The Combined Effect of Hydroxyurea and X-rays on Chinese Hamster Cells *in Vitro*¹

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SUMMARY

Hydroxyurea inhibits DNA synthesis in Chinese hamster cells and is selectively toxic for cells actually in DNA synthesis (i.e., in S phase) at the time of exposure to the drug. Synchronized cells in G1 inhibited from entering S by hydroxyurea show a complex response to X-radiation. Cells become steadily more sensitive for a period of about 4 hr and then recover about a normal G1 sensitivity in approximately a further 4 hr. They never achieve the high survival level normally found for uninhibited cells in late S, but they will reach this level quite rapidly if the hydroxyurea is removed prior to exposure. The sensitization is, therefore, completely reversible. Hydroxyurea has no effect if present only during exposure at room temperature. Additional sensitization is obtained if the drug is present after exposure which increases for a period of about 4 hr. This postirradiation sensitization is independent of the time in the cycle at which irradiation takes place.

These results with synchronized populations enable the effects of hydroxyurea on asynchronious populations to be predicted. The most sensitive condition occurs when irradiation is given about 4 hr after the administration of hydroxyurea and the drug is kept on subsequently.

The results indicate that hydroxyurea in combination with X-rays can be a most effective toxic agent for Chinese hamster cells. If these results are applicable to in vivo situations, combination therapy with hydroxyurea given systemically and X-rays delivered locally to tumors may be most effective. However, in view of some of the problems in tumor control that cannot be evaluated in *in vitro* experiments with suitable animal tumor systems are needed.

INTRODUCTION

Hydroxyurea, an inhibitor of DNA synthesis, has been shown in earlier work with Chinese hamster cells to be selectively toxic for cells in the DNA synthetic phase (S) of the generation cycle (8) and to sensitize inhibited cells to X-rays (8). In view of the suggestions made by this author (8, 10) that a combination of this drug with X-ray treatment in *in vivo* may be more effective in the therapy of tumors than either agent alone, further experiments on the sensitization of cells to X-rays by hydroxyurea seemed desirable. This paper reports experiments on Chinese hamster cells grown in culture using hydroxyurea before and/or after X-irradiation.

MATERIALS AND METHODS

Cells. The cell line employed was a subline of the V79 line of Chinese hamster (*Cricetulus griseus*) which was originally derived from female lung tissue. The subline, V79-285B, initially included only cells of a single near-diploid composition of 23 chromosomes, but in the later stages of the investigation significant aneuploidy developed.

The cells were grown in log phase cultures on 85-mm plastic Petri dishes using EM-15 medium, which is similar to HU-15 but without NCTC 109 (3). Cultures were maintained at 37°C in a humid atmosphere of CO₂ and air. In log phase, the generation cycle lasts about 10 hr subdivided into a DNA synthetic period of 6 hr, G1 of 1.5 hr, G2 of 1.5 hr, and mitosis of 0.5 to 1.0 hr, as measured by the labeled metaphase technic.

Cell Synchrony. Synchronous populations of cells at or near division were obtained by harvesting log phase cells under controlled conditions (13), using a modification of the selection technic of Terasima and Tolmach (16). Tests were applied to ensure that the population was ergodic (7). Synchronous cells in suspension were then inoculated into plastic dishes and re-incubated at 37°C for treatment throughout the next cycle. Time was measured from the time of reincubation. The first cycle often lasted 11.5–12 hr rather than 10 hr, mainly because of lowered incubator temperatures due to frequent sampling. This method of synchrony yields small microcolonies containing, during the first cycle, an average of ~2 cells per colony. In general, the results given below have not been corrected for this cellular multiplicity.

X-radiation. Cell cultures were irradiated at room temperature on a rotating table 65 cm below the target of a 250 Kvp X-ray machine, half value layer 0.9 mm Cu, exposure rate 110 R/min. The ratio of absorbed dose to exposure (calculated, and measured with a ferrous sulfate dosimeter) was 0.945 rads/R. During the delivery of X-ray doses and subsequent rinsing operations the cultures spent a total time of 10–30 min at room temperature.

