

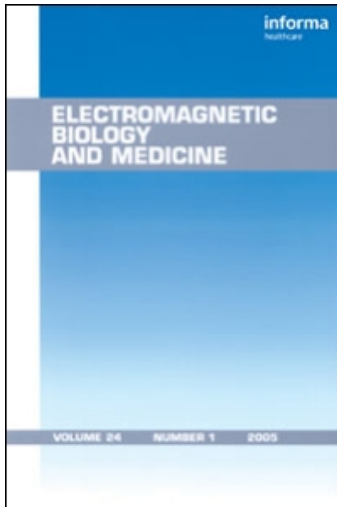
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SHORT COMMUNICATION

EFFECT OF ANODALLY GENERATED SILVER IONS ON FIBROSARCOMA CELLS

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ABSTRACT

The use and potential side effects of electrical parameters for possible tumor control are discussed. The conversion of tumor cells to normal cells by regenerating tissues is proposed as a possible alternate method. Preliminary observations indicating that anodally generated silver ions may have a similar action on human fibrosarcoma tumor cells in culture are reported.

INTRODUCTION

The recent paper "Tumor Bioelectric Potential and Its Possible Exploitation for Tumor Growth Retardation," by Miklavcic *et al.* (1), ably reviews the history of the concept of electrically treating cancer. I would add only that positive polarity DC was first used clinically on human uterine tumors in the late 1800s by Franklin M. Martin (2, 3). Using 75 mA, Martin noted prompt local anesthesia and prolonged relief from pain and bleeding, which he ascribed to the "acid" chemical reaction produced by the local electrolysis at the anode. He subsequently dropped all such experimentation upon discovering (using fertile chicken eggs) that the galvanic current was a "feticide."

Most of the recent experiments cited by Miklavcic *et al.* (1) have also used positive-polarity DC based on the concept that cell growth was biologically controllable by electrical factors, such as polarity, alone and little attention was paid to the electrochemical events occurring at the electrodes. Electrolysis always accompanies the passage of any level of direct current, with very different electrochemical events occurring at the anode compared with the cathode and, in part, dependent on the composition of the metallic anode. Miklavcic *et al.* (1) present a summary of these complex

events associated with stainless steel anodes. The extent and rate of electrolysis are a function of the applied voltage and current, and many of the reaction products, such as H^+ ions or certain metallic ions derived from the anodal material, are toxic to cells. However, in living tissues, such factors as the circulation rate and buffering capacity of the extracellular fluid prevent any toxic effects at low voltages and correspondingly low rates of electrolysis. With higher voltages, these factors are unable to prevent local toxic effects due to acidic pH shifts or actual production of gaseous hydrogen. Nordenstrom, for example, observed "gas cavities at sites of electrodes" in his lung cancer patients treated with 10 volts delivered to the anode (4). Therefore, for any implanted DC electrode system there will be a "low" voltage zone in which functionally significant biological effects will predominate (5). The voltage zone separating these two effects will vary depending on a number of factors, particularly the metallic composition of the electrodes. Since there is this major difference in the biological effect of low versus high voltages, I propose that the voltage at which the change in effect occurs be assigned a specific name, such as "overt" or "toxic" electrolysis. Miklavcic *et al.* attributed the tumor necrosis they observed to such toxic electrolysis, a conclusion I concur with. However, they proposed that this might be the basis for possible therapeutic use, a conclusion that I believe has a basic flaw.

The technique of inserting an electrode into a tumor and passing a current adequate to produce toxic effects within the tumor requires that completion of the electrical circuit occur with the current returning to the cathode. In this pathway, electrical parameters would drop below toxic electrolysis levels, and the effect of such current on peripheral tumor tissues or adjacent tumor masses must be considered. In 1981, I reported that DC parameters well below toxic electrolysis levels significantly stimulated tumor cell mitosis *in vitro*, equally at both the anode and the cathode when stainless steel was used as the electrode material (6). I concluded that electrical currents below this level of electrolysis were highly stimulatory to tumor cells in a non-polarity-dependent fashion. I know of no reported attempts to duplicate this work. Nevertheless, I believe it raises an important question regarding the use of simple DC electrical parameters as a therapeutic technique for cancer.

