Radiation Protection in the World of Modern Radiobiology: Time for A New Approach

R. E. J. Mitchel and D. R Boreham

Radiation Biology and Health Physics Branch, AECL, Chalk River Laboratories,
Chalk River ON. Canada, K0J 1P0

INTRODUCTION

All current radiation risk estimates and all radiation-protection standards and practices are based on the so-called "Linear No-Threshold Hypothesis". This LNT hypothesis is in turn, based mainly on epidemiological data of humans exposed to high doses and dose rates but is considered to also apply at low doses and dose rates, with a two-fold reduction in risk. The hypothesis states that risk is linearly proportional to dose, without a threshold. This hypothesis therefore predicts that:

- every dose, no matter how low, carries with it some risk
- risk per unit dose is constant
- risk is additive
- risk can only increase with dose
- biological variables are insignificant compared to dose

In this paper, we will present the results of some of our low dose and/or low dose rate experiments with low LET radiation in human and rodent cells, and in animals, and determine if the results support or reject the LNT hypothesis. However, in order to properly compare the predictions of the LNT hypothesis with the actual experimental data, several biological and physical considerations must be kept in mind. Although low dose radiation exposure can potentially generate different kinds of biological risk, the risk of most concern is cancer, which is the end-point that will be addressed here.

In considering radiation induced cancer, two biological points are important. First, cancer arises from changes in a single cell. This point is important because it defines the limits of the meaning of "low dose". Unlike the concept of whole body dose, where dose is averaged over all cells in the body, a single cell is the smallest volume that is relevant for carcinogenic risk. The lowest possible dose is, therefore, that dose which can be deposited in a single cell. However, cancer formation is a multi-step process (Figure 1)(1) and although we usually consider radiation as acting on normal cells, it may act on cells which are at any point in that process. This second biological point is relevant because some or all of the multiple changes required to produce a cancer will occur after the exposure, and their rate of occurrence defines the latent period, the time between exposure and the appearance of cancer. While one measure of risk is the frequency of cancer, another is the amount of lifespan lost, as determined by the latent period.

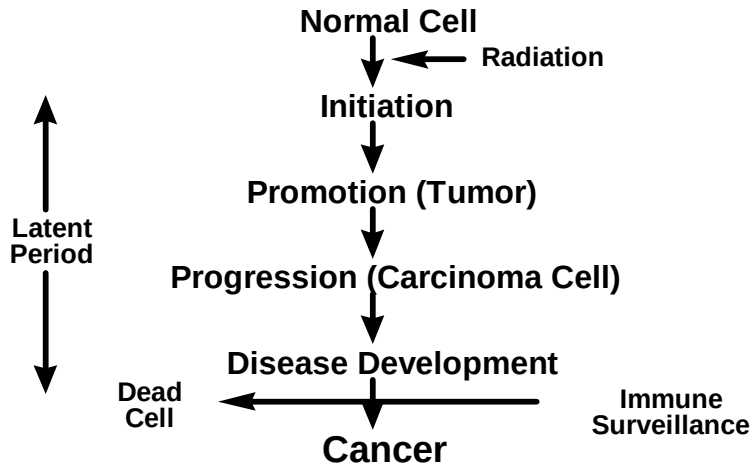

Figure 1. Multi-step process of carcinogenesis

It is also important to recognize some physical characteristics of radiation:

- radiation deposits energy, and damage, in tracks
- the smallest dose a cell can receive is that deposited by a single track
- at total doses which are less than one track/cell, not all cells are hit, i.e., some cells receive no dose; however, those that are hit still receive the dose deposited by one track.

While the lowest possible dose to a cell is that deposited by one track, the actual dose depends on the nature of the radiation. For example, a single alpha particle track can deposit tens of cGy while a single ^{60}Co-γ ray will deposit, on average, about 1 mGy. The experiments presented here describe mainly gamma-ray exposure and therefore the minimum possible dose to a cell is about 1 mGy.

If we consider the potential biological outcomes of a radiation exposure to a normal cell, the first step depicted in Figure 1, there are three general biological outcomes of DNA damage as shown in Figure 2(1).

