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The Cellular Differences Between Acute and Chronic Neutron and
Gamma Ray Irradiation in Mice[†]

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1. INTRODUCTION

It has been well established that even small doses of radiation will shorten the life expectancy of animals, and that in general the causes of death are the same for the irradiated as for the normal animals. When x or γ rays are compared with neutrons in their ability to shorten the life span, some interesting differences appear. All available data from different laboratories on the shortening of the life span by x or γ rays on the one hand and neutrons on the other, have been compared.¹ In spite of the obvious difficulties in comparing such data, if one expresses dose in terms of the LD 50/30 dose required for acute survival, one can pool the data from other laboratories and plot them on a single graph without excessive error. Results of such a compilation for single acute exposures are shown for x or γ rays in Figure 1 and for neutrons in Figure 2.

The striking thing about these two graphs is their similarity and the consistency of the data from different laboratories when plotted in this way. Except for a perturbation at the very low doses in the x-ray curve, the two curves appear to be straight lines with about the same slopes. This means that the RBE for single doses is the same for late effects as for acute effects, i.e. about 2.3 for neutrons as compared to γ rays.

However, when one turns to chronic irradiation one finds quite a different story. It is possible to plot the available data on a single consistent graph provided the neutron experiments are plotted on 1/10th the scale as the γ -ray experiments. This is shown in Figure 3. It thus appears that the RBE for chronic neutron irradiation is 10, which is quite

different from the value found from acute irradiation studies.

From the slope of the curve of Figure 3 at zero dose, it is possible to compute the slope of the curves of Figures 1 and 2 at zero dose, since the results of chronic irradiation can be considered as the sum of a great many single doses. When this is done for neutrons, it is found that the computed slope agrees with the slope of the line in Figure 2 within the experimental error. In this case the effect of the chronic irradiation is indeed the sum of the effects of many small acute irradiations. Thus one can, with considerable assurance, state that the late effects of neutrons are a linear function of the dose down to the lowest doses.

When one makes the same extrapolation for γ rays, a completely different picture is obtained. The slope of the curve of Figure 1 as extrapolated from Figure 3 gives a slope at zero dose of only about 25% of that as judged from higher doses. This means that in order to reconcile these two sets of data, it is necessary to postulate that small doses of γ rays are proportionately only about $1/4$ as effective as are large doses. Thus the curve of Figure 1 has been drawn with a slope at zero dose only $1/4$ that at higher dose, and this slope is indicated by the broken line. It is seen that the experimental points fit this modified curve equally as well as they would a straight line.

Thus a basic difference exists in the reaction of the animal to these different radiations and different radiation regimens. Whereas these differences can be demonstrated most clearly in the life-shortening effects which they produce, there is every reason to believe that they could be demonstrated also for all radiobiological reactions. It is the purpose

of this paper to attempt to explain these differences in terms of basic cellular reactions.

In recent years a method has been developed for estimating the amount of chromosomal damage present in somatic cells of mice.² It consists in scoring the chromosome aberrations seen at anaphase in regenerating liver cells. Since liver cells in the normal mouse rarely divide, the method constitutes a way of unmasking the chromosomal damage existing in the cell. It is found first that these aberrations increase steadily with age (Fig. 4).² Following a single dose of x rays there is a very rapid rise in aberrations followed by a very slow return toward normal (Fig. 4).² The increase in aberrations is roughly proportional to the x-ray dose. When mice are given a single dose of neutrons, there is likewise a spectacular sudden increase in aberrations, but the values stay high for more than a year (Fig. 5),³ which is in marked contrast to the situation with x rays. A constant feature of all such curves following a large dose of any kind of radiation is the slow rise in aberrations which takes place after the initial rise, and this will be discussed later. When mice are exposed to chronic γ irradiation, it is found that they develop chromosome aberrations faster than the controls, but only about 25% as fast as one would compute from the single dose responses assuming a one-hit phenomenon (Fig. 6).³ It will be recalled that chronic irradiation is only about 25% as effective in shortening the life span as is the same dose applied acutely. This indicates that partial healing of chromosomes takes place following a very small dose of radiation, but little if any healing after large doses.

Thus there is a strong correlation between the amount of life

shortening produced by a given radiation or dosage regimen, and the amount of chromosomal damage produced. These and other experiments have been summarized by Curtis⁴ and taken together, constitute strong support for the somatic mutation theory of natural and radiation induced aging.

