Enhanced In Vitro Selective Toxicity of Chemotherapeutic Agents for Human Cancer Cells Based on a Metabolic Defect [1,2]

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ABSTRACT—A metabolic defect that is prevalent in human cancer cell lines was exploited to selectively kill these cells without killing cocultured normal human fibroblasts. Methionine dependence, a metabolic defect seen only in cancer cells or immortalized cell lines in vitro, precludes the cells from growing in media in which methionine is replaced by its immediate precursor, homocysteine, a condition that allows the growth of all normal cell strains tested. The methionine-dependent cells become reversibly blocked in late S-G_2 (i.e., late-S and G_2 phases) under the above condition, a block that was exploited for selective chemotherapy against these cells. In cultures that were initiated with equal amounts of cancer cells and human diploid fibroblasts, substitution of homocysteine and doxorubicin for methionine in the culture medium followed by methionine repletion with vincristine was totally effective at selectively eliminating a methionine-dependent human sarcoma and 3 methionine-dependent human carcinomas. The above protocol was nearly totally effective against a partially methionine-independent revertant of the sarcoma. The chemotherapeutic procedure used was not lethal to normal cells growing alongside the tumor cells and was ineffective when conducted totally in methionine-containing medium. The optimal procedure was 10^{-10} *M* doxorubicin in methionine-free, homocysteine-containing medium for 10 days followed by 2×10^{-7} *M* vincristine in methionine-containing, homocysteine-free medium for 1 day, in turn followed by drug-free methionine-containing, homocysteine-free medium. These results demonstrate the potential for treatment of solid tumors with chemotherapy based on metabolic differences between normal and tumor cells.—JNCI 1986; 76:629–639.

With a few exceptions, chemotherapy of solid human tumors has been largely unsuccessful. Chemotherapeutic drugs in their current usage are generally highly toxic substances, cytotoxic to normal and tumor cells alike, and lead to poor therapeutic indices with insufficient killing of tumor cells and troublesome side effects. For success with relatively slow-growing solid tumors, it is necessary to design chemotherapy that takes advantage of metabolic differences between tumor and normal cells.

Chemotherapeutic agents fall into a number of classes. One class consists of drugs that interfere with DNA synthesis and have their greatest effect on cells in the S-phase of their cycle, while another comprises drugs that interfere with mitotic-spindle formation and have their greatest effect on cells in the M-phase of the cell cycle. An example of the first type is doxorubicin (*1*), an anthracycline antibiotic that affects the S-phase by intercalating into DNA, although it has some effect on other phases of the cell cycle; an example of the second is vincristine (*2*), an alkaloid that interferes with microtubule formation and thereby affects the M-phase, although it too has some effect on other phases of the cell cycle. Any procedure that selectively increases the percentage of tumor cells in either the S- or M-phase would improve the therapeutic index of one or the other agent, while one that selectively increases the percentage of tumor cells in both phases sequentially could take advantage of both classes of drugs.

Met dependence is a phenomenon found only in transformed cells that are "immortal" (*3, 10, 11*). To date, no normal unestablished cell strain, meaning a cell culture derived from normal tissue that has maintained its normal character and has not become immortalized, has been found to be Met dependent [(*3, 4, 10, 11*); Lechner J: Personal communication]. Met dependence is defined as the inability or greatly reduced ability of a cell to grow when the Met in the media is replaced by its immediate precursor Hcy to give Met^-Hcy^+ medium. Of 25 human cell lines from a broad range of human tumors tested in our laboratory, including carcinomas, sarcomas, and neurologic tumors, 13 were found to be absolutely Met dependent in that they were totally unable to grow in Met^-Hcy^+ medium, while 11 others had varying amounts of growth-rate reduction [(*5*); Wallace CD, Hoffman RM: Unpublished results]. Only 1 cell line out of the 25 had its growth rate unaffected by the switch from Met^+Hcy^- to Met^-Hcy^+ medium. Normal cells in Met^-Hcy^+ medium grow quite well and indeed animals have thrived when the Met in their diet has been replaced by Hcy (*6*).

When Met-dependent cells are placed in Met^-Hcy^+ medium, the cells go through stages in which the percentage of cells in the S-phase increases until after 9 days when the cells are reversibility arrested in the late S-G_2 (i.e., late-S and G_2 phases) stage, thus synchronizing them for immediate entry into mitosis when Met is

ABBREVIATIONS USED: Hcy=homocysteine; Met=methionine.

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again added to the medium (*7*). In this study, we take advantage of this cell-cycle block of Met-dependent tumor cells incubated in Met^-Hcy^+ media to selectively eliminate the tumor cells from a mixed population with normal Met-independent cells by the use of the cell cycle-dependent drugs doxorubicin and vincristine.