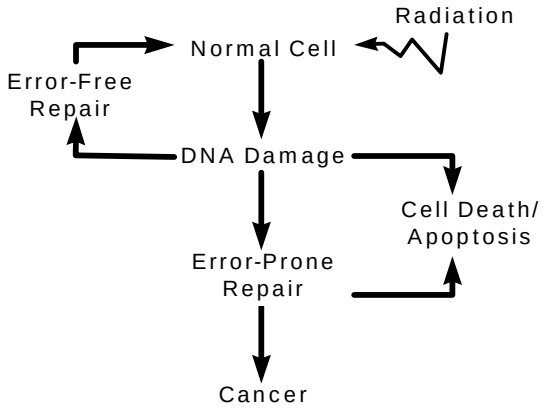

Figure 2. Possible outcomes of a cellular radiation exposure in a normal cell.

When DNA damage is created as a result of one or more tracks of radiation through a normal cell, the cell will attempt to repair that damage. If the repair is successful and the DNA restored to its original state, i.e., an error-free repair, then the cell is also restored to normal. In this case, there is no resulting consequence to the cell and hence no resulting risk. An alternate possibility is that the cell recognizes that it cannot properly repair the damage, and as a consequence activates its genetically encoded cell death process, called apoptosis. Again, in this case, no risk of carcinogenesis results since dead cells do not produce cancer. The third possible outcome of the DNA damage is repair which avoids cell death but which is error prone; that is, results in a mistake that creates a mutation. At this point, the cell may still activate its apoptotic cell death program but could also simply resume dividing. While the vast majority of radiation-induced mutations do not create the potential for cancer, there are some that do and it is these mutations that represent the risk. Of the three possible outcomes, therefore, only one creates an “initiated” cell (Figure 1) and subsequently a risk of carcinogenesis. It is useful to remember that the LNT hypothesis predicts that risk is influenced only by dose, and hence predicts that the relative proportions of these three biological possibilities must be constant. If they were not constant, then risk would vary with their relative proportions, i.e., not as a function of dose.

EXPERIMENTAL RESULTS AND DISCUSSION

(I) Cellular studies

One consequence of a radiation exposure of cells is breakage of chromosomes, which indicate DNA double strand breaks. The competence of the cells at repairing such breaks can be measured using the micronucleus (MN) assay. Most radiation-induced MN contain unrepaired pieces of chromosomes. Counting the frequency of micronuclei after an exposure therefore provides a measure of the ability of cells to repair broken chromosomes (and therefore DNA double strand breaks) in response to radiation damage.

We have tested the influence of low doses and low dose rate exposures on the ability of human skin cells to repair radiation breaks in chromosomes (2).

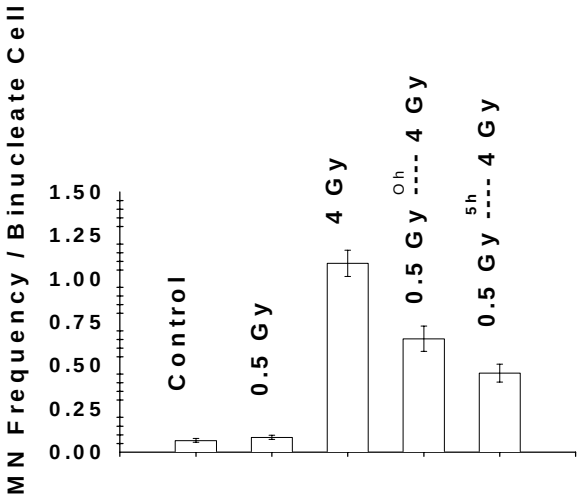

Figure 3. Repair of broken chromosomes in human fibroblasts

Figure 3 shows the MN frequency in cells exposed to a moderate dose (0.5 Gy) delivered at a low dose rate (2.5 mGy/min) and then immediately (0h) to a high dose (4 Gy) delivered at a high dose rate (1.8 Gy/min). The LNT hypothesis predicts that the consequences of the two doses would be additive and yet the experiment shows that they are not. The combined exposure resulted in less broken chromosomes than the single acute 4 Gy exposure alone, and when the doses were separated by a 5 h incubation, the resulting MN frequency was even less. This experiment indicates that the low dose rate exposure had stimulated the cells to increase their ability to repair broken chromosomes, such that the consequences of the second large exposure were reduced. It is apparent from this experiment that biological variables are important in determining the consequences of radiation exposures and that the risk is not proportional to dose, results that do not support the LNT hypothesis.