Hydroxyurea Addition and Removal. Hydroxyurea, freshly prepared by dissolving the powder in EM-15, was added to the cultures at selected times in the cell cycle and the cultures were irradiated at suitable intervals thereafter. Unless otherwise indicated, hydroxyurea was removed immediately after...
irradiation by rinsing once with 14 ml of buffered saline (Puck's saline A). Petri dishes were refilled with 10 ml of fresh medium and incubated at 37°C for 8–10 days before staining and counting visible colonies. After a 14-ml rinse, no residual hydroxyurea effects could be detected. The final concentrations of hydroxyurea in the medium on the dish ranged between 0.5 mM and 2.5 mM, but was 1 mM unless otherwise stated. These concentrations were sufficient to inhibit DNA synthesis in these cells but allowed protein and RNA synthesis to proceed unchecked (10). [Note: A complete bibliography on hydroxyurea is extensive so only essential references have been quoted. Other references will be found in Reference 8 and other earlier work (e.g., 18, 19).]

RESULTS

Hydroxyurea Present during X-ray Exposure Only. The X-ray survival (s) of Chinese hamster cells, when hydroxyurea was added to G1 cells (1 hr after synchronization) just prior to irradiation at room temperature and removed immediately afterward, is shown in Chart 1. The survival curve has parameters $n \approx 3.6$ and $D_0 \approx 160$ rads, where $s = 1 - \left(1 - e^{-D/D_0}\right)^n$ for dose $D$. The presence of the drug during exposure has no effect upon the X-ray survival of these cells.

Hydroxyurea Present before X-ray Exposure. Hydroxyurea was added to synchronized cultures during G1 (i.e., at 1 hr after synchronization). Its effect upon the population was to kill those few cells that were already in S and to inhibit those about to enter S (8). Other cells were not affected. After different lengths of exposure to the drug, cells received a dose of 710 rads and the drug was removed immediately thereafter. The result of two independent experiments is shown in Chart 2. Curve A shows the effect of hydroxyurea alone, Curve B the effect of X-rays on cells inhibited by hydroxyurea. In the latter case, cells became increasingly sensitive to X-rays (by a factor of 10 or so) until about 5 hr; then survival increased again to a level considerably below the normal maximum in untreated cells exposed to X-rays and approximately the same as the initial G1 sensitivity. The decrease and subsequent increase take place in the total absence of DNA synthesis, as shown by pulse labeling with tritiated thymidine (10). If the drug was removed immediately after exposure, the latter part of Curve A was not corrected for the eventual hydroxyurea toxicity. At the foot of the graph the times for the survival curves of Chart 3 are indicated.
drug was removed at 5 hr or at 8 hr, so that DNA synthesis could proceed, the survival level rose to the full value normally observed in uninhibited cells during late S (12). Thus, the observed initial sensitization was completely reversible if the drug was removed. X-ray survival curves for cells exposed at 1 hr to hydroxyurea were performed at the times indicated on Chart 2 by Survival I, II, III, i.e., for hydroxyurea exposures of duration 1, 4 and 8 hr. These are shown in Chart 3. The first survival curve, I, after 1 hr in hydroxyurea, showed slight sensitization compared with Chart 1 (n ~ 4.8 and D0 ~ 140 rads vs n ~ 3.6 and D0 ~ 160 rads); but an appreciable change in D0 (to ~ 100 rads) was found for cells after 4 hr exposure to hydroxyurea (Curve II). After an 8 hr exposure to hydroxyurea the cells were less sensitive again, Curve III exhibiting a D0 similar to that of I with a higher n (about 9.5). Although there may be some changes in the shoulder of these survival curves the main effect of the drug is to alter D0 from the normal 160 rads (Chart 1) to about 100 rads at the most sensitive time (~4 hr exposure). This sensitivity was lost again on further exposure to the drug and after 8 hr exposure cells were no more sensitive to X-rays than uninhibited cells initially.