MATERIALS AND METHODS

Cell lines chosen were HOS-1A, a clone of the human Met-dependent osteogenic sarcoma HOS (*8*); HOS-1A-R3, a partially Met-independent revertant derived from HOS-1A (Wallace CD, Hoffman RM: Unpublished results); PC3-1C (Wallace CD, Hoffman RM: Unpublished results), a clone of the human Met-dependent prostatic carcinoma PC3 (*9*); A2182-5D, a clone of the Met-dependent lung cancer A2182 [(*5*); Wallace CD, Hoffman RM: Unpublished results]; MCF-7, a human Met-dependent breast cancer cell line (*5*); and FS-3, a normal diploid fibroblast cell strain derived from human foreskin. The FS-3 strain was a gift from Dr. Jerry A. Schneider. Cells were grown in Eagle's minimum essential medium containing 10% fetal bovine serum and supplemented with either 100 μM L-Met or 100 μM L-Hcy thiolactone and 100 μM folic acid and 1.5 μM hydroxocobalamin, selective conditions that distinguish Met-dependent cells from Met-independent cells (*3, 4, 5, 7, 10, 11*).

Cells were seeded in 6-well cluster plates in Met^+Hcy^- medium. Each well contained 2×10^4 cells of normal fibroblast strain FS3 plus 2×10^4 cells of one of the tumor cell types. After 24 hours' incubation, groups were divided in the following manner. One control group for each tumor cell type was maintained in Met^+Hcy^- medium. Another control group was kept in Met^-Hcy^+ medium for 10 days and then placed in Met^+Hcy^- medium. A third group was placed in Met^+Hcy^- medium containing 10^{-10} M doxorubicin for 10 days and then switched to drug-free Met^+Hcy^- medium, while a fourth group had 10^{-10} M doxorubicin in Met^-Hcy^+ medium for 10 days before being switched to drug-free Met^+Hcy^- medium. Groups 5 and 6 were incubated in Met^+Hcy^- and Met^-Hcy^+ medium, respectively, for 10 days, treated with Met^+Hcy^- medium containing 2×10^{-7} M vincristine for 24 hours, and then placed in drug-free Met^+Hcy^- medium for the remainder of the experiment. Groups 7 and 8 were incubated in Met^+Hcy^- and Met^-Hcy^+ medium, respectively, containing 10^{-10} M doxorubicin. After 10 days they were treated for 24 hours with Met^+Hcy^- medium containing 2×10^{-7} M vincristine and then placed in drug-free Met^+Hcy^- medium for the duration of the experiment. Except as otherwise noted, the experiment was conducted for a total of 31 days and the medium was replaced at 3- to 4-day intervals. Photomicrographs of the cells were taken at various time periods; after the experiment was completed the cells were fixed in methanol, stained with Giemsa's stain, and photographed again.

RESULTS

Figure 1 shows the effect of various treatments on the growth of Met-dependent human sarcoma HOS-1A cocultured with human diploid fibroblast FS-3. All procedures conducted solely in Met^+Hcy^- medium (groups 1, 3, 5, 7) proved inadequate at selectively preventing the proliferation of HOS-1A cells (fig. 1A–D). Placement of the cell coculture in Met^-Hcy^+ medium (group 2) selectively reduced the rate of tumor-cell growth but did not eliminate the tumor cells (fig. 1E). Met^-Hcy^+ medium containing 10^{-10} M doxorubicin (group 4) did not greatly enhance the selective effect (fig. 1F). The use of Met^-Hcy^+ medium followed by 2×10^{-7} M vincristine in Met^+Hcy^- medium (group 6) was more selective against the tumor cells but still did not completely eliminate the tumor cells (fig. 1G). The combination chemotherapy of 10^{-10} M doxorubicin in Met^-Hcy^+ medium followed by 2×10^{-7} M vincristine in Met^+Hcy^- medium for 24 hours and then drug-free Met^+Hcy^- medium (group 8) was the only procedure that resulted in a culture completely free of HOS-1A cells while still allowing proliferation of the normal fibroblasts (figs. 1H, 3D).

In data not shown, use of 10^{-7} M vincristine for 4 days resulted in less tumor-cell kill and more normal-cell toxicity than 2×10^{-7} M vincristine for 1 day in a procedure equivalent to that performed on group 8 as described above.

Figure 2 shows the effect of various treatments on a coculture of HOS-1A-R3, a partially Met-independent revertant of HOS-1A able to grow to a certain extent in Met^-Hcy^+ media (data not shown), with a normal diploid fibroblast strain FS-3. As with HOS-1A, no procedure in Met^+Hcy^- medium selectively eliminated the HOS-1A-R3 tumor cells (groups 1, 3, 5, 7; fig. 2A–D). Use of Met^-Hcy^+ medium alone (group 2) had much less effect on HOS-1A-R3 than on HOS-1A cells, as would be expected for a partially Met-independent cell line (figs. 1E, 2E). Met^-Hcy^+ medium containing 10^{-10} M doxorubicin (group 4, fig. 2F) was somewhat better than Met^-Hcy^+ medium itself (fig. 2E) and better than Met^-Hcy^+ followed by 2×10^{-7} M vincristine in Met^+Hcy^- medium (group 6, fig. 2G), but neither procedure resulted in much tumor-cell kill. Once again the combination procedure of Met^- Hcy^+ medium with 10^{-10} M doxorubicin followed by 1 day of 2×10^{-7} M vincristine in Met^+Hcy^- medium, in turn followed by drug-free Met^+Hcy^- medium, gave the best result (group 8, fig. 2H). Although the treatment did not completely eliminate the tumor cells, very few tumor cells remained compared to the number of normal cells.