The data in Figure 3 showed that moderate doses at low dose rates reduce the DNA damaging consequences of a subsequent exposure, a result not consistent with the predictions of the LNT hypothesis. Figure 4 shows that the same result occurs at 1 mGy, the lowest γ dose possible in a single cell since it represents, on average, a single track per cell (3). The figure also shows that higher doses, representing multiple tracks/cell, produce the same result as one track/cell when those tracks from the high doses are delivered at a low dose rate (3 mGy/min) i.e., spaed out in time. In all cases the cells were given 3h after the first (adapting) exposure to allow resistance to dvelop.

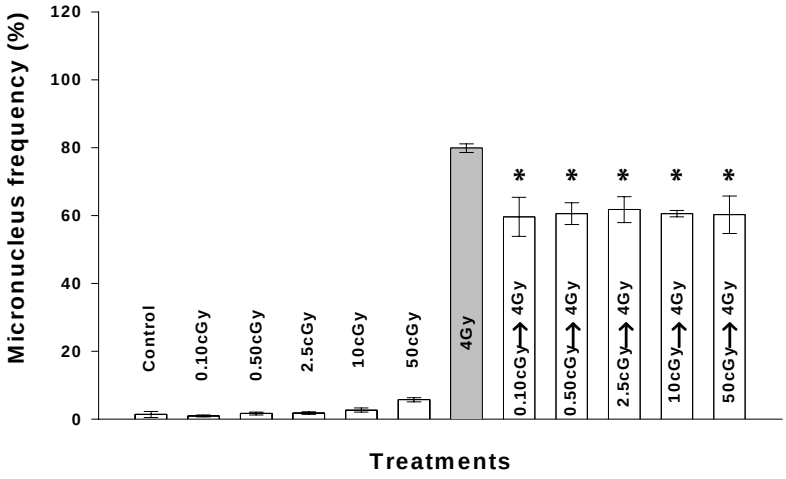

Figure 4. Ability to repair broken chromosomes in cells adapted by exposure to low doses

While the data shown in Figures 3 and 4 do not appear to be consistent with some of the predictions of the LNT hypothesis, they do not directly contradict the hypothesis since only repair competence and not cancer risk was measured. When we examined whether these radiation adapted cells in Figure 4 applied their increased repair competence uniformly to each chromosome, we found that the cells now displayed a bias in the repair of broken chromosomes (Figure 5) (4).

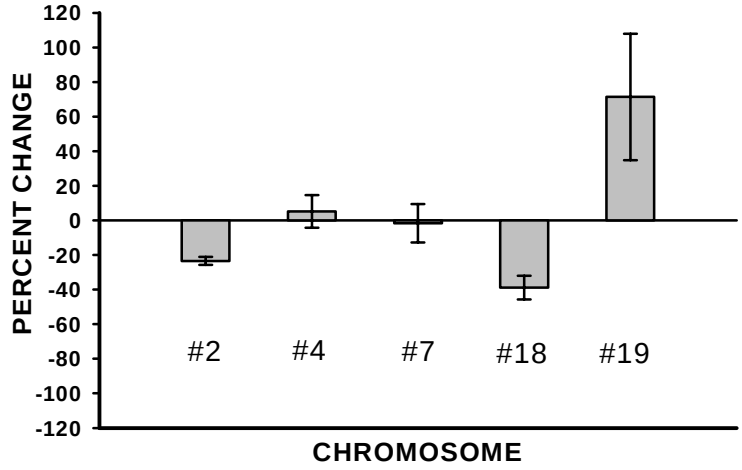

Figure 5. Change in the frequency of broken chromosomes in micronuclei which form after exposure of radiation adapted cells.

Less broken pieces of chromosomes 2 and 18 appeared in micronuclei after a 4 Gy exposure when cells were first given a prior 10 cGy dose, indicating that the adapted cells now preferentially repaired breaks in these chromosomes. In contrast, chromosome 19 was more frequently left unrepaired while repair of chromosomes 4 and 7 was unchanged . It appears, therefore, that low dose exposure to radiation altered the risk to some chromosomes and by implication, to the genes on those chromosomes. This result points out the difficulty in estimating the implications for risk of any measure of mutation in one specific gene or chromosome. A low dose may increase, decrease, or cause no change in repair of a specific chromosome and therefore of the genes on that chromosome. Consequently, translating that observation to the risk of cancer is not straight forward. In addition to the DNA repair being biased for or against some chromosomes or genes, the radiation-induced increase in repair competence could reflect either error-free repair, which would decrease risk, or error-prone repair, which would increase risk (Figure 2). Using an assay which just measures rejoining of broken chromosomes does not distinguish between these possibilities.

