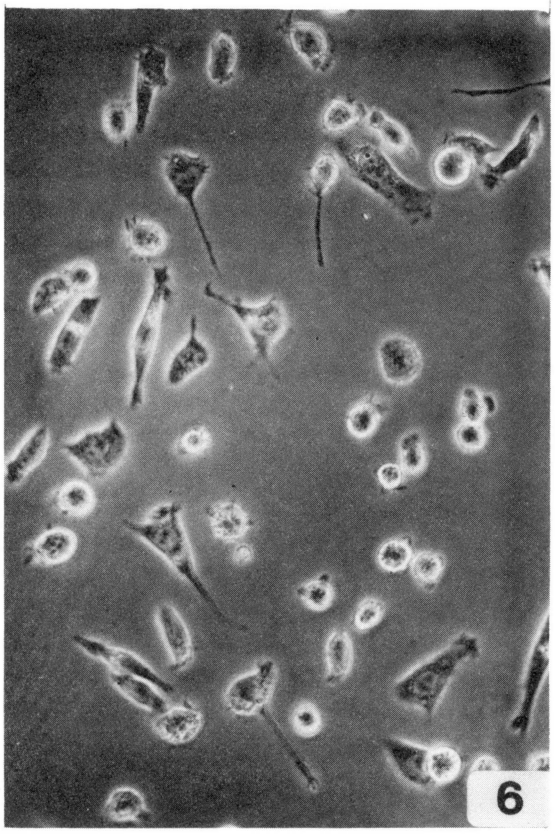
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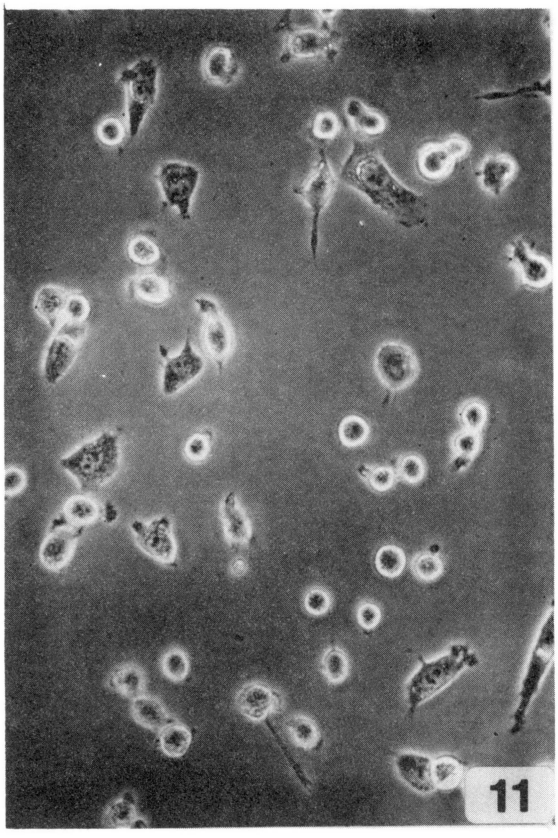
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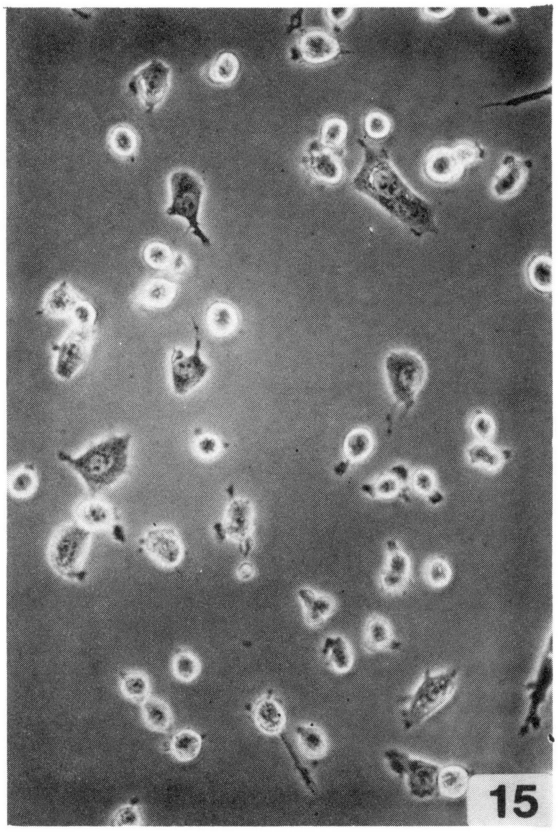
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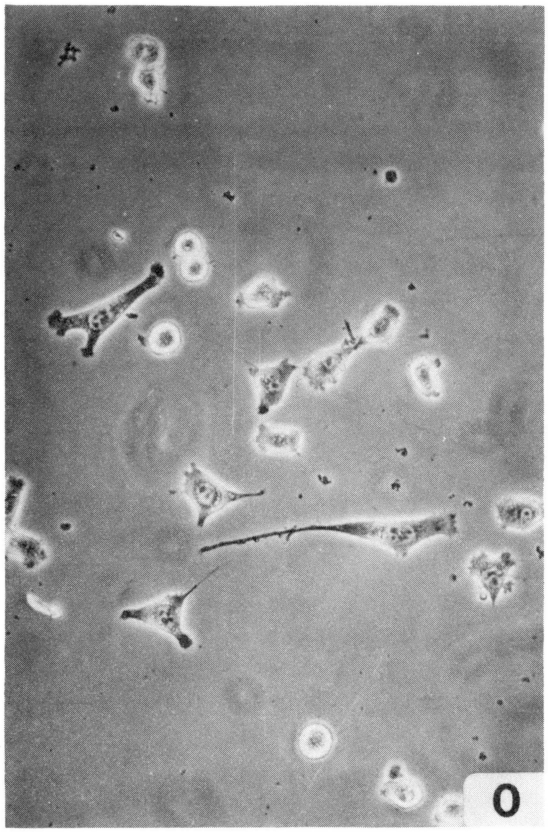
Figure 17. S-180 Cells Prior to Treatment