The Met-dependent human prostate carcinoma cell line PC3-1C proved highly susceptible to Met^-Hcy^+ medium (group 2) when cocultured with normal diploid human fibroblast strain FS-3. While no treatment in Met^+Hcy^- medium (groups 1, 3, 5, 7) had any effect, hardly any PC3-1C cells remained after incubation in Met^-Hcy^+ medium; and the use of either chemotherapeutic agent in combination with Met^-Hcy^+ medium

(groups 4, 6) resulted in total tumor-cell kill while allowing the normal cells to grow (data not shown).

Figure 3 contains photomicrographs taken at different time periods during the combination chemotherapy of human osteogenic sarcoma HOS-1A growing alongside normal human diploid fibroblast strain FS-3. Figure 3A shows the cells 1 day after plating and indicates the presence of cells of each type. Figure 3B shows the cells 3 days after the removal of vincristine in treatment that involved 10 days in Met^-Hcy^+ medium containing 10^{-10} *M* doxorubicin and a subsequent switch to Met^+Hcy^- medium and 2×10^{-7} *M* vincristine for 24 hours, in turn followed by drug-free Met^+Hcy^- medium (group 8). The treatment had little effect on the normal cells. Figure 3C shows the culture described in figure 3B after another 10 days and demonstrates the presence of a confluent monolayer of normal cells and the absence of colonies of tumor cells. Figure 3D is a photomicrograph of the stained culture described in figures 3B and 3C, at the end of the experiment on day 31, showing once again the healthy state of the normal cells and absence of any tumor cells. Figure 3E is a photomicrograph of the stained cells of group 1 in drug-free Met^+Hcy^- medium showing complete overgrowth by the HOS-1A tumor cells over the FS-3 normal diploid fibroblasts. Figure 3F is a photomicrograph of the stained cells of group 7, treated with Met^+Hcy^- medium containing doxorubicin followed by the same medium with vincristine, in turn followed by the same medium without either drug, and shows colonies of HOS-1A tumor cells on a background of FS-3 normal cells.

Figure 4 contains photomicrographs of the culture that was initiated with an equal number of human breast cancer MCF-7 cells and normal diploid human fibroblasts. Figure 4A is a photomicrograph of group 1 in drug-free Met^+Hcy^- medium and shows great overgrowth by the cancer cells. Figure 4B is a photomicrograph of group 2 in Met^-Hcy^+ medium for 13 days followed by Met^+Hcy^- medium for 14 days and shows a large number of tumor cells on a layer of normal fibroblasts. Figure 4C is a photomicrograph of group 8, the total treatment—Met^-Hcy^+ medium with 10^{-10} *M* doxorubicin for 13 days, 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hours, and drug-free Met^+Hcy^- medium for 13 days—and shows healthy normal fibroblasts and the absence of tumor cells.

In data not shown, we have found that the human lung tumor cell line A2182-5D behaved exactly as the above-mentioned breast cancer cell line MCF-7, osteogenic sarcoma cell line HOS-1A, and prostate carcinoma cell line PC3-1C in that they too could be selectively eliminated from a coculture with normal cells by the Met-dependent chemotherapy.

DISCUSSION

When Met-dependent cells are incubated in Met^-Hcy^+ medium, the percentage of cells in S-phase increases; eventually they become reversibly blocked in late S-G_2 as detected by cell-sorting experiments measuring DNA content (7). With HOS-1A cells, this can be seen by an increase in the incorporation of tritiated thymidine into DNA relative to the amount of proliferation when the cells are incubated in Met^-Hcy^+ medium. Somewhat surprisingly, the same result was found for the partially Met-independent revertant HOS-1A-R3, although to a lesser extent than for its Met-dependent parent, HOS-1A. Although the Met-independent revertant is able to grow in Met^-Hcy^+ medium, its rate of growth is reduced relative to the rate of growth of fully normal cells in the same medium (Stern P, Hoffman R: Unpublished results).

After the percentage of cells in the S-phase is increased over time, Met-dependent cancer cells in Met^-Hcy^+ medium finally are reversibly arrested at the late S-G_2 stage (7) as mentioned above. This synchronization was expected to make the cancer cells in Met^-Hcy^+ medium more sensitive to a drug affecting DNA synthesis and then to a mitotic-spindle drug such as vincristine when the cells entered mitosis as a result of being placed once again in Met^+Hcy^- medium. However, the potentiation of the effect of vincristine due to synchronization would be lost after as little as one cell cycle. It was therefore thought that a single large dose of vincristine in a short time period would be more effective than a smaller dose given over a longer time period, and such was found to be the case. For normal cells, conversely, the lack of synchronization in Met^-Hcy^+ medium was expected to make them less susceptible than the tumor cells to doxorubicin and then to vincristine given over a short time period after the switch to Met^+Hcy^- medium; and such was found to be the case.