If hydroxyurea was present before the X-ray exposure of G2 cells or mitotic cells, the drug killed any contaminating S cells present but no effect of the drug was observed upon X-ray survival. In Chart 4, survival of cells irradiated immediately prior to harvesting for synchrony purposes (14) was the same whether the cells were exposed to high specific activity tritiated thymidine (HSA-TdR-3H, which, of course, did not inhibit cells from entering S) or hydroxyurea. Presumably, the absence of an effect is because there is no inhibition of any cellular process in G2 or mitotic cells.

The effect of hydroxyurea upon the X-ray response of Chinese hamster cells in S was complicated by the fact that hydroxyurea alone kills S cells. However, when X-rays (710 rads) were also given to cells exposed to the drug, survival of S cells decreased more rapidly and its magnitude was reduced by about a decade (Chart 5). During this time, survival after X-rays alone was essentially constant (Chart 5).

Chart 3. Survival curves for G1 cells exposed at 1 hr to 1 mM hydroxyurea (HU) for 1 hr (I), 4 hr (II), and 8 hr (III). The drug was removed immediately after exposure. The data are not corrected for cellular multiplicity which in this experiment was high (>2).

Chart 4. Survival curves for G2-mitotic Chinese hamster cells, with and without hydroxyurea (HU). For one set of points (O) S cells were removed by exposing the plates to be harvested for 1 hr to 20 μc/ml of high specific activity tritiated thymidine (HSA-TdR-3H, 14.4 c/m mole). In the other (X) the plates to be harvested were exposed to 1 mM hydroxyurea for 1 hr. Both sets were irradiated immediately prior to harvest. This method depends upon the plating efficiency remaining constant for each harvest. The range indicated on two of the points is the range of plating efficiencies observed on control harvests and does not refer to the uncertainty of the data for each point, which is much less.
Hydroxyurea and X-ray Effects on Cells

Hydroxyurea Present after Exposure. When hydroxyurea (1 mM) was added to G1 cells after exposure to 710 rads of X-radiation, the top curve (panel A) of Chart 6 was obtained. Cells became more sensitive with duration of exposure to the drug only up to about 4 hr of exposure. Thereafter, the number of survivors was essentially constant and there were no significant undulations in sensitivity comparable to those in Chart 2. The survival level after 4 hr is about a decade below that of G1 cells and similar to that at the trough (5 hr) of Curve C, Chart 2. The survival curve at this time therefore may well be similar to that of Curve II, Chart 3.

The effect of hydroxyurea on irradiated G2 or mitotic cells is more difficult to examine. However, irradiated cells harvested from plates at or close to mitosis and plated into medium containing either HSA-TdR-3H or hydroxyurea show little difference providing the exposure is short. If the exposure is prolonged, some cells begin to move into S and are killed by HSA-TdR-3H or inhibited (and therefore sensitized) by hydroxyurea. I have not detected any postirradiation effect of hydroxyurea on G2 or mitotic cells.

For cells in S, the situation was again complicated by the toxic effect of the drug, shown in Chart 6, panel B. Cells irradiated with 710 rads of X-radiation were not killed initially by 1 mM hydroxyurea as were unirradiated cells, presumably because the lethal effect of hydroxyurea on S cells is related in some way to the rate of DNA synthesis and the latter is depressed (as measured by thymidine-3H uptake) following 710 rads to about half the normal rate (11). Eventually, however, the combination of X-rays and hydroxyurea was more damaging than either agent alone.

Hydroxyurea Present Both before and after X-ray Exposure. When cells are in G2 or mitosis, no additional effects are to be expected because there is essentially no interaction between the drug and the cells. Experiments on G2 or mitotic cells exposed to hydroxyurea for periods of 1–2 hr have shown no effect of the drug upon X-ray response. Longer exposures to the drug...
are some more difficult to study since the cells progress from
G₂ to mitosis and through a relatively short G₁, after which
inhibition begins.