An alternative approach to the biological control of cancer has long been neglected. In 1877, Cohenheim postulated that cancer cells could be caused to mature into normal nonmalignant cells if their local environment could be made into a duplicate of the embryonic state (7). In 1948, Rose and Wallingford postulated that since the process of amphibian limb regeneration involved the dedifferentiation of the local cell population, it constituted such an embryonic environment, and they used this system to evaluate Cohenheim's hypothesis (8). They reported that frog renal cancer cells implanted into the limbs of salamanders grew, metastasized, and ultimately caused the death of the host animal unless the limb was amputated *through* the implant site. In this case, as regeneration of the limb began, the residual frog cancer cells were re-differentiated, along with the normal cells present, and were incorporated into the regenerating limb as normal tissue cells. In 1962, Seilern-Aspang and Kratochwil reported the successful induction of a primary malignant tumor in the salamander (9). A year later they reported the regression of this cancer with the induction of local regeneration (10). Shortly thereafter, Rose's experiment was duplicated and confirmed by Mizell using radioisotopically labeled Lucke-tumor cells implanted in salamanders (11).

In 1978, these experiments were the subject of an editorial by Wolsky (12), urging further investigation of the link between the dedifferentiation of regenerating cells and cancer. At the time, however, cancer was still viewed as primarily an irreversible metabolic disease and the link with dedifferentiation was obscure. Now that cancer is known to be a genetic disorder resulting from activation or derepression of previously inactive oncogenes (13), the link with dedifferentiation has become more tenable. Since dedifferentiation may be viewed as the total deprogramming of the entire genetic code, it may permit subsequent reprogramming to take place in the normal configuration of oncogene suppression. Since regeneration appears to be, at least in part, dependent on local, very low-strength, negative electrical polarity (14), I postulated that this electrical environment was a possible cause for the tumor cell dedifferentiation. Unfortunately, as noted above (6), I found that mammalian tumor cells appear to respond to such an environment by increasing their mitotic rate.

Some years prior to that experiment, I had developed an electrochemical technique for the treatment of recalcitrant local infection based on the release of silver ions from silver anodes placed directly in the wound and energized well below predetermined toxic electrolysis levels. Clinical studies demonstrated the desired antimicrobial effect but also revealed a growth stimulatory action despite the positive polarity (15,16). This stimulatory effect was subsequently determined to result from the production of dedifferentiation of normal mammalian fibroblast cells apparently by the electrically generated silver ions (17). The effect of this technique on human cancer cells has been evaluated in a preliminary fashion and the results appear worth reporting in the overall context of the electrical control of tumors.

METHODS

Human fibrosarcoma cells (ATCC-HT-1080) were grown on glass microscope cover slips in triwell plastic culture dishes with modified Eagle's media with 10% fetal calf serum and Hepe's buffer. The dishes were modified so that current could be passed through two of the chambers, isolating the positive and negative environments and providing a control chamber (Fig. 1). Embedded electrodes were 28-gauge 99.99 fine silver. Equal aliquots of stock cultures were added to each chamber and incubated at 37 °C for 24 h. Phase contrast microscopic visual inspection was then made to ensure that the cultures were free of contamination and in the log phase of growth. For the next 24 h direct current averaging 360 nA was passed through the two electrodes. Visual examination was again performed to ensure that there was no gas formation or overt, toxic cellular effects, and the cultures were incubated for an additional 24 h without current flow before photographing and harvesting by removal of the cover slips. The cells were subsequently fixed and stained *in situ* with Giemsa-Jenner and the cover slips fixed to standard glass microscope slides for examination and photographs.

RESULTS

Of 10 separate experiments, two were lost to contamination and eight were carried to completion. The cellular effects observed at 72 h in all eight successful cultures were

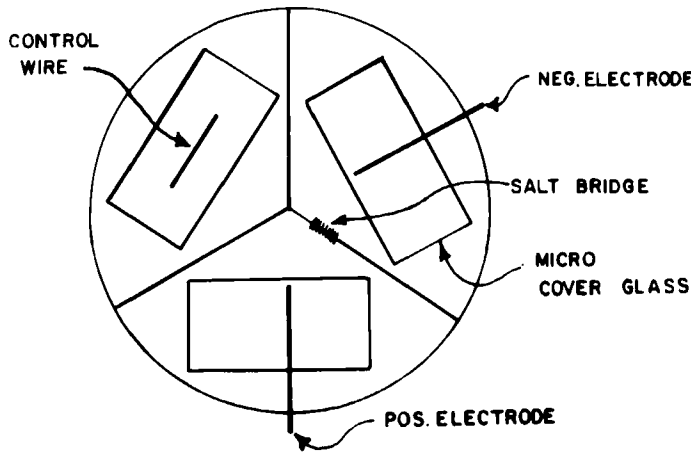


FIGURE 1. Schematic view of the modified, plastic, triwell culture dishes. In preparation, straight segments of wire were cut, and one end was heated and passed through the wall of two chambers over previously inserted glass cover slips. The wires were fixed in place with a drop of epoxy cement applied to their insertion point on the outside of the dish. After drying, the dishes were gas sterilized and allowed to equilibrate for 1 week. Aliquots of prepared media were placed in each chamber, followed by aliquots of cell suspension and incubation at 37°C.