Figure 18. 1 mM EGTA (in HBS) - 1 Minute of Treatment

Figure 19. 1 mM EGTA (in HBS) - 8 Minutes of Treatment

Figure 20. 1 mM EGTA (in HBS) - 15 Minutes of Treatment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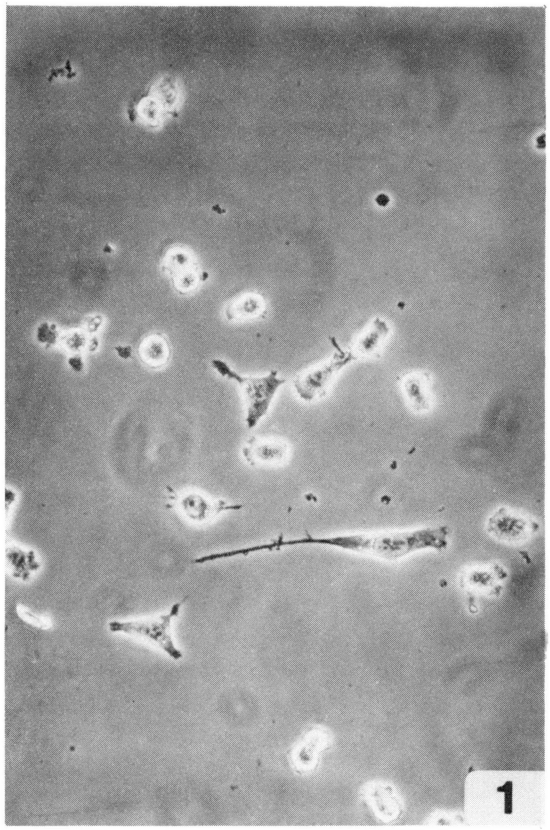
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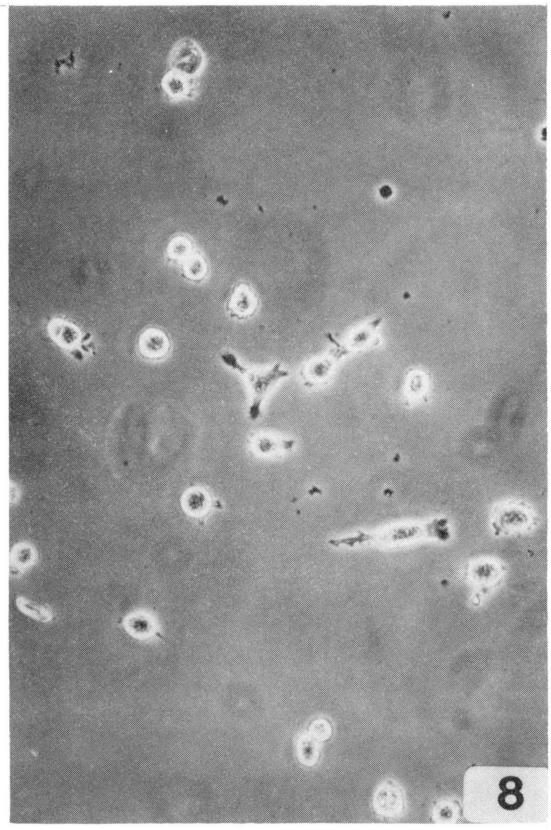
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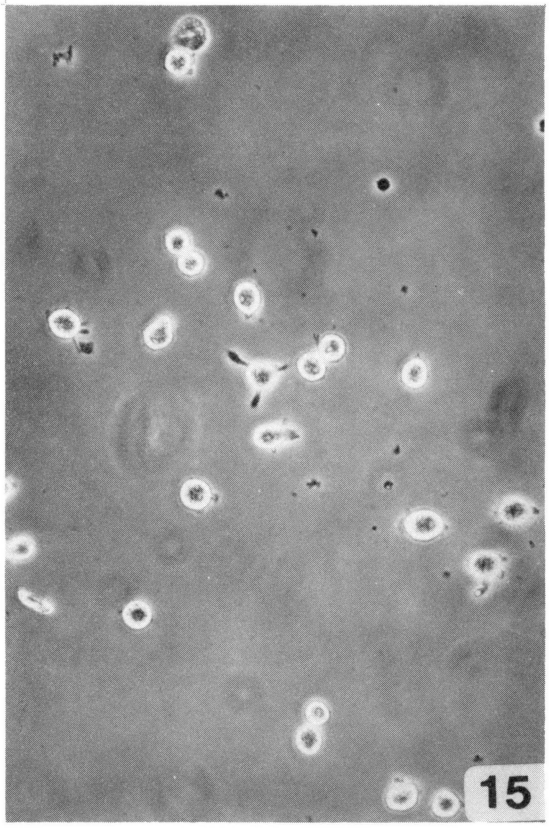
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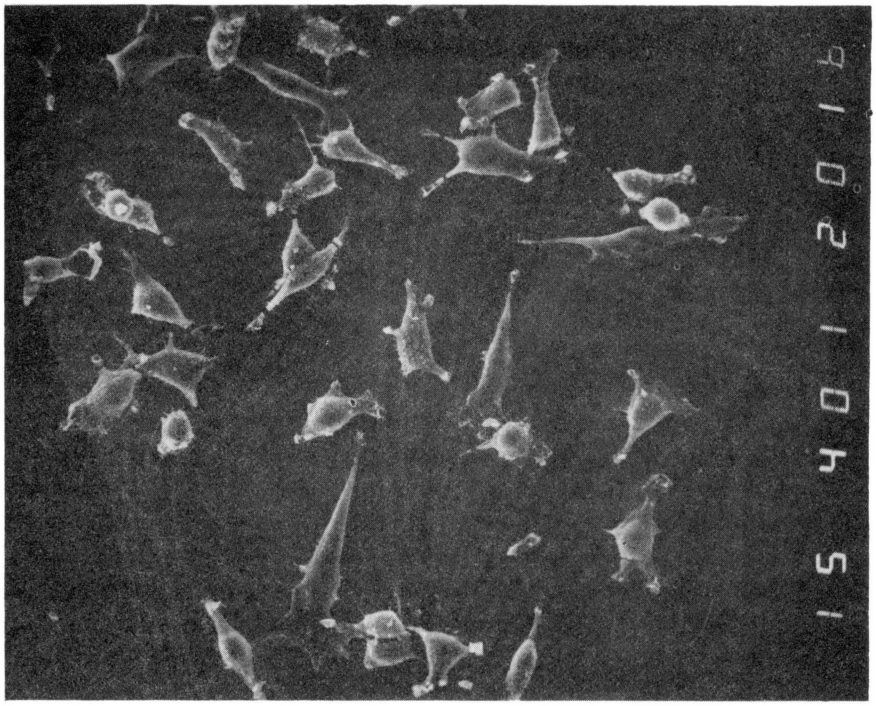
Figure 21. Growth Medium Control. Typical population of cells. X400.