The substitution of Hcy for Met in the culture medium (Met^-Hcy^+ medium) has thus been shown to greatly increase the in vitro selective toxicity of a chemotherapeutic procedure involving doxorubicin in Met^-Hcy^+ medium followed by Met^+Hcy^- medium containing vincristine for a Met-dependent human sarcoma, 3 Met-dependent carcinomas, and a partially Met-independent revertant of the above sarcoma. Further work to be done would include the extension of the technique to other tumor cells including those in primary culture, the testing of other possible chemotherapeutic agents for Met-dependent chemotherapy, and the attempt at such treatment of human tumors implanted in "nude" mice by replacement of the Met in the diet with Hcy. Toward the last goal, it may be necessary to deplete serum Met with a methioninase. However, in studies we have performed we have shown that normal fibroblasts as well as cancer cells growing in Met-free Hcy-containing medium secrete Met into the medium (Stern P, Hoffman R: Unpublished data). The fact that the procedure is effective in vitro despite the excretion of Met into the medium indicates that enzymatic depletion may not be required in vivo.

In all cases, but for lung cancer cells A2182-5D, the normal fibroblasts were dividing until after the completion of the therapy phase, after which they became confluent (data not shown). Thus in these experiments, the fibroblasts can serve as a model for dividing normal

cells. In the experiment with lung cancer A2182-5D cells, the normal fibroblasts became confluent prior to the addition of vincristine, at which time the culture still contained cancer cells (data not shown); in this case, at least for the vincristine treatment, the normal fibroblasts served as a model of nondividing cells in vivo.

This work demonstrates the potential of administering chemotherapy on the basis of metabolic differences between normal and cancer cells. Perhaps this approach will finally lead to effective chemotherapy for many types of solid tumors that are refractory to current chemotherapy methods that, although potentially effective for high growth-fraction leukemias and lymphomas, are not effectve in many types of solid tumors, possibly in part because their growth fraction is lower than that of many types of normal tissues.

REFERENCES

(1) ARCAMONE F, FRANCESCHI G, PENCO S, et al. Adriamycin (14-hydroxydaunomycin), a novel antitumor antibiotic. Tetrahedron Lett 1969; 13:1007-1010.

(2) SVOBODA GH. Alkaloids of *Vinca rosea*. IX. Extraction and characterization of leurosidine and leurocristine. Lloydia 1961; 24:173-178.

(3) HOFFMAN RM. Methionine dependence in cancer cells—a review. In Vitro 1982; 18:421-428.

(4) COALSON DW, MECHAM JO, STERN PH, et al. Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells. Proc Natl Acad Sci USA 1982; 79:4248-4251.

(5) MECHAM JO, ROWITCH D, WALLACE CD, et al. The metabolic defect of methionine dependence occurs frequently in human tumor cell lines. Biochem Biophys Res Commun 1983; 117:429-434.

(6) DUVIGNEAUD V, DYER HM, HARMON J. The growth-promoting properties of homocystine when added to cystine-deficient diet and the proof of structure of homocystine. J Biol Chem 1933; 101:719-726.

(7) HOFFMAN RM, JACOBSEN SJ. Reversible growth arrest in simian virus 40-transformed human fibroblasts. Proc Natl Acad Sci USA 1980; 77:7306-7310.

(8) EVA A, ROBBINS KC, ANDERSEN PR, et al. Cellular genes analogous to retroviral *onc* genes are transcribed in human tumor cells. Nature 1982; 295:116-119.

(9) KAIGHN M, NARAYAN KS, OHNUKI Y, et al. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 1979; 17:16-23.

(10) HOFFMAN RM. Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. Biochim Biophys Acta 1984; 738:49-87.

(11) ———. Altered methionine metabolism and transmethylation in cancer. Anticancer Res 1985; 5:1-30.

