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Review article

Muller's Nobel Prize data: Getting the dose wrong and its significance

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ABSTRACT

This paper evaluates the significant historical paper of Muller and Mott-Smith (1930), which successfully disputed the proposal of Olson and Lewis (1928) that background ionizing radiation is the driving mechanism of evolution. While the present analysis supports the general conclusion that background radiation is not a quantifiable factor affecting evolution, the paper reveals methodological errors and questionable conclusions in the Muller and Mott-Smith (1930) paper, which may have impacted the acceptance of the linear non-threshold (LNT) model. Most importantly, this paper reveals that in Muller's (1927) Nobel Prize research he used a treatment exposure (total dose) that was 95 million-fold greater than the average background exposure, a value far greater than the 200,000 fold reported by Muller and Mott-Smith (1930). Such a large exposure rate discrepancy may be historically important as it may have led to the over-reliance on Muller's research in support of the derivation and use of the LNT single-hit model.

1. Introduction

Olson and Lewis (1928) hypothesized that the mechanism of evolution was background (cosmic and terrestrial) ionizing radiation. This was mediated by the linear non-threshold dose response and applied to all species. While this hypothesis initially received support, Muller and Mott-Smith (1930) asserted that background radiation could only account for about 1/1300th of the mutations in the control group of Muller's (1927) groundbreaking research in the fruit fly. They concluded that other unknown factors (Harman, 1962) were contributing to the mutation incidence and the dominant mechanism was not background radiation. The present paper was initially intended to probe the theoretical question of whether a single electron-ion pair, produced via ionization, could initiate the process of carcinogenesis and how Muller addressed this concept when he formulated the dose-response Proportionality Rule to describe a linear dose response in 1930. This assessment led to a detailed re-analysis of the Muller and Mott-Smith (1930) paper and other relevant literature. During this process, questions emerged over the widely cited statements of Muller that his Nobel Prize research used a treatment exposure rate about 200,000-fold greater than background. The 200,000-fold value had often been discussed within a framework of what this meant for extrapolation to low dose. However, the present paper demonstrates that Muller actually used a treatment exposure rate of at least 95 million-fold, rather than 200,000 fold, greater than background, resulting in a 475-fold discrepancy between actual and reported values. The present paper, therefore, re-examines how Muller and Mott-Smith (1930) calculated the treatment exposure rate relative to background and then explores its possible implications for the adoption of the LNT concept.

2. Muller's dose calculation

The conclusion of Muller and Mott-Smith (1930) that background radiation could not be the mechanism of evolution was derived by comparing the radiation exposure in the control and treatment groups, along with their respective mutation rates. An analysis of their approach raises concerns about their procedures and conclusions, as follows. Muller and Mott-Smith (1930) employed a continuous 42-min X-ray exposure at 81.4 r (roentgen) per minute (or 1.36 r/second), yielding a total dose of approximately 42 min x 81.4 r/min = 3420 r during this exposure/treatment period. They converted the dose into the number of ion pairs produced per cm^3 of air, with 1 r corresponding to an electrostatic unit charge in air of 2.1×10^9 ion pairs per cm^3. This value was multiplied by 3420 r to yield 7.12×10^{12} ion pairs per cm^3 for the total ion pairs generated in each cm^3 of air by the 3420 r X-ray treatment over the 42-min period.

The total ion-pair estimate of 7.12×10^{12} was supported by an alternative derived value, constructed in the same paper (Muller and Mott-Smith, 1930). This second ion-pair estimate was based on the number of photoelectrons produced in air per cm^3/sec via Muller's (1927) specific experimental X-ray generating procedure taking into account X-ray intensity, distance from flies, screening method, voltage, current and absorption coefficient of the air. It yielded 8.2×10^{12} ion pairs per cm^3 of air, in reasonably close agreement for the two

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estimation procedures. Muller and Mott-Smith (1930) then used the 7.12×10^{12} ion pairs per cm^3 of air in later comparisons.

Muller and Mott-Smith (1930) then stated the number of ion pairs/cm^3/second in the control group air was "very unlikely to be greater than 30 ion pairs/cm^3/second." While Muller and Mott-Smith (1930) failed to provide a reference to support this statement, similar estimates have been reported by others (e.g. Timofeeff-Ressovsky, 1931; Giles, 1940 - 50 ion pairs per cm^3/sec; Blackett, 1935 - between 20 and 30 ion pairs/cm^3/sec).

