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# IN VIVO MUTANT T CELL FREQUENCY WITHIN ASSIGNED DOSE GROUPS IN ATOMIC BOMB SURVIVORS CARRYING EXTREME DOSE-SPECIFIC VALUES OF CHROMOSOME ABERRATION FREQUENCY

## 各特定線量群中で例外的な染色体異常頻度を示す原爆被爆者における生体内突然変異T細胞の頻度の線量群別解析

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# IN VIVO MUTANT T CELL FREQUENCY WITHIN ASSIGNED DOSE GROUPS IN ATOMIC BOMB SURVIVORS CARRYING EXTREME DOSE-SPECIFIC VALUES OF CHROMOSOME ABERRATION FREQUENCY

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## SUMMARY

The objective was to investigate whether there is a positive association between frequencies of HPRT$^-$ T cell mutations and chromosome aberrations among atomic bomb survivors at a given dose. This would provide evidence regarding whether individuals vary in their sensitivity to radiation. The approach was to compare T cell mutant frequencies (Mf) among survivors who have unusually high- and low-chromosome aberration frequencies, in relation to their radiation dose. Within each of four dose groups (1-99, 100-199, 200-299, and 300+ rad) equal numbers of survivors were selected who have unusually high and unusually low chromosome aberration frequencies for the dose group. The total numbers selected from each of the dose groups were 8, 8, 8, and 16, respectively. Within each dose group, the mean doses for the high- and low-aberration subjects were very similar. The difference between T cell Mf of the high- and low-chromosome aberration groups was tested by a t-test on the logarithms of the frequencies, stratifying on dose groups. The Mf of the high-aberration group was significantly higher than that of the low-aberration group (p=0.01). However, at least part of this positive association may be explained by imprecision in the dose estimates. This is because among survivors at the same estimated dose it can be expected that those with high-aberration frequencies may have higher true doses than those

## 要 約

本研究の目的は，特定の線量を受けた原爆被爆者における HPRT$^-$ T細胞突然変異と染色体異常の間に正の相関関係があるかどうかを調べることであった．これにより，放射線感受性に個人差があるか否かを知ることができるであろうと考えた．方法としては，染色体異常率が異常に高いか又は低い被爆者におけるT細胞突然変異体頻度（Mf）を，放射線量との関連で比較した．四つの線量群（1-99，100-199，200-299，300＋ rad）別に，染色体異常頻度が異常に高い被爆者及び異常に低い被爆者を同数ずつ選択した．各線量群に選択された総数はそれぞれ８名，８名，８名及び16名であった．各線量群内における染色体異常頻度の高い者と低い者の平均線量は極めて類似していた．染色体異常高頻度群と低頻度群との間のT細胞 Mf の差に関しては，線量別に層化した頻度の対数に対してｔ検定を行った．高異常頻度群の Mf は低異常頻度群の Mf より有意に高かった（p＝0.01）．しかし，この正の相関関係の少なくとも一部は線量推定値の不正確性により説明できる．すなわち，推定線量が同じでも，異常頻度が高い者の

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with low-aberration frequencies. Presently not enough is known about how to determine whether it could entirely explain the positive association seen between frequencies of mutation and chromosome aberration among those at similar estimated doses.

## INTRODUCTION

The most prominent late health effect of A-bomb radiation has been shown to be an increase in cancer mortality.[1] Persistent genetic damage caused by A-bomb radiation on the somatic cells has also been demonstrated by the elevated frequency of lymphocytes carrying chromosome aberrations in the survivors.[2] In order to detect injuries of somatic cell genes at the level of specific loci, Langlois et al[3] measured the frequency of variant erythrocytes lacking the expression of glycophorin A and reported a similar increase of such variants in the survivors. For the same purpose we have applied the measurement of the frequency of hypoxanthine phosphoribosyltransferase-deficient ($HPRT^-$) mutant T cells because the mutant nature can be further characterized in this method by propagating the cells in vitro. In the previous report,[4] we showed that the frequency of mutant T cells increased as the radiation dose or the frequency of lymphocytes with chromosome aberrations increased. Furthermore, a slightly higher correlation coefficient was observed between Mf and chromosome aberration frequency than that between Mf and radiation dose.