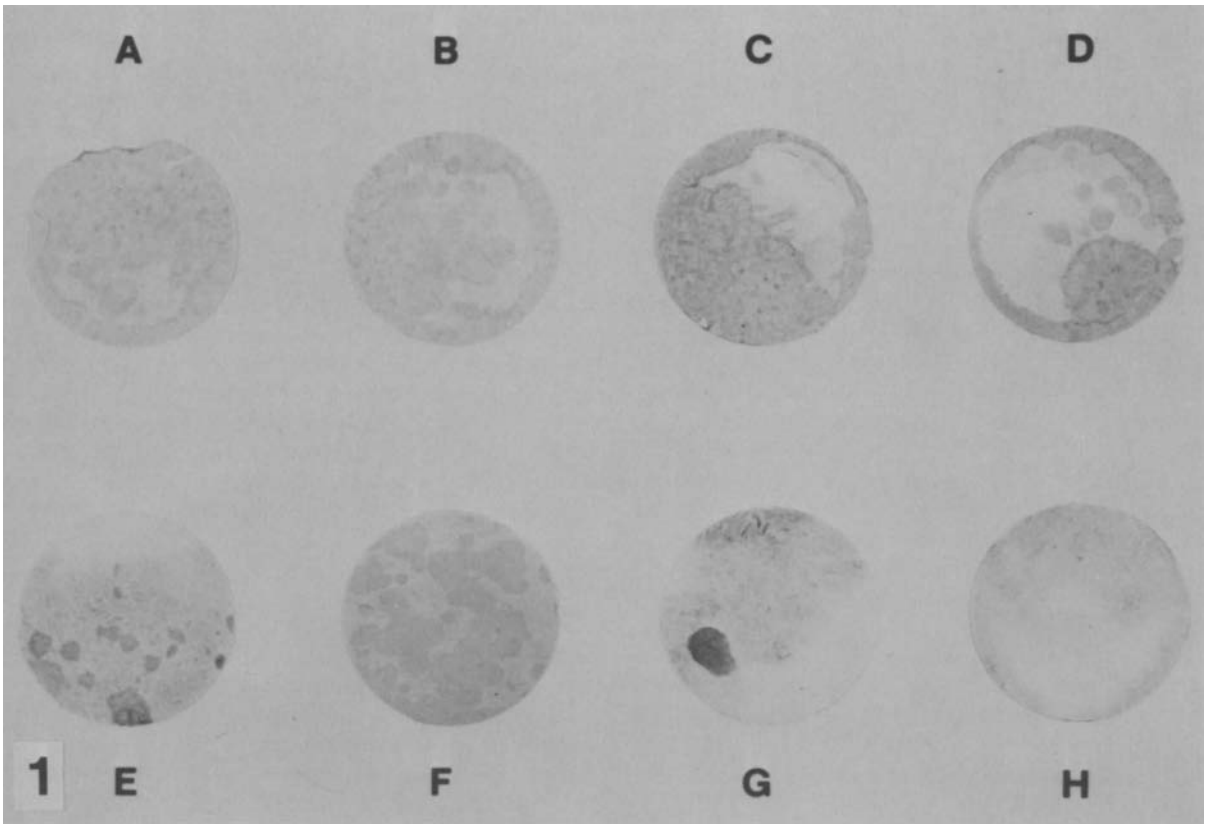


FIGURE 1A–H.—Fixed and stained HOS-1A Met-dependent human osteogenic sarcoma cells cocultured with FS-3 human diploid normal fibroblasts. After treatment, the cells were fixed with methanol and stained with Giemsa's stain on day 31 of the experiment or at day 20 after the last treatment. A) Drug-free Met^+Hcy^- medium (group 1) (*see* fig. 3E for a photomicrograph). *Note* that the majority of the cells are tumor colonies. B) 10^{-10} *M* doxorubicin in Met^+Hcy^- medium for 10 days followed by drug-free Met^+Hcy^- medium (group 3). *Note* that the majority of the cells are tumor colonies. C) Drug-free Met^+Hcy^- medium for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- medium (group 5). *Note* that the majority of the cells are tumor colonies. D) 10^{-10} *M* doxorubicin in Met^+Hcy^- medium for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- medium (group 7) (*see* fig. 3F for a photomicrograph). *Note* that the majority of the cells are tumor colonies. E) Drug-free Met^-Hcy^+ medium for 10 days followed by drug-free Met^+Hcy^- medium (group 2). *Note* some darkly stained tumor colonies present on a lighter background of normal fibroblasts. F) 10^{-10} *M* doxorubicin in Met^-Hcy^+ medium for 10 days followed by drug-free Met^+Hcy^- (group 4). *Note* the majority of the cells are tumor colonies. G) Drug-free Met^-Hcy^+ for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- medium (group 6). *Note* the presence of one darkly stained tumor colony on a lighter background of normal fibroblasts. H) 10^{-10} *M* doxorobicin in Met^- Hcy^+ for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- for 24 hr followed by drug-free Met^+Hcy^- medium (group 8) (*see* fig. 3D for a photomicrograph). *Note* the absence of dark tumor colonies, confirmed by photomicrograph 3D.