In order to understand the impact on cancer risk of these changes in cellular DNA repair which result from low doses, some measure more closely related to cancer risk is required. Using an assay that measures the frequency at which rodent cells in tissue culture are transformed into cancer cells, we repeated the experiments shown in Figures 3 and 4. Some of the results are shown in Table 1 and provide a direct measure of changes in carcinogenic risk as a result of the low dose radiation exposures (5).

Treatment	**Transformation Frequency** ($\times 10^{-4}$)
Control	3.7
4 Gy (high dose rate)	41
100 mGy (low dose rate) + 4 Gy (high dose rate)	16

Table 1: Reduction in the risk of radiation-induced malignant transformation by a prior chronic exposure.

The data show that a large (4 Gy) high dose rate (2 Gy/min) exposure increased the transformation frequency about 10-fold over the spontaneous frequency in these cells. However, a 100 mGy low dose rate exposure (2.4 mGy/min) immediately before the 4 Gy exposure did not further increase risk, as predicted by the LNT hypothesis, but actually decreased risk by 2- to 3-fold. This result is therefore contrary to current assumptions of risk from multiple exposures, and suggests that low dose rate exposures are protective against subsequent exposure. The result is consistent with the concept that low doses stimulate cells to increase their

capacity for an error free type of DNA repair (Figure 2) and the cells then selectively apply that repair to damage in those chromosomes and genes (Figure 5) which would otherwise create a risk of cancer formation.

While the data in Table 1 showed that the combined risk of the two exposures was less than that of the single exposure alone, it can also be seen that the net risk is still about 4-fold higher than the inherent spontaneous risk in the absence of radiation. This experiment therefore does not preclude the possibility that low doses or low dose rate exposures by themselves elevate the risk above that which occurs as a result of spontaneous (non-radiation-induced) cellular events.

The LNT hypothesis ultimately predicts that any dose, no matter how small, increases the risk of cancer. Using the rodent cell transformation assay, we directly tested that prediction, and the results are depicted in Table 2 (6).

Treatment	**Transformation Frequency** ($\times 10^{-3}$)
Control	1.8
1.0 mGy	0.62
10 mGy	0.39
100 mGy	0.49

Table 2: The influence of low doses delivered at low dose rate (2.4 mGy/min) on the risk of spontaneous malignant transformation.

Those data show that at an average of one track per cell (1 mGy) the risk of spontaneous transformation was reduced from that which occurred spontaneously in the absence of radiation exposure. The data also show that higher doses, up to 100 mGy delivered at a low dose rate, produced the same 3-4 fold reduction in spontaneous transformation risk.

These DNA repair and cell transformation assays in human and rodent cells clearly indicate that a single ionizing radiation track, or multiple tracks if received intermittently in time, stimulate an error-free DNA repair process. That repair system increases the probability of correctly repairing either radiation-induced or spontaneous DNA damage, and therefore reduces the overall risk of either radiation-induced or spontaneous transformation to malignancy. These results are inconsistent with the LNT hypothesis and argue strongly that the hypothesis should be rejected..

The above experiments tested the predictions of the LNT hypothesis for two of the three possible outcomes of a radiation exposure of a normal cell. The influence of a low dose on the third possibility, death by apoptosis, has also been tested. Comparing the extent of apoptosis induced in human lymphocytes by exposure to 2 Gy with the extent of apoptosis induced by 2 Gy in cells pre-exposed 6 h earlier to 10 cGy, the pre-exposed cells showed about a 20% increase in the number of apoptotic cells. This data is based on lymphocytes taken from 26 individuals (7-9).

These results show that low doses amplify the probability of apoptotic cell death resulting from a second exposure. This sensitization of cells to radiation-induced cell death increases the probability that a cell will die rather than survive with a mutation, an outcome that is believed to reduce cancer risk in the whole organism. Having examined all three possible biological outcomes, the effect of a low dose exposure on cancer risk in a normal cell appears quite clear from the above cellular studies. Low doses or doses delivered at low dose rate reduce rather than increase risk in normal cells, a result that contradicts the LNT hypothesis.