Although there exists a clear dose response, a considerable scatter in aberration frequencies for individual donors was observed.[5] The reason for this scatter remains to be resolved. The present study was designed to further investigate the relationship between Mf and aberration frequency by measuring Mf of the survivors who showed outlying values of aberration frequency. The results will be mainly discussed in relation to the variability of radiation sensitivity among individuals which has been suggested by several in vitro experiments (reviewed by Setlow[6]).

## MATERIALS AND METHODS

Sampling sources were A-bomb survivors who were participants in the RERF Adult Health Study at Hiroshima, for whom tentative dose estimates (T65D) were available, and for whom chromosome aberration frequencies in peripheral blood lymphocytes had been previously measured. As shown in Figure 1, the exposed subjects (exposed to 1 rad or more) in this study were selected from those

方が低い者より真の線量が高いと予想されるからである．推定線量が同程度の被爆者の Mf と染色体異常との間に見られる正の相関関係が，線量推定値の不正確性により完全に説明できるかどうかは現在のところ情報が十分でない．

## 緒　言

原爆放射線が健康に及ぼす最も顕著な後影響は癌死亡率の増加であることが示されている．[1] また，原爆放射線によって体細胞に持続性の遺伝的影響が生ずることは，被爆者において染色体異常を有するリンパ球の頻度の増加が見られることによっても証明されている．[2] Langlois ら[3] は，特定の遺伝子座レベルで体細胞障害を検出するため，グリコフォリンAの発現を欠失した変異赤血球の頻度を測定し，この種の変異型も被爆者において同様に増加していることを報告した．同じ目的で，我々は hypoxanthine phosphoribosyltranferase 欠損（$HPRT^-$）突然変異T細胞の頻度を測定した．この方法を用いてT細胞を試験管内培養することにより，突然変異の性質をより詳細に調べることができるからである．前報[4] においては，放射線量の増加又は染色体異常を有するリンパ球の頻度の増加に伴い突然変異T細胞頻度が増加することを示した．また，Mf と染色体異常頻度との間の相関係数は，Mf と放射線量の場合より若干高いことを認めた．

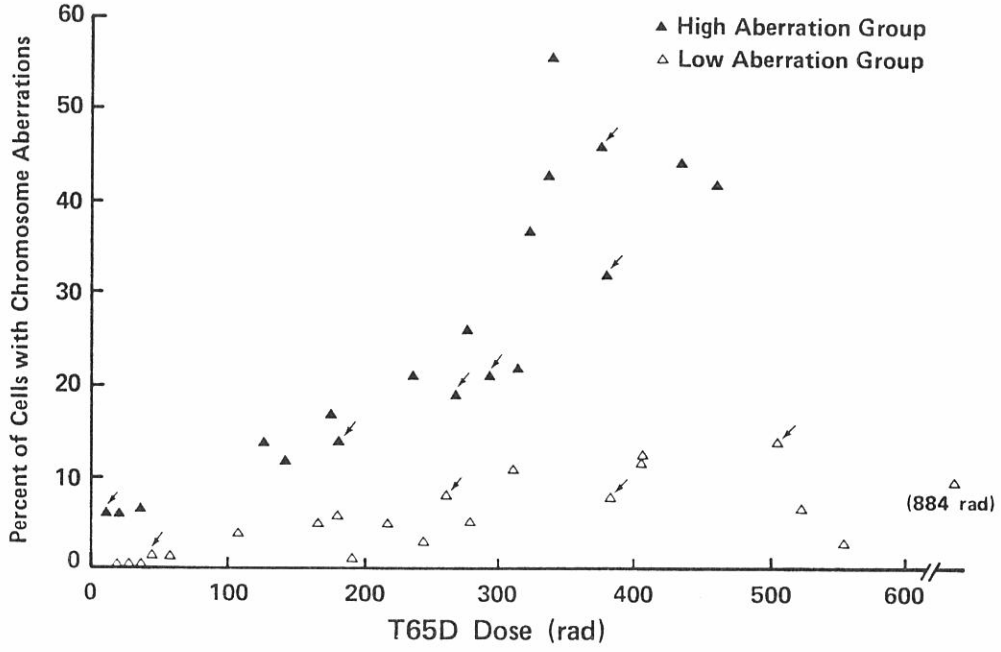
明瞭な線量反応関係が存在するものの，各血液供与者における異常頻度の間に大きなばらつきが認められた．[5] このばらつきの原因は不明である．本研究は，極端に偏った染色体異常頻度を示す被爆者の Mf を測定し，Mf と異常頻度との間の関係を更に詳細に調べるため企画されたものである．得られた結果については，幾つかの試験管内実験により示唆された（Setlow[6] の検討による）放射線感受性の個人差との関連で主として考察する．

