Effects of the Antineoplastic Alkaloid Acronycine on the Ultrastructure and Growth Patterns of Cultured Cells

Ping Tan and Nelly Auersperg

Cancer Research Centre and Department of Zoology, University of British Columbia, Vancouver 8, British Columbia, Canada

SUMMARY

Acronycine, a lipophilic, antineoplastic alkaloid, was found to cause swelling and destruction of Golgi complexes and, less consistently, of mitochondria, in suspension cultures of L5178Y murine leukemia cells and in cell layer cultures of cervical carcinoma (C-4 II), melanoma (HFH-18), and SV40-induced hamster tumor cells. Delayed effects of the drug include cellular swelling, binucleation, reduced adhesion to substrata and to other cells, changes in colonial morphology, interference with melanosome dispersion, and cessation of mitotic activity by viable cells at a reduced cell density. The cytological effects of acronycine differ from those of cyclic adenosine 3',5'-monophosphate and also, at least chronologically, from those of cytochalasin B. It would appear that the alkaloid acts primarily on membranous organelles and that its delayed effects might be due, at least in part, to interference with the structure, function, and/or turnover of cell-surface components.

INTRODUCTION

Acronycine is an alkaloid with chemical features unrelated to those of currently used antitumor agents (10). It is effective against a variety of experimental neoplasms (18), but its particular interest lies in its capacity to inhibit the growth of some solid tumors that are resistant to more established kinds of chemotherapy (14). In suspension cultures of L5178Y murine leukemia cells, acronycine inhibits the incorporation of uridine and thymidine into nucleic acids (10) but, as shown in the preceding paper (6), the drug does not directly interfere with RNA or DNA synthesis. Rather, it appears to inhibit the entry of extracellular nucleosides into the intracellular precursor pools. Information regarding cytological effects of acronycine has been limited to the observations that, unlike the Vinca alkaloids, the drug does not cause metaphase arrest in monolayer cultures (18) and that, in suspension cultures of leukemia cells, it inhibits population growth and causes binucleation through interference with cytokinesis (10).

This report describes the cytological and ultrastructural changes in suspension cultures of L5178Y mouse leukemia cells that are induced in vitro by acronycine under conditions identical to those used by Dunn et al. (6), as well as the effects of acronycine on the growth patterns on solid substrata and on the ultrastructure of the carcinoma cell line (C-4 II (1), the melanoma line HFH-18 (12), and line SV/HT, derived from an SV40-induced hamster tumor. Because acronycine causes binucleation in L5178Y cells in a manner reminiscent of cytochalasin B (5), a comparison was made of the effects of the 2 drugs on the growth pattern in cultures of line C-4 II and on the contraction of suspended sheets of C-4 II cells in vitro, a process previously shown to be cytochalasin B sensitive (2). Line HFH-18 was chosen in particular for observation of effects of acronycine on cell pigmentation, and the SV/HT line was chosen because its rapid growth rate was more comparable to that of the L5178Y leukemia cells than were the slower proliferative rates of the other 2 lines. Initial observations suggested that acronycine caused growth inhibition at a reduced cell density, as well as a change from epithelial to fusiform cell shapes. Since these changes had both been reported previously as responses of cultured cells to cyclic 3',5'-AMP (11), the effects of this compound on cell growth were compared to those of acronycine in all 3 cell lines.

The ultrastructural and cytological observations in this study indicate that acronycine alters membranous organelles, and they suggest further that the delayed effects of the drug may be due, at least in part, to changes in the region of the cell surface.

MATERIALS AND METHODS

Chemicals

Acronycine (a gift from Eli Lilly and Company, Indianapolis, Ind.) was dissolved in ethanol at a concentration of 12.0 mg/ml. Appropriate amounts of this stock solution were added to vigorously stirred culture medium immediately prior to experimentation. Cytochalasin B (a gift from Dr. S. B. Carter, Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England) was added to culture media as a stock solution of 1.0 mg of dimethyl sulfoxide. Dibutyryl cyclic 3',5'-AMP was purchased from Schwarz/Mann (Orangeburg, N. Y.).

