Increase in Dry Mass of Ehrlich Ascites Tumor Cells after Treatment with Nitrogen Mustard

HUN LEE, VICTOR RICHARDS, AND ARTHUR FURST
(Surgery Department, Presbyterian Medical Center, San Francisco, California)

SUMMARY

The increase in mean dry mass per cell in the surviving population of the Ehrlich ascites tumor cells after treatment with HN2 was studied with the aid of the interference microscope. The carcinoma cells in animals treated with a single dose (2.5 mg/kg) of HN2 5 days after tumor transplant showed no change in mean dry mass during the first 8 hours after treatment. Starting sometime between the 8th and the 24th hour, the mean dry mass per cell increased at a uniform rate of about 250 arbitrary units every 80 hours for five of the six treated animals and about 15 arbitrary units every 18 hours for the sixth animal, in spite of the suppression of mitotic indices from the 3rd hour to the 6th day post-treatment. Control animals showed no significant change in either dry mass values or mitotic index.

Changes in dry mass of individual cells can now be conveniently followed by measurements with the interference microscope (1). Using this technic, we have shown that the Ehrlich ascites carcinoma cells, after treatment with rabbit antiserum, decreased in mean mass per cell (17). The opposite effect was noted after treatment with the carcinostatic agent, phenazine-di-N-oxide (16). We have chosen to study HN2—methylbis(2-chlorethyl)amine hydrochloride—a "radiomimetic" agent (6), for a comparison between the effects of HN2 and x-radiation. A comparison of the two might be helpful in elucidating the action of these agents on tumor cells.

X-radiation at a whole-body dose level of 1250 r has been shown to have no effect on the rate of RNA and protein syntheses of the Ehrlich ascites cells. Mitosis, however, has been completely inhibited for 24 hours and DNA synthesis reduced in rate. The net result was an increase of cell volume and dry mass (2, 7, 9, 11, 13). Following HN2 treatment, suppression of mitosis and an increase of cell size or volume occurred in mammalian cells (15), various protozoa (5, 10, 12), and some bacteria and yeast (20). However, the dry mass changes have not been systematically studied as after x-radiation. This study, with the interference microscope, reports the effect of HN2 on the dry mass changes in Ehrlich ascites cells in relation to mitotic index.

MATERIALS AND METHODS

Eight-week-old male, Swiss-Diablo mice, weighing between 18 and 20 gm., were given inoculations of 0.1 ml. of undiluted Ehrlich ascites fluid which contained between 10 and 15 X 10^6 cells. The hypotetraploid Ehrlich ascites tumor used was originally obtained from Doctor T. Hauschka. After 3 or 4 years of transplantation from generation to generation the cells were found to be predominantly diploid, but tetraploid and octoploid cells were present in increasing numbers. The animals were then randomized and divided into four groups. A single intraperitoneal (I.P.) injection of 2.5 mg/kg of HN2 (Merck), freshly prepared for each animal, was administered on the 5th day following tumor inoculation to the treated groups. The study was carried out as follows: Four groups of animals were used.

Group 1 (four animals).—One drop (0-hour sample) of ascites fluid was first aspirated from an animal bearing a 5-day tumor, and then the drug was administered. The injection was completed within 2 minutes after the HN2 solution was pre-
pared. Hourly ascites fluid samples were taken subsequently for 8 hours.

**Group II (six animals).**—After the first sample was taken the drug was given. A sample was taken every 24 hours subsequently until the death of the animal, for five of the six animals. In the case of the sixth animal, the tumor regressed 6 days after treatment, and the animal survived. Sampling for this animal became extremely difficult 6 days after treatment because very little ascites fluid was available. Data from samples taken after the 6th day were not reliable and were discarded.

**Group III (four animals).**—No treatment was given. Samples were taken hourly for 8 hours in the same manner as in Group I, serving as their control.

**Group IV (four animals).**—No treatment was given. Samples were taken daily in the same manner as in Group II, serving as their control.

Thirty cells were selected at random from each 1-drop sample of ascites fluid for the measurement with the interference microscope. Inflammatory cells or any cell of similar size were not measured. Tumor cells were generally the largest cells present. An AO interference microscope with a half-shade eyepiece was used for the dry mass cell determination. Technics of the interference microscopy and slide preparation, as well as formulae for calculations, were described in our previous publications (16, 17). A smear was made from each sample of ascites fluid, stained with Feulgen, and counter-stained with Fast Green for the mitotic index study.