Figure 22. Growth Medium Control. Individual cell. X1100.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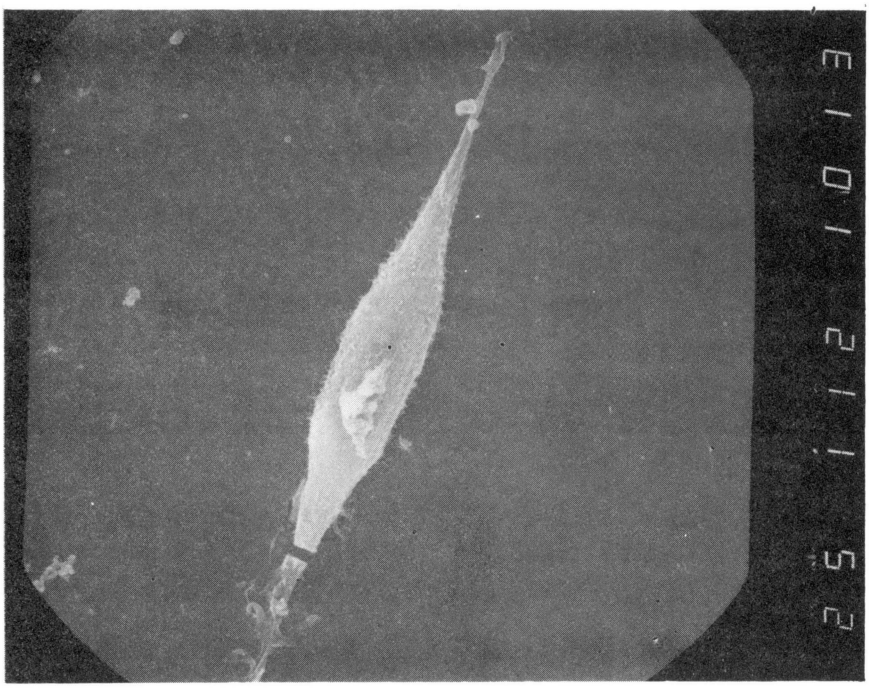
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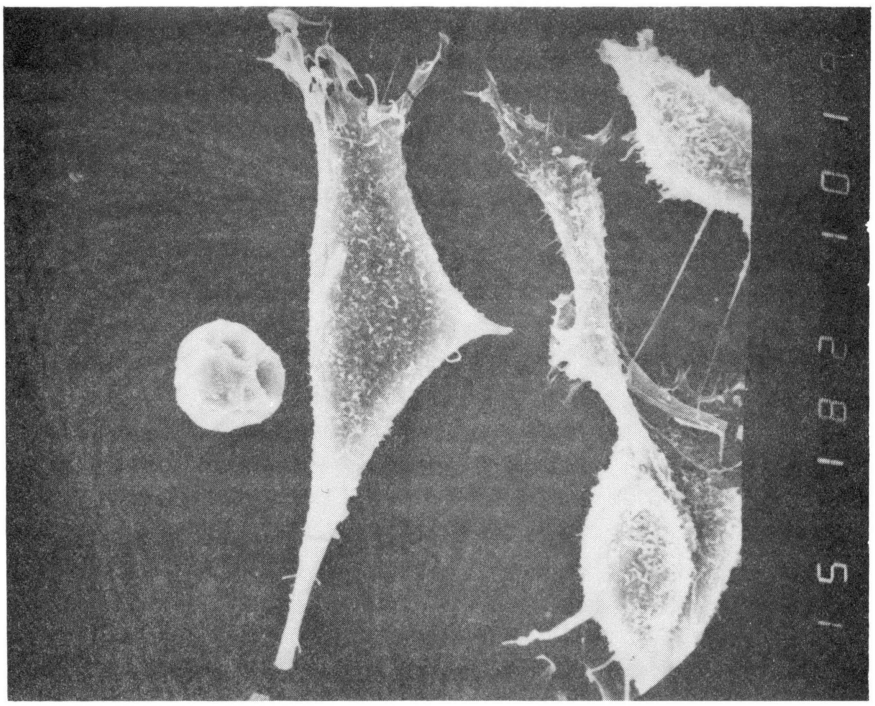
Figure 23. Hepes Buffered Saline Control. Cells after ten minutes of treatment. X1800.

Figure 24. 3 µg/ml Ionophore A23187 (in HBS + 0.06% DMSO). 15 minutes of treatment. Note ridges on cell surface. X4000.



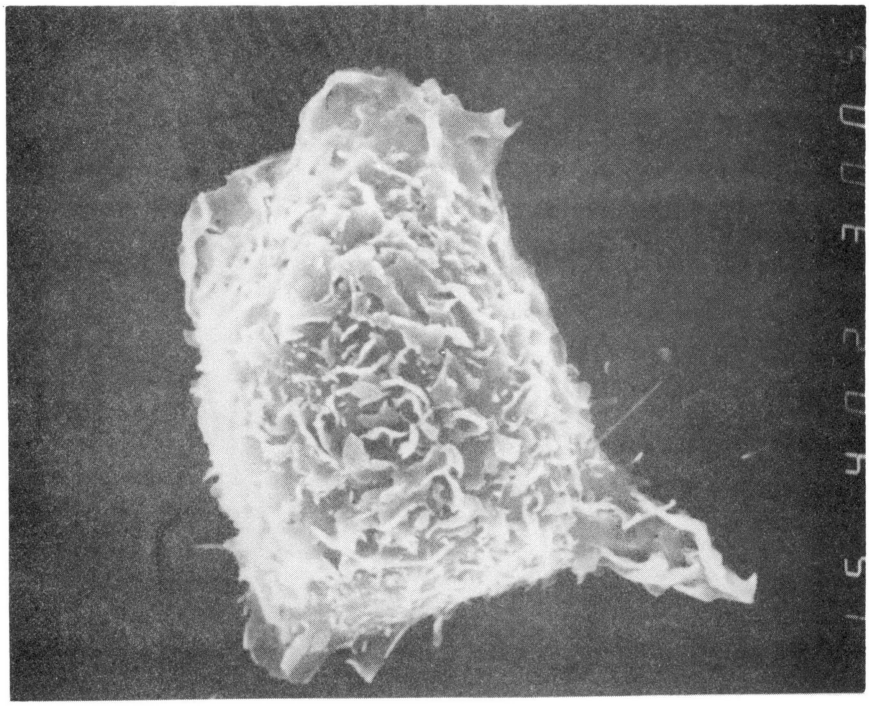
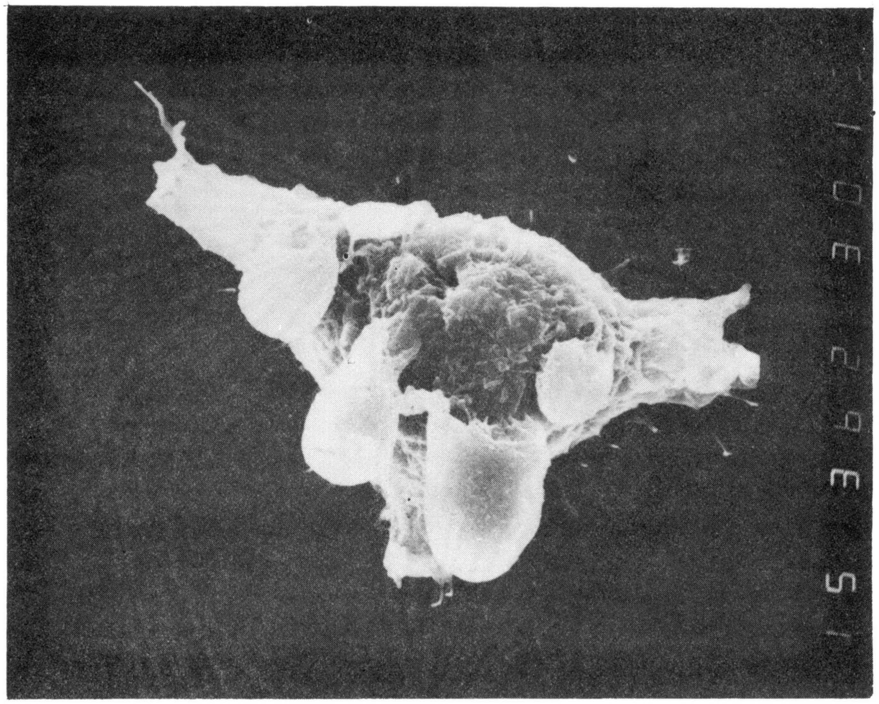


Figure 25. 3 µg/ml Ionophore A23187 + 1 mM $CaCl_2$ (in HBS + 0.06% DMSO). The cell has been treated for fifteen minutes. X3600.

