Title

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Inactivation of Mammalian Cells at Different Stages of the Cell Cycle as a Function of Radiation Linear Energy Transfer

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ABSTRACT

Recent studies have demonstrated the possible therapeutic advantage of beams of high linear energy transfer particles in terms of the increased biological effectiveness, loss of the shoulder in the survival curves, and the lowered oxygen enhancement ratio. This study was performed to indicate the response of mammalian cells to different LET radiation as a function of the time of irradiation in the cell cycle.

Chinese hamster cells (V79) were grown in cell culture, and were synchronized without the use of drugs by selective shakeoff of mitotic cells from the culture. After the cells had reattached to a surface they were irradiated with X rays (150 kV) or with Carbon, Neon, or Argon ions at the Berkeley HILAC at different times during the cycle of growth. The colony forming ability was measured after six days. In addition, aliquots of the cells were used to determine the labelling index, mitotic index, and colony multiplicity as a function of time.

The X ray survival curves of the cells exhibited a sensitive period in the G1 stage, a more resistant period during the late S stage, and an increased sensitivity after the S stage of the cycle. The cells irradiated with Carbon, Neon or Argon exhibited no such response and remained equally sensitive throughout the experimental period. The survival curves with these ions had an absence of a shoulder throughout the life cycle.

Since the age response of these mammalian cells appears constant throughout the cycle the results suggest a further possible therapeutic value of high LET beams in that cell kinetics are much less important for the timing of radiation exposure. In addition, these results have obvious implications to considerations of the effects of heavy primary cosmic ray particles on astronauts in long term space flight.

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INTRODUCTION

One hope of those who perform radiobiological research using cultured mammalian cells is that the results will lead to significant improvement in radiotherapeutic procedures. The discovery of the reduction of oxygen enhancement ratio using intermediate and high LET radiation offers a solution to the problem of reduced radiosensitivity of anoxic cells within tumours. Recently much attention has been paid to a rather large radiosensitivity variation of mammalian cells in terms of their age in the mammalian cell generation cycle. It is not difficult for one to imagine situations in which certain tumour cells because they are in a resistant age in the cell cycle are more resistant than the normal slowly dividing or nondividing cells in close proximity to the tumourous tissue. If there were a significant reduction in the age response of mammalian cells to radiation of high LET this might be important because of its obvious therapeutic implications. For this reason we have performed studies of the inactivation of mammalian cells as a function of LET and the position of the cell in the cell cycle.

IRRADIATION OF ASYNCHRONOUS MAMMALIAN CELLS

It has been known for several years as results of the works of Barendsen (1) and Todd (2) and also Deering and Rice (3) that the survival curves of mammalian cells change from those having a shoulder-type curve where Elkind-Sutton type recovery occurs with low LET radiation to single hit type curves with high LET radiation. These experiments studied induced reproductive death of cultured cells from both normal and abnormal cell tissue. The same change from shouldered to exponential type survival curves is seen when hamster cells in culture are irradiated as a function of the linear energy transfer of the radiation. In Fig. 1 is seen the data obtained by Skarsgard et al. (4) which has been verified in our laboratory quite recently by Bird and Burki (5). We see in this figure

that the radiosensitivity of the cells increases up to a maximum sensitivity in the range of linear energy transfer of 100 to 200 kev/micron. This pattern of killing effectiveness as a function of LET is therefore quite similar in cultured human and hamster cells, indicating that the asynchronous cell killing studies are generally the same in different species.

IRRADIATION OF SYNCHRONOUS MAMMALIAN CELLS

A. Gamma- and X-Radiation Exposure

The age response pattern for two types of human cells is now known due to work with cultured HeLa S3-91V cells by Terasima and Tolmach (6) and human kidney T cells by Vos et al. (7). For cultured mouse cells Whitmore (8) with L cells and also Burki (9) for L5178Y cells have determined the age response patterns. The general characteristics of the human cell age response to low LET radiation is a resistant period in the G1 stage followed by gradually increasing sensitivity toward the end of the G1 stage and the beginning of the S stage with a rapidly increasing resistance to radiation through the S stage culminating in maximum resistance of the cells in the last part of the S stage. This is followed by a rapidly increasing sensitivity during the G2 stage of the cycle to a most sensitive mitotic cell population. In the case of the mouse cells there is a variation which again shows radiosensitive and radioresistant portions of the cell cycle although it appears different from human cell variation.

Sinclair and Morton (10) have shown the general response for cultured hamster cells has only an increase in resistance through the S period with the G1 and G2 stages similar in radiosensitivity to low LET radiation although metaphase cells may be quite sensitive as shown by Dewey (11). We have performed experiments which yield the same general survival pattern for hamster cells in terms of age in the life cycle at the time of radiation as obtained by Sinclair and Morton (10), Dewey et al. (11), and Hall et al. (12). In order to perform these experiments a synchronous population of cells is used. This synchronous population is obtained by a shaking method similar to Sinclair (10) which yields mitotic cells that are selectively detached from growing surfaces. To monitor the synchrony of these experiments pulse labels of tritiated thymidine are given to cultures of the synchronous cells to determine using radioautographic methods the progress of the cell through the cell life cycle. In addition mitotic index and colony multiplicity are also monitored. In Fig. 2 is given the labeling pattern and colony multiplicity after shakeoff for V79-S171 Chinese hamster cells synchronized in our laboratory. The survival curves which we obtained at different ages after synchrony are shown in Fig. 3. One may see from this data that the survival curves vary throughout the cell life cycle but most dramatically in the DNA synthesis period. This data may be summarized by determining the survival at different ages in the cell cycle to the same dose of low LET radiation. Such results are seen in Fig. 4 which is a summary of four different experiments in which cells were exposed to 950 rads of 145 kVp X rays at different times after shakeoff.