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Cell Culture

Maintenance

L5178Y murine lymphocytic leukemia cells (9) were grown in suspension culture (rotation at 4 rpm) in Fischer's medium supplemented with 10.0% horse serum, 50 units of penicillin per ml, and 50 µg of streptomycin per ml. Stationary cell layer cultures of human cervical carcinoma line C-4 II (1), mouse melanoma line HFH-18 (12), and SV/HT, a line of SV40-induced hamster tumor cells (Catalog No. TT 101; Flow Laboratories, Inc., Rockville, Md.) were grown in Waymouth's Medium 752/1 supplemented with 10% fetal calf serum, 100 units of penicillin per ml, and 100 µg of streptomycin per ml. They were subcultured periodically with 0.12% crystalline trypsin/Ca++- and Mg++-free balanced salt solution.

Experimental

L5178Y Cells. Acronycine was added to suspension cultures to a final concentration of 6.0 µg per ml of medium. The cells, at an initial concentration of 300 x 10^3/ml, were rotated at 4 rpm at 37°, and after 1, 2, 4, 6, 8, and 24 hr, samples were fixed for electron microscopy. Concurrently, cell density was determined by hemocytometer counts, the percentage of binucleated cells was determined by examination of smears, and incorporation of 3H-labeled uridine was determined as described previously (10), for correlation of the ultrastructural changes with the biochemical effects of the drug (6).

Lines C-4 II, HFH-18, and SV/HT. For light microscopic examination of growth patterns and cytology, cultures were grown on glass coverslips and, for electron microscopy of cells fixed in situ, they were grown on polyester plastic sheeting (8). Series of culture tubes in which cells had not yet reached confluency were divided into groups with culture media that contained 1 of the following: (a) no additive (control group); (b) 3, 6, 12, or 24 µg of acronycine per ml; (c) 10 mM dibutyryl cyclic 3',5'-AMP; or (d) 1.0 or 10.0 µg cytochalasin B per ml (line C-4 II only). Media were changed daily for 3 days, and cultures were fixed for light and electron microscopy after 24, 48, and 72 hr.

For comparison of the effects of acronycine and cytochalasin B on the contraction of suspended epithelial sheets in vitro (2), fragments of confluent C-4 II cultures were detached from the substratum, centrifuged briefly, and resuspended in fresh medium, with either (a) no additives (control); (b) 10.0 µg of cytochalasin B per ml; or (c) 6, 12, or 24 µg of acronycine per ml. The cultures were incubated for up to 24 hr in culture dishes that had been coated with 1% agar to prevent readhesion of the cells and were photographed at intervals; samples were fixed for electron microscopy after 5 and 22 hr.

Cytology

Smears from L5178Y cultures were fixed with 100% methanol and stained with Wright's stain. Cultures of lines C-4 II, HFH-18, and SV/HT were fixed in Carnoy's fixative and stained with 0.25% toluidine blue-Veronal acetate buffer at pH 4.5. Mitotic indexes and the percentage of binucleated cells were estimated in these preparations by differential counts in at least 6 microscopic fields in replicate cultures (3000 to 4000 cells total) for each sample.

Electron Microscopy

Cultures grown on plastic sheeting were fixed in 2.5% glutaraldehyde in Millonig's buffer (16), pH 7.3, at room temperature for 30 min, postfixed with 1% OsO4 for 15 min, and further processed as described previously (1). L5178Y cells were treated identically, except that these cells were collected by centrifugation prior to fixation and were embedded in agar following OsO4 fixation. For light-microscopic examination of plastic-embedded material, sections 0.2 to 0.5 µm thick were stained with 1% toluidine blue-1% borax.