## RESULTS

**Group I.**—Thirty cells were measured from each hourly sample. The value of mean dry mass per cell (in arbitrary units) for each hour was calculated and plotted against time in hours. From the curve (Chart 1) it can be seen that little or no significant changes in cell mass took place during the first 8 hours in this treated group. This curve was similar to the curve of the control group (Group III).

**Group II.**—Thirty cells were again measured from each daily sample. Five of the six animals in this group responded to the treatment with a similar rate of increase in the mean dry mass; in the sixth animal a greater rate of increase of the cell mass was found, but the values for this animal returned to normal. Therefore, the mean dry mass of the average of the first five animals and the mean dry mass of the sixth animal are represented separately by the curves in Chart 2. Each of the first five animals died on a different day after the single dose of the drug. Thus, the daily mean dry mass for 0–6th day was calculated from 150 cells; for the 7th and 8th days the data were from 129 cells and for the 9th day were the result of 90 cells. The mean dry mass values after the 9th day from the last two survivals were not included, nor were the values from the sixth animal after mean dry mass of the cells returned to normal.

As shown in Chart 2, the mean dry mass per cell of all the experimental animals increased approximately at a uniform rate until the death of the animal, or a peak was reached before the values returned to normal. Linear curves could be constructed by inspection according to data on the increasing dry mass values (broken lines). The rate of increase of the five-animal curve was approximately 250 arbitrary units (mean dry mass at the 0 hour) every 80 hours. The rate of increase of the sixth animal was much greater, being 325 arbitrary units (mean dry mass at the 0 hour) every 18 hours. After a peak of 1121 arbitrary units was reached, which was about 5 times that of the original, the values returned to normal in 3 days. The greater rate of increase of a single animal, however, may not bear much significance. The control group (Group IV) showed no significant change in the cell mass (Chart 2).

The slide smears made from each sample and stained with both Feulgen and Fast Green showed that accompanying the increase of mean dry mass
there was an apparent increase in both cell size (Figs. 1–4) and number of disintegrated cells; at the same time there was a decrease in number of cells per unit volume of ascites fluid. The mitotic indices from each sample of the four groups of animals were calculated by counting the number of cells in mitosis per 1000 cells in the stained smears. A percentage was derived from the counts for each sample and plotted against time (in hours or days, Chart 3). The curves of the treated groups demonstrated a significant drop of indices at the 3d hour after treatment and reached the lowest value at the 5th- to 6th-hour period, which persisted till the 8th hour. A slight recovery began sometime between the 8th and 24th hours—this index was maintained at the same approximate level until the 5th day. Significant recovery began sometime between the 5th and 6th day, and complete recovery was reached on the 8th day. The control groups also showed considerable variation in mitotic index from hour to hour and from day to day, and, although the values were slightly lower than those reported by Okada and Roberts (19), at no time was it as low as that of the treated animals during the period from 3d hour to the 6th day. A breakdown of the mitotic index of the treated groups

![Chart 3](https://cancerres.aacrjournals.org/content/canres/21/10/1110.full)