## 材料及び方法

広島放影研の成人健康調査の対象である原爆被爆者中，暫定推定線量（T65D）が判明しており，しかも末梢血リンパ球の染色体異常頻度が以前に測定されていた者から今回の調査対象者を抽出した．図1に示すように，染色体異常頻度と T65D 被曝線量との

FIGURE 1 FREQUENCY OF LYMPHOCYTES WITH CHROMOSOME ABERRATIONS AND T65D DOSES AMONG 40 EXPOSED SURVIVORS WHO WERE SELECTED FOR THIS STUDY

図1 本研究に選択した被爆者40名における染色体異常リンパ球頻度及びT65D線量




Small arrows indicate the individuals who were not included in the data analysis (seven individuals declined to come to RERF and the other three individuals showed low CEs).

矢印はデータ解析に含めなかった者を示す(7名は非受診, 3名は低いCEを示した).

whose chromosome aberration frequency deviated from the expected value calculated from the linear fit between aberration frequency and T65D exposure dose. One set of the subjects (high-aberration group) was selected from those whose aberration frequency was near the higher end of the distribution and the other set (low-aberration group) from those whose aberration frequency was near the lower end of the distribution.

This sample selection was based on four dose groups: 1-99, 100-199, 200-299, and 300+ rad. Usually four cases of the high or low-aberration group were selected from each dose group except for the 300+ rad group, from which eight cases of each group were selected. Thus, in total, 40 exposed cases were selected. The controls of this study were the same as those that were used in the previous study,[4] which were randomly selected from those who were exposed distally to the bomb and whose T65D estimates were less than 1 rad.

間の関係が直線的であると仮定して計算した期待値から逸脱した頻度を示す被爆者(1 rad 以上)を選択した. 調査群の一つ(高異常頻度群)は, 異常頻度分布の上限に近い者から選び, 他の群(低異常頻度群)は異常頻度分布の下限に近い者から選択した.

標本抽出は次の四つの線量群別に行った. すなわち, 1-99, 100-199, 200-299, 及び300+ radである. 各線量群から高異常頻度及び低異常頻度の者をそれぞれ4名ずつ選択した. ただし, 300+ rad 群からは, それぞれ8名選んだ. このようにして被爆者を合計40名選択した. 本研究の対照者は, 前回の研究[4]で用いたものと同一であり, 遠距離被爆者でT65D推定値が1 rad 未満の者から無作為に選択した.

The method for measuring the mutant T cell frequency has been reported previously.[7] Briefly, an average of 1 cell or $10^5$ fresh lymphocytes per well were inoculated with feeder cells into the wells without or with 2.5 $\mu$g/ml thioguanine (TG), respectively. The cells were cultured with the medium containing phytohemagglutinin (PHA) and interleukin 2 (IL2). After 15 days, the presence or absence of the lymphocyte colonies was determined by observing each well by an inverted microscope and by measuring [$^3$H]-thymidine incorporation. Cloning efficiency (CE) was calculated from the proportion of colony-negative wells, assuming a Poisson distribution of the cells with the ability to form colonies. Mf was obtained by dividing the CE of TG-selected cells by the CE of nonselected cells.

