[CANCER RESEARCH 36, 905-916, March 1976]

The Effects of Methyl [5-(2-Thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate, (R 17934; NSC 238159), a New Synthetic Antitumoral Drug Interfering with Microtubules, on Mammalian Cells Cultured *in Vitro*[1]

M. J. De Brabander,[2] R. M. L. Van de Veire, F. E. M. Aerts, M. Borgers, and P. A. J. Janssen

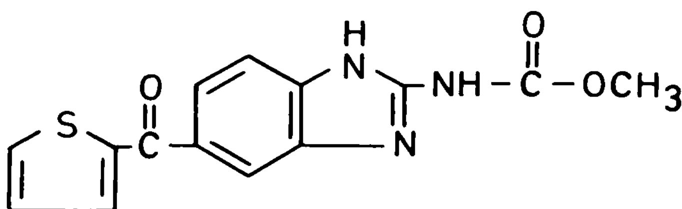
Laboratories of Oncology and Cell Biology, Janssen Pharmaceutica Research Laboratories, B-2340 Beerse, Belgium

SUMMARY

Ultrastructural investigations on mammalian cells cultured *in vitro* show that R 17934, a new synthetic anticancer drug, interferes with the structure and function of microtubules, both in interphase and mitotic cells. The activity of this compound in a wide range of experimental tumor systems can thus be explained partly as a direct antimitotic effect and partly as the disintegration of the normal subcellular organization of the nondividing cells. Preliminary investigations in experimental animals show that malignant cells are more susceptible to the antimicrotubular effect of R 17934 than are the nonmalignant cells of the host.

INTRODUCTION

R 17934 (methyl [5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbamate; M.W. 301.31; Janssen Pharmaceutica) is a new experimental antitumoral compound with the following structural formula:



R 17934 has been found to inhibit completely the growth of transformed C3H mouse embryonal cells (MO_4[3]) *in vitro* (M. J. De Brabander, unpublished data) and to be active against a wide range of experimental tumor systems (L1210, MO_4-sarcoma, Melanoma B16, Lewis lung carcinoma, P388 leukemia, and LSTRA leukemia) (1, 2).[4]

This report gives a description of its effects on different mammalian cells cultured *in vitro*, as assessed by methods combining time-lapse phase-contrast observation and ultrastructural techniques.

MATERIALS AND METHODS

Cell Lines and Culture Procedures. MO is a C3H mouse embryonal cell line of epithelioid character that shows contact inhibition of movement and mitosis in culture. Upon gaining confluency, the cells form transparent sheets of large polygonal cells. Inoculation of a large number of cells ($>10^7$) does not produce tumors in the syngeneic animal (18). MO_4 was derived from the MO cell line by transformation with the Kirsten strain of murine sarcoma virus. It shows absence of contact inhibition in culture and forms multilayered cultures of pleiomorphic cells in the superconfluent state. Minimal cell inocula ($<10^2$) produce invasively growing fibrosarcomatous tumors in the syngeneic animal. The MO and MO_4 lines were a gift from Dr. A. Billiau (Rega Institute, Leuven, Belgium), who described their origin (6).

Primary embryonal fibroblast cultures were established by trypsin treatment of C3H embryonal carcasses.

Melanoma B16 cultures were derived by trypsin treatment of s.c. tumors. HeLa cells and cultures of human embryonal skin fibroblasts were obtained from Dr. A. Billiau.

The MO, MO_4, and B16 lines and the human embryonal skin fibroblasts were grown in Eagle's minimal essential medium supplemented with nonessential amino acids and 10% fetal bovine serum, in a humidified atmosphere of 5% CO_2 in air at 37°.

HeLa cells were cultured under the same conditions in Eagle's and Earle's media supplemented with lactalbumin hydrolysate and 10% calf serum.

Phase-Contrast Observation. For observation with an inverted phase-contrast microscope (Reichert-Biovert) and for cell counting in quantitative experiments, the cells were seeded in plastic Petri dishes (Falcon Plastics, Oxnard, Calif.) of 6-cm diameter. For time-lapse microcinematography, the cells were seeded in culture chambers consisting of 2 coverglasses on a steel frame.

Ultrastructural Observation. For ultrastructural observation, the cells were cultured in Falcon plastic Petri dishes of 6-cm diameter. At the appropriate moment, the cells were rinsed twice with 0.9% NaCl solution at room temperature and were fixed in 5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) at room temperature for 15 min. Postfixation was done in a mixture of 4% glutaraldehyde in 0.1 M sodium

[1] This study is supported by a grant from the Instituut tot aanmoediging van het wetenschappelijk onderzoek in nijverheid en landbouw, Belgium.

[2] To whom requests for reprints should be addressed, at Laboratory of Oncology, Janssen Pharmaceutica, Turnhoutsebaan, 30, B-2340 Beerse, Belgium.

[3] Abbreviations used are: MO_4, transformed mouse embryonal cell line; MO, nontransformed mouse embryonal cell line.

[4] L. Desplenter. R 17934, a New Synthetic Antitumoral Compound: Effects on Transplantable Tumors in Mice. Janssen Research Products Information Service (report obtainable on request).

Received June 16, 1975; accepted October 12, 1975.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

cacodylate and 1% osmium tetroxide in 0.05 M Veronal for 1 hr at 0°. After being stained with 0.5% uranyl acetate (40 min), the cultures were rinsed with sodium cacodylate buffer supplemented with 0.22 M sucrose (minimum 1 hr), dehydrated in a graded series of ethanol, and embedded in Epon in the Petri dishes. After polymerization at 50° (48 hr), the plastic dish was removed and cells were selected by observation with the phase-contrast microscope. The cells were either reembedded in flat molds in Epon for transverse sectioning or a hardened Epon capsule was glued to the opposite site for flat sectioning. Ultrathin sections were stained with uranyl acetate and lead citrate before examination in a Philips EM 300 electron microscope.

Experiments. With the use of a time-lapse microcinematographic apparatus and a phase-contrast microscope, the general morphology and behavior of MO and MO_4 cells were recorded before and after treatment with R 17934 (1 μg/ml). In different experiments, the cells were filmed up to 72 hr after treatment. The reversibility after washing the cultures was filmed up to 72 hr. In several experiments, the cells had been allowed to phagocytose colloidal carbon (Pelikan 611/1431a; Günther Wagner, Hannover, Germany) so that endocytic vacuoles could be visualized easily. Confirmation of the findings with other doses was done using still photographs taken at regular time intervals: 1, 2, 3, 4, 5, 6, 7, 8, and 24 hr and 2, 3, 4, 5, 6 days, etc. until 14 days. This was done on MO and MO_4 cells using the following doses: 0.001, 0.01, 0.02, 0.04, 0.08, 0.1, 1, and 10 μg/ml, With 1 μg of R 17934 per ml, the effects on the other cell lines were recorded with phase-contrast photography using the same times as above.

For quantitative differential cell counts, the cells were seeded in plastic Petri dishes at a density of 5000/sq cm. After 24 hr, some randomized fields were marked (minimum, 4) and the cells were counted at a magnification of ×160. At this magnification, each field contains approximately 50 cells at 0 hr. Immediately thereafter, the test solutions were added and the same fields were counted again at the times indicated in the charts.

Ultrastructural observations are based on the following experiments. MO and MO_4 cells were treated with R 17934 (1 μg/ml), or with the solvent and were fixed after 5, 10, 20, 40, 80, and 120 min; 5, 24, 48, and 72 hr; and 5 days. With different doses of R 17934 (0.01, 0.04, 0.1, 1, or 10 μg/ml), the cells were fixed after 2, 24, and 48 hr. Reversibility was studied on MO cells using a 48-hr treatment with 0.1 μg/ml followed by extensive washing (3 times), further incubation in normal medium, and sampling after 2, 4, 24, and 48 hr.

Confirmation on the other cell lines was done with R 17934 (1 μg/ml) and sampling after 2, 24, and 48 hr.

All previous experiments were performed in an identical manner with colchicine. In the experiments with a single dose, 1 μg/ml was used. The doses of colchicine used were: 100, 10, 1, 0.1, 0.04, 0.02, 0.01, and 0.001 μg/ml. Reversibility was studied using 0.1 μg/ml. For ultrastructural studies on MO cells with vinblastine and vincristine, the following concentrations were used: 10, 1, 0.1, 0.01, and 0.001 μg/ml. The cells were fixed after 24 and 48 hr.

As a rule, each observation was done on a minimum of 50 cells sectioned in a semiserial fashion from bottom to top. To this was added a variable number of transverse sections through the cells.