B. Neutron and Boron Ion Exposures

Hall (12) has recently synchronized F strain hamster cells using hydroxyurea and compared the age response after exposure to 14.6 Mev neutrons (120 Mev cm^2-gm^{-1}, see Bewley (13)) with that obtained using 250 kVp X rays as a function of cell age. The variation in survival as a function of age was similar in both cases. Sinclair (14) has determined the age response of V79 Chinese hamster cells to the broad spectrum of fast neutrons from the Janus Reactor. (Average LET is 480 Mev cm^2-gm^{-1} as determined by Bewley (13)). He found that the variation in survival as a function of age was reduced by about 50% in his experiments. Elkind (15) has recently discussed certain unpublished data of Skarsgard in which CH2B2 hamster cells were synchronized using the Whitmore (8) window technique and irradiated using Boron ions at an average track segment LET of 127 kev/micron. These data indicate that an age variation still exists although it is probably only approximately 40% of that variation seen with X rays at this survival value. These results are seen in Fig. 5.

C. High LET Radiation

We have obtained data for several ions in the high LET range as a function of the age of the cell after synchrony, which was obtained by non-chemical means as in our experiments with low LET radiation. The dosimetry and apparatus for heavy ion irradiation has been previously reported by Todd et al. (16). In Fig. 6 is given the survival curve which we obtained from cells irradiated with Argon ions of 2,000 kev/micron at different ages in the cell life cycle. One can see from this data that the survival curves are the same throughout the cell life cycle. The shoulders are due to the fact that these survival curves are uncorrected for the cellular multiplicity which in these experiments was equal to the experimentally determined extrapolation number, about 1.7. We have obtained similar experimental results for Carbon and Neon ions, 190 and 650 kev/micron respectively, and in Fig. 7 is shown the response of mammalian cells as a function of cell age through the experimental period for carbon ion irradiation. One can see that this period of time (see Fig. 4) corresponds in our experiments to a time in which the survival of the cells after X ray exposure varies by a factor of 3, while the survival after carbon ion irradiation varies at most 30%.

DISCUSSION

The age response of hamster cells may be summarized at this time in terms of LET variation as is shown in Fig. 8. In this figure is given the percent variation in survival as a function of cell age for available data as a function of the linear energy transfer of the radiation used. It is clear that for low and intermediate LET irradiation there is an age dependent variation in cell killing. This is in contrast to the results obtained for high LET irradiations performed with Carbon, Neon and Argon ions where this data indicates that the survival of cells is independent of the biochemical events occurring in the cell cycle. It is also important to note that, using radiation of an LET range where variation in survival as a function of age occurs, it is important that the cell

life cycle survival kinetics still be considered. It is also clear from this data that additional work is necessary in the intermediate LET range to determine how steeply the age survival variation changes as a function of LET in the 48 to 190 kev/micron range.

POSSIBLE USEFULNESS TO RADIATION THERAPY

Because of the similarity of the data between human cells and Chinese hamster cells using asynchronous populations, as a function of different LET, it is expected that the age response to carbon and higher LET irradiations will not exist for human cells. However this is a very important experiment to be performed. For the intermediate range of LET we could expect differences between changing age variation of hamster cells and human cells due to the additional resistant peak in the G1 stage of human cells.

In localized tumours we now have 3 major considerations which might be of advantage for potential therapy with intermediate and high LET beams: (a) physical advantages of focusing the beam, (b) advantages in a lower oxygen enhancement ratio, and (c) the biological advantage of reduced age variation in cell killing. In the case of high LET radiation this would make at least the radiation sensitivity of tumour cells and normal cells the same. This is not necessarily the case with low LET radiation. For intermediate LET radiation the age response still must be considered and in addition the effects of radiation-induced parasynchrony as a result of G2 blocks may be quite important. Skarsgard (4) has argued that mitotic delay phenomena are strongly enhanced using high LET radiation. In addition, Scaife (17) has obtained mitotic delay results with human kidney cells which indicate that in low LET ranges the mitotic delay may vary. For intermediate LET these fractionation kinetics might be put to advantage by judicious dose fractionation schedules. However, for high LET radiation we expect that these results may not be important since fractionation, even if it induced parasynchrony, would not be expected to give different amounts of killing than acute exposure. There is some rather interesting data based on two dose fractionation experiments with high LET radiation by Skarsgard (15) in which there appears to be actual potentiation of radiation killing with fractionated doses of high LET radiation. Although this may be a result of the chemical synchronization methods used and technical difficulties in his experiments it is clearly a point which should be examined in future studies because of the possible therapeutic advantage.