## RESULTS

Of the 40 exposed survivors selected, peripheral blood was obtained from 33 individuals. Seven people declined to come to RERF. Results from three cases whose CE of nonselected cells was less than 0.25 were not included for data analysis to avoid possible overestimation of Mf, a procedure also followed in the previous report.[4] These 10 persons who were not included in the present study are indicated in Figure 1. The mean radiation doses (T65D) for the persons analyzed in the high- and low-aberration groups were 248 and 273 rad, respectively. The significance test to compare the Mf between high- and low-aberration groups was made in essence by a t-test stratifying on dose groups. Figure 2 gives a display of the data which corresponds closely to the nature of the test. The analysis was done using the logarithm of Mf, since the variation within each of the nine groups of Figure 2 (two groups in each dose category and the control group) is much more homogenous on the log scale. Formally the test was done as a two-way analysis of variance, with factors dose group and aberrtion group, except that the variance within the control group was also included in the estimate of individual variation. The aim of the test is to determine whether the consistently higher logarithmic Mf means seen within each dose group constitute a statistically significant result when evidence is combined over the dose groups. In this sense there is a significantly higher Mf in the high-aberration group, compared to the low-aberration group (p=0.01, two-sided test, t=2.68 on 41 df). (The significance is more extreme if analysis is done without using logarithms, but this is less

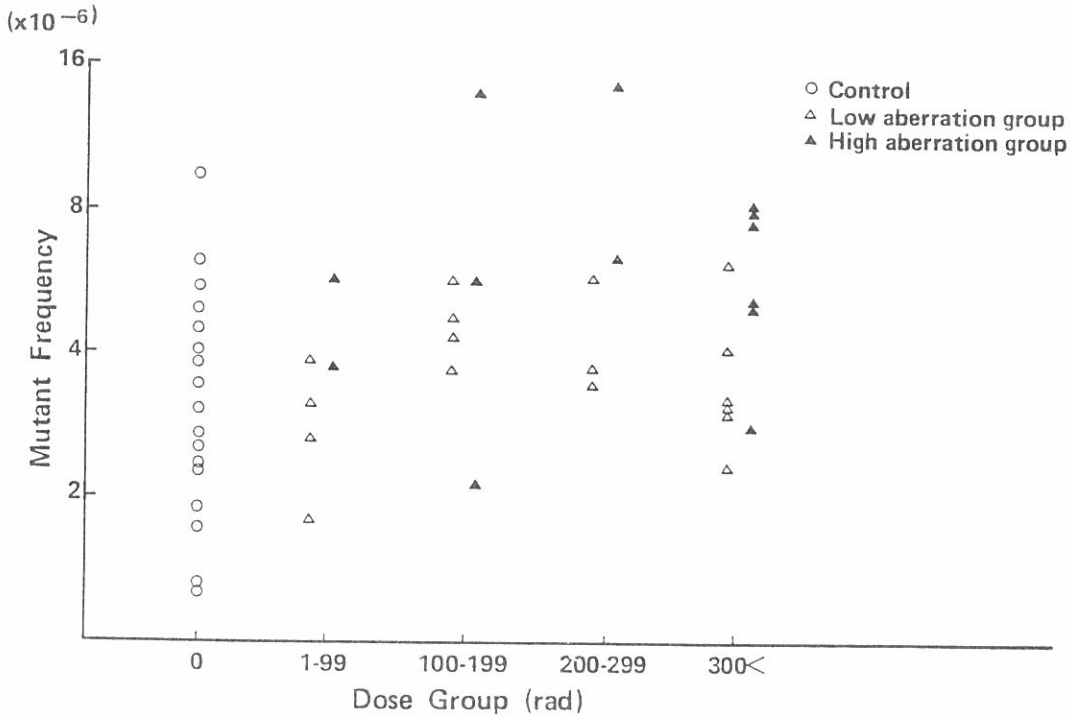
突然変異T細胞頻度の測定方法については以前に報告した.[7] 簡単に述べると次のとおりである. 1穴当たり平均1～$10^5$個の新鮮リンパ球とフィーダー細胞を入れ, 2.5 $\mu$g/ml のチオグアニン(TG)を加えないか又は加えて, phytohemagglutinin(PHA)及びインターロイキン2(IL2)を含む培地で培養した. 培養15日後, 倒立顕微鏡を用いて各穴を観察し, 〔$^3$H〕チミジンの取り込みを測定してリンパ球コロニーの有無を調べた. コロニー形成能を有する細胞はPoisson分布を示すものと仮定し, コロニー無形成の穴の割合からクローニング効率(CE)を計算した. TG選択細胞のCEを非選択細胞のCEで割り, Mfを求めた.