Muller and Mott-Smith (1930) then used the 30 ion pairs/cm^3/sec value for the background rate and summated it over a 14-day fruit fly reproductive period (i.e., from germ cell of parent to germ cell of offspring). Since there are 1.2×10^6 s in 14 days, this value times 30 ion pairs/cm^3/sec yields 3.6×10^7 ion pairs per cm^3 of air for total background ion pairs produced over a 14-day period.

Muller and Mott-Smith (1930) then compared the mutation rate in the control using the summated 14-day ion pairs background value 3.6×10^7 with the 42-min treatment group exposure 7.12×10^{12}. (They neglected to consider that the treated flies would also receive the 14-day background exposure during the time of treatment and in the remaining 13 days, 23 h and 18 min of the 14-day period). Employing this numerical comparison Muller and Mott-Smith (1930) reported that the treated flies received 200,000-fold ($7.12 \times 10^{12}/3.6 \times 10^7$) greater exposure over the 14-day period than the control group.

Muller and Mott-Smith (1930) then noted that the control mutation rate was about 1/150 of the treated group where the exposure was claimed to be 200,000-fold higher. They believed that the control group mutation rate was therefore far greater than would have been expected by the background radiation alone using the linear dose response model. They determined that background radiation could only account for 1/1300 of the mutations observed in the controls. This was determined by dividing the 200,000 by 150 = 1300. Thus, Muller and Mott-Smith (1930) rejected the Olson and Lewis (1928) proposition.

3. Concerns with Muller – Mott-Smith (1930) approach

In Muller's X-ray treatment of the fruit flies, the dose rate was 81.4 r/min or 1.36 r/sec. Since 1 r/sec produces 2.1×10^9 ion pairs/cm^3/sec, the background radiation level that produces 30 ion pairs/cm^3/sec is $30 \div (2.1 \times 10^9) = 14.3 \times 10^{-9}$ r/sec. Therefore, the ratio of the treatment dose rate to the background value is $1.36 \div (14.3 \times 10^{-9}) = 95 \times 10^6$. This indicates that the total exposure used by Muller **during the exposure period of 42 min** (bolded for emphasis) exceeded that of the controls by 95 million-fold.

Note that the Muller and Mott-Smith (1930) paper used 30 ion pairs/cm^3/second in the denominator when calculating X-ray exposure relative to background, and this value was adopted in the present paper to be consistent with their calculations. However, Muller and Mott-Smith (1930) stated that this value was very likely to be an overestimate. If a value of 15–20 ion pairs/cm^3/second (as generally consistent with Blackett, 1935) had been used, a relative increase over background would have been proportionately greater than the 95 million-fold value reported here. Furthermore, the use of such a lowered background estimate would have yielded an annual background exposure in the 225–300 mrem range per year.

Muller and Mott-Smith (1930) used a figure of 200,000 fold as they calculated the total number on ion pairs produced over 14 days for the control group and compared this to the treatment group value of only 42 min. They failed to consider that background exposures would have also occurred for the treatment group during the entire 14-day period. They also only considered total dose and not dose rate which profoundly differed during the treatment period between the control and treatment groups.

In retrospect, the control group displayed a mutation rate about 1/150 of the treatment group but had an exposure that was 95 million fold lower than the treatment group during the treatment time period.

This re-analysis of the Muller and Mott-Smith (1930) procedure argues that their approach was inappropriate, failing to 1) compare exposure differences between the control and treatment groups during the exposure time (i.e., 42 min); 2) failing to account for differences in dose rates between background and treatment; and 3) failing to acknowledge that the treatment group also received the identical background exposure of the control during the 42 min treatment period [i.e., control 75,600 ion pairs (30 ion pairs/sec x 60 s/min x 42 min)] and over the remaining reproductive period. As a result, the conclusions of Muller and Mott-Smith (1930) need to be reconsidered.

First, the major conclusion of Muller and Mott-Smith (1930) that background radiation is not likely to be the mechanism of evolution would in fact become even more strongly supported since other unknown factors at that time would appear to be far more dominant in producing mutations in mature spermatozoa than background radiation. These "unknown factors" would later become explained by endogenous reactive oxygen species (Harman, 1962; Pollycove and Feinendegen, 2003).