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FIGURE LEGENDS

Fig. 1. The RBE as a function of LET for killing mammalian cells as determined by Skarsgard (4). RBE is calculated based on D_o doses.

Fig. 2. Percent cells labelled with H^3TdR and colony multiplicity as a function of time after synchronization of V79 Chinese hamster cells.

Fig. 3. Survival curves of V79 hamster cells after irradiation with X rays at different times after obtaining synchronized cells.

Fig. 4. Survival of V79 hamster cells after 950 rads of X rays at different times after synchronization.

Fig. 5. Survival of CH2B2 hamster cells exposed to 380 rads of 127 kev/micron Boron ions at different times in the cell cycle.

Fig. 6. Survival curves of V79 hamster cells exposed to Argon ions at 2000 kev/micron at different times after obtaining synchronized cells.

Fig. 7. Survival of V79 hamster cells after a single exposure to 250 rads of 190 kev/micron Carbon ions. Results of two different experiments.

Fig. 8. The relative percent survival variation in the life cycle is given as a function of the LET of the radiation. The relative percent survival variation is the amount of variation in survival of cells in their life cycle obtained with one type of radiation compared to the amount of variation using X rays at the same survival level.

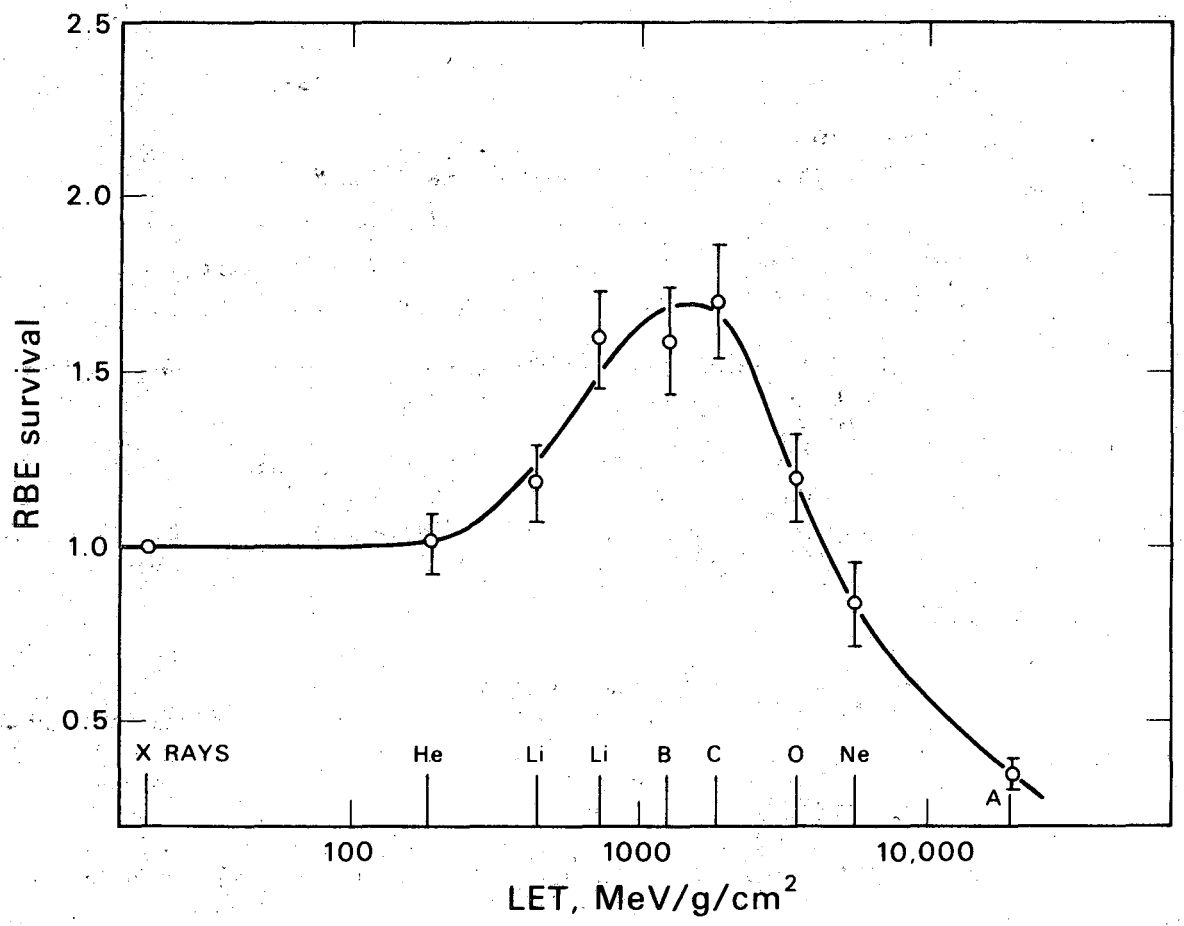

2.5
2.0
1.5
1.0
0.5
RBE survival
X RAYS
He
Li
Li
B
C
O
Ne
A
100
1000
10,000
LET, MeV/g/cm²
DBL 713-5692