## 結　果

選択した被爆者40名のうち, 33名から末梢血を採取した. 7名は非受診者であった. Mfの過大推定を避けるため, 非選択細胞のCEが0.25未満であった3例はデータ解析から除外した. これは, 前回の報告書[4]でも採用した方法である. 本研究に含めなかったこれら10名は図1に示した. 解析を行った対象者の平均放射線量(T65D)は高異常頻度群で248 rad, 低異常頻度群で273 radであった. 高異常頻度群と低異常頻度群のMfを比較するための有意差検定は, 基本的には線量群別に層化したt検定によって行った. 図2のデータは, 実施した検定の特徴を反映したものである. 図2の9群(各線量区分で2群ずつと対照群)における群内変動は対数を用いるとより均一になるので, Mfの対数を用いて解析を行った. 正式には検定は, 線量群と異常群を要素として用いた分散の両側検定を行ったが, それぞれの変動の推定には対照群内の分散も含めた. 検定の目的は, 各線量群に一貫して見られる高い対数Mf平均値が, 全線量群を合計した場合に, 統計学的に有意な結果となるかどうかを決定することである. この意味で, 低異常頻度群と比較して, 高異常頻度群に有意に高いMfが認められる(p=0.01, 両側検定, df 41のときt=2.68). (対数を使用しないで解析を行うと有意性は更に顕著になるが, これは適当ではない.)染色体

FIGURE 2 MUTANT FREQUENCY OF CONTROLS (EXPOSED TO LESS THAN 1 rad) AND TWO GROUPS OF EXPOSED SURVIVORS (EXPOSED TO 1 rad OR MORE)

図2 対照者(被曝線量1 rad 未満)及び二つの被爆群(被曝線量1 rad 以上)の突然変異体頻度




The logarithmic values of Mf are plotted based on the four dose groups. Mf of the high-aberration group was significantly higher than that of the low-aberration group (p=0.014), which was tested by a stratified t-test. Mf of the exposed survivors was significantly higher than that of controls. (p=0.0078).

四つの線量群別にMfの対数値をプロットした．高異常頻度群のMfは低異常頻度群より有意に高かった(p=0.014)が，これは層化t検定により調べたものである．被爆者のMfは対照者のMfより有意に高かった(p=0.0078)．

appropriate.) The antilogarithm of the parameter estimate for the comparison of chromosome aberration groups indicates that the Mf for the high-aberration group is 66% higher than for the low-aberration group.

異常群間の比較のためにパラメーター推定値の真数をみると，高異常頻度群のMfは低異常頻度群のMfより66%高い．

The extent of the effect is greater than might be inferred from inspection of Figure 2, because of the logarithmic scale there. Similarly, the Mf dose response is greater than might be inferred by inspection of Figure 2. The Mf of the entire exposed group is very much significantly higher than that of the control group (p=0.0078). The control group, on which chromosome aberration data were not available, plays no major role in the investigation here, except for the combination of it with the other

図2では対数尺度を用いたため，その図から示唆されるよりは影響は大きい．同様に，Mfの線量反応も図2が示唆するものより大きい．被爆群全体のMfは対照群のMfより極めて有意に高い(p=0.0078)．対照群は染色体異常のデータを示さず，今回の解析でlog(Mf)における実験的誤差の推定のため他のデータを組み合わせて用いた以外に主要な役割を

data for estimation of experimental error in log(Mf). The mean CE of nonselected cells in the controls, low-aberration group, and high-aberration group was 0.43, 0.49, and 0.43, respectively, indicating that the significant difference between the mean Mfs was not attributable to the lower CE in the high-aberration group.