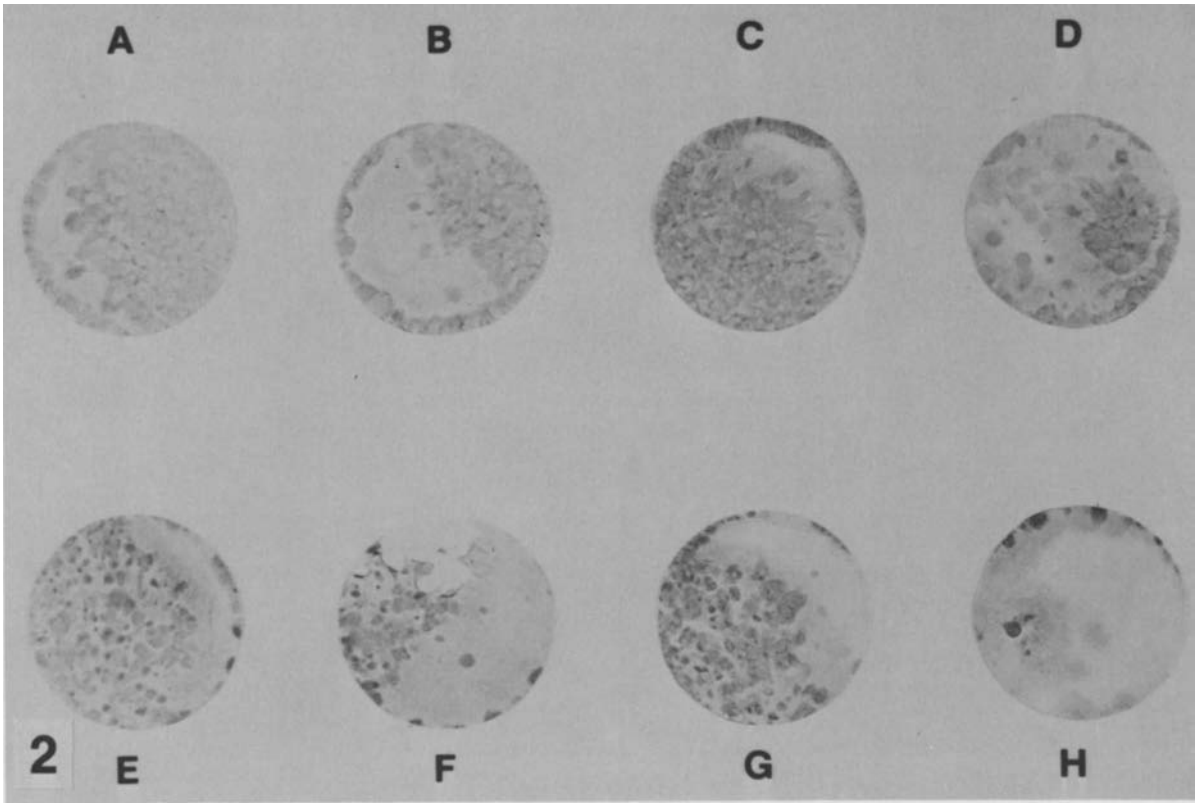
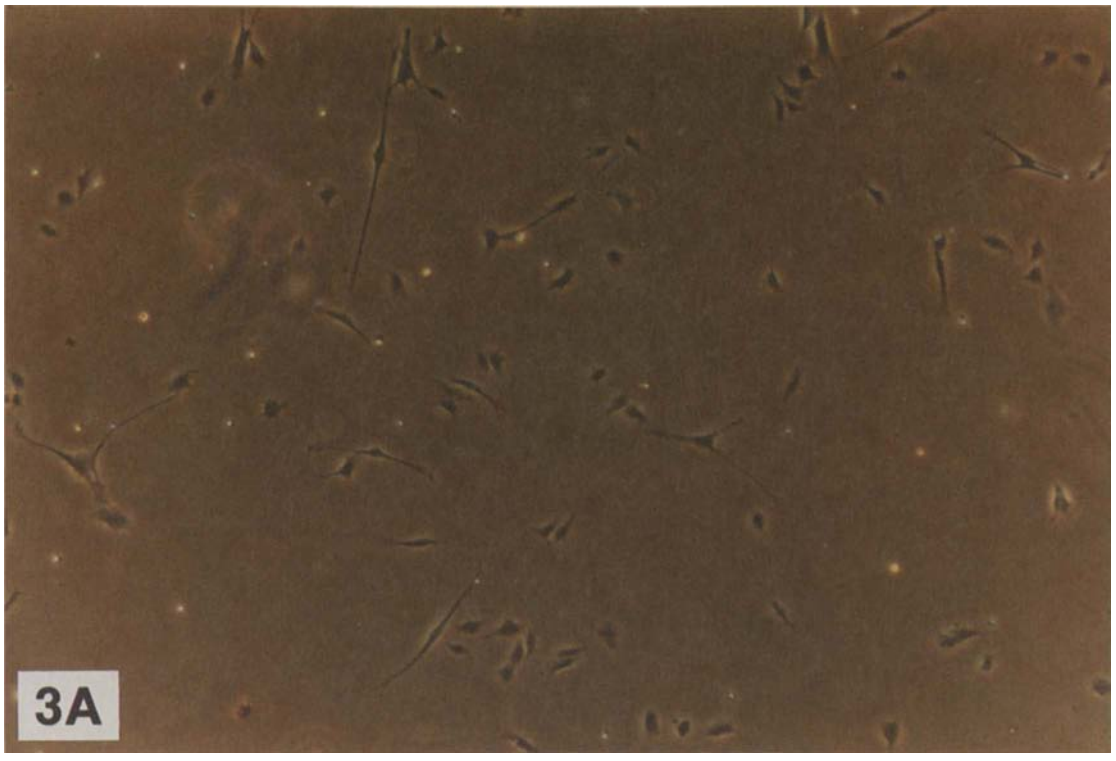


FIGURE 2A-H.—Stained HOS-1A-R3 partially Met-independent osteogenic sarcoma cocultured with FS-3 human diploid normal fibroblasts. After treatment, the cells were fixed with methanol and stained with Giemsa's stain on day 31 of the experiment or at day 20 after the last treatment. A) Drug-free Met^+Hcy^- medium (group 1). *Note* large area of darkly stained tumor colonies on a lighter background of normal fibroblasts. B) 10^{-10} *M* doxorubicin in Met^+Hcy^- medium for 10 days followed by drug-free Met^+Hcy^- medium (group 3). *Note* large area of darkly stained tumor colonies on a lighter background of normal fibroblasts. C) Drug-free Met^+Hcy^- medium for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- medium (group 5). *Note* large area of darkly stained tumor colonies on a lighter background of normal fibroblasts. D) 10^{-10} *M* doxorubicin in Met^+Hcy^- medium for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- medium (group 7). *Note* large area of darkly stained tumor colonies on a lighter background of normal fibroblasts. E) Drug-free Met^- Hcy^+ for 10 days followed by drug-free Met^+Hcy^- medium (group 2). *Note* large area of darkly stained tumor colonies on a lighter background of normal fibroblasts. F) 10^{-10} *M* doxorubicin in Met^- Hcy^+ medium for 10 days followed by drug-free Met^+Hcy^- medium (group 4). *Note* fewer colonies of tumor cells. G) Drug-free Met^-Hcy^+ for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- medium for 24 hr followed by drug-free Met^+Hcy^- (group 6). *Note* large area of darkly stained tumor cells on a lighter background of normal fibroblasts. H) 10^{-10} *M* doxorubicin in Met^-Hcy^+ medium for 10 days followed by 2×10^{-7} *M* vincristine in Met^+Hcy^- for 24 hr followed by drug-free Met^+Hcy^- medium (group 8). *Note* that very few colonies of tumor cells remain.