Fig. 1

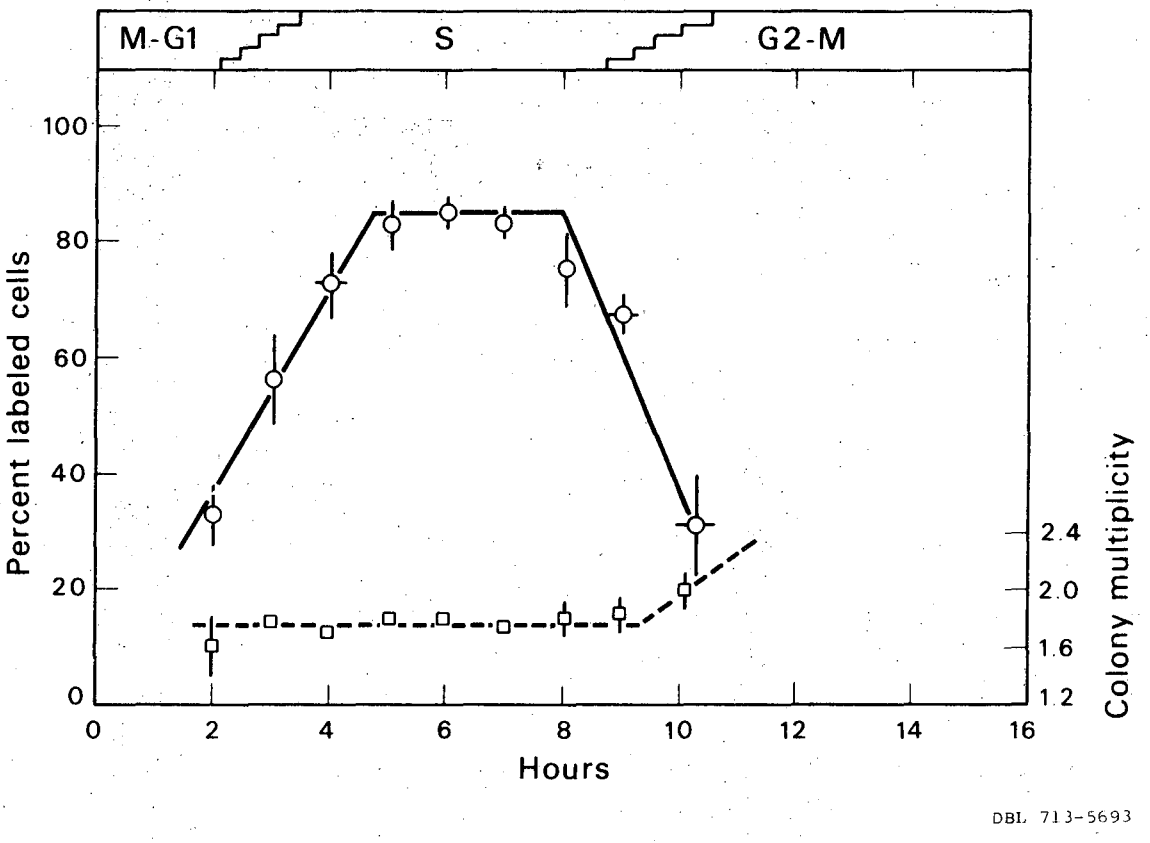

M-G1
S
G2-M
Percent labeled cells
Colony multiplicity
Hours
0
20
40
60
80
100
1.2
1.6
2.0
2.4
0
2
4
6
8
10
12
14
16
DBL 713-5693