RESULTS

L5178Y Suspension Cultures. In these experiments, 6 µg of acronycine per ml completely inhibited population growth within 2 hr, reduced uridine incorporation by about 60% in the 1st hr, and resulted in a gradual increase in the proportion of binucleated cells from 4 hr on, in a manner comparable to the effects observed by Gout et al. (10).

Light microscopically, the shape of the control cells was rather irregular, and most nuclei appeared indented, with single large nucleoli on the concave side (Fig. 1). In response to acronycine, the shape of the cells became progressively rounder, suggesting cell swelling. After 4 hr of treatment, some cytoplasmic vacuoles appeared, and the number of cells with this feature increased on prolonged incubation. Binucleated cells appeared after 4 hr of treatment and, in smears, the nuclei of these cells appeared to be of equal and normal size, usually with a single nucleolus (Figs. 2 and 3). At 24 hr, most of the cells were dead or disintegrating (Fig. 4).

Electron microscopically, the cytoplasm of the control cells contained numerous mitochondria and polysomes, occasional fat droplets and vacuoles, and few microfilaments, Golgi complexes, and strands of rough endoplasmic reticulum. Most of the particulate organelles, as well as centrosomes and microtubules, were concentrated in the region of the nuclear indentation. The nuclear chromatin was dispersed, and 1 large nucleolus was usually located close to the nuclear membrane on the side of the indentation. The plasma membrane was irregular in outline, with some microvilli, and otherwise was unremarkable.

Acronycine exerted its most striking effects on the Golgi complexes and mitochondria, while causing less definite and less consistent alterations of plasma membranes. The Golgi complexes were inconspicuous in untreated cells, and consisted of 4 to 6 flat stacked cisternae, with some coated vesicles present in the adjacent regions (Fig. 5). After only 1 hr of acronycine treatment, the Golgi complexes were larger, with dilated cisternae and some membrane breakdown (Fig. 6). This damage and swelling ap-
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appeared to be progressive and, at hr 4, 6, 8, and 24, were so advanced that the Golgi complexes often appeared as aggregates of vesicles, sometimes with little discernible membrane material (Figs. 7 to 9). Coated vesicles, dilated smooth-walled vesicles, and some lipid droplets were usually found in the region of the damaged Golgi bodies, but their number did not seem to differ much from that of the controls.

The mitochondria in control cells were of average size, varied in shape, and usually possessed a few well-formed cristae in a dense matrix (Fig. 10). Acronycine produced progressive mitochondrial swelling which corresponded closely, both in location and timing, to the increasing cytoplasmic vacuolation observed light microscopically. This change varied in intensity among cells after the same period of treatment, although it tended to be similar in all mitochondria of any single cell. After 1 hr of acronycine treatment, changes in mitochondria were inconsistent, but from hr 2 to 24, there was a progressive decrease in the matrix density; the inner compartments gradually expanded, the cristae unfolded and grew shorter, and finally most of them could still be seen after 8 hr of treatment.

Due to swelling of the cells, pinocytotic pits and vesicles were often 2 or 3 times as large as those in the control cells. However, most of their outer membranes remained intact and continuous even after 24 hr of treatment (Fig. 13).

The only change noted on the plasma membrane was an inconsistent reduction in the number of microvilli, possibly due to swelling of the cells. Pinocytotic pits and vesicles could still be seen after 8 hr of treatment.

Acronycine did not appear to alter the appearance, number, or position of microtubules, microfilaments, centrioles, endoplasmic reticulum, or nuclear membranes, and no change was observed in the nucleus and nucleolus.

Cell Layer Cultures. Cells growing on glass seemed considerably more resistant to acronycine than were the L5178Y cells. While most of the latter cells were dead as a result of exposure to 6 µg of acronycine per ml for 24 hr, SV/HT, HFH-18, and C-4 II cells survived for 3 days in concentrations up to 24 µg/ml, and effects of the drug on growth characteristics or growth rates became obvious only after 24 hr or more of incubation. SV/HT cells (generation time, 15 to 17 hr) were most sensitive and C-4 II cells (generation time, 24 to 48 hr) were least sensitive to acronycine toxicity. Thus, over a 3-day period, 12 µg of the drug per ml caused lysis of most cells in SV/HT cultures, while it resulted in only a moderate reduction in final cell density in C-4 II cultures. The effect on HFH-18 cells was intermediate (Figs. 14 to 16).