Figure 26. 3 µg/ml Ionophore A23187 + 1 mM $CaCl_2$ (in HBS + 0.06% DMSO). Treated cell after fifteen minutes. Note cell debris in background. X1600.



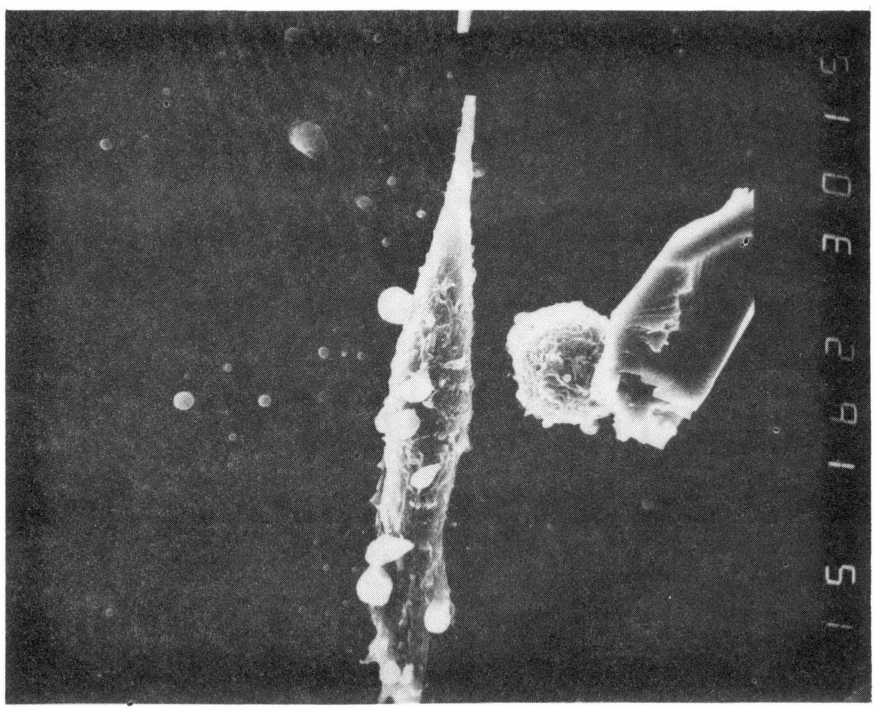
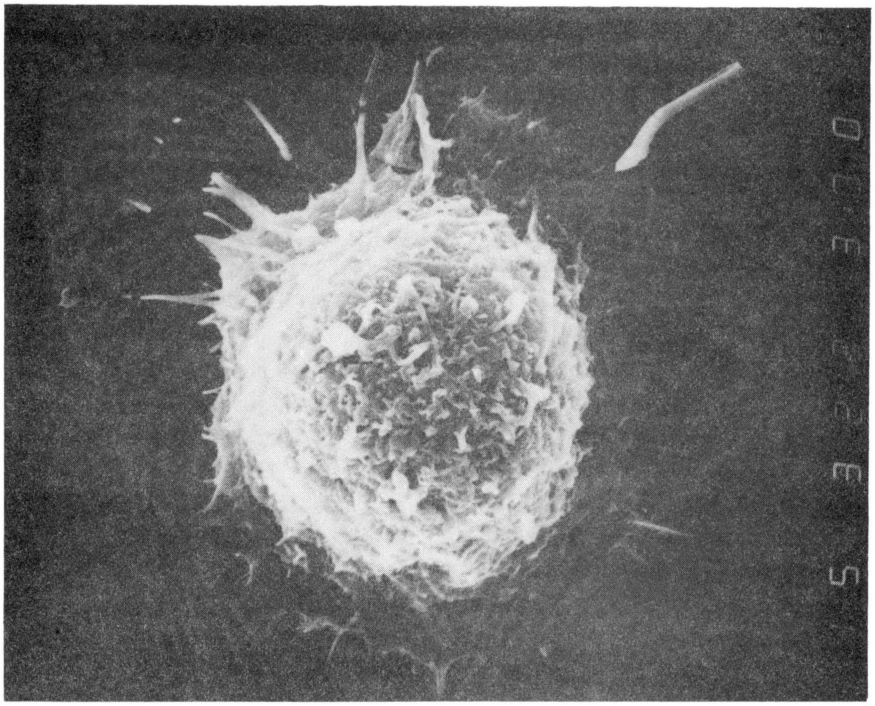


Figure 27. 3 µg/ml Ionophore A23187 + 1 mM EGTA (in HBS + 0.06% DMSO). Cell treated for five minutes. X3200.

Figure 28. 3 µg/ml Ionophore A23187 + 1 mM EGTA (in HBS + 0.06% DMSO). Cell treated for fifteen minutes. X3200.