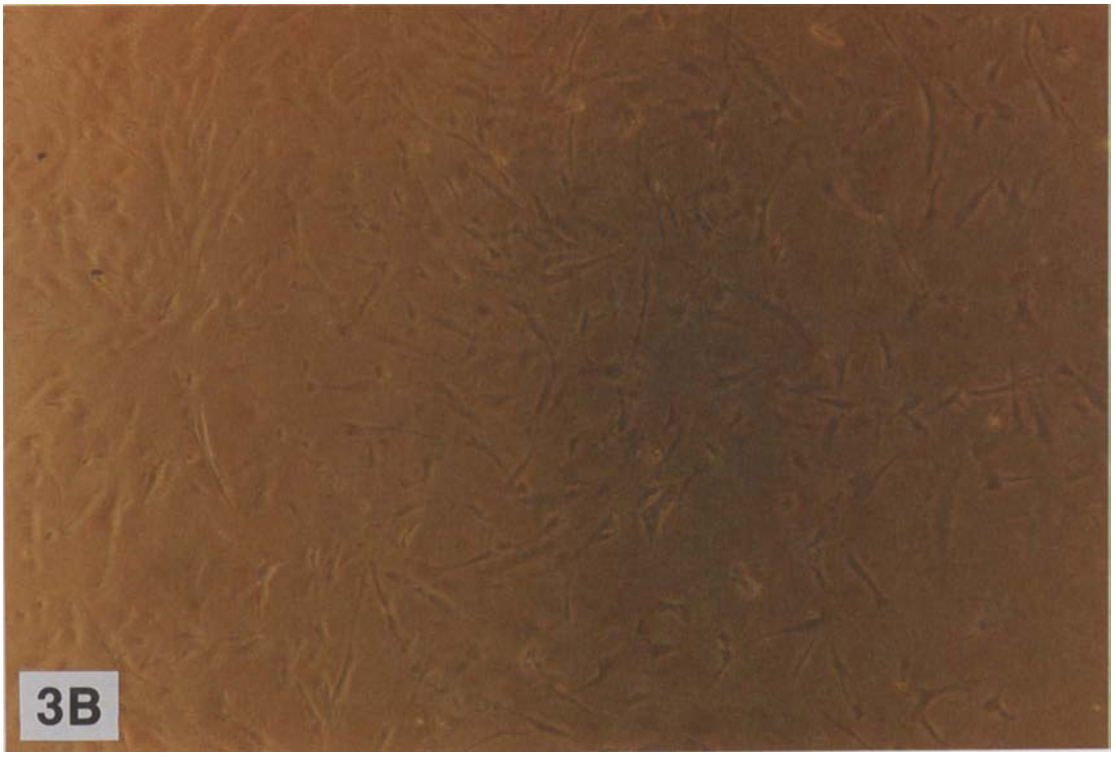


FIGURE 3A.—Photomicrograph 24 hr after seeding. Compact cells are tumor cells. Elongated cells are normal fibroblasts.
FIGURE 3B.—Treatment was with $10^{-10}M$ doxorubicin in $Met^{-}Hcy^{+}$ medium for 10 days followed by 2×10^{-7} *M* vincristine in $Met^{+}Hcy^{-}$ medium for 24 hr followed by $Met^{+}Hcy^{-}$ medium for 3 days when photograph was taken. Tumor cells are absent; normal fibroblasts are proliferating.