The quantitative results obtained with colchicine, vinblastine, and vincristine are published elsewhere (11).

Compounds. R 17934 was dissolved in dimethyl sulfoxide (5 mg/ml) and was further diluted in complete culture medium. The control cultures received the appropriate amount of dimethyl sulfoxide.

The same procedure was followed for vinblastine and vincristine (obtained as a gift from Eli Lilly & Co., Indianapolis, Ind.). Colchicine (Sigma Chemical Co., St. Louis, Mo.) and 5-fluorouracil (Sigma) were diluted immediately in complete culture medium.

RESULTS

For the sake of clarity, the effects on MO and MO_4 cells (which reacted in an identical fashion) will be described first and the data obtained with other cell lines will be summarized later.

MO and MO_4 Cell Lines. When MO cells were treated with an active dose of R 17934 (0.04 to 10 μg/ml), the following sequence of events took place. Within 10 to 20 min, the cells lost their normal stretched, bipolar appearance and became more or less rounded with undulating membrane activity distributed all over the cell periphery (Fig. 1). Consequently, directional cell migration was largely hampered. The saltatory movements of intracellular particles and endocytic vacuoles were also immediately halted, and by periodic bursts of cytoplasmic mass streaming, these organelles lost their normal perinuclear location and became distributed randomly throughout the cytoplasm within 2 to 5 hr.

At the ultrastructural level, this correlates in time with the gradual disappearance of cytoplasmic microtubules (Figs. 4 and 5). After 10 min, the majority of microtubules disappeared with the 1-μg/ml dose of R 17934. No microtubules were left after 20 min. None of the other cellular organelles seemed to be directly affected. The distribution, however, altered concomitantly with the disappearance of cytoplasmic microtubules. The Golgi area, consisting of groups of parallel cisternae and saccules normally forms a compact zone in close apposition to the nucleus, surrounding the centriolar complex. Within 2 to 5 hr after initiation of the treatment, individual Golgi organelles, each consisting of normal-looking cisternae and saccules, were dispersed over the entire cytoplasm in a randomized fashion (Figs. 2, 3, and 6). This was also true for the lysosome-like structures that are normally located in a perinuclear rim. The centriolar complex, normally situated in the center of the Golgi zone, became dislodged too, and it was often found somewhere near the plasma membrane in the cell periphery (Fig. 6).

Subsequent to the disappearance of cytoplasmic microtubules and the phenomena described above, a temporary accumulation or hypertrophy of smooth endoplasmic vesicular material occurred (Figs. 6 and 7), often concentrated in patches around or in the vicinity of dispersed Golgi organelles. This reached a maximum after 2 to 5 hr and then subsided; it was followed by a gradual hypertrophy of the rough endoplasmic reticulum and the appearance of spe-

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

cialized structures such as intracytoplasmic confronting cisternae and annulated lamellae (9). The 1st solitary annulated lamellae appeared after 20 min. Elaborated complexes gradually increased in size and number, reaching a maximum after 24 to 48 hr.

Cytoplasmic filaments (~100 Å in diameter) seemed not to be affected *per se*. However, they accumulated in the form of large bundles, curving throughout the cytoplasm (8). This accumulation also was gradual, reaching a maximum after ~48 hr.

Apart from the altered spatial distribution, no effects of R 17934 on other organelles were apparent.

When control MO cells in culture entered mitosis, they rounded up completely, remaining attached to the substratum only by very fine filopodia. Within 45 min, the normal metaphase-anaphase-telophase transition was observed, after which a cleavage furrow separated the 2 daughter cells that readhered onto the substratum. In treated cultures, the initial rounding was identical to that in control cells; however, no normal metaphase or anaphase distribution of the chromosomes was observed (Fig. 1). At the ultrastructural level, microtubules or an intact spindle were completely absent (Fig. 9). The double chromosomes, which had 2 well-defined centromeres, were scattered throughout the cytoplasm, intermingled with the remainder of the other cellular organelles.

After approximately 5 hr, the pseudomitotic cells began to form multiple furrows (Fig. 1), each of which was comparable to a normal cleavage furrow, showing a circular rim of subplasmalemmal microfilaments. During this "lobulated" phase, new nuclear membranes appeared between and around the chromosomal groups that were dispersed between the different lobes. Finally, individual chromosomes or small clumps began to decondense; each was wrapped individually within a separate nuclear membrane (Fig. 10). After approximately 12 hr, the pseudomitotic phase ended. The cells readhered onto the substratum and were seen to have many (up to 40) micronuclei, each containing 1 or several nucleoli (Fig. 1). Thus, both chromosomal separation and cytokinesis failed to occur and the cells became polyploid with the genetic material distributed over several individual nuclei.

Prolonged treatment (72 hr to 5 days) resulted in the appearance of necrotic cells which detached from the substratum (see also quantitative results). In general, an accumulation of lysosome-like structures was apparent. This was, however, also the case in the control cultures and seems to be a general phenomenon in aging cell cultures.

The foregoing phenomena could be reproduced with all doses of R 17934 between 0.04 and 10 μg/ml. The only difference seemed to be the rate at which the initial morphological alteration was produced. With higher doses, this was accomplished somewhat earlier (10 min with 10 to 1 μg/ml); with lower doses, it was accomplished somewhat later (30 to 60 min with 0.1 to 0.04 μg/ml). This could be due to a concentration-dependent entry of the compound in the cells. No difference from untreated cells was visible with doses below 0.01 μg/ml.

When the drug was withdrawn from the cells and replaced by normal growth medium, they gradually resumed their normal stretched morphology. This coincided with the reappearance of cytoplasmic microtubules and the reorganization of the subcellular compartmentalization. The Golgi cisternae regrouped around the centrioles in a perinuclear position. Filament accumulations and annulated lamellae, however, remained present for a long time (more than 48 hr). In multinucleated cells, reversal of the treatment was followed by gradual fusion of the individual nuclei (Fig. 1), which can result in the formation of 1 normal nucleus. Another way in which this was accomplished was by multipolar mitoses resulting in the formation of 3 or 4 daughter cells, some with 1 nucleus and some with several nuclei.

Other Cell Lines. The effects on interphase cells are largely the same in all cell lines tested to date. Major differences, however, appeared during the mitotic phase. Indeed, HeLa cells (Fig. 11) and melanoma B16 cells became necrotic during the abortive mitotic phase, and prolonged treatment resulted in the extermination of the culture. Human embryonal skin fibroblasts and primary cultures of embryonal mouse fibroblasts became polyploid (by nondisjunction). However, most cells that underwent mitosis showed only 1 nucleus. In a few exceptions 3 to 4 large nuclei or a lobulated nucleus were present.

Quantification of the Effects in MO Cells. For quantitative experiments, the MO line was chosen since these cells show absence of overlapping, which is essential for the technique used (direct counting of cells under the phase-contrast microscope).

Within a dose range of R 17934 from 10 μg/ml down to 0.04 μg/ml, the effect was essentially the same (Chart 1). Higher doses could not be used because of the relative insolubility of the compound. Threshold doses, 0.04 to 0.02 μg/ml, showed a partial effect. Below 0.01 μg/ml, there was no effect whatsoever and growth of the culture, expressed either as cell number or total protein content, was unaffected. With doses above 0.04 μg/ml, each abortive mitosis produced 1 multimicronucleated cell. This became apparent from the analysis of the graphs and was also shown by observation of more than 500 individual cells in culture. Moreover, pretreatment of the cells with 5-fluorouracil, which blocks the cells in a point of the cycle prior to mitosis (S phase), completely abolished the multinucleation response (Chart 1). This was also the case in cultures in which mitosis had ceased by postconfluent contact inhibition. The effects on interphase cells, however, were identical to what has been described above.

Chart 2 shows that continuous treatment was necessary in order to interfere with the mitotic events. However, the cultures were able to recover, even after prolonged treatments, when the majority of the cells had become multinucleated (Chart 3). The speed of recovery became slower, however, with prolongation of the treatment. Which part of growth resumption is due to the remaining mononucleated cells and which part is due to nuclear fusion cannot be determined exactly from these data. However, individual multinucleated cells were seen to be capable of undergoing mitosis and producing viable daughter cells.