(II) Animal studies

Several investigators have examined the adapting effects of low doses in tissues taken from irradiated animals but there is little data examining the effect of low doses on tumor or cancer risk. We have reported the results of two such investigations in mice. One examines the influence of low doses on tumor frequency, a measure of the risk of the first “initiation” step (Figure 1). Another examines the effect on tumor latency, a measure of the speed at which the multiple steps are proceeding i.e., a measure of the risk of “lost days of life."

Table 3 shows the results of an experiment to investigate the influence of *in vivo* β-irradiation of mouse skin on the frequency of non-malignant skin tumors, produced by exposure to a chemical carcinogen followed by exposure to a chemical tumor-promoting agent (10). The experiment showed that skin irradiation 24 h prior to treatment with a DNA damaging chemical carcinogen reduced tumor frequency by about 5-fold. This result is consistent with the cell-based studies described above. It implies that the radiation exposure stimulated an error-free DNA repair system that was able to recognize and remove much of the chemically produced DNA damage.

Initiation Treatment	Tumors per Animal
methyl-nitro-nitroso guanidine	2.04
β-radiation	0
β-radiation + 24h + methyl-nitro-nitroso guanidine	0.39

Table 3: Protection by β-irradiation (50 cGy) against chemical initiation of skin tumors in mice.

While all the above experiments measured the effects of low doses on risk by measuring the frequency of DNA damage or of transformation, only one experiment has been reported that investigated the influence of low, adapting doses on tumor latency (11). That data is summarized in Table 4 and shows the influence of a prior low dose exposure delivered at low dose rate (8 mGy/min) on the latency of myeloid leukemia induced in mice by a subsequent exposure to a large dose, also delivered at that low dose rate.

Treatment	Average Lifespan (Days)	Life Lost (Days)
Control	727	0
1.0 Gy	486	241
0.1 Gy, 24h, 1.0 Gy	578	149

Table 4: Extension of latency period in mice developing acute myeloid leukemia.

The table shows that the leukemia latent period was significantly extended by the prior exposure, such that the loss of lifespan was reduced by about 40%. Interestingly, the frequency of leukemia in this experiment was unchanged. These two animal experiments show that the risk of both tumor frequency and latency can be influenced by a low dose. While both results indicate a net protective effect, the experiments suggest that the nature of the outcome may be influenced by the specific nature of the cancer risk.

(III) Uncertainties

The data describing the responses of normal cells and normal animals to low doses of low LET radiation, and the influence of those responses on cancer risk, are convincing and show that low doses reduce rather than increase risk. On the other hand, the influence of genetics and genetic variation in individuals, as well as the response to high LET radiation is less clear. Experiments are in progress to address those uncertainties.

In the human apoptosis data quoted above, we described a low dose as causing about a 20% increase in apoptosis in lymphocytes subsequently exposed to a high dose of radiation, and therefore reducing the risk. However, the 26 normal individuals whose lymphocytes showed this response clearly segregated into two groups. Lymphocytes from 18 individuals showed a 27.5 ± 5.7 % increase while lymphocytes from 8 other individuals averaged only 7.0 ± 3.0 % increase (8). If this difference is representative of genetic variation in the population, then the extent of the risk reduction from a low dose exposure may also be variable.

We are using animal studies to examine the impact of genetic variation, and the role of the so called “cancer risk” genes on radiation risk. We wish to understand, for example, the relative magnitude of the risk introduced by genetic factors alone, versus the risk of a radiation exposure. We are particularly interested in genetic defects which impact on DNA repair and apoptosis. The “cancer risk” gene Trp 53 is important for control of both the repair of DNA damage and apoptosis after radiation exposure. Mice heterozygous for a defect in this gene (i.e., one normal copy and one defective copy) appear normal but spontaneously develop cancer and die much earlier than normal mice that have two good copies of the gene. We have shown that the risk of early death resulting from one defective copy of this gene, in the absence of any radiation exposure, is approximately equivalent to a 4 Gy acute whole body exposure to a normal animal. Thus, the risk of life shortening resulting from the genetic defect alone is far greater than from the risk of any likely radiation exposure in normal individuals. Early results indicate that the incremental effects of high doses on life shortening in the heterozygous mice are similar to those in the normal mice, indicating that radiation exposure does not produce unexpected effects in mice who are predisposed to cancer because they carry this “cancer risk” gene.