When cells are in G₁, the X-ray response varies in the pres-
ence of the drug (Chart 2); consequently, it is of interest to
know whether inhibited sensitive cells are further sensitized by
hydroxyurea present afterward. The answer is given by the
results of the experiments shown in Chart 7. When hydroxy-
urea was kept on after irradiation, cell survival was further im-
paired (by a factor of a decade or so, after 710 rads). The
kinetics of the postirradiation effect of the drug look quite
similar no matter when the irradiation takes place (i.e., Curves
C, D, and E are similar in shape). To demonstrate this further,
G₁ cells exposed to hydroxyurea at 1 hr were irradiated at
2, 5, and 9 hr and hydroxyurea was removed at intervals there-
after. The results are shown in Chart 8, plotted in terms of
relative survival. These show that the kinetics of the post-
irradiation effect were essentially the same irrespective of the
stage of irradiation. The decline in survival was initially ex-
ponential with \( T₁ \sim 1.1-1.2 \) hr, but after about 4 hr no further
sensitization by the drug occurred. [At much longer times the
hydroxyurea toxicity (9) would obscure further sensitization
effects.] At 2 hr, 2.5 mm hydroxyurea was only slightly more
effective than 1 mm (presumably because inhibition of DNA
synthesis was complete with the lower concentration of drug)
but, in this example, 0.5 mm hydroxyurea was appreciably
less effective (\( T₁ \sim 1.8 \) hr initially) and survival leveled off at
a higher value. In a further experiment at 9 hr hydroxyurea
(1 mm) was removed during exposure and replaced imme-
diately thereafter. The response was the same as given by
Curve A of Chart 8, indicating no effect during exposure solely,
as in Chart 1.

Survival curves for cells sensitized by hydroxyurea both be-
fore and after exposure are given in Chart 9. In each, 1 mm
hydroxyurea was added to cells in G₁ (1 hr after synchro-
nation) and kept on for 4 hr after irradiation. Irradiation oc-
curred at 2 hr after synchronization (i.e., 1 hr in hydroxy-
urea), Curve I, and 5 hr after synchronization (i.e., 4 hr in hydroxy-
urea). Chart 7. Effect of hydroxyurea (HU) (1 mm) added to G₁
cells and present both before and after 710 rads X-radiation.
Curve A, normal response of untreated synchronous cells to 710
rads X-radiation. Curve B, survival of synchronous cells to which
1 mm HU was added at 1 hr, following 710 rads X-radiation (drug
removed immediately after); compare with Chart 2, Curve C.
For Curves C, D, and E irradiation was given after 1 hr ex-
posure to HU, 4 hr exposure to HU, and 7 hr exposure to HU,
respectively, and HU kept on afterwards and removed at differ-
ent times.

Chart 8. Postirradiation effect of hydroxyurea (HU). Relative
survival against duration of postirradiation exposure to HU.
Curve A (initial \( T₁ \sim 1.2 \) hr) fits all the data, except that for
0.5 mm HU, (Curve B, initial \( T₁ \sim 1.8 \) hr). ○, 1.0 mm HU, 710
rads at 2 hr; △, 1.0 mm HU, 710 rads at 5 hr; □, 1.0 mm HU, 710
rads at 9 hr; ●, 0.5 mm HU, 710 rads at 2 hr; ▼, 2.5 mm HU,
710 rads at 2 hr.
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Chart 9. Effect of 1 mM hydroxyurea (HU) present both before and after upon X-ray survival. Curve I, 1 mM HU added at 1 hr, X-rays given at 2 hr, HU removed after 4 more hr. Curve II, 1 mM HU added at 1 hr, X-rays given at 5 hr, HU removed after 4 more hr.

At small doses the survival is about the same, but an appreciable difference appears after 300 rods or more. Curve I has parameters \( n \approx 2.5 \) and \( D_0 \approx 120 \) rods, and Curve II, \( n \approx 6.8 \) and \( D_0 \approx 80 \) rods. Curve II represents the most sensitive condition for G, Chinese hamster cells exposed to hydroxyurea and X-rays. If the cells were exposed to hydroxyurea prior to irradiation for a longer period (e.g., 9 hr), survival is higher and the postirradiation kinetics are essentially the same. The experiment of Chart 9 was conducted with the same population of cells as those of Chart 3, and somewhat larger than normal shoulders were observed in this set of data, partly because of higher cellular multiplicity and partly because of an increase in percentage of tetraploid cells in the population.