identical. Cells from three of the eight positive silver chambers were continued in subculture (with media changes every other day) for periods up to 35 days after the initial 72-h treatment. All three subcultures demonstrated the same cellular behavior.

The HT-1080 cells in the control chambers were typically pleomorphic, attached to the glass substrate, and the cultures were still actively growing at the end of the 72-h incubation (Fig. 2). In the negative polarity chambers the HT-1080 cells demonstrated a marked increase in growth rate compared with the control chamber (Fig. 3), confirming the previous report (6) in which stainless steel electrodes were used. In the positive silver chambers the growth rates appeared to have been slowed, possibly because of the cessation of mitosis shortly after the beginning of current passage. These cells were markedly changed in morphology into free-floating, rounded cells with deepening basophilic cytoplasm and eccentric nuclei (Fig. 4). Microscopic observation during current passage revealed the morphological change to begin adjacent to the electrode and the spread to involve all cells in the chamber by the end of the third 24-h period. Retention of these cells in culture for periods up to 35 days demonstrated an initial tendency to clump together but remain free-floating. This was followed by a period of total inactivity for up to an average of 25 days with no mitotic activity or morphological changes. At that time the cells slowly regained their original HT-1080 morphology and, thereafter, resumed their normal mitotic rate. Each experimental run produced the same results in the control, negative, and positive chambers as noted above.

DISCUSSION

In prior experiments utilizing this same technique on 3T6 mammalian fibroblasts, the same results were obtained in the anodal silver chamber (17) both at 72 h and in

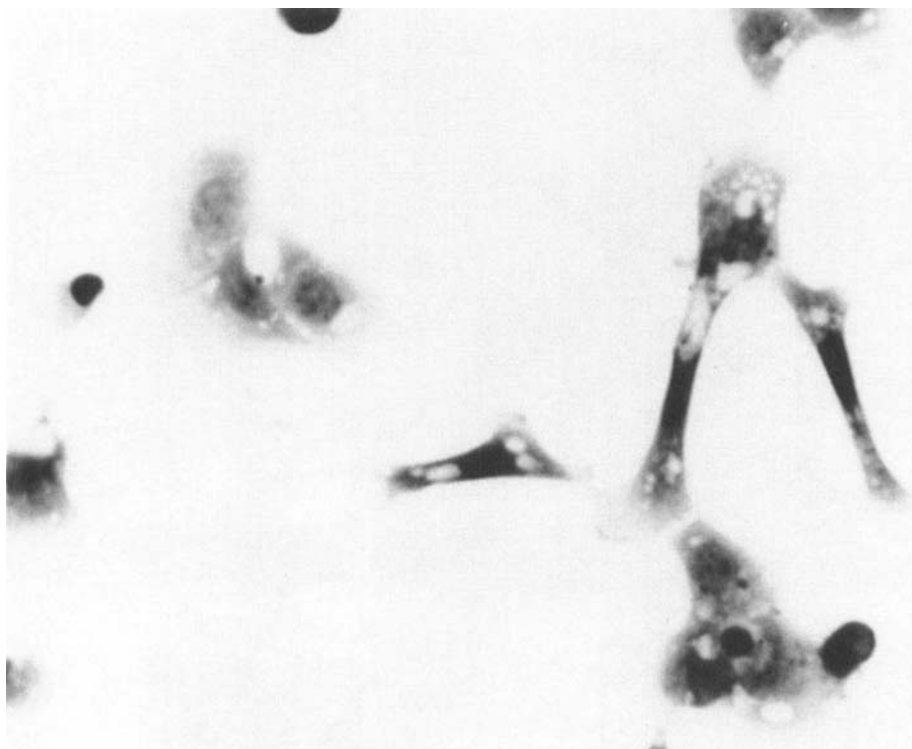


FIGURE 2. Photomicrograph (200 \times), of stained HT-1080 cells from a control chamber at the end of 72-h incubation. The morphology and growth rate are typical of these cells.

continued culture. I postulate that in each case the quiescent cells in continued culture were equivalent to dedifferentiated cells of the regenerating blastema and would have had the ability to redifferentiate if provided with an appropriate induction signal. Recent clinical trials with the anodal silver technique in cases of full-thickness skin loss have revealed the regeneration of anatomically normal, full-thickness skin with specific characteristics appropriate to the area and accompanied by peripheral nerve regeneration (18).