Comparison with Other Compounds. Since the foregoing results suggested that R 17934 interferes with the structure and function of microtubules, both cytoplasmic and mitotic,

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

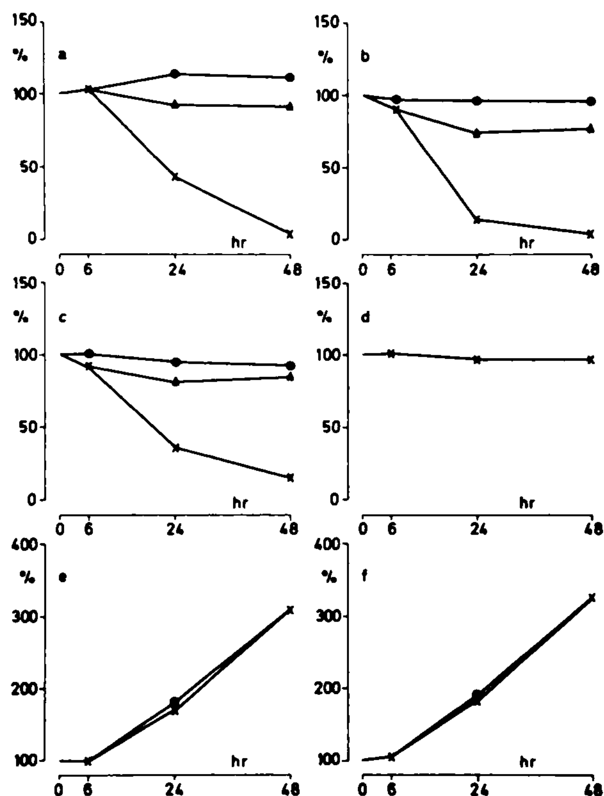


Chart 1. Quantification of the effects of R 17934 on MO cultures. For technical details see "Materials and Methods." R 17934 was added immediately after 0 hr. The results are expressed as percentages of the number of cells before treatment (0 hr) and the different classes are superimposed on each other. ×; percentage of normal mononucleated cells; ▲, percentage of multimicronucleated cells (plus [×]); ●, percentage of mitotic cells (plus [×] and [▲]); *a*, effect of R 17934 (10 μg/ml); *b*, effect of R 17934 (0.156 μg/ml); *c*, effect of R 17934 (0.039 μg/ml); *d*, effect of R 17934 (1.25 μg/ml) on an MO culture treated concomitantly (added 16 hr before 0 hr) with 5-fluorouracil (10 μg/ml). *e*, effect of R 17934 (0.01 μg/ml); *f*, control culture.

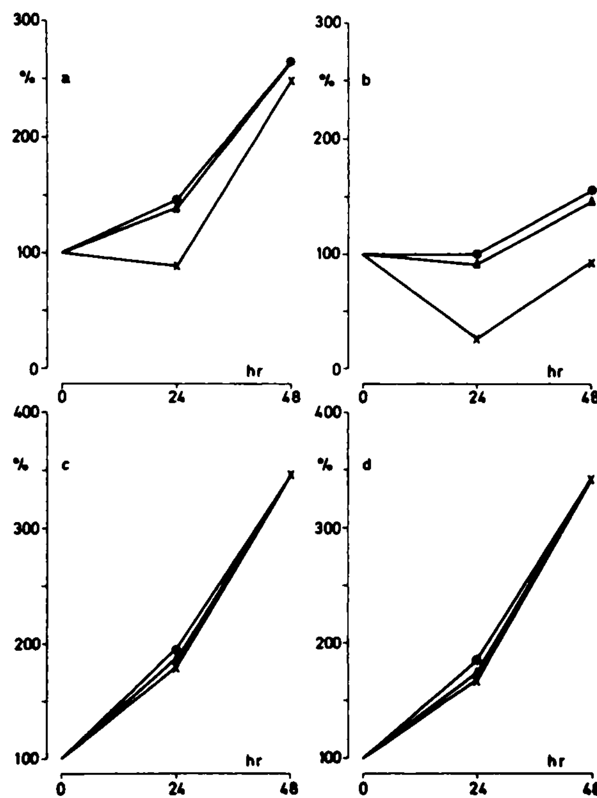


Chart 2. Reversibility of the effects of R 17934 and colchicine on MO cells. The experimental set-up and symbols are the same as for Chart 1. *a*, effect of colchicine (0.1 μg/ml); the compound was present from 0 hr until 4 hr, then, the cultures were washed twice during 15 min and further incubated in normal growth medium; *b*, effect of colchicine (0.1 μg/ml) present from 0 to 8 hr. *c*, effect of R 17934 (0.1 μg/ml) present from 0 to 8 hr; *d*, growth of the control culture. Continuous incubation with either colchicine (0.1 μg/ml) or R 17934 (0.1 μg/ml) showed graphs identical to Chart 1, *a* to *c*.

the standard antitubulins (colchicine, vinblastine, and vincristine) were tested in the same system. The results can be summarized very briefly. With minor differences, both the qualitative and quantitative effects are identical to those seen with R 17934. The differences are the following. Vinblastine and vincristine produced paracrystalline tubulin precipitates within the cytoplasm with doses above 1 μg/ml (8, 17). From a quantitative point of view, the lowest active doses were: colchicine, 0.04 μg/ml; vinblastine, 0.008 μg/ml; and vincristine, 0.02 μg/ml. With regard to reversibility, vinblastine and vincristine behaved identically to R 17934 but colchicine differed in that 4-hr incubation was sufficient to produce multinucleation after 24 hr (Chart 2).

Combination of threshold doses of R 17934 with colchicine, vinblastine, or vincristine had synergistic effects (Chart 4). Combination of colchicine with either vinblastine or vincristine showed synergistic effects also, although they are known to be bound to a different site of tubulin (24).

DISCUSSION

The results show that R 17934 can be regarded as a rather specific antitubulin, a compound that interferes with the structure and function of microtubules through inhibition of tubulin polymerization into normal microtubules (19). The effects of the standard antitubulins (colchicine, vinblastine, and vincristine) on the same cells were almost identical and are comparable to those described previously by other authors (4, 5, 7, 12, 14, 16, 20, 22, 23). Moreover, we have shown that R 17934 is able to inhibit completely the polymerization of rat brain tubulin *in vitro* (11).

The rather high degree of specificity of R 17934 as an antitubulin is shown by the lack of nonspecific toxic effects at doses that are several log units higher than the lowest active one.

From an experimental point of view, R 17934 could prove to be of value as an additional tool in studying the functions and structure of microtubules. The high degree of reversibility with R 17934 suggests that binding is not as tight as with colchicine. This could make certain experiments (*e.g.*, reversibility after short incubation times) easier than with colchicine.

Although synergistic action is shown with other antitubulins when combining subactive doses, this does not mean that the drugs act at the same binding site. Indeed, the same synergism is shown for vinblastine and colchicine although they are known to bind at different sites (24).

The results of microtubule disappearance can be summarized as a disappearance of directional subcellular organelle movements and a loss of the normal spatial organization and compartmentalization of the cell. For interphase

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

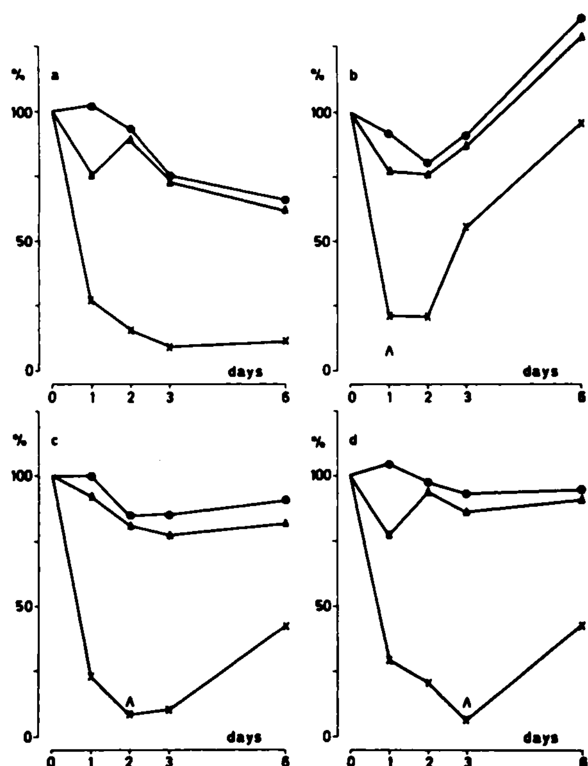


Chart 3. Reversibility of the effects of R 17934 (0.04 µ/ml) on MO cultures after prolonged incubation times. The experimental set-up and the meaning of the symbols are the same as for Chart 1. In *a*, the cultures were incubated for the whole observation period with R 17934 (0.04 µg/ml). In *b*, *c*, and *d*, the cultures were washed (*arrow*) after 24, 48, and 72 hr respectively, and further incubated in normal growth medium. Control cultures had reached a plateau at 400 to 600% of the starting value after 6 days.

cells, this is shown by the arrest of the centripetal movement of endocytic vacuoles and by the randomization of structures that normally assume an ordered distribution in the cell. Examples are the altered distribution of the undulating membrane, the Golgi complex, and the lysosomes. This confirms previous observations on the essential role of microtubules in these phenomena (4, 5, 14, 19, 20, 23). R 17934 induced the formation of annulated lamellae and filament bundles as do the other antitubulins from which it differs widely in its chemical structure. This has strengthened our belief that these phenomena are not side effects of these drugs but are directly related to microtubule disintegration (8, 9). We have previously suggested that this could be a reactive hyperproduction of organelles that are normally functionally linked with microtubules (8, 9).