Effect of Hydroxyurea on an Asynchronous Population. It is possible from the foregoing to predict the effect of hydroxyurea upon an asynchronous population. If given 4 hr prior to exposure, 1 mM hydroxyurea will not only kill the S cells (\( \sim 60\% \) of the population) but will also produce maximum sensitization which can only be further enhanced by keeping hydroxyurea on for 4 hr (or more) afterward. The results of an experiment to test this are given in Chart 10. Curve I is for 2-hr attached “asynchronous cells” given X-rays only; Curve II is for 2-hr cells given X-rays followed by 1 mM hydroxyurea added immediately and kept on for 4 hr; Curve III is for 2-hr cells to which 1 mM hydroxyurea was added, then X-rays were given 4 hr later and the hydroxyurea was removed; Curve IV is for 2-hr cells to which 1 mM hydroxyurea was added, then X-rays were given 4 hr later and hydroxyurea was removed after an additional 4 hr. In Curves II, III, and IV between 65 and 75% of the population were killed as a result of the hydroxyurea exposure alone. The four survival curves in Chart 10 have approximate multitarget parameters (\( n \) and \( D_0 \)):

- Curve I: 4.7, 218 rods
- Curve II: 1.5, 190 rods
- Curve III: 2.4, 155 rods
- Curve IV: 1.6, 115 rods

From the work with synchronized cells, survival Curve IV was expected to be the most sensitive, as indeed it is. The parameters of the curve, while not exactly those to be expected from the synchronized cell work (\( D_0 \) is higher and \( n \) lower), are at least of the same order. [A complication in later experiments with this subline of hamster cells resulted from a gradual change in ploidy. By the time of the experiments of Chart 10, an increased incidence of aneuploid cells had occurred and this seemed to be responsible for a more resistant survival curve (Chart 10, Curve I) than formerly found (9).]

In any event, the combination of hydroxyurea + X-rays with the timing that leads to survival Curve IV, i.e., 4 hr pre-
and postirradiation exposure to hydroxyurea, is very effective in preventing proliferation of a hamster cell population.

DISCUSSION

The presence of hydroxyurea during irradiation has no effect upon X-ray survival. Its presence both before (depending upon duration) and after X-irradiation increases the lethal effect of X-radiation, provided cells are either in DNA synthesis (S) or about to enter it (late G1) and therefore inhibited. The drug has no effect on G2 or mitotic cells whether irradiated or not. [In our system, cells in G2 or mitosis move through a short G1 quickly; consequently it is difficult to be completely certain that hydroxyurea exerts no postirradiation effect on these cells.]

The sensitization effect of the drug upon X-ray response is the result of either its inhibitory action on G1 cells or its lethal action on S cells. DNA synthesis is the process involved. Do other inhibitors of DNA synthesis do likewise? To test this point, experiments were performed with this cell line using excess thymidine at 3 mM or 7.5 mM to inhibit DNA synthesis. This agent is not toxic at these levels unless exposure is continued for a generation time (12). The fact that DNA synthesis is inhibited by these levels of thymidine has been demonstrated in these cells by adding the inhibitor to synchronized cells in G1 (at 1 hr) and relieving it after a further 8 hr (i.e., 9 hr after synchronization). During this interval uninhibited cells would pass through their S period. Pulse labeling relieved cells at intervals after 9 hr with HSA-TdR-3H demonstrates that over 90% of the cells pass through the S period between 10 and 15 hr but the grain count per cell is reduced compared with normal. It is concluded that excess thymidine inhibits DNA synthesis in these cells but, possibly, not as completely or as rapidly as does hydroxyurea. In cells from the same line, Elkind et al. (2) came to the same conclusion using a different method of assay for inhibition of DNA synthesis. Excess thymidine had the same general effect on X-ray response as did hydroxyurea. An example is shown in Chart 11. The effect of inhibition with excess thymidine (Curve B) is the same in form but somewhat less in magnitude than that of hydroxyurea in Chart 2. Curves C and D compare the postirradiation effect of 7.5 mM thymidine with 1 mM hydroxyurea upon G1 cells inhibited with excess thymidine and then given 710 rads at 4 hr. The same result is obtained, a postirradiation sensitization lasting 4–5 hr, but excess thymidine is somewhat less effective than hydroxyurea. In another experiment, 7.5 mM thymidine added to G1 cells given 710 rads showed essentially no postirradiation effect for about 4 hr, after which survival declined, a rather different result from that shown in Chart 6A for hydroxyurea. Thus there appear to be differences in the nature of the postirradiation effect of excess thymidine and hydroxyurea as well as in degree. This may be because excess thymidine may inhibit DNA synthesis less rapidly and less completely than does hydroxyurea. On the other hand, the route by which inhibition occurs may be involved (21–23). At this point, hydroxyurea stands out as a very effective DNA inhibitor resulting in large changes in X-ray survival both pre- and postirradiation.