In all 3 cell lines, acronycine caused a gradual reduction in cell proliferation which, by 72 hr of treatment, led to an almost complete cessation of mitotic activity at cell densities considerably lower than those of comparable control cultures. However, although proliferation had ceased, virtually all cells remaining in the cultures after 3 days of treatment were alive by eosin exclusion. Changes in mitotic index as well as in the proportion of binucleated cells with acronycine treatment were compared in lines C-4 II and SV/HT. As shown in Chart 1, treated C-4 II cells showed a significant reduction in mitotic activity from 48 hr on but showed no change in the proportion of binucleated cells. In SV/HT cultures, mitotic activity was similarly reduced by acronycine and, in addition, there was a small but significant increase in the proportion of binucleated cells from hr 48 (Fig. 14).

Cytoplasmic vacuolation, similar to that observed in L5178Y cells and thought to be evidence of mitochondrial swelling, was prominent in SV/HT cells, occurred sporadically in HFH-18 cells, and was seen only rarely in C-4 II cells (Fig. 14). In all 3 cell lines, acronycine caused a gradual reduction in cell flattening and in adhesion to glass that was first noted after about 24 hr of treatment and that was accompanied by a change of the cells to fusiform and, eventually, round shapes. Again, SV/HT cells were most sensitive and C-4 II cells least sensitive to this effect of acronycine (Figs. 14 to 16). While in cultures of lines SV/HT and HFH-18 the alteration in cellular morphology did not seem to be accompanied by any specific change in

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Chart 1. Effects of acronycine treatment on the percentage of cells in mitosis and of binucleated cells in cultures of lines SV/HT and C-4 II. Mean ± S.E. of 6 to 8 microscopic fields (500 cells/field) per sample.
intercellular relationships, in the C-4 II cultures it was associated with a striking rearrangement in colonial morphology. As the symmetrical, cuboidal cells of C-4 II cultures became fusiform as a result of acronycine treatment, they retracted from the substratum, drew together, and aligned parallel to one another into narrow ridges (Fig. 16). Thus, intercellular contact appeared to be maintained while cell-substratum adhesion diminished. Ultrastructurally, those parts of the cell surfaces that were rendered free, i.e., non-adhesive, as a result of retraction from the glass, had changed from a flat conformation to one characterized by numerous microvilli and resembled the free surfaces of control cells (1). Contact between adjacent cells was also altered by acronycine. In control cultures, adjacent cells adhered by desmosomes and dense junctions and in addition adhered across unmodified 200 Å intercellular spaces over most of the areas between these specialized structures (Figs. 17 and 18). In treated cultures, intercellular adhesion was maintained by the desmosomes only, with cell surfaces between these structures widely separated (Figs. 19 and 20). Swollen or damaged Golgi complexes were present in all preparations of C-4 II cells, but the degree of damage rarely reached that observed in L5178Y cells. No consistent change was observed in the mitochondria.

In the HFH-18 melanoma cultures, the changes in adhesiveness and cell shape in response to acronycine were not associated with an obvious quantitative change in pigmentation, but there was a striking rearrangement in melanin distribution. Whereas in control cultures, pigment granules were usually dispersed throughout the cytoplasm, in acronycine-treated cells, pigment was present in large coarse aggregates which, with increasing concentration of the drug and duration of treatment, became increasingly concentrated in the perinuclear regions (Fig. 23). Ultrastructurally, the perinuclear pigment aggregates in treated cells represented dense accumulations of melanosomes in the Golgi regions (Fig. 24). The internal structure and size of individual melanosomes in these aggregates seemed comparable to those of control cells, although the limiting membranes as well as the shapes often seemed less well defined and less regular (Figs. 25 and 26). Furthermore, the Golgi regions in acronycine-treated cells contained (in addition to the large number of melanosomes) disorganized membranous material which seemed to include swollen and disintegrating Golgi cisternae. Acronycine caused an increase in the proportion of abnormal (both swollen and condensed) mitochondria in the melanoma cells, but this change was inconsistent and never reached the degree observed in L5178Y cells.