In mitotic cells too, microtubule disintegration results in a loss of directional organelle movement (in this case, the chromosomes) and in the disappearance of the normal spatial organization. The latter is shown by the scattered distribution of the chromosomes, intermingled with other organelles that are normally excluded from the mitotic spindle. The production of multiple orderless furrows in MO and MO_4 cells, instead of 1 furrow in the equatorial plane, could be another indication of the organizing function of microtubules.

The final outcome, however, of microtubule disintegration in mitotic cells apparently depends on the type of cell line used. HeLa cells and melanoma B16 cells rapidly become necrotic through pyknosis as is the case for most mammalian cells (13). Some cell types are obviously able to regain the interphase stage after having spent a long time in the abortive mitotic phase. Since cell division fails to occur, these cells have become polyploid. In some cells (mouse embryonal fibroblast and human embryonal fibroblasts), the chromosomes are reenveloped into 1 nucleus. In other cells (MO, MO_4), the chromosomes are reenveloped separately or in small groups, giving rise to multimicronucleated cells, in a manner similar to that described by other authors (12, 22). This phenomenon can be exploited as a simple test system for quantitative studies with antitubulins (11).

The compound has been shown to be active against many experimental tumors and leukemias, namely, MO_4 sarcoma (M. J. De Brabander, unpublished material), L1210, P388, melanoma B16, Lewis Lung, and LSTRA leukemia (1, 2).[4]

It has been shown in the L1210 system that R 17934 produces a relative "metaphase arrest" and that synchronization experiments yield promising results (1).[4] However, it is far from certain that the only, or even the major, antitumoral effect of R 17934 lies in its ability to interfere with the normal mitotic events. A lack of correlation between the mitotic rate and the antitumoral effect has also been found for the *Vinca* alkaloids (21). It is indeed worthwhile stressing that interphase cells are also very strongly attacked by antitubulins. The metabolic events that depend on a normal spatial organization of the cell or on normal intracellular

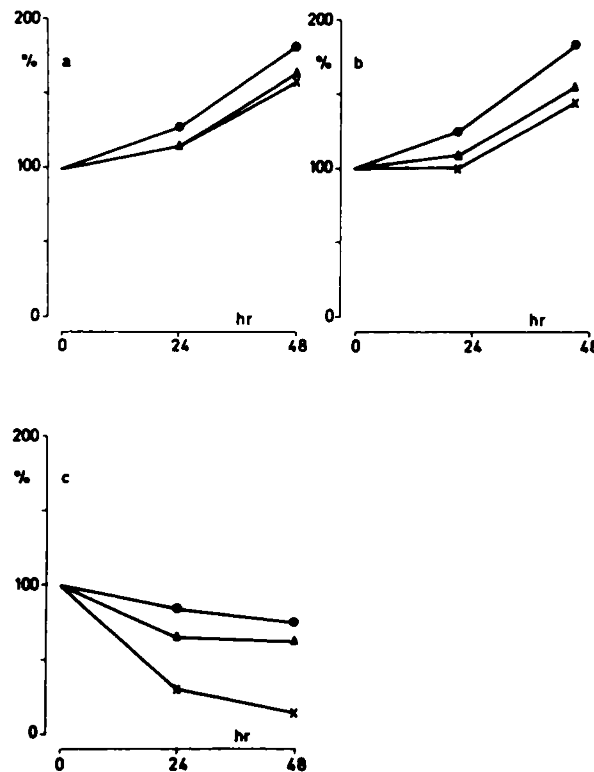


Chart 4. Effect of a combination of threshold doses of R 17934 and colchicine on MO cultures. The experimental set-up and the meaning of the symbols are the same as for Chart 1. In *a* and *b*, the effect of continuous treatment with either R 17934 (0.02 µg/ml) or colchicine (0.02 µg/ml) is shown. In *c*, the effect of combined treatment. [R 17934 (0.02 µg/ml) and colchicine (0.02 µg/ml)] is shown.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

organelle movements should be strongly affected. The interference with directional cell movement could have consequences for the invasive and metastatic behavior of a tumor *in vivo*. Finally, both the alteration of the "fluid mosaic" behavior of the cell membrane (3) and the altered turnover of the cell coat (15) could seriously change the interaction with immune and nonimmune host defense mechanisms.

Our own ultrastructural investigations on the L1210 system which will be published separately (10) have shown that *in vivo* too microtubules disappear both in mitotic and interphase L1210 cells after treatment with R 17934. A single i.p. treatment with R 17934 (40, 80, or 160 mg/kg) 5 days after i.p. inoculation with 10^5 L1210 cells induced a complete loss of microtubules in L1210 cells for up to 30 hr. Even with the highest dose (160 mg/kg) the microtubules in the nonmalignant leukocytes and macrophages that were present in the ascitic fluid were apparently unaffected. The same was true for the mesothelial and interstitial cells lining the peritoneal cavity. This shows that *in vivo* the microtubules of tumoral cells are apparently more susceptible to the lytic action of R 17934 than are the microtubules of nonmalignant cells, which contrasts with the lack of specificity *in vitro* (MO *versus* MO_4). However, an *in vitro* culture system is still highly artificial, especially since the cells that are used are mostly of embryonic origin. Moreover, by the routine passage procedures they are in some way forced to behave as continuously dividing tumoral cells.

The rather high degree of specificity of R 17934 *in vivo* could explain the low degree of bone marrow toxicity that has been observed.[5] Finally, R 17934, unlike the standard antitubulins, has been shown to be devoid of neurotoxicity.[6]

ACKNOWLEDGMENTS

The skillful assistance of J. Van Reempts and L. Leyssen in preparation for electron microscopy and photography, of V. Van Kesteren in reviewing the manuscript and of M. Van de Ven in typing the manuscript is greatly appreciated.

REFERENCES

1. Atassi, G., Schaus, C., and Tagnon, H. J. R 17934-NSC 238159: A New Antitumor Drug II. Effect on Mitotic Cycle of L1210 Leukemia Cells *In Vivo* and Synergism with Cytosine Arabinoside (NSC 63878). European J. Cancer, *11:* 609-614, 1975.
2. Atassi, G., and Tagnon, H. J. R 17934-NSC 238159: A New Antitumor Drug. I. Effect on Experimental Tumors and Factors Influencing Effectiveness. European J. Cancer, *11:* 599-607, 1975.

[5] R. Marsboom. Acute and Subacute Intravenous and Intramuscular Toxicity of Micronized R 17934 in Dogs. Janssen Research Products Information Service, in preparation (report obtainable on request).

[6] P. Laduron and P. Van Gompel. Effect of R 17934 on the Catecholamine Content in Rat Tissues. Janssen Research Products Information Service (report obtainable on request).