In order to produce the effects prior to irradiation, DNA alone must be inhibited. If cycloheximide, which, in addition to DNA synthesis, inhibits protein synthesis and, to a lesser extent, RNA synthesis, is used, X-ray survival is not modified in the same way (12). Nor does hydroxyurea have this effect if cycloheximide is also present. Thus, the inhibition of DNA synthesis only is responsible for this sensitization (12). A possible preirradiation sensitization due to DNA inhibitors was evident in the earlier work of Terasima and Tolmach (17) using 5-fluorodeoxyuridine in HeLa cells. The experiment was apparently not continued sufficiently long to observe a subsequent rise in survival, such as that shown in Chart 2, if it occurs in HeLa cells. In later work by Phillips (5) in the same laboratory, a rise in survival was detected with 5-fluorodeoxyuridine but not with hydroxyurea, and the effect was not investigated further; consequently, its presence or absence in HeLa cells still remains to be established. More recently, in

![Chart 11](chart11.png)
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L cells, Terasma and Fujiwara (15) have also observed a subsequent rise in survival after hydroxyurea. These changes clearly have important fundamental implications concerning the relationship between X-ray survival and biochemical processes in the cell, but, from the practical point of view, they indicate that timing of the X-ray exposure relative to drug administration may be important for the optimum effect.

The postirradiation sensitization of HeLa cells with hydroxyurea and with 5-fluorodeoxyuridine was previously reported by Phillips and Tolmach (6) and is apparently characteristic of DNA inhibitors. They found the effect to persist about 5 hr after exposure except that, in the case of hydroxyurea, killing continued for a longer period. In more recent work from the same laboratory (20), differences have been detected in the postirradiation effect in HeLa cells of various DNA inhibitors, including hydroxyurea. Hydroxyurea exerts an effect greater in magnitude and longer in duration (up to 18 hr) than, for example, excess thymidine. In hamster cells, as noted above, the effect of hydroxyurea is also greater in magnitude than for excess thymidine, but the effect does not appear to persist longer. However, it is more difficult to examine the effects of hydroxyurea after irradiation in hamster cells than in HeLa cells since the agent is lethal to hamster cells synthesizing DNA. This may mask any other effects. Furthermore, in view of the already reported difference in the action of hydroxyurea upon the S cells of hamster and HeLa (4, 8) (see Addendum), other differences are also to be expected. What is of primary importance in this work is that these agents can exert an even greater effect if present both pre- and postirradiation, depending upon appropriate timing.

The sensitization of cells to X-irradiation by hydroxyurea in particular is sufficient in magnitude to be important practically. $D_0$ may change from 200–220 rads for untreated cells to as low as 80–100 rads for treated cells and there may also be some reduction in shoulder (Chart 10). In a Chinese hamster asynchronous population in which 60% or more of the cells are in S and are killed by the drug anyway, the total effect (Chart 10) is large. For example, after a modest dose of 300 rads, survival is 0.65 in the absence of hydroxyurea, while in its presence survival may be only 0.03. Although exact timing is of some importance to obtain the maximum killing effect, there is no situation in which adding hydroxyurea to a Chinese hamster cell population increases survival.

The results most applicable to cells in vivo are those in which hydroxyurea is present both before and after irradiation. In tumors, the cell population is likely to be a mixture of cells in various phases of the cell cycle. For this reason alone careful experiments in appropriate in vivo tumor situations in animals, which are capable of cell kinetic analysis, should be undertaken.