In none of the 3 cell lines did acronycine-induced changes in growth pattern resemble the effects of 10mM dibutyryl cyclic 3',5'-AMP. Treatment with the latter compound over 3 days resulted in a relatively minor reduction in cell density, in continuation of mitotic activity, and in normal or exaggerated melanosome dispersion. Cell flattening and adhesion to glass were exaggerated rather than reduced, and cells tended to assume irregular rather than fusiform shapes, in keeping with observations in other culture systems (13). The response of the C-4 II cells to cytochalasin B also differed from that to acronycine. The contraction upon suspension of C-4 II cell sheets, shown previously to be inhibited by cytochalasin at 10.0 μg/ml within 1 hr (2), was not interfered with by acronycine at up to 24.0 μg/ml over 24 hr of treatment (Fig. 21). However, while in control preparations this contraction involves the appearance of a dense cortical microfilament layer adjacent to the basal plasma membrane with the latter thrown into irregular folds, in acronycine-treated cell sheets the filament bands seemed less well defined, and the basal cell surfaces were characterized by many microvilli, as are normally found only on the apical surfaces of C-4 II cells (Fig. 22). Cytochalasin B treatment (1.0 μg/ml for 72 hr) of C-4 II cell layers resulted in binucleation or multinucleation of about 50% of cells. No change to fusiform cell shapes, loss of adhesiveness to glass, ridge formation, or mitotic inhibition was observed. However, cytochalasin B at 10.0 μg/ml did cause cell separation and a reduction in adhesiveness and mitotic activity but caused no ridge formation.

DISCUSSION

In contrast to the inhibition of uridine uptake, which occurs within minutes after the addition of acronycine (6), changes in the shape and adhesiveness of the cells studied, as well as the inhibition of cytokinesis, of melanosome dispersion, and of population growth, were observed only after treatment periods of the order of hours or days. It would appear therefore that these changes represent secondary effects which result from acronycine damage inflicted on the cells earlier, a sequence similar to that proposed by Gout et al. (10) as the basis for binucleation of acronycine-treated L5178Y cells. The fact that Golgi complexes and, to a lesser extent, mitochondria were visibly abnormal after only 1 hr of treatment indicates that the membranes of these organelles may be primary sites of acronycine action. The extensive swelling of L5178Y cells suggests further that plasma membrane function may also be altered by the drug. However, cell membranes did not seem to be affected indiscriminately. By ultrastructural criteria, nuclear membranes, outer mitochondrial membranes, and strands of endoplasmic reticulum remained intact throughout acronycine treatment.