The work reported here applies these same methods to mice subjected to either acute or chronic neutron irradiation.

2. MATERIALS AND METHODS

The mice used in these experiments were from the Charles River CD1 strain. They were approximately 8 weeks of age at the start of the experiment, and were divided into 5 groups and treated as follows:

Group 1--60 mice irradiation in perforated Teflon tubes at the Brookhaven Medical Research Reactor with a single dose of thermal neutrons of a flux of $7.54 \times 10^9 \text{ N}_0/\text{cm}^2/\text{sec}$ for a total dose of 192 rads at a dose rate of 55 rads/min.

Group 2--72 mice irradiated in perforated Teflon tubes at the Brookhaven Medical Research Reactor for 2 hours per day and 4 days a week at a thermal neutron flux of $8.85 \times 10^6 \text{ N}_0/\text{cm}^2/\text{sec}$ giving a dose of 31 rads per week.

Group 3--60 mice irradiated in perforated Lucite containers with a dose of 290 rads of γ rays from a Co^{60} source, with a dose rate of 83 rads/min.

Group 4--65 mice irradiated in perforated Lucite tubes with a small Co^{60} source for 2 hours per day, 4 days a week, at a rate of 5.44 rads/hr giving a dose of 43.5 rads/week.

Group 5--72 untreated control mice, placed in perforated Teflon tubes 2 hours per day, 4 days a week.

Every 15 days, 5 animals from each group were given a dose of CCl_4 and sacrificed 72 hours later. Liver cell squashes were prepared by the method previously described² and 150 anaphase and telophase figures were scored for each slide.

3. RESULTS

The results of the neutron portion of the experiment are shown in Figure 7 which also shows the theoretical build-up curve assuming a single hit reaction. It is clear from this experiment that the experimental points fall almost perfectly on the one-hit curve and therefore there is no chromosome healing following even very small doses of neutrons.

The results of the γ -ray experiment are shown in Figure 8. This part of the experiment was performed under conditions identical to those of the neutron experiment so it acted as a rigid control. The results are in complete accord with the earlier experiment shown in Figure 6.

4. DISCUSSION

These results clearly give a cellular basis for the differences between acute and chronic neutron or γ -ray irradiation in mice. For neutrons there is no chromosomal recovery following any dose of radiation, while for γ rays there is chromosomal recovery, the magnitude of which depends on the dose.

The reason for this almost certainly lies in the differences in

linear energy transfer (LET) between the two radiations. Gamma rays produce very sparse ionizations, so the ionization events occurring within individual chromosomes will be widely separated in space and time. It takes two or more such events within a certain region and within a certain time to produce a lasting chromosome aberration. Otherwise the single event is healed and no permanent damage results. From this, it follows that the permanent damage resulting from γ irradiation will follow a multi-hit curve, and this is just what is found here.

On the other hand, the ionization density along a neutron track is so high that if a chromosome is hit at all, it produces two or more closely spaced ionizations leading to a permanent chromosome aberration. This explains the single hit curve found here and the lack of chromosome healing.

These results and this explanation are in entire accord with the results of cytological studies in plants where it is possible to see the chromosomes much better. Such studies (see Neary et al.⁵) have shown that neutrons give an almost perfect one-hit curve, while x or γ rays give multi-hit curves which vary according to the particular type of chromosome aberration which is being scored.

Following a single large dose of radiation, and especially following neutron irradiation, the percentage of aberrations may increase for some days or weeks (Figs. 4 and 5). This is a consistent finding and the explanation for it probably lies in the fact that cell division is delayed following irradiation. Since the only cells which can be scored are ones which undergo division, a higher percentage of normal cells will undergo

division following stimulation by partial hepatectomy and thus the percentage abnormalities will be scored too low. As the damaged cells recover, they can be forced into division and thus the percentage of abnormalities will rise.

We have as yet no accurate measurement of the absolute chromosomal damage produced as a function of dose for large single doses of these different radiations, and indeed such would be quite difficult to obtain since the response differs qualitatively as well as quantitatively. However, there is a very strong impression that the chromosome damage due to a single dose of neutrons is about twice as great as the same rad dose of γ rays. This would indicate that the RBE for acute effects as well as chronic effects can be explained on the basis of chromosome damage. It is attractive to postulate that this is due to the ability of chromosomes to partially heal following even large doses of x rays, and the inability to heal following neutron irradiation. This offers an explanation for the differences in RBE between different radiations and dosage regimens entirely on the basis of the ability of chromosomes to heal following x or γ irradiation.