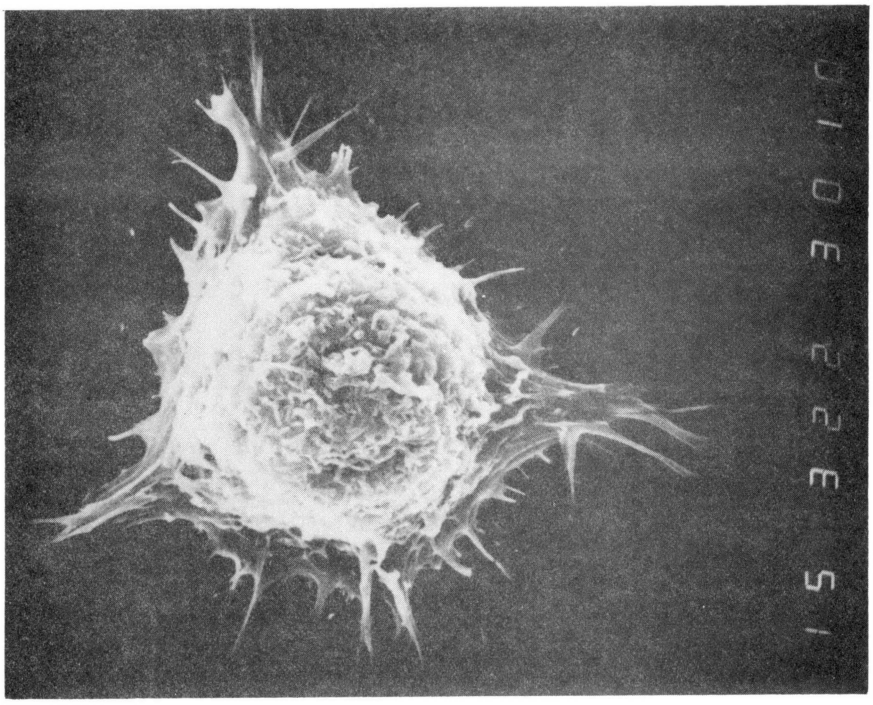
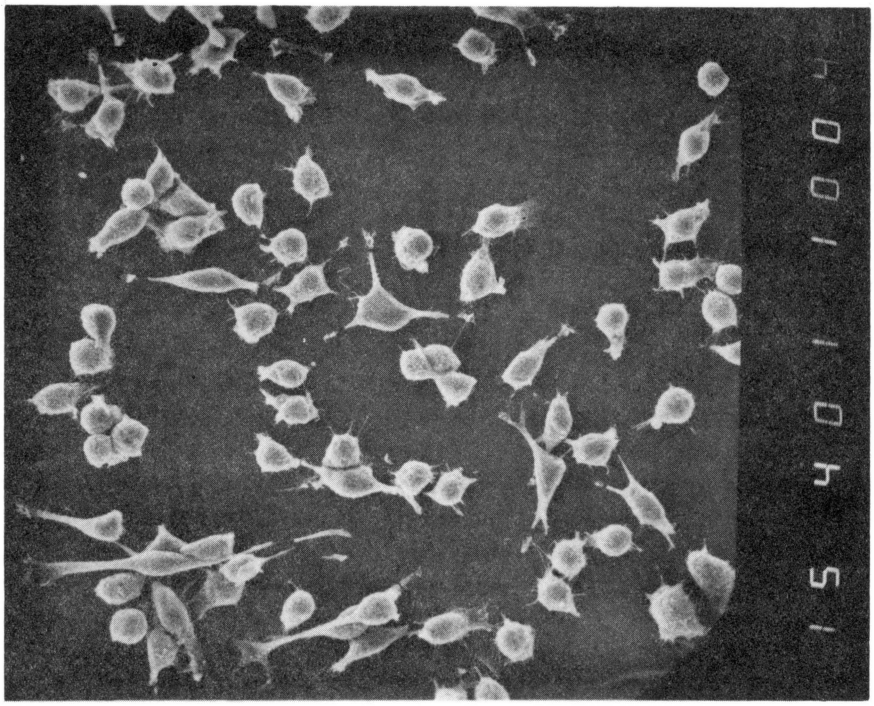


Figure 29. 1 mM EDTA (in HBS). Population of cells after four minutes. X400.

Figure 30. 1 mM EDTA (in HBS). Individual cell treated for four minutes. X4000.


IS 40101004

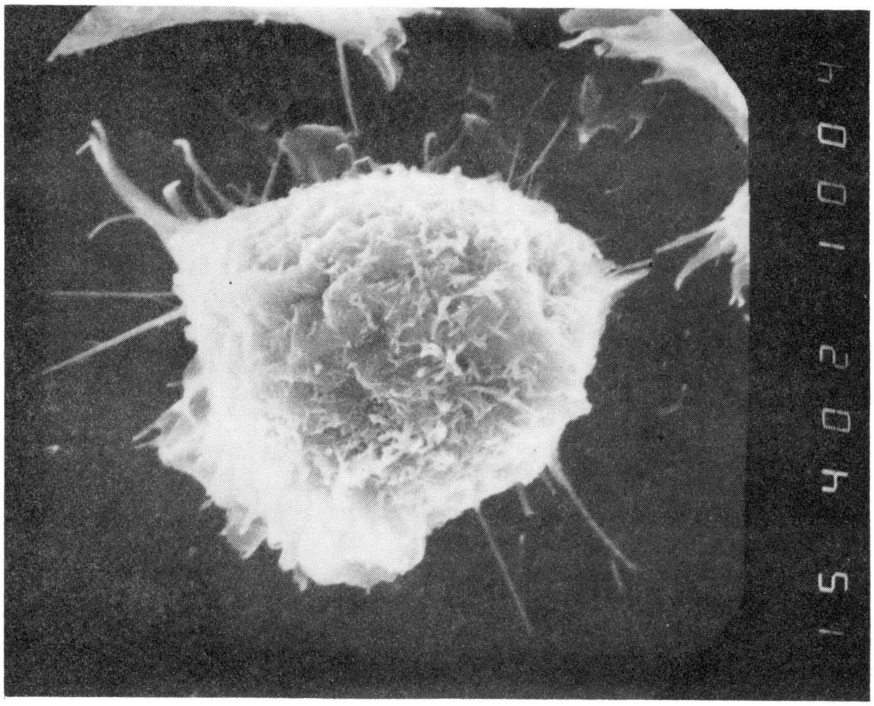
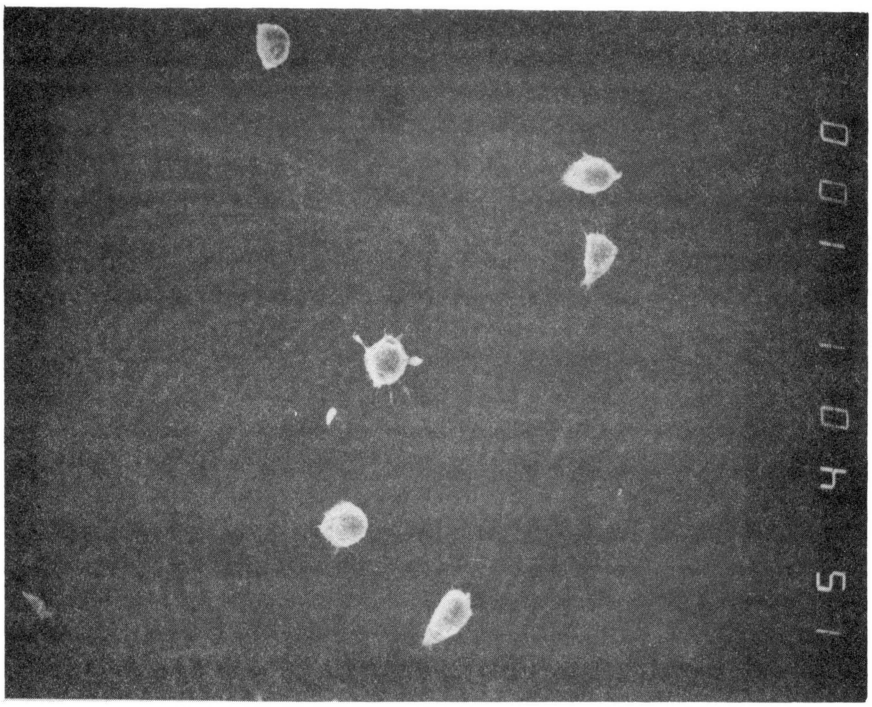

IS 40201004

Figure 31. 1 mM EDTA (in HBS). Population of cells after fifteen minutes of treatment. X400.