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean dry mass/cell on 0 day</th>
<th>Mean dry mass/cell on 3d day</th>
<th>Mean dry mass/cell on 6th day</th>
<th>Mean dry mass/cell on 9th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>246 (± 82, δm = 6)</td>
<td>456 (± 181, δm = 14)</td>
<td>737 (± 350, δm = 28)</td>
<td>1002 (± 638, δm = 67)</td>
</tr>
<tr>
<td>3</td>
<td>224 (± 91, δm = 16)</td>
<td>1123 (± 643, δm = 117)</td>
<td>230 (± 83, δm = 15)</td>
<td>284 (± 123, δm = 3)</td>
</tr>
<tr>
<td>6</td>
<td>224 (± 91, δm = 16)</td>
<td>1123 (± 643, δm = 117)</td>
<td>230 (± 83, δm = 15)</td>
<td>284 (± 123, δm = 3)</td>
</tr>
<tr>
<td>9</td>
<td>224 (± 91, δm = 16)</td>
<td>1123 (± 643, δm = 117)</td>
<td>230 (± 83, δm = 15)</td>
<td>284 (± 123, δm = 3)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The fact that the protein and RNA syntheses of Ehrlich ascites cells were not affected during the...
period of suppression of mitosis by x-radiation was established by Klein et al. (13), Kelly et al. (11), and Caspersson et al. (2). HN2, the alkylating agent, was reported as suppressing mitosis in Walker carcinoma 256 by Koller and Casarini (15) and in other organisms by various authors (5, 10, 12, 18, 20). Koller and Casarini noticed that, besides cytological changes, the cells increased in size and volume after treatment. Cobb (3) noticed the same phenomenon of increase of cell size in a variety of human cancer cells grown in tissue culture after exposure to a few “radiomimetic” agents. However, the effect of these agents on the dry cell mass has not been systematically studied. Our results showed that after a single dose of HN2 (2.5 mg/kg) the mean dry mass per cell of Group II animals did increase continuously at a uniform rate during a long period of suppression of mitosis. This is compared with results found after x-radiation. If we consider the increase in cell number as increase in mass, then a comparison between the growth rate of cell number and the growth rate of cell mass can be made. In this case, the rate of increase of the cell mass for five of the six animals seemed low as compared either with the normal growth rate of the number of the Ehrlich ascites cells themselves in the same mouse strain, for the same tumor age, and the same dose of cell inoculum, as reported by Gross, Furst, and Gross (8) in their work of N-methylformamide-resistant cell strain development; or with the mean generation time of Ehrlich ascites cells for the inoculum sizes of 17.5 X 10^6 and 1.8 X 10^6, as carefully calculated by Klein and Révézé (14). The rate of growth of total cell number as interpolated from the growth curve of the untreated controls in the work of Gross, Furst, and Gross (8), was about 34 hours for a doubling of the cell population on the 5th day after the inoculation of the tumor—the same day we chose for drug treatment. As reported by Klein and Révézé (14), the mean generation time for the inoculum of 17.5 X 10^6 cells was 34.8 hours and that for the inoculum of 1.8 X 10^6 cells was 27.4 hours for the approximately 5-day-old tumor. The rate of increase of mean cell mass of HN2-treated Ehrlich ascites cells did not follow the normal growth rate of the cell population. Mass increase was neither logarithmic, as shown by the normal growth curve constructed by Gross, Furst, and Gross, nor in cube-root fashion as reported by Klein and Révézé, but rather in arithmetic progression—though it must be noted that our inoculum differed from those of Klein and Révézé.

We found that the value of the mean dry cell mass of two of the six animals in the treated Group II returned to original after it reached a maximum (the return to normal values for one of the two animals occurred after the 9th day and therefore was not shown in the chart). Since the mitotic rate had not recovered at this time, the fragmentation and hence dying-off of these large nondividing cells shifted the value of the mean dry mass back to normal values.

We believe that the fate of most of the large nondividing cells is one of disintegration, in agreement with the findings of Koller and Casarini (15), because we did find that some of these large cells were actually in the process of disintegrating. It should be noted that this report concerns the growth and fate of surviving cells in a depleted population of viable cells, because the total number of cells, both large and small, per unit volume were markedly decreased. This may lead some to believe that the dry mass increase of the surviving cells is due to the availability of more nutrition in a thinning population. This is not likely, however, since the last survivals returned to normal mean dry mass values.

Since the rate of DNA synthesis and cell division is parallel in the Ehrlich ascites tumor (21), are we not approaching what Cohen and Barner (4) discuss in killing bacteria? Effective killing of bacteria results by unbalanced growth in which RNA and protein syntheses continue while DNA synthesis is blocked. Under such condition bacterial cells enlarge in size, fail to divide, and die.

REFERENCES


**Figs. 1-4.—Photomicrographs from phase microscope. The smears were stained with Feulgen and counter-stained with Fast Green. Notice the changes in cell size. Mag. ×380.**

Fig. 1.—Cells before treatment.

Fig. 2.—24 hours after treatment.

Fig. 3.—5 days after treatment.

Fig. 4.—9 days after treatment.
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