3. Berlin, R. D., Oliver, J. M., Ukena, T. E., and Yin, H. H. Control of Cell Surface Topography. Nature, *247:* 45-46, 1974.
4. Bhisey, A. N., and Freed, J. J. Ameboid Movement Induced in Cultured Macrophages by Colchicine or Vinblastine. Exptl. Cell Res., *64:* 419-429, 1971.
5. Bhisey, A. N., and Freed, J. J. Altered Movement of Endosomes in Colchicine-treated Cultured Macrophages. Exptl. Cell Res., *64:* 430-438, 1971.
6. Billiau, A., Sobis, H., Eyssen, H., and Van den Berghe, H. Non-Infectious Intracysternal A-type Particles in a Sarcoma-Positive, Leukemia-Negative Mouse Cell Line Transformed by Murine Sarcoma Virus (MSV). Arch. Ges. Virusforsch., *43:* 345-351, 1973.
7. Buckley, I. K. Subcellular Motility: A Correlated Light and Electron Microscopic Study Using Cultured Cells. Tissue Cell, *6:* 1-20, 1974.
8. De Brabander, M., Aerts, F., Van de Veire, R., and Borgers, M. Evidence against Interconversion of Microtubules and Filaments. Nature, *253:* 119-120, 1975.
9. De Brabander, M., and Borgers, M. The Formation of Annulated Lamellae Induced by the Disintegration of Microtubules. J. Cell Sci., *19:* 331-340, 1975.
10. De Brabander, M., Van de Veire, R., Aerts, F., Geuens, G., Borgers, M., Desplenter, L., and De Crée, J. R 17934: A New Anti-Cancer Drug Interfering With Microtubules. Effects on Neoplastic Cells Cultured *In Vitro* and *In Vivo*. Proceedings of the International Symposium on Microtubules and Microtubular Inhibitors, Beerse (Belgium). *In:* M. Borgers and M. De Brabander (eds.), Microtubules and Microtubular Inhibitors, pp. 509-521. Amsterdam: ASP Biological and Medical Press B. V., 1975.
11. De Brabander, Van de Veire, R., Aerts, F., Geuens, S., and Hoebeke, J. A New Culture Model Facilitating Rapid Quantitative Testing of Mitotic Spindle Inhibition in Mammalian Cells. J. Natl. Cancer Inst., in press.
12. Deig, E. F., Starr, T. J., and Church, K. K. Micronuclei Formation in Tissue Cultured Cells Treated with Colchicine. Proc. Soc. Exptl. Biol. Med., *111:* 248-250, 1962.
13. Deysson, G. Antimitotic Substances. Intern. Rev. Cytol., *24:* 99-148, 1968.
14. Freed, J. J., and Lebowitz, M. M. The Association of a Class of Saltatory Movements with Microtubules in Cultured Cells. J. Cell Biol., *45:* 334-354, 1970.
15. Ginsel, L. A., Daems, W. Th., and Debets, W. The Effect of Colchicine on the Glycocalyx-containing Vesicles and Tubules in the Absorptive Cells of Cultured Human Small Intestine. Proceedings of the International Symposium on Microtubules and Microtubular Inhibitors, Beerse (Belgium). *In:* M. Borgers and M. De Brabander (eds.), Microtubules and Microtubular Inhibitors, pp. 187-198. Amsterdam: ASP Biological and Medical Press B. V., 1975.
16. Krishan, A., Hsu, D., and Hutchins, P. Hypertrophy of Granular Endoplasmic Reticulum and Annulate Lamellae in Earle's L Cell Exposed to Vinblastine Sulfate. J. Cell Biol., *39:* 211-216, 1968.
17. Marantz, R., Ventilla, M., and Shelanski, M. Vinblastine-induced Precipitation of Microtubule Protein. Science, *165:* 498-499, 1969.
18. Mareel, M., De Ridder, L., De Brabander, M., and Vakaet, L. Characterization of Spontaneous, Chemical and Viral Transformants of a C3H/3T3-type Mouse Embryo Cell Line by Transplantation Into Young Chick Blastoderms. J. Natl. Cancer Inst., *54:* 923-929, 1975.
19. Margulis, L. Colchicine-sensitive Microtubules. Intern. Rev. Cytol., *34:* 333-361, 1973.
20. Robbins, E., and Gonatas, N. K. Histochemical and Ultrastructural Studies of HeLa Cell Cultures Exposed to Spindle Inhibitors with Special Reference to the Interphase Cell. J. Histochem. Cytochem., *12:* 704-711, 1964.
21. Rosner, F., Hirshaut, Y., Grünwald, H. W., and Dietrich, M. *In Vitro* Combination Chemotherapy Demonstrating Potentiation of Vincristine Cytotoxicity by Prednisolone. Cancer Res., *35:* 700-705, 1975.
22. Starr, T. J. Morphological and Biochemical Integrity of Colchicine-induced Psittacosis-infected Micronucleated Cells. Texas Rept. Biol. Med., *21:* 412-421, 1963.
23. Vasiliev, J. M., Gelfand, I. M., and Guelstein, V. I. Initiation of DNA Synthesis in Cell Cultures by Colcemid. Proc. Natl. Acad. Sci. U. S., *68:* 977-979, 1971.
24. Wilson, L. Microtubules as Drug Receptors: Pharmacological Properties of Microtubule Protein. Ann. N. Y. Acad. Sci. *253:* 213-231, 1975.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

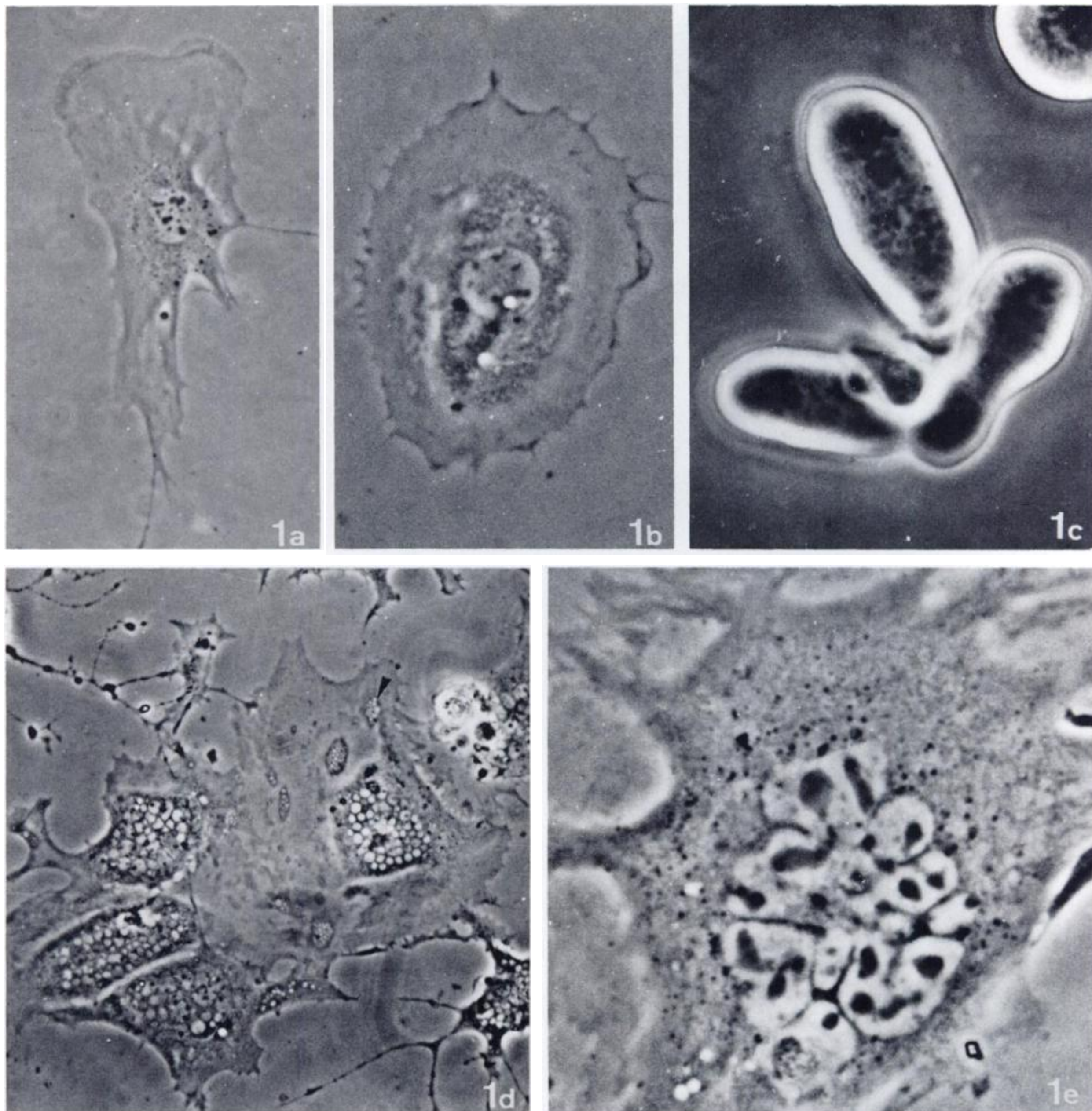
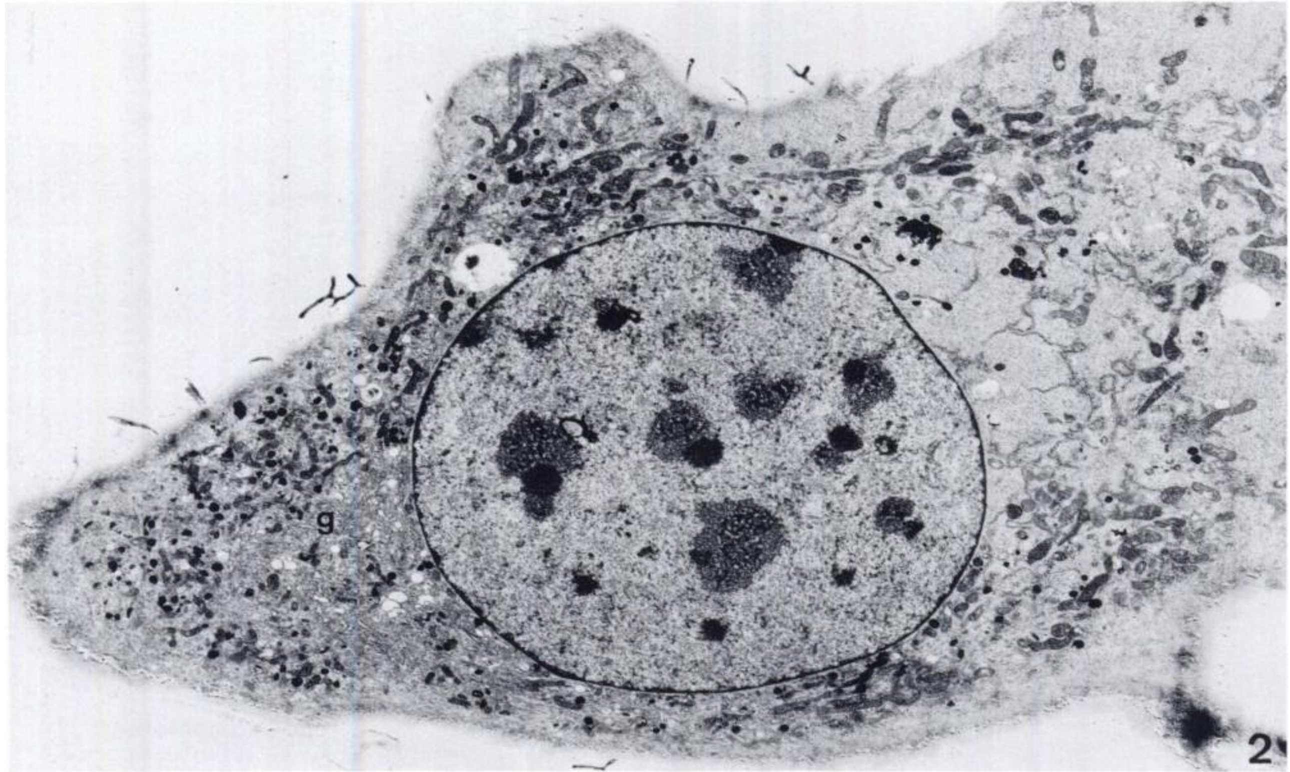