Fig. 2

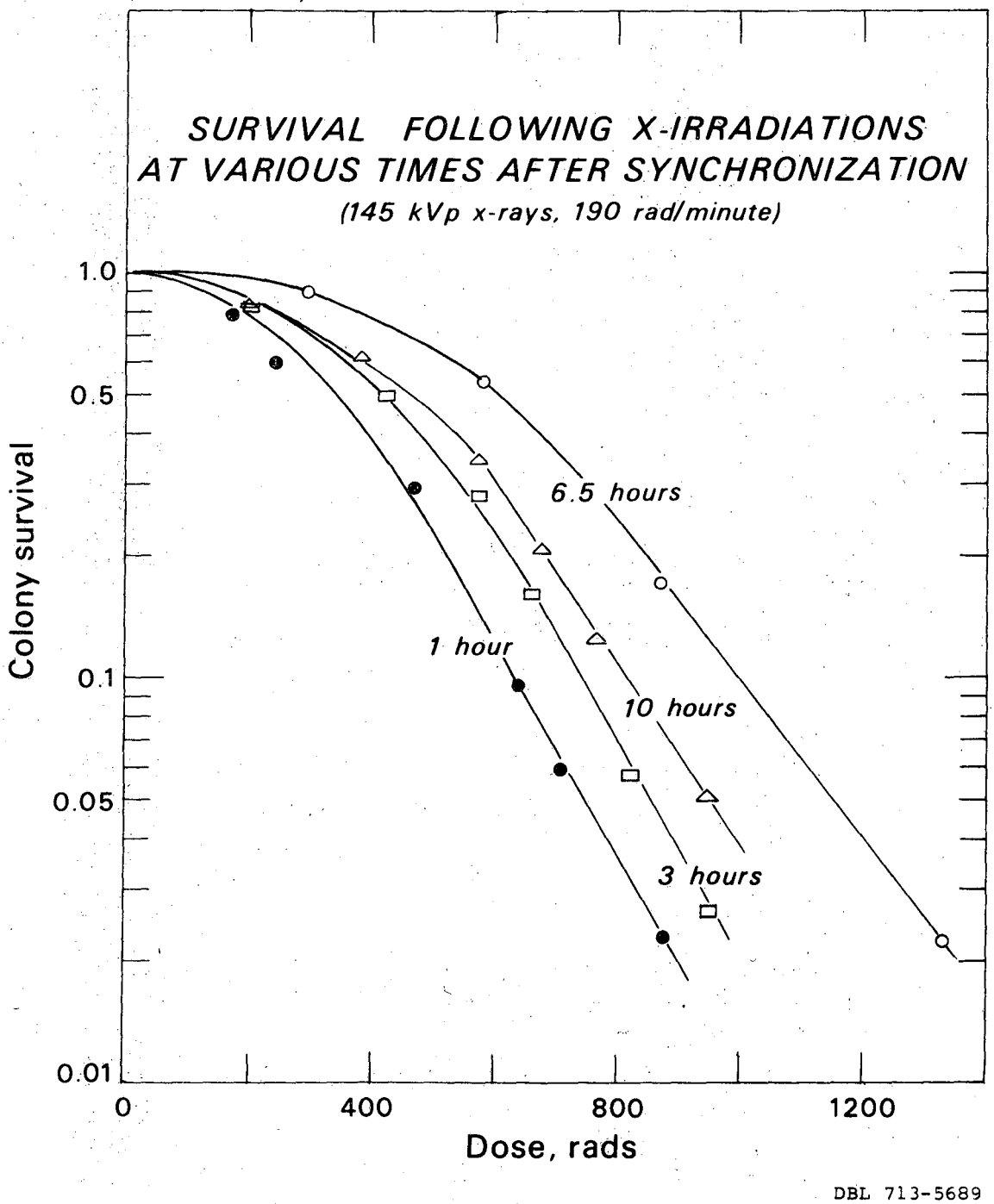
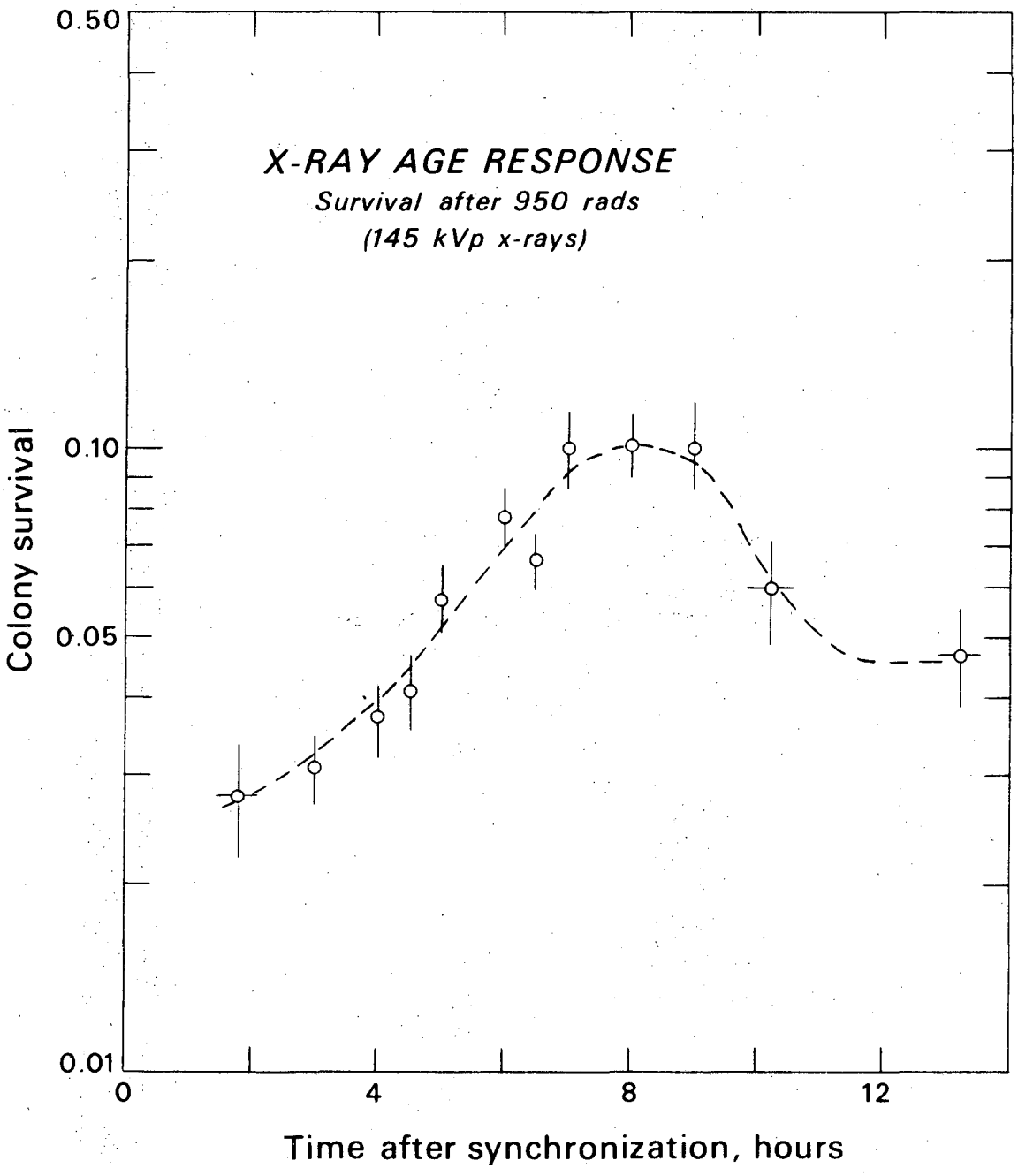