Figure 32. 1 mM EDTA (in HBS). Single cells treated for fifteen minutes. X4000.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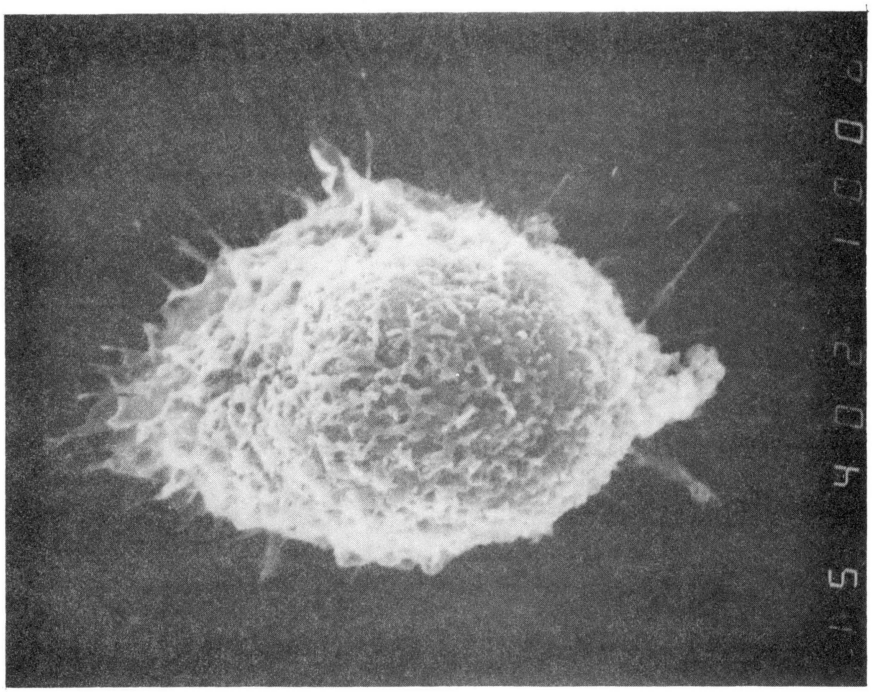
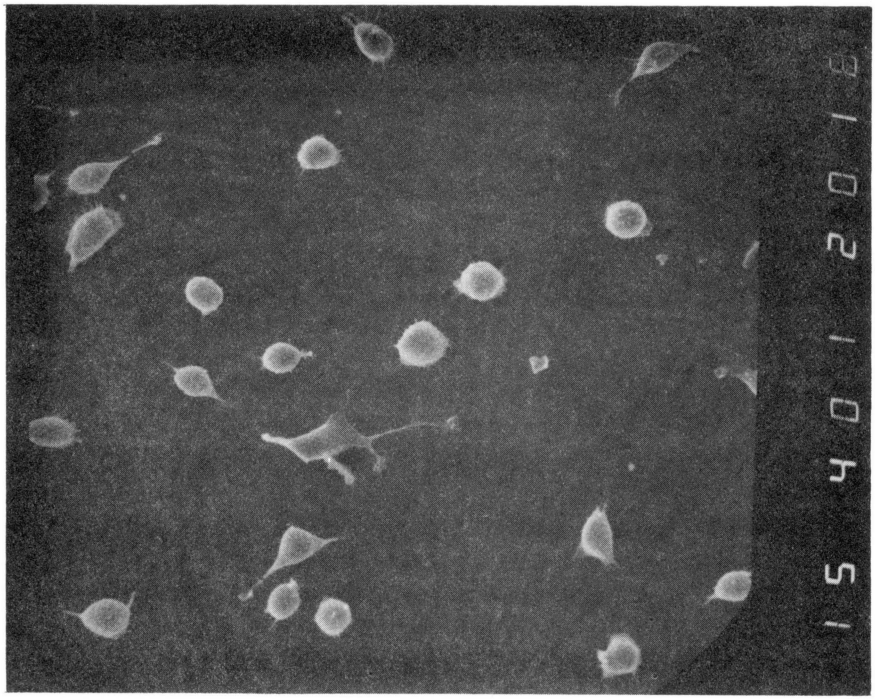

15 40 20 00

Figure 33. 1 mM EGTA (in HBS). Population of cells treated for fifteen minutes. X400.

Figure 34. 1 mM EGTA (in HBS). Individual cell treated for fifteen minutes. X4000.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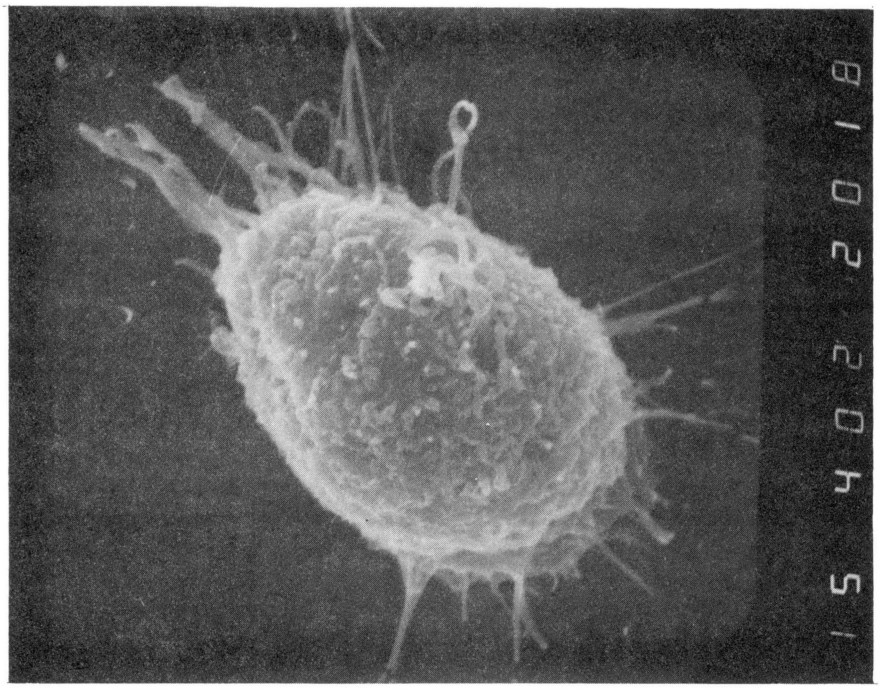
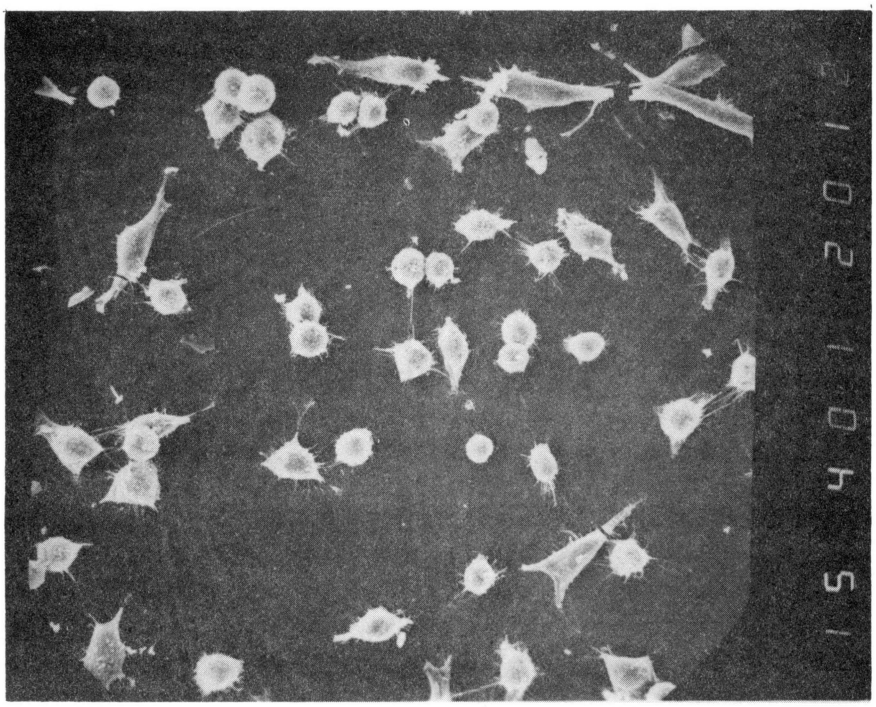