Fig. 1. *a*, phase-contrast picture of an untreated MO cell. Note the bipolar character of the cell with undulating membrane activity at the top and retraction fibers at the bottom. × 300. *b*, phase-contrast picture of an MO cell treated for 2 hr with R 17934 (1 µg/ml). Undulating membrane activity is distributed all around the cell periphery. × 460. *c*, phase-contrast picture of a "lobulated" mitotic MO cell [24-hr treatment with R 17934 (1 µg/ml)]. The chromatin is dispersed throughout the different lobes. × 400. *d*, phase-contrast picture of multimicronucleated MO cells (24-hr treatment with R 17934 (1 µg/ml)]. Note also the peripheral clustering of endocytic vacuoles (*arrow*). × 380. *e*, phase-contrast picture of an MO cell treated for 48 hr with R 17934 (1 µg/ml) and subsequently replaced in normal growth medium for 24 hr. The individual micronuclei have started to fuse with each other. × 140.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022



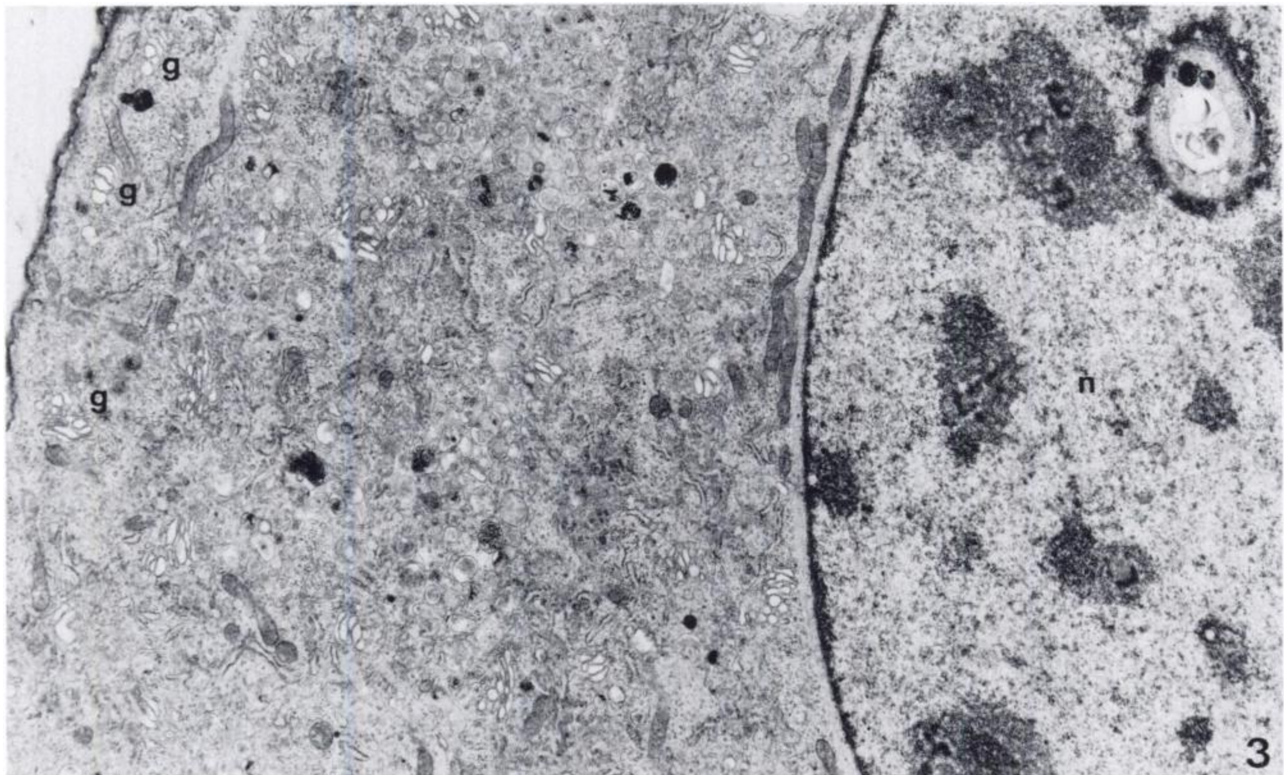
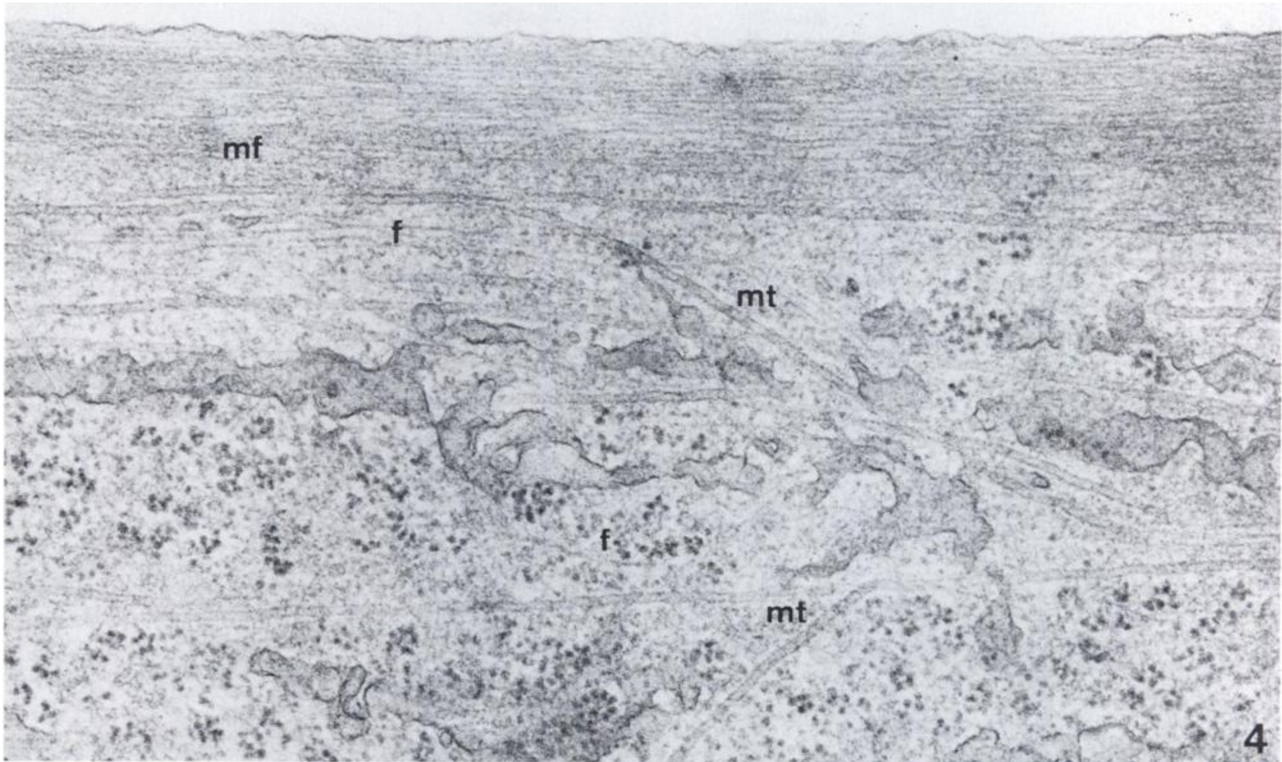
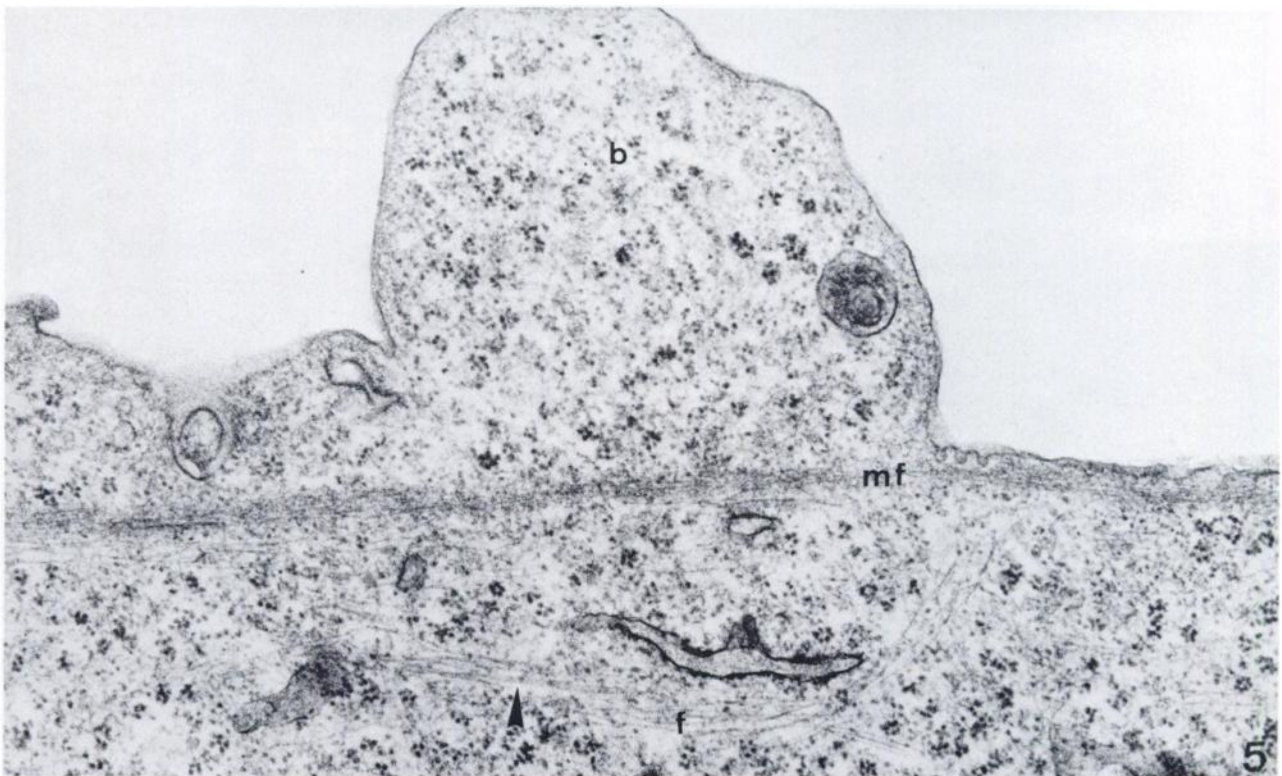