Second, Muller and Mott-Smith (1930) never considered how comparable the germ cell mutations from low dose background radiation and other factors may be. They also made the assumption that the nature of the genetic change at background was similar to that induced at the massively higher dose and dose rate, an assumption now known to be incorrect.

Third, there is no basis for ignoring the background exposure in the treatment group. By doing so, this inappropriately and significantly reduced the difference in summated ion pair exposure between control and treatment groups, when they should have been cancelled out.

Fourth, in 1930 Muller believed that total dose, rather than dose rate, was the only consideration for deriving mutation damage. However, the massively larger doses could induce mutations via multiple and alterative mechanisms including profound chromosome damage responses, affecting bursts of transposable elements (Ratner et al., 2001), massive inflammatory responses (Colotta et al., 2009) amongst other possible factors. Thus, the naïve approach for using a simple proportionality rule (as Muller proposed in 1930 as well) was not an accurate guide to estimate damage from background risks of 30 ion pairs per cm^3/sec versus a total dose some 95 million fold greater.

Fifth, the data of Muller and Mott-Smith (1930) and the present analysis indicate that the background dose of radiation is not likely to have any demonstrable genetic impact in this biological model. Instead of accounting for about 1 in 1300 of the mutations as Muller and Mott-Smith (1930) suggested, this would be reduced to about 1 in 633,333 (i.e., 1 in 95,000,000 divided by 150). If these quantitative findings with the fruit fly have the capacity for generalization they may be useful in reassessing estimates of background radiation mutation in ecological and human risk assessment.

Sixth, while Muller and Mott-Smith (1930) assumed a linear dose response to assess background radiation effects on mutation, Giles (1940) failed to observe chromosome aberrations in *Tradescantia* when the dose (i.e., radium capsule-gamma source) exceeded background by 1000-fold for 24 h. In this experiment, there were nearly 1600 control and 1344 treatment group chromosomes assessed; the mutation incidence was less in the radium treated tissue than controls by 26%.

As a final reflection on this now nearly 90-year-old paper, it had a major and correct impact on blunting the hypothesis of Olson and Lewis (1928). However, it is not known how the field may have responded to the findings of Muller and Mott-Smith (1930) had they acknowledged that the exposure for Muller's Nobel Prize research was some 95 million fold greater than background. A recognition of this extremely high dose rate may have introduced more caution in the estimation of responses at very low doses, something that Muller's Proportional Rule and its LNT-single hit extension a few years later failed to do (Calabrese, 2015, 2019).

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References

Blackett, P.M.S., 1935. La Radiation Cosmique. Hermann, Paris (cited in Giles (1940).
Calabrese, E.J., 2015. On the origins of the linear no-threshold (LNT) dogma by means of untruths, artful dodges and blind faith. Environ. Res. 142, 432–442.
Calabrese, E.J., 2019. The linear no-threshold (LNT) dose response model: A comprehensive assessment of its historical and scientific foundations. Chem. Biol. Inter. 301, 6–25.
Colotta, F., Allavena, P., Sica, A., Garlanda, C., Mantovani, A., 2009. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 30 (7), 1073–1081.
Giles, N., 1940. Spontaneous chromosome aberrations in Tradescantia. Genetics 25, 69–87.
Harman, D., 1962. Role of free radicals in mutation, cancer, aging, and the maintenance of life. Radiat. Res. 16, 753–763.
Muller, H.J., 1927. Artificial transmutation of the gene. Science 66 (1699), 84–87.
Muller, H.J., Mott-Smith, L.M., 1930. Evidence that natural radioactivity is inadequate to explain the frequency of “natural” mutations. Proc. Natl. Acad. Sci. Unit. States Am. 16, 277–285.
Olson, A.R., Lewis, G.N., 1928. Natural radioactivity and origin of species. Nature 121, 673–674.
Pollycove, M., Feinendegen, L.E., 2003. Radiation-induced versus endogenous DNA damage: possible effect of inducible protective responses in mitigating endogenous damage. Hum. Exp. Toxicol. 22, 290–306.
Ratner, V.A., Bubenshchikova, E.V., Vasilyeva, L.A., 2001. Prolongation of MGE *412* transposition induction after γ-irradiation in an isogenic line of *Drosophila melanogaster*. Genetics 37 (4), 384–391.
Timofeeff-Ressovsky, N.W., 1931. Die bisherigen Ergebnisse der Strahlengenetik, vol. 5. Erg Med Strahlenforschung, Leipzig, pp. 129–228.