SURVIVAL FOLLOWING X-IRRADIATIONS
AT VARIOUS TIMES AFTER SYNCHRONIZATION
(145 kVp x-rays, 190 rad/minute)
Colony survival
1.0
0.5
0.1
0.05
0.01
0
400
800
1200
Dose, rads
6.5 hours
1 hour
10 hours
3 hours
DBL 713-5689

Fig. 3



DBL 713-5691

Fig. 4

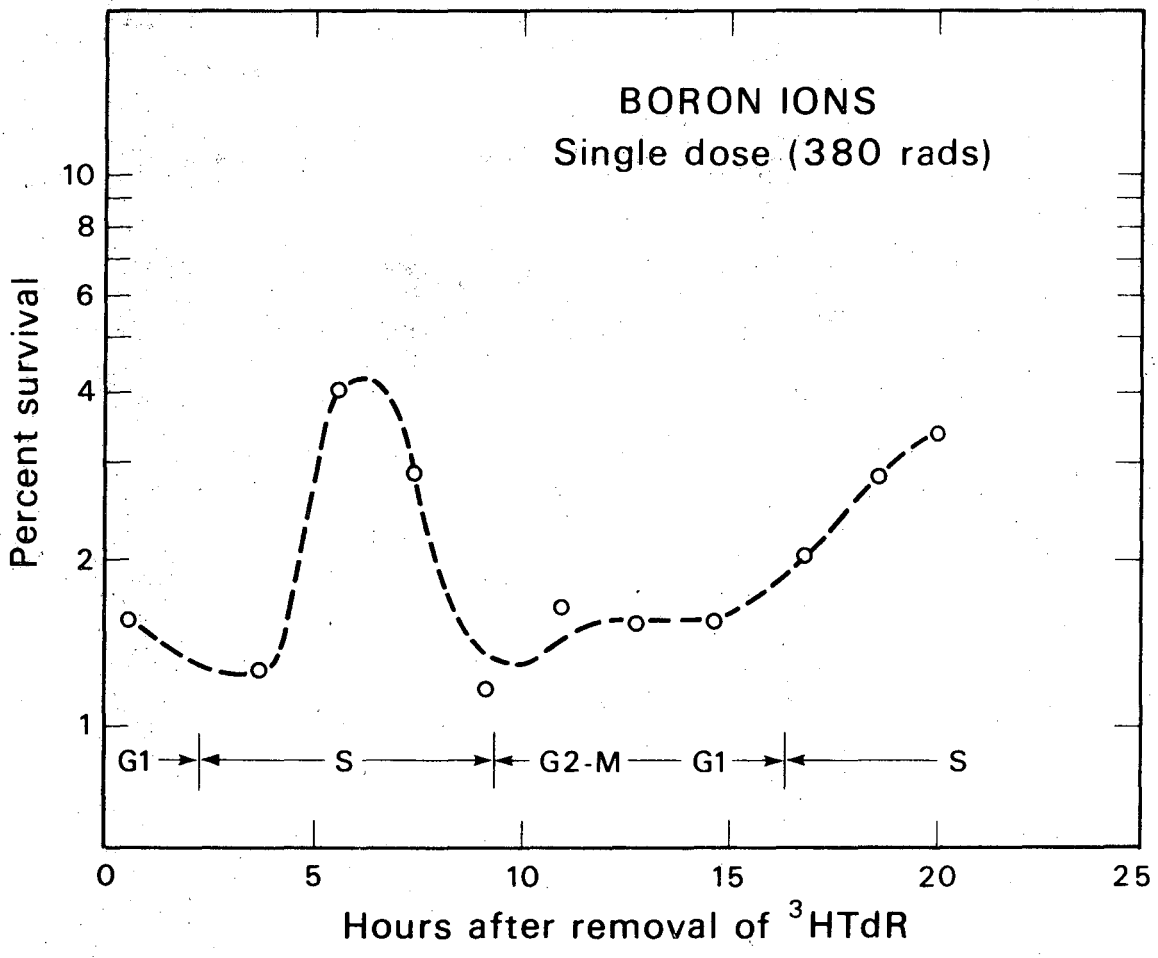

BORON IONS
Single dose (380 rads)
Percent survival
10
8
6
4
2
1
G1
S
G2-M
G1
S
0
5
10
15
20
25
Hours after removal of ^{3}HTdR
XBL 714-624