Fig. 2. General view of an untreated MO cell, sectioned parallel to the culture surface. A concentrated Golgi area (*g*) is located at 1 side of the nucleus. × 3800.

Fig. 3. MO cell treated for 5 hr with R 17934 (1 μg/ml). Individual Golgi organelles (*g*) are distributed throughout the cytoplasm and often assume a peripheral location. Note also the presence of patches of smooth vesicular material. *n*, a piece of the nucleus. × 7600.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022





Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

Fig. 4. The organization of the 3 types of filamentous structures in the cortical region, in an untreated MO cell. The subplasmalemmal region is occupied by a sheet of microfilaments (*mf*). The orientation of both microtubules (*mt*) and filaments (*f*) is largely parallel to the plasma membrane in the subcortical region. Deeper into the cytoplasm the orientation of these structures becomes more randomized. × 53,000.

Fig. 5. The cortical region of an MO cell treated for 20 min with R 17934 (1 µg/ml). Microtubules have disappeared. *Arrow*, a possible remnant. A normal subplasmalemmal sheet of microfilaments (*mf*) is present as well as 100-Å filaments (*f*). Note also the presence of a large bleb. × 36,200.

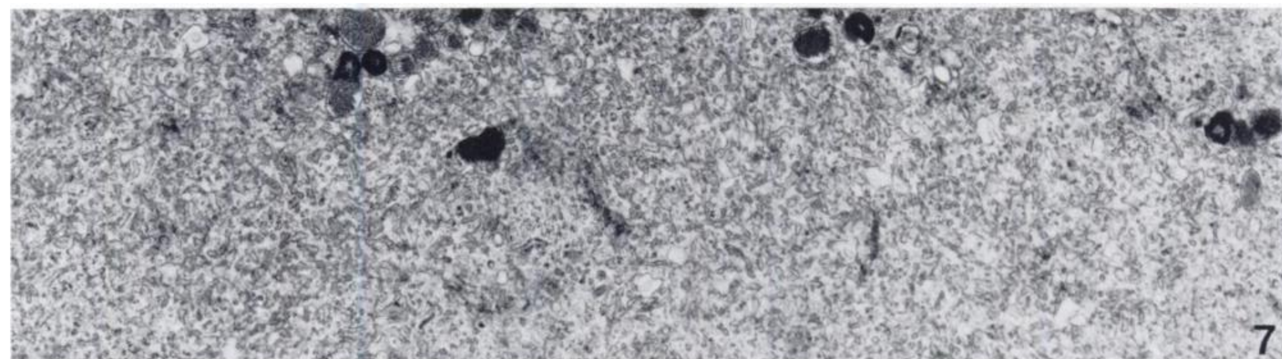
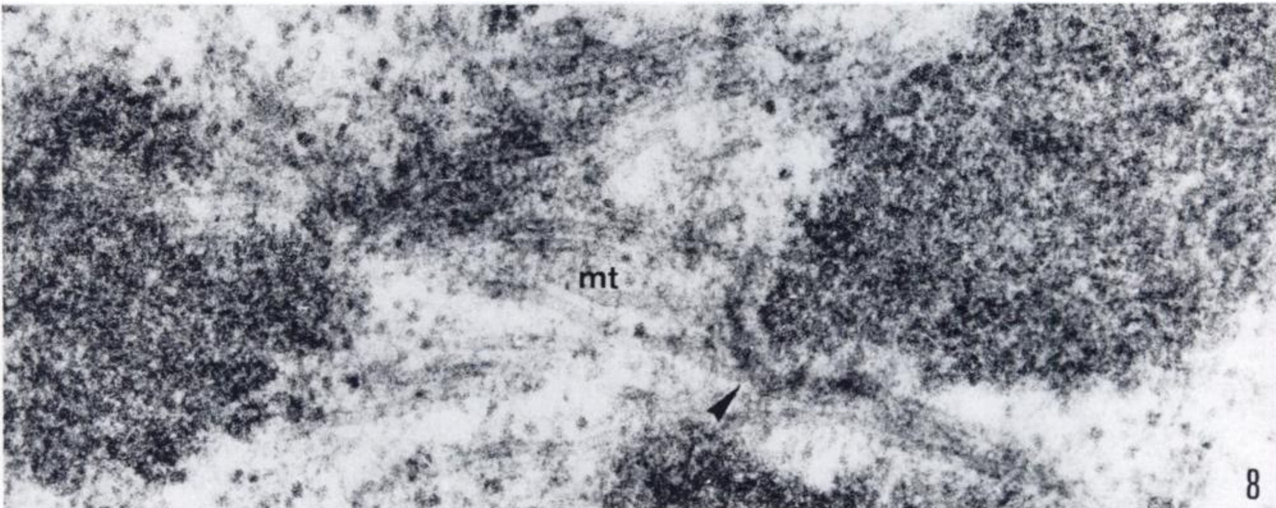


Fig. 6. MO cell treated for 5 hr with R 17934 (1 μg/ml). The centriolar complex (c) and many Golgi organelles have assumed a peripheral position near the plasma membrane. *Asterisks*, patches of smooth vesicular material in the vicinity of dispersed Golgi organelles. × 37,700.