Hydroxyurea, when administered to humans in therapeutic doses, reaches a high level in the blood and other fluids for only a comparatively short time. Beckloff et al. (1) showed that 2 hr after 80 mg/kg the concentration in serum exceeded 2 mM, remained above 1 mM for the first 4 hr, and above 0.5 mM for the first 8 hr. Unfortunately, there is no information available concerning the level of hydroxyurea actually attained in tumors and whether this is higher or lower than that in surrounding normal tissues. Although it seems clear that hydroxyurea must be present in adequate concentration in order to achieve growth limiting properties in certain tumors (18, 19), precise information in specific instances would seem to be one essential factor involved in a decision to undertake combination therapy. If the levels attained in serum are also, or could be, attained in tumors, combination therapy with hydroxyurea and X-rays judiciously timed should be more effective than either agent alone. However, since this combination would also be most damaging to normal cell renewal systems, satisfactory therapy would depend, as in normal radiotherapy, in delivering as high an X-ray dose as possible to the tumor and as little as possible to other tissues.

In view of the many unknown factors attendant upon the use of hydroxyurea in combination with X-rays for the therapy of tumors, it is to be hoped that further investigation will be concerned with the resolution of these uncertainties in suitable animal tumor systems.

ACKNOWLEDGMENTS

I wish to acknowledge the excellent technical assistance of Grace Raeser and E. F. Tarka in these studies.

ADDENDUM

In a recent paper by J. H. Kim et al. Cancer Res. 27: 1301–1305, 1967, it was shown that hydroxyurea selectively kills HeLa cells in S phase, as previously shown for Chinese hamster cells (8). A high concentration of hydroxyurea was required, however. Differences in action of the agent upon the two cell lines may, therefore, be less significant than implied in Ref. 4.

REFERENCES


Announcements

SYMPOSIUM ON AMPHIBIAN TUMORS

An International Symposium: Biology of Amphibian Tumors will be held at the Royal Orleans Hotel in New Orleans, Louisiana, October 28-30, 1968, under the sponsorship of Tulane University, the National Cancer Institute, and the Cancer Association of Greater New Orleans, Inc. Further information can be obtained from:

Dr. Merle Mizell
Department of Biology
Tulane University
New Orleans, Louisiana 70118

SECOND CARIBBEAN CANCER CONGRESS

The Second Caribbean Cancer Congress will be held in Kingston, Jamaica, from November 24-27, 1968. It will review recent advances in research and treatment of malignant disease with special emphasis on the problems relating to the Caribbean and Latin American countries. Internationally renowned guest lecturers will present papers at the conference. For further information write to:

Dr. Kenneth A. McNeill
Secretary General
Second Caribbean Cancer Congress
5 Tangerine Place
Kingston 10
Jamaica, West Indies

THIRTEENTH ANNUAL CLINICAL CONFERENCE

"Breast Cancer, Early and Late" will be the subject of the Thirteenth Annual Clinical Conference, sponsored by The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, November 21-22, 1968.

The conference, which is to convene at the Shamrock-Hilton Hotel, will cover a full range of diagnostic and therapeutic concepts of breast cancer. The Heath award, conferred annually on a physician or scientist who has made an outstanding contribution to the better care of the cancer patient through clinical application of basic research, will be awarded at the final session.

Further information can be obtained from:

Dr. E. C. White, Chairman
The University of Texas
M. D. Anderson Hospital
and Tumor Institute
Houston, Texas 77025

Erratum

In the article "The Combined Effect of Hydroxyurea and X-rays on Chinese Hamster Cells in Vitro" by Warren K. Sinclair (Cancer Res., 28: 198-206, February 1968), the following corrections should be noted. On page 202, Chart 7 appears erroneously over the legend for Chart 8 and Chart 8 appears erroneously over the legend for Chart 7. On page 199, second column, line 9, Curve B should read Curve C. On page 202, first column, line 15 and in the legend to Chart 8, line 6, 9 hr should read 8 hr. Also, on page 202, legend to Chart 7, line 5, "following" should be "followed by."
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