## DISCUSSION

A significantly higher HPRT mutant T cell frequency has been observed in the high-aberration group than in the low-aberration group, adjusted for estimated dose. Note that the estimated radiation doses of the two groups were similar. The relationship between radiation-induced chromosome aberrations and gene mutations has been investigated in several laboratories by in vitro radiation experiments. Evans and Vijayalaxmi[8] showed similar dose-response curves for the production of autoradiographically detected azaguanine-resistant lymphocytes and the chromosome aberrations after X-irradiation of peripheral blood lymphocytes. The relationship was further investigated by the karyotypic analysis of the radiation-induced HPRT$^-$ mutants. Thacker and Cox[9] reported that 5%-10% of HPRT$^-$ mutant diploid fibroblasts induced by X-irradiation carried visible abnormalities of the X chromosome. Vrieling et al[10] studied the X ray-induced HPRT$^-$ CHO cells and showed that 3 of 19 mutants carried visible deletions of the long arm of the X chromosome although they found that many (12 of 19) mutants possessed large deletions of the HPRT gene. More recently, Muir et al[11] analyzed 17 X ray-induced mutant human T cell clones and found that only 1 clone carried an X chromosome abnormality. These in vitro results indicate that the HPRT$^-$ mutants arise by HPRT gene mutations rather than by gross changes of chromosome structure in X-irradiated samples. This is also supported by an in vivo study. We have analyzed 49 mutant T cell colonies obtained from eight proximally exposed A-bomb survivors; structural abnormalities of the long arm of X chromosome were found in only 2 colonies. On the other hand, approximately 50% of the colonies showed abnormality of the autosomes.[12]

Based on the above argument, the results obtained here may suggest that radiation sensitivity for the induction of chromosome aberrations and gene mutations may vary among individuals. However, it should be noted that at least part of this effect

果たしていない．対照群，低異常頻度群及び高異常頻度群における非選択細胞の平均CEはそれぞれ0.43，0.49及び0.43であり，平均Mf間の有意差が高異常頻度群における低いCEによるものではないことを示す．

## 考　察

推定線量を補正すると低異常頻度群に比べて高異常頻度群に有意に高いHPRT突然変異T細胞頻度が認められた．両群の推定放射線量が類似していることに注目すべきである．放射線誘発染色体異常と遺伝子突然変異との関係については，幾つかの研究所で，試験管内放射線実験が行われている．Evans及びVijayalaxmi[8]は，末梢血リンパ球のX線照射後に，オートラジオグラフィーで検出されたアザグアニン耐性リンパ球と染色体異常が，同様の線量反応曲線を示すことを報告した．この関係は，放射線誘発HPRT$^-$突然変異の核型分析によって更に研究されている．Thacker及びCox[9]は，X線により誘発されたHPRT$^-$突然変異二倍体線維芽細胞の5％～10％が明瞭なX染色体異常を有すると報告した．Vrielingら[10]はX線誘発HPRT$^-$ CHO細胞を調べ，突然変異細胞19個中3個が明瞭なX染色体長腕欠損を有することを示したが，突然変異細胞の多く（19個中12個）は，HPRT遺伝子に大きな欠損箇所があることを発見した．Muirら[11]は最近の研究で17個のX線誘発突然変異ヒトT細胞クローンを分析し，その中の一つのみにX染色体異常を認めた．これらの試験管内研究結果は，X線照射標本におけるHPRT$^-$突然変異が，染色体構造の肉眼的な変化のためではなく，むしろ，HPRT遺伝子の突然変異によって生ずることを示唆するものである．これは生体内研究によっても支持されている．我々は近距離原爆被爆者8名から得た49個の突然変異T細胞コロニーを解析した．その中の二つのコロニーにおいてのみX染色体長腕の構造異常が観察されたが，全コロニーの約50％に常染色体異常が認められた．[12]