Fig. 5

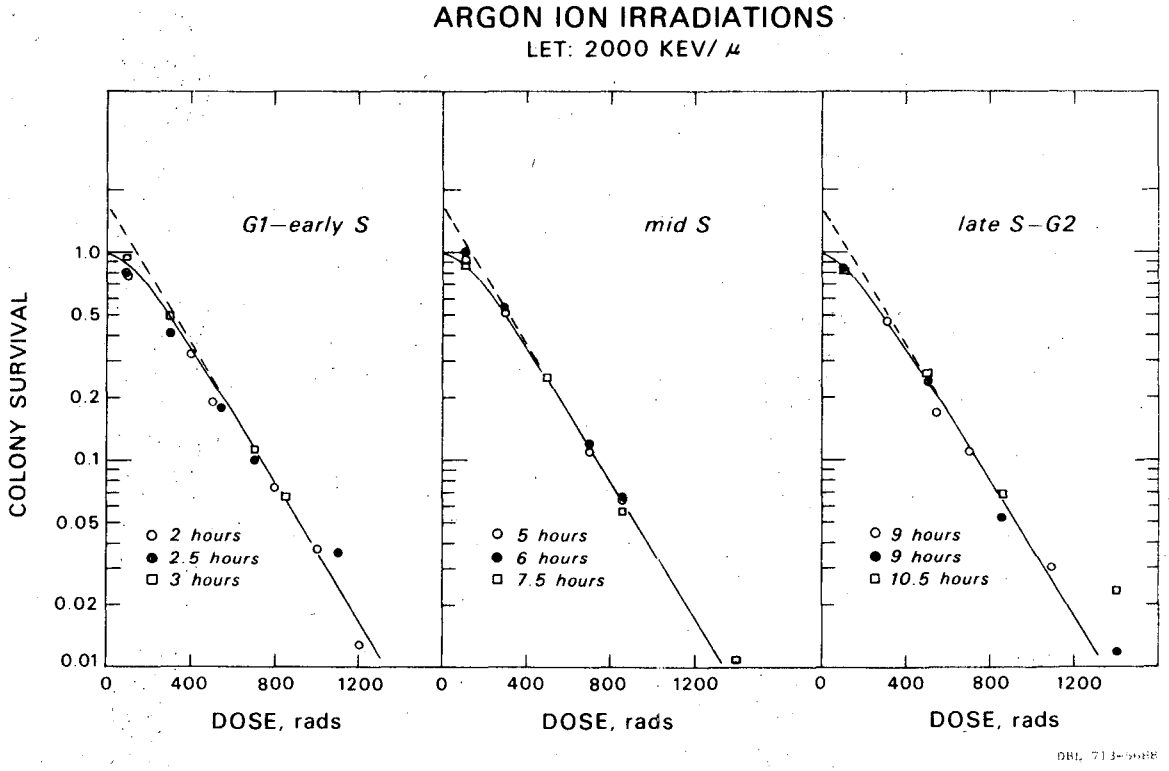

ARGON ION IRRADIATIONS
LET: 2000 KEV/ μ
COLONY SURVIVAL
1.0
0.5
0.2
0.1
0.05
0.02
0.01
G1—early S
2 hours
2.5 hours
3 hours
mid S
5 hours
6 hours
7.5 hours
late S—G2
9 hours
9 hours
10.5 hours
0
400
800
1200
DOSE, rads