Duplication of these experiments both *in vitro* and *in vivo* with anodes composed of several other metals (gold, platinum, stainless steel) did not yield similar results, except for the growth stimulation in both the anode and cathode chambers. Therefore, I believe the anodal effect described is due to the release of silver ions from the silver anode. The exact mechanisms involved in this action of anodally generated silver ions on both normal fibroblasts and fibrosarcoma cells can only be speculated upon at this time. The size of the silver ion appears to preclude passage through the intact cell membrane, and an action on some membrane-spanning receptor such as the *trk* proto-oncogene (19,20) appears more likely.

CONCLUSION

Anodally produced silver ions appear to produce dedifferentiation of human fibroblasts, *in vitro*, and *in vivo*, and some human cancer cells, *in vitro*. In view of



FIGURE 3. Photomicrograph ($200\times$) of stained HT-1080 cells from a negative chamber at the end of the 72-h experiment. In comparison with Figure 2, the increase in the growth rate is apparent.

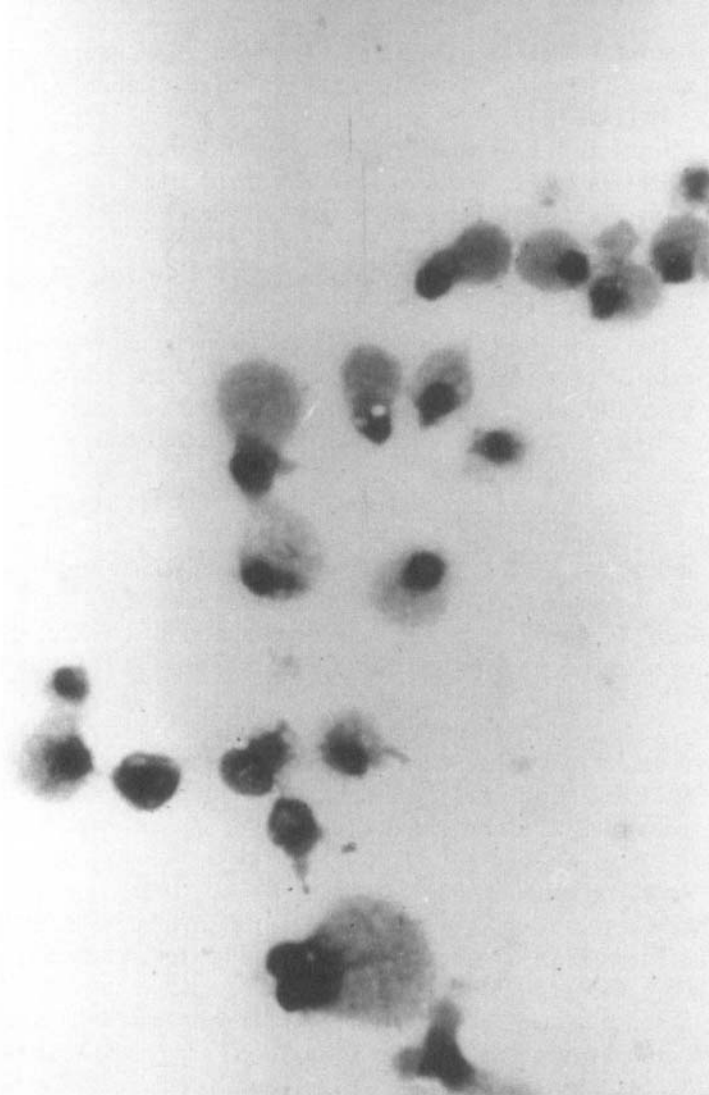


FIGURE 4. Photomicrograph (200 \times) of stained HT-1080 cells from a positive silver chamber at the end of the 72-h experiment. The alteration in morphology is apparent and is similar to that observed with 3T6 fibroblasts under the same condition.

the reports that dedifferentiation of animal cancer cells *in vivo* results in their reversion to normal cell types, further studies on the anodal silver technique, and other similar electrochemical methods, appear justified.

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