It may be significant that the Golgi apparatus normally plays a role in most of the functions that showed delayed effects of acronycine treatment. Golgi complexes are involved in plasma membrane formation and in cell coat synthesis, and therefore the decreasing adhesiveness of the treated cells could have been due to a breakdown in the normal turnover of cell surface constituents. It is interesting that in C-4 II cultures, acronycine interfered preferentially with cell-substratum adhesion and with intercellular adhesion over nonspecialized regions, while it had less effect on intercellular adhesion by desmosomes, i.e., specialized junctions thought to possibly involve calcium bonding (3, 4). Acronycine treatment prevented melanosome dispersion in HFH-18 cells. Both microtubules and microfilaments are required for the normal distribution of pigment in melanocytes (15), but this study provided no evidence of acronycine effects on either of these organelles. However,
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the limiting membranes of the pigment granules are of Golgi origin and, even in the absence of a definite visible change in melanosome structure, submicroscopic abnormalities of these membranes might prevent the interactions with filamentous organelles that are necessary for melanosome movements. The observation that the action of acronycine on cytokinesis may be delayed for more than a complete cell cycle (10) and the delayed appearance of binucleated cells in these experiments suggest that acronycine might interfere with the production of some factors required for cytoplasmic cleavage. Cytokinesis involves a rapid increase in cell surface area, possibly through the assembly of preformed membrane subunits (17), which are again of Golgi origin. Alternatively, acronycine-induced changes in cell coat or plasma membrane structure, similar to those responsible for the cell swelling or reduction in adhesiveness, might interfere with normal furrow formation. The failure of acronycine to induce the accumulation of cells with more than 2 nuclei indicates that, at doses which prevent cytokinesis, progress of the cells through earlier stages of the replication process is also inhibited within less than 2 cell cycles. The percentage of binucleated cells obtained was proportional to the growth rates in different types of cultures. It would appear, therefore, that in rapidly proliferating cell populations, binucleation resulted because cytokinesis was interfered with when nuclear division still proceeded to completion while, in slowly replicating cultures, nuclear division came to a standstill prior to or at the same time as cytokinesis, resulting in nondividing, mononucleated cells. The ability of acronycine to produce binucleated cells resembles the action of cytochalasin B, a mold metabolite of unrelated structure. It has been suggested that cytochalasin B acts through interference with microfilament function, and recently there has been evidence that the drug may also have direct effects on the plasma membrane (7). While in the present study the immediate response of cells to acronycine and to cytochalasin B differed, the 2 drugs do produce a number of common end results; both alter the uptake of extracellular nucleosides (6, 7), reduce cell adhesiveness, cause binucleation, and inhibit melanin dispersion. It is possible that some of the differences in cellular responses to the drugs in this study were due to the particular timing of the observations, since most of the effects common to the 2 compounds tend to occur more rapidly with cytochalasin B than with acronycine.

Mitochondrial swelling was a less consistent acronycine-induced change, but it was very prominent in the fast-growing lines L5178Y and SV/HT. Whether and to what degree mitochondrial damage and resulting energy depletion were responsible for the various late effects of the drug is unknown, but at the level of individual cells, there was no consistent relationship between the extent of mitochondrial swelling and the degrees of other types of acronycine-induced changes.

The results of this study indicate that the delayed cellular changes and eventual growth arrest caused by acronycine can be accounted for, at least in part, by alterations of the cell periphery. The cytotoxicity of acronycine, as of many other antineoplastic agents, was proportional to the proliferative rate of different tumor cell populations in vitro. It may be of importance, however, in view of the problems encountered in the treatment of slow-growing tumors, that while cultured cells with slow growth rates were relatively resistant to acronycine in terms of survival, they did undergo growth arrest and changes in surface characteristics and growth patterns. It is possible that, in vivo, similar acronycine-induced alterations in cell properties might interfere with tumor invasion and might render slow-growing tumors more susceptible to additional modes of therapy.

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REFERENCES

Figs. 1 to 4. Progressive structural changes in L5178Y cells treated in suspension culture with 6 µg of acronycine per ml. Plastic embedding; alkaline toluidine blue staining, × 800.

Fig. 1. Control. Irregular cell shapes; indented nuclei with single large nucleoli.

Fig. 2. Four hr of treatment. Onset of binucleation (b) and of cytoplasmic vacuolation (v) which probably represents mitochondrial swelling.

Fig. 3. Eight hr of treatment. Cells are round, suggesting swelling; cytoplasmic vacuoles are more prominent.

Fig. 4. Twenty-four hr of treatment. Cells and nuclei are highly swollen and disintegrating.

Figs. 5 to 13. Progressive changes in Golgi complexes and mitochondria of L5178Y cells treated in suspension culture with 6 µg of acronycine per ml.