Fig. 6

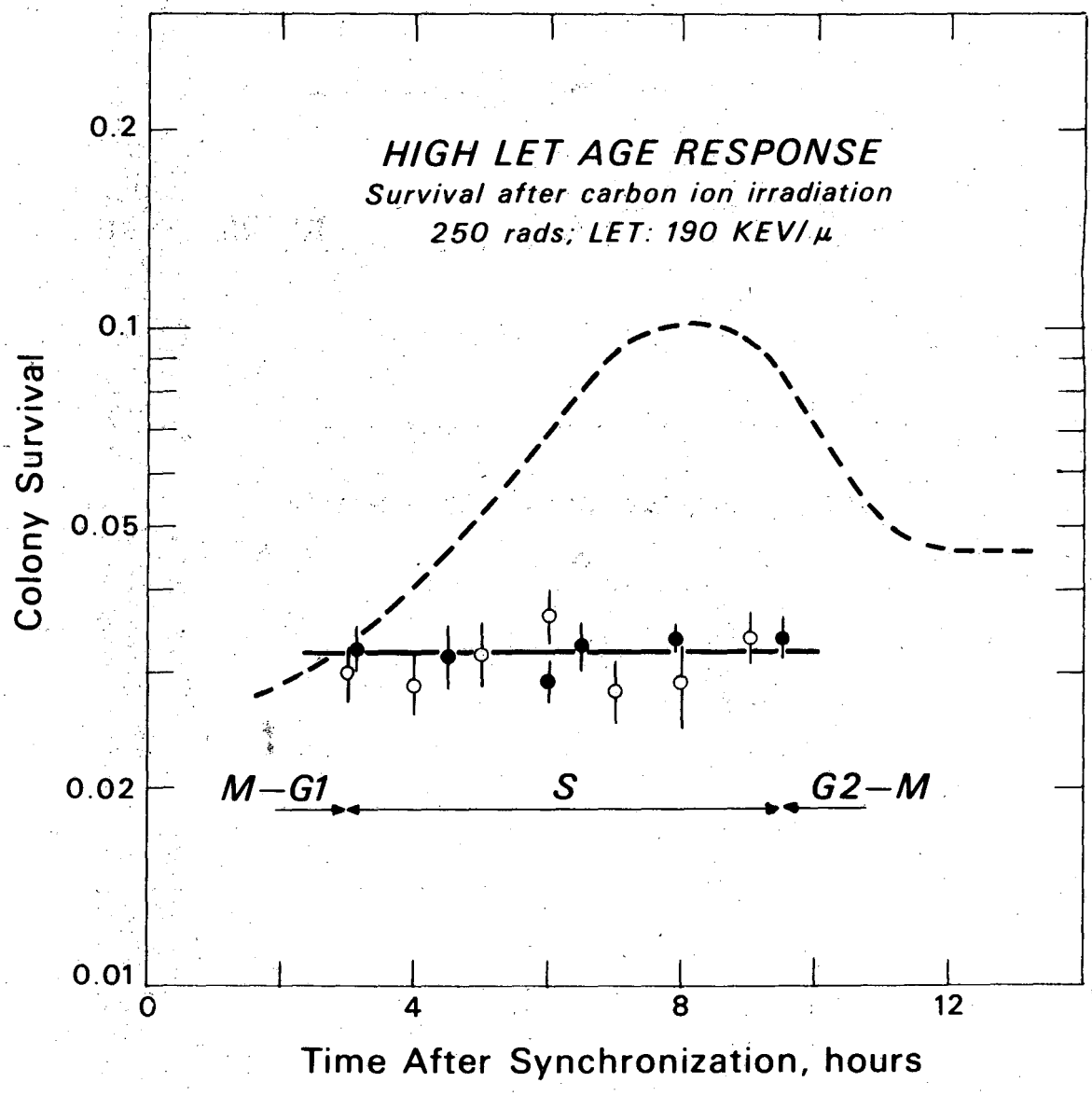

HIGH LET AGE RESPONSE
Survival after carbon ion irradiation
250 rads; LET: 190 KEV/μ
Colony Survival
0.2
0.1
0.05
0.02
0.01
M—G1
S
G2—M
0
4
8
12
Time After Synchronization, hours
DBL 713-5690

Fig. 7

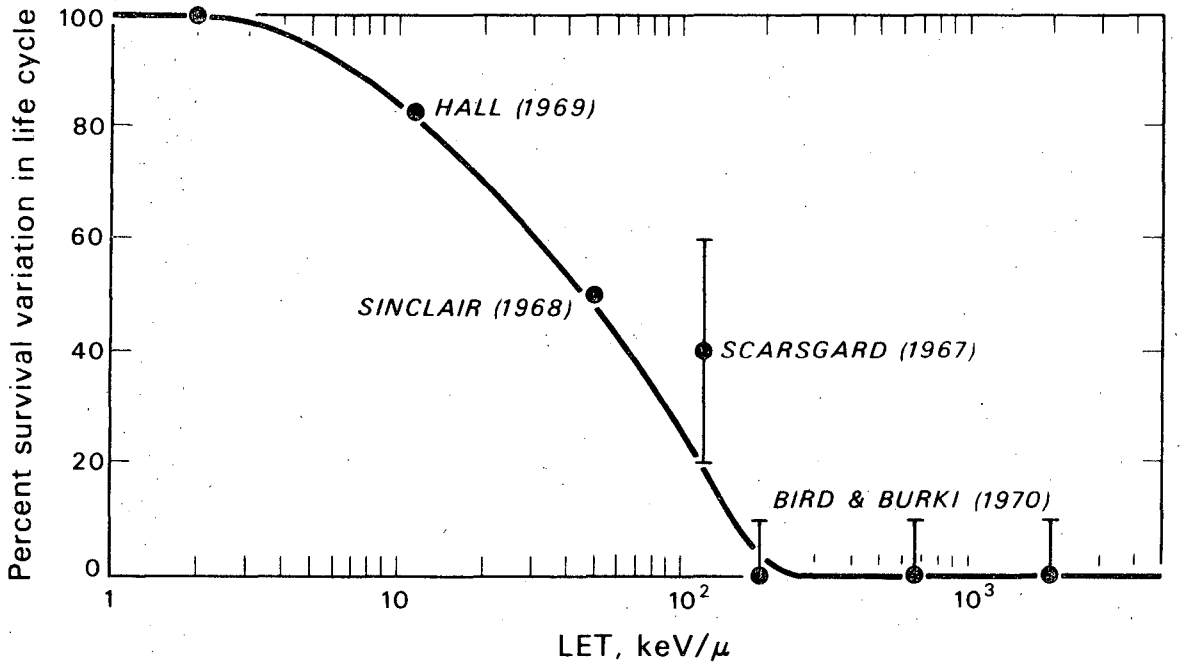

Percent survival variation in life cycle
100
80
60
40
20
0
1
10
10^2
10^3
LET, keV/μ
HALL (1969)
SINCLAIR (1968)
SCARSGARD (1967)
BIRD & BURKI (1970)

Fig. 8

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