15 40 20 18

Figure 35. 1 mM $CaCl_2$ (in HBS). Population of cells treated for fifteen minutes. X400.

Figure 36. 5 mM $CaCl_2$ (in HBS). Cell treated for five minutes. X3600.



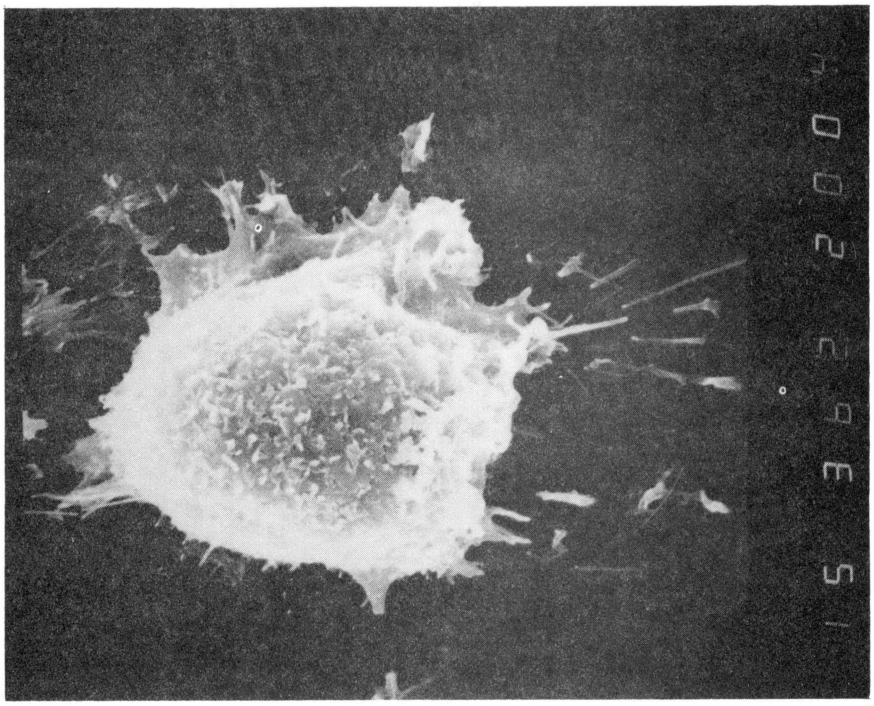
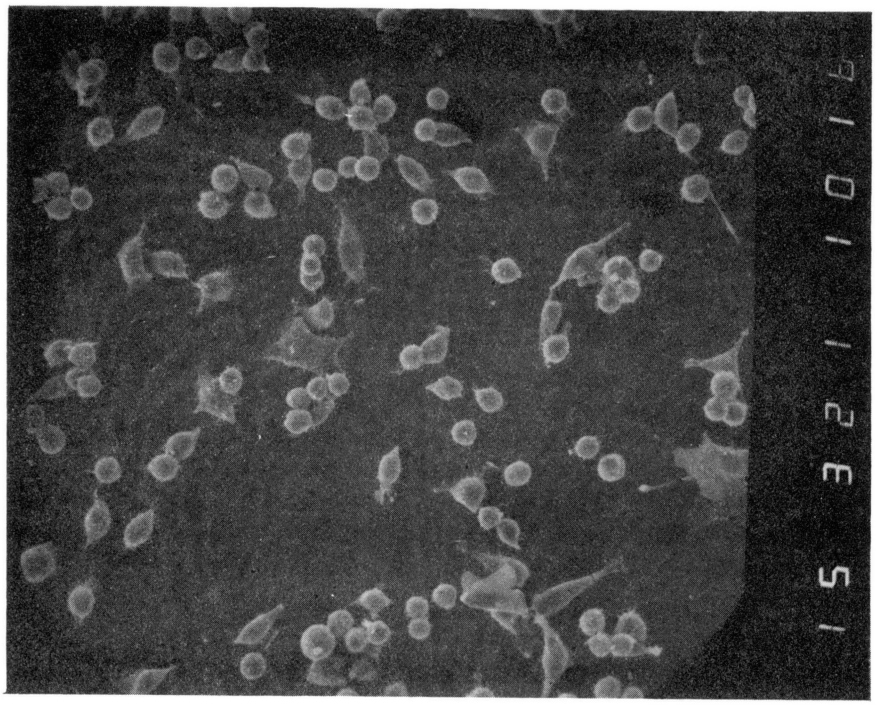


Figure 37. 5 µg/ml Trypsin (in HBS). Population of cells treated for fifteen minutes. X320.

Figure 38. 5 µg/ml Trypsin (in HBS). Individual cell treated for fifteen minutes. X4000.