Fig. 5. Control; Golgi complex. Note that magnification is comparable to that of Figs. 6 to 8. × 21,000.

Fig. 6. One hr of treatment. Some swelling and membrane breakage in the Golgi cisternae. × 23,000.

Fig. 7. Four hr of treatment. The Golgi region is occupied by numerous dilated vesicles and swollen cisternae with broken membranes. × 21,000.
Fig. 8. Six hr of treatment. Membranous components of the Golgi complex are barely discernible, and the cisternae are replaced by irregular, dilated spaces. × 23,000.

Fig. 9. Eight hr of treatment. Remnants of the Golgi complex and of associated structures occupy most of the nuclear indentation (N, nucleus). × 7,500.

Fig. 10. Control; mitochondria. × 21,000.

Fig. 11. Two hr of treatment. Early mitochondrial swelling. × 21,000.

Fig. 12. Six hr of treatment. Extensive mitochondrial swelling; loss of cristae. × 22,000.

Fig. 13. Twenty-four hr of treatment. In spite of extreme swelling and loss of internal structure, outer mitochondrial membranes seem to remain intact. × 22,000.
Figs. 14 to 16. Effects of 72 hr of treatment with 12 µg of acronycine per ml on the growth pattern of cell layer cultures. Carnoy fixation; toluidine blue staining. a and b, × 80; c, × 200.

Fig. 14. Line SV/HT: a, control; b and c, treated, showing reduced cell density, fusiform cell shapes, binucleation (b), and cytoplasmic vacuolation (arrows).

Fig. 15. Line HFH-18: a, control; b and c, treated, showing reduced cell density, aggregation of pigment (arrow), and fusiform cell shapes.

Fig. 16. Line C-4 II: a, control; b and c, treated, showing somewhat reduced cell density and retraction of fusiform cells from substratum into ridges.
Figs. 17 to 20. Effects of 72 hr of treatment with 12 μg of acronycine per ml on intercellular adhesion in C-4 II cultures.

Fig. 17. Control. Closely adherent cells. Section tangential to substratum. Plastic embedding; toluidine blue staining, × 600.

Fig. 18. Same specimen as in Fig. 17. Cells adhere by desmosomes (d) and by intervening unmodified cell surfaces (arrows), × 20,000.

Fig. 19. Treated culture. Cells are widely separated and adhere by intracellular bridges. Section tangential to substratum. Plastic embedding; toluidine blue staining, × 600.

Fig. 20. Same specimen as in Fig. 19. Intercellular adhesion is limited to desmosomes (d), × 21,600.

Fig. 21. Line C-4 II. Cell sheets suspended for 24 hr in control medium (a); medium with 10 μg cytochalasin B per ml (b); or medium with 24 μg acronycine per ml (c). The contraction seen in a is prevented by cytochalasin B but not by acronycine. Living Cells, × 60.

Fig. 22. Same specimen as in Fig. 21c. Numerous microvilli on the inner (basal) surface of the contracted, acronycine-treated C-4 II cell sheet. × 8,400.
Fig. 23. Effect of 12 µg of acronycin per ml on HFH-18 melanoma cells. a, control; b, 48 hr of treatment, partial pigment aggregation; c, 72 hr of treatment, complete aggregation of pigment in the perinuclear region. Carnoy fixation; toluidine blue staining. × 200.

Fig. 24. Same preparation as Fig. 23c. Pigment is contained in melanosomes which are limited to the Golgi region. Note microtubules radiating from centrioles (c); membranous material (m), possibly of Golgi origin. × 21,000.

Fig. 25. HFH-18 cell. Control. Normal melanosomes. × 39,000.

Fig. 26. HFH-18 cell; 72 hr treatment with 12 µg of acronycin per ml. Melanosomes seem to be of normal size but of less regular shape. Note limiting membranes of melanosomes, and membranous material, possibly of Golgi origin, in lower left-hand corner. × 44,000.
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