Following a single dose of x rays there is a slow return of the damaged liver cells toward normal levels (Fig. 4) while for neutrons the damage seems to persist almost indefinitely (Fig. 5). It would seem entirely reasonable to suppose that a cell containing severe chromosomal damage would divide perhaps only once and then the daughter cells would either die or their chromosome damage become invisible by the methods employed here. However, this appears not to be the case. One might explain the slow return to normal following γ irradiation on this basis, because

there is a low normal cell division in mouse liver. But the neutron treated mice appear to show no recovery, which they should if this explanation were correct. The other explanation for this is the possibility that chromosome healing may be continuing in mice even several months after a single dose of γ rays, whereas there is no healing following neutron irradiation. This might follow from the results of chronic irradiation reported here, but it seems an excessive extrapolation from the data. Thus this phenomenon remains unexplained at the present time.

Previous communications¹ have pointed out that the correlations between chromosome aberrations and life shortening gives strong support to the idea that mutations in somatic cells are responsible for the aging of the animals. The present results further strengthen this somatic mutation theory of aging.

5. SUMMARY

Data from many laboratories indicate that for mice the RBE of large single doses of γ rays or neutrons is about the same for shortening of the life span as it is for acute lethality. However, for chronic irradiation the RBE is quite high for shortening of the life span, due to the fact that very small doses of neutrons are proportionately just as effective as large doses, while for γ rays, small doses are proportionately only about $1/4$ as effective as large doses.

The reason for this difference has been investigated by scoring the chromosome aberrations in regenerating liver cells in mice subjected to both chronic and acute neutron and γ -ray irradiations. The results

show that for γ rays, very small doses are proportionately only about $1/4$ as effective in producing chromosome aberrations as are large doses. However, for neutrons, large and small doses are proportionately equally effective. This result adequately explains the differences in RBE for life shortening and at the same time gives strong support for the somatic mutation theory of aging.

The present work seems to indicate that for relatively large doses, equal rad doses of neutrons produce about twice the chromosome damage as do γ rays. This would indicate that the RBE for acute effects can also be explained on the basis of chromosome damage.

Following single doses of γ rays, the percentages of abnormal chromosomes falls slowly over a period of many months, but following a single dose of neutrons the aberration frequency may be as high as 90% and stay this high for at least a year. There is no ready explanation for this difference in reaction to these two radiations.

6. ACKNOWLEDGEMENT

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FIGURE LEGENDS

FIG. 1. Survival time for mice as percentage of control vs. dose expressed as a percentage of the LD 50/30 for single doses of x or γ rays. Data taken from various laboratories. (From Curtis¹)

FIG. 2. Survival time for mice as percentage of control vs. dose expressed as a percentage of the LD 50/30 for single doses of neutrons. Data taken from various laboratories. (From Curtis¹)

FIG. 3. Survival time for mice as percentage of control vs. dosage rate in rads/week for chronic irradiation with either γ rays or neutrons. Data taken from various laboratories. (From Curtis¹)

FIG. 4. Chromosome aberrations in liver cells of mice following a single dose of 700 rads of x rays. (From data by Stevenson and Curtis²)

FIG. 5. Chromosome aberrations in liver cells of mice following a single dose of 300 rads of fast neutrons. The mice were of a different strain from those shown in Figure 4, so absolute values cannot be compared between these two experiments. (From data by Curtis and Crowley³)

FIG. 6. Chromosome aberrations in liver cells of mice subjected to chronic γ -irradiation and acute x-irradiation. The dotted line gives the rate of build-up which would be expected for the chronically irradiated mice if chronic irradiation were as effective in causing chromosome damage as is acute irradiation. Since the experimental curve for chronic irradiation is far from this, it must be concluded that chromosome healing takes place following small doses of γ -radiation. (From Curtis and Crowley³).

FIG. 7. Chromosome aberrations in liver cells following acute and chronic thermal neutron irradiation. The dotted line gives the rate of build-up which would be expected for the chronically irradiation mice assuming chronic irradiation to be as effective in causing chromosome damage as is acute irradiation. Since the experimental points fall very close to the theoretical curve, it must be concluded that the two forms of radiation are equally effective.

FIG. 8. Chromosome aberrations in liver cells following acute and chronic γ -irradiation. This experiment is similar to the one shown in Figure 6, but repeated under conditions identical to those of the thermal neutron experiment shown in Figure 7.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8