S1 E2 1101 6

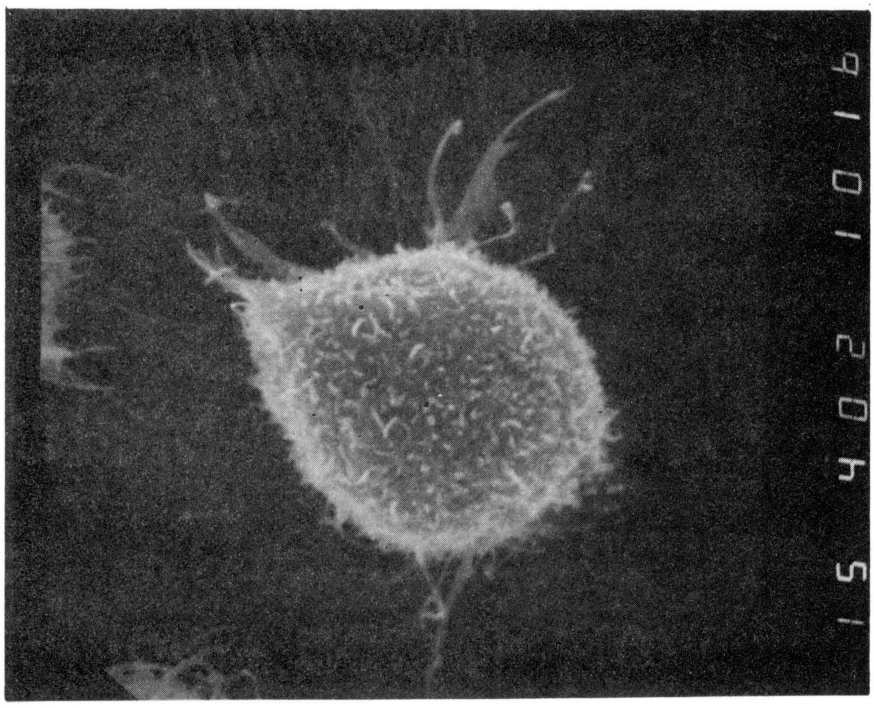
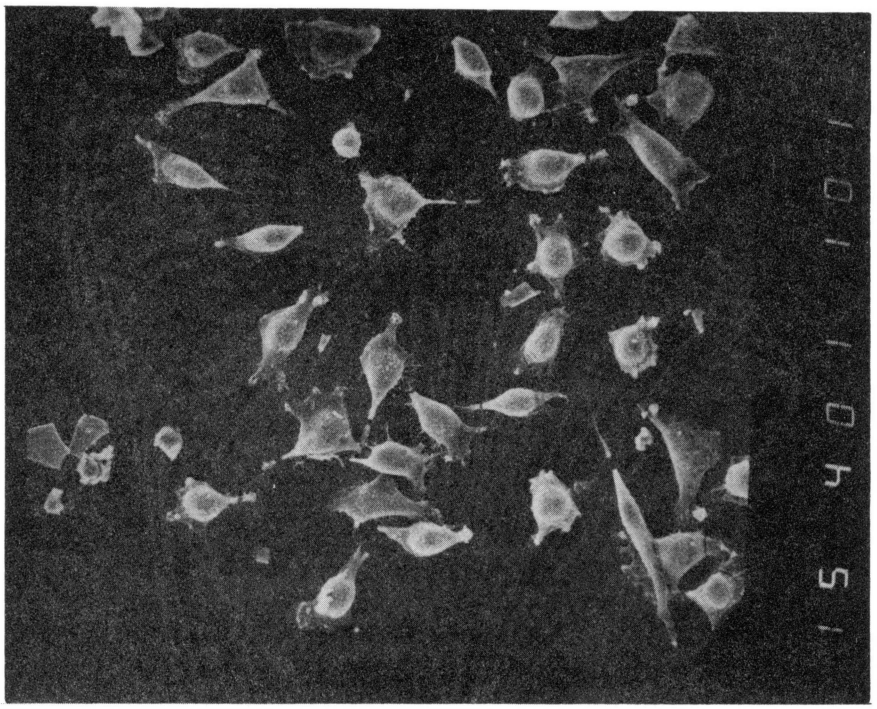
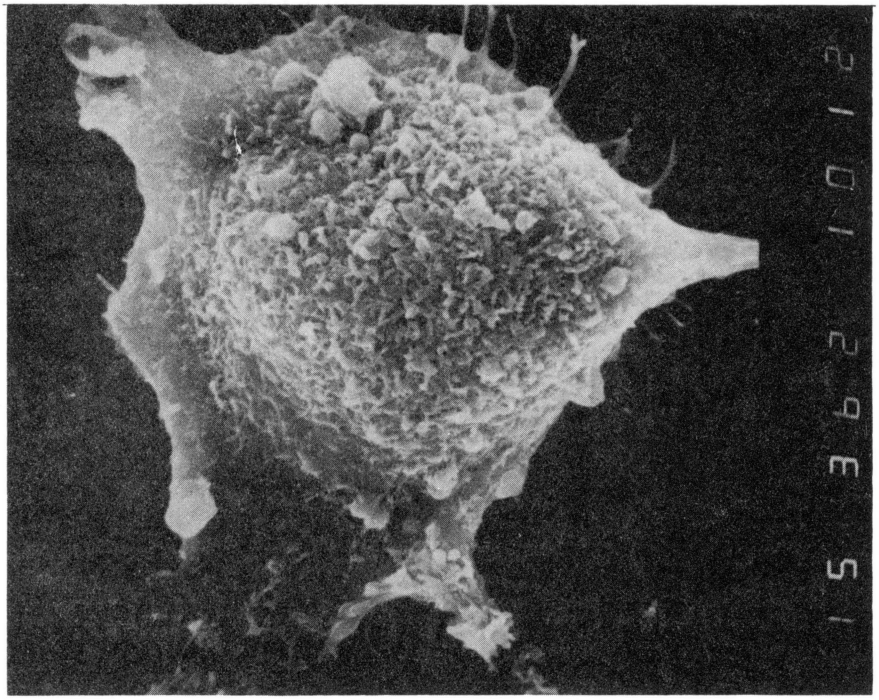

S1 4D 2D 1016

Figure 39. 10 mM Azide (in HBS). Population of cells treated for fifteen minutes. X400.

Figure 40. 10 mM Azide (in HBS). Cell treated for fifteen minutes. X3600.





VITA

Sandra Cantwell McGuire

Candidate for the Degree of

Master of Science

Thesis: MORPHOLOGICAL CHANGES IN S-180 TUMOR CELLS INDUCED BY VARIOUS PERTURBING AGENTS AS DETERMINED BY USING TIME-LAPSE CINEPHOTOMICROGRAPHY AND SCANNING ELECTRON MICROSCOPY

Major Field: Microbiology

Biographical:

Personal Data: Born in Oklahoma City, Oklahoma, July 10, 1952, the daughter of Thomas L. and Loretta D. Cantwell. Married Wayne R. McGuire, December 30, 1976.

Education: Graduated from Southeast High School, Oklahoma City, Oklahoma, in May, 1970; received the Bachelor of Science degree in Microbiology from Oklahoma State University in 1975; completed the requirements for the Master of Science degree in Microbiology at Oklahoma State University in December, 1977.

Professional Experience: Research assistantship in Biochemistry at Oklahoma State University from January, 1977, to December, 1977.

Membership in Professional Organizations: American Society for Microbiology, and Oklahoma Academy of Science.