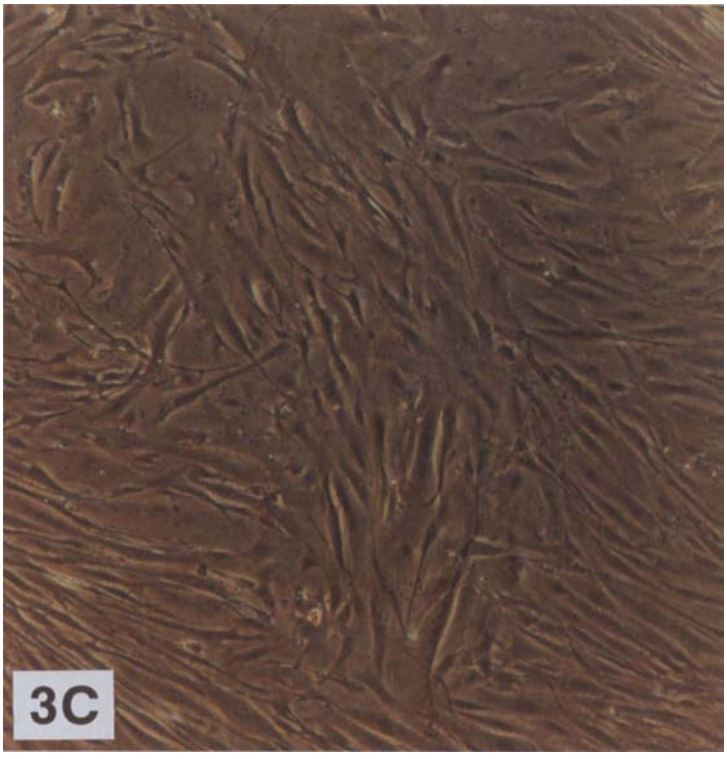


FIGURE 3C.—Ten days later than in fig. 3B. Fibroblasts have formed a confluent layer.

FIGURE 3D.—Photomicrograph of fixed and stained cells shown in fig. 1H. Tumor cells are absent, but fibroblasts appear normal and confluent.

FIGURE 3E.—Photomicrograph of fixed and stained cells shown in fig. 1A. The field is filled with tumor cells.
FIGURE 3F.—Photomicrograph of fixed and stained cells shown in fig. 1D. Colony of tumor cells is growing on a layer of normal fibroblasts.

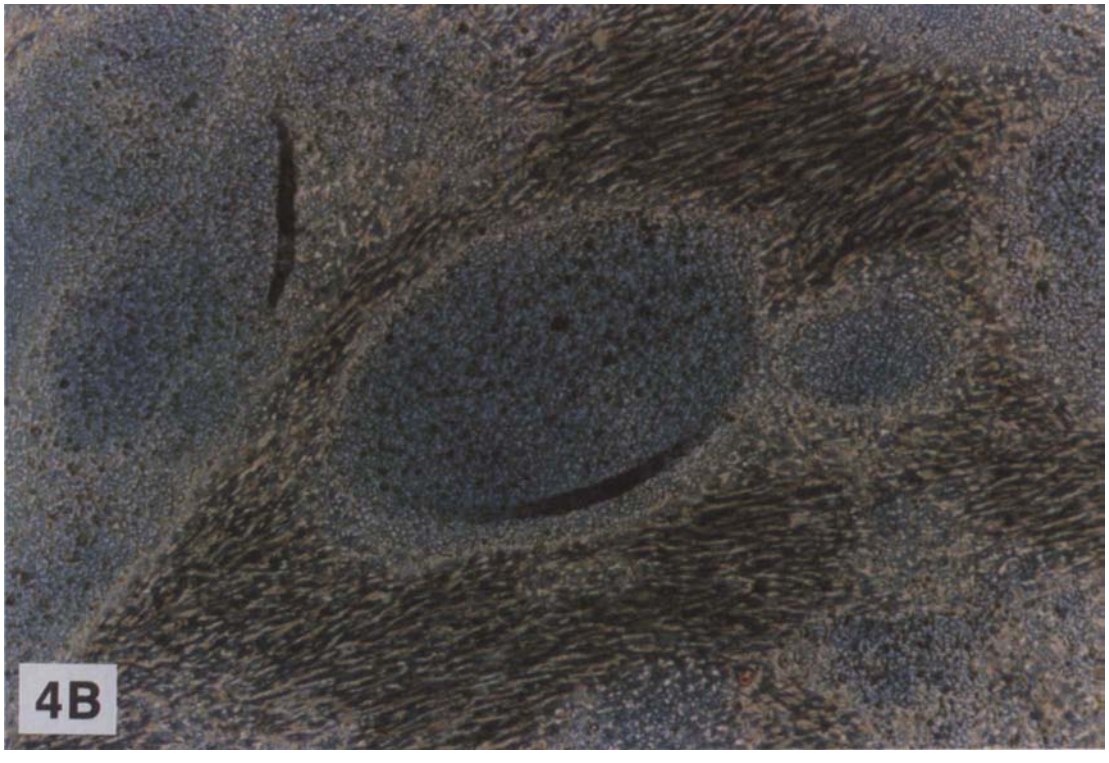


FIGURE 4A-C.—Photomicrographs of Giemsa-stained MCF-7 Met-dependent human breast cancer cells cocultured with FS-3 normal diploid human fibroblasts.

FIGURE 4A.—Drug-free $Met^{+}Hcy^{-}$ for 27 days (group 1). The field is filled with tumor cells.
FIGURE 4B.—Culture in drug-free $Met^{-}Hcy^{+}$ medium for 13 days, followed by drug-free $Met^{+}Hcy^{-}$ medium for 14 days (group 2). Colonies of tumor cells are visible on a background of normal fibroblasts.

FIGURE 4C.—Treatment with 10^{-10} *M* doxorubicin in $Met^{-}Hcy^{+}$ medium for 13 days, followed by 2×10^{-7} *M* vincristine in $Met^{+}Hcy^{-}$ medium for 24 hr followed by drug-free $Met^{+}Hcy^{-}$ medium for 13 days as described above (group 8). Tumor cells are absent and fibroblasts appear normal.