Fig. 7. A large accumulation of smooth vesicular material in an MO cell treated for 5 hr with R 17934 (1 μg/ml). × 12,700.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022



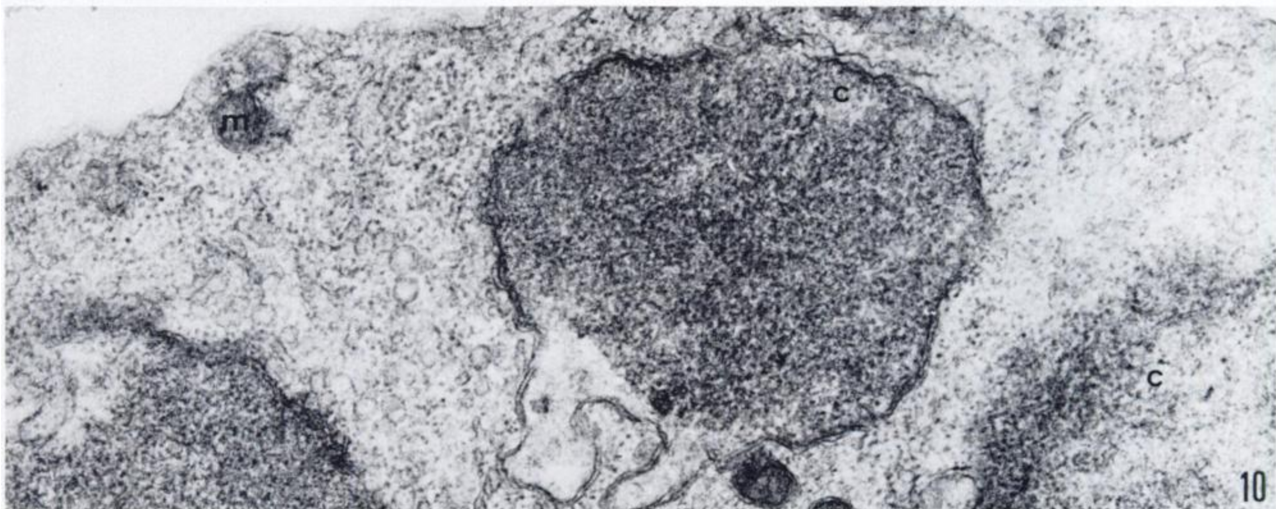


Fig. 8. Spindle microtubules (*mt*) in a mitotic MO cell. *Arrow*, a centromere region. × 82,000.

Fig. 9. The centromere region of a nondisjuncted chromosome in a mitotic MO cell treated for 5 hr with R 17934 (1 μg/ml). Note the complete absence of microtubules and the presence of mitochondria (*m*) in the vicinity of the chromosome. × 31,000.

Fig. 10. Chromosomes (*c*) that are being enveloped individually in a new nuclear membrane during the lobulated phase of the abortive mitosis in an MO cell treated for 24 hr with R 17934 (1 μg/ml). Note the presence of other organelles, vesicles, and mitochondria (*m*) between the chromatin masses and their peripheral location near the plasma membrane. × 34,800.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022

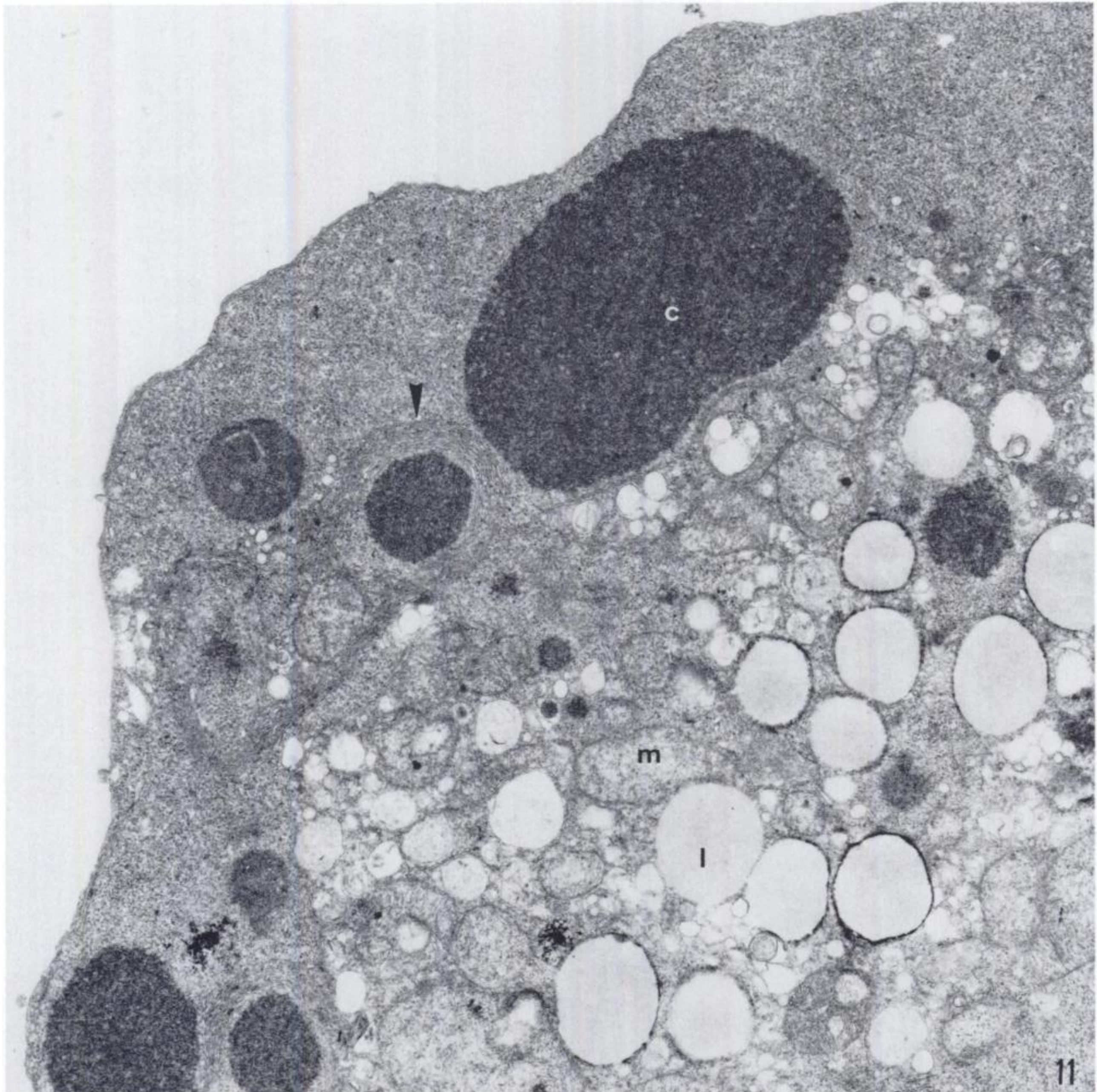


Fig. 11. Mitotic HeLa cell treated for 24 hr with R 17934 (1 μg/ml). Dispersed chromatin masses (c) are intermingled with membrane whorls (*arrow*), prenecrotic accumulations of lipid vesicles (*l*) and damaged mitochondria (*m*). × 14,5000.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/36/3/905/2396641/cr0360030905.pdf by guest on 25 August 2022