Another area of uncertainty is the effect of low doses of high LET radiation. However, it is important to recognize that the lowest possible cellular dose, i.e., that from a single track, is of the order of tens of cGy, much

higher than that from a low LET radiation exposure. Recent published data suggest that a single alpha track can induce so called “bystander” effects, changes in gene activity or even DNA damage in cells adjacent to the cell actually receiving the radiation track. We have previously reported similar responses to low LET radiation where human lymphocytes exposed to low doses of gamma radiation, such that not all cells were traversed by a radiation track, secreted a factor that caused gene activation in other unexposed cells (12). Whether ‘bystander” cells are at higher or lower risk as a result of such events is unknown. However, in an animal study examining the risk of lung carcinogenesis from inhaled uranium ore dust, an alpha emitter, we observed that the frequency of lung tumors was not related to lung dose but was instead directly proportional to dose rate (13). This result implies that the risk of lung cancer from this high LET exposure was determined only by the rate of DNA repair, a process seen above to be inducible by low doses.

CONCLUSIONS AND RECOMMENDATIONS

None of the predictions of the LNT hypothesis, as it applies to cancer risk from low or chronic doses of low LET radiation, are supported by the above data in human or rodent cells. The limited data in animals also indicates that the observed responses are not consistent with the hypothesis. The protective responses observed in mammalian cells and in animals are consistent with those seen in lower eukaryotes, including yeast, indicating that they are evolutionarily conserved (14) and lending credence to the idea that such responses are the normal and expected consequences of low dose exposures.

Scientific advancement depends upon the testing of hypotheses. When the data do not support the hypothesis being tested, that hypothesis must be rejected and replaced with a new testable hypothesis. Since, at low doses and dose rates, there are no data in the literature that support the LNT hypothesis for cancer risk, and considerable evidence contradicting it, including the evidence given above, then this hypothesis must therefore be rejected. It is time for a new risk based approach to radiation protection, firmly linked to the actual biological responses.

Abandoning the LNT hypothesis as an appropriate model to estimate risk and to form the basis of radiation protection at low doses and dose rates will result in considerable difficulties for regulatory agencies and for radiation-protection practices. For example, the basic principle in the nuclear industry of dose minimization known as ALARA, *as low as reasonably achievable*, can now, in some cases, be seen as increasing rather than decreasing risk. These are real, practical problems and will not be resolved overnight. It is incumbent upon the scientific community to inform and convince politicians, regulators and the public of the biological facts concerning radiation responses to low doses so that these difficulties can be overcome. In this regard we must be mindful of the uncertainties, but this is not an argument for the “precautionary principle.” Given the actual data, it would be more logical to argue that it may be imprudent and incautious <u>not</u> to expose radiation workers to low doses of radiation, when there is a possibility that such exposures, while doing no harm, will protect them against the known harmful effects of higher doses to which they might later be exposed. Equally, it could well be argued that it may be unethical to protect workers and the public against small doses of radiation which may reduce their inherent spontaneous risk of carcinogenesis.

Given the uncertainties, it is important to increase research efforts in this field; in particular, in animal test systems. Such research is required to determine the extent and limitations of the benefit on cancer risk at the organ and whole animal level, and particularly to clarify the genetic questions.

Abandoning the LNT hypothesis as the basis for radiation protection practices at low doses will require adoption of an alternate hypothesis. An interim hypothesis that could be used as a practical radiation-protection guideline would be the assumption of a linear-threshold response for cancer risk. While this hypothesis is also not consistent with the observed benefits at low doses, and is not a risk based approach, it at least removes the concept of risk at those doses and could serve until the limits of the benefits are better defined. Given the accelerating advances in molecular and cellular biology, in the very near future we will have the ability to easily assess individual responses to radiation. The radiation protection community must recognize this incoming tide of information, and accept the need to move toward a risk based radiation management practice, which for workers will be based on individual responses to low doses. Such a practice will inevitably become the standard. Risk estimates and dose limits for the public will necessarily continue to be population based, but must also recognize that the underlying principles currently used are incorrect.

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