上述の考察に基づくと，今回得られた結果は，染色体異常及び遺伝子突然変異の誘発における放射線感受性に個人差があることを示唆するものかもしれない．しかし，このような所見の少なくとも一部は

will be due to imprecision of estimated doses. That is, among survivors at the same estimated dose there is a range of true doses, and the separation of these survivors according to unusually high and low chromosome aberration rates will to some extent imply higher true doses for the former group in comparison to the latter. Another way of saying this is that the dosimetry estimate of dose combined with the chromosome aberration finding should provide a somewhat better estimate of an individual's true dose than that based on the dosimetry system alone. It is difficult to estimate how much of the apparent effect seen in Figure 2 may be due to this difficulty.

推定線量の不正確さに起因することに注意すべきである．すなわち，同じ推定線量を付与されている被爆者でも，その真の線量に一定の範囲があり，染色体異常が異常に高率である被爆者及び異常に低率である被爆者に分類できることから，前者の真の線量は後者に比べて高いであろうと推測される．換言すれば，線量計算方式のみに基づいて線量を求めるよりも，線量計算値と染色体異常所見を組み合わせることによって個人の真の線量について幾分かより正確な推定値が得られると考えられる．図2に見られる見かけ上の影響が，どの程度にこの問題のために生じたかを推定することは困難である．

Radiation sensitivity among normal individuals has been investigated by the colony-forming assay using fibroblasts[13-15] and T lymphocytes,[16-18] in which the sensitivity to the killing effect of ionizing radiations is tested. The sensitivity has been usually represented as a $D_0$ value, the dose necessary to reduce the survival rate to 0.37. Wide range of the distribution of the normal sensitivity among tested individuals has been reported depending on the study. Fibroblasts and T lymphocytes showed a similar range of the distribution. However, these results using cultured cells cannot simply be interpreted by a variability of the individual radiation sensitivity. Whether different kinds of cells from the same person show a similar sensitivity or not has not been tested. Even fibroblasts obtained by repeated biopsies from the same individual showed some differences, although the differences gave a somewhat narrower distribution than that for the cell strains obtained from different individuals. If such reactions of cell strains to the killing effects of ionizing radiations represent the radiation sensitivity of individuals and the variability of the sensitivity exists, it has been suggested that A-bomb radiation could have eliminated those persons who are unusually sensitive to radiation.[19,20] However, the radiosensitivity of cells from the control population was not different from that of the exposed survivor population when skin fibroblasts were studied.[21]

健常人の放射線感受性については，線維芽細胞[13-15]及びTリンパ球[16-18]を用いて，コロニー形成法により，電離放射線の致死効果に対する感受性が測定されている．感受性は，通常生存率を0.37に減少させるのに必要な線量 $D_0$ 値で表される．それぞれの研究の間で正常な感受性に広範な個人差が見られた．線維芽細胞とTリンパ球における個人差は同じような分布を示した．しかし，培養細胞を用いて得られたこれらの結果は，放射線感受性の個人差のためであると単純に解釈することはできない．同一の被検者から得た各種の細胞が同様の感受性を示すか否かが調べられたことはない．同一の被検者から反復生検によって得られた線維芽細胞さえも若干の差異を示すと認められているが，その差異の分布範囲は異なる被検者から得た細胞株の場合に比べてやや狭い．電離放射線の致死効果に対する細胞株のこのような反応が個人の放射線感受性を反映するとすれば，また，感受性の差が存在するとすれば，原爆放射線被曝によって放射線感受性が異常に高い者が死亡した可能性があるとの推論がある．[19,20] しかし，皮膚線維芽細胞の研究[21]では，対照集団と被爆集団との間に細胞の感受性に差異は認められていない．

Wide distribution of the induction of mutations at the HPRT locus in T lymphocytes by $\gamma$ rays has also been presented by Sanderson et al.[22] Because only the dose-response curve showing the mean ±SE at several doses of 11 healthy individuals has been provided, precise estimation of the distribution is not possible. It will need an extensive study including repeated experiments using lymphocytes from the

また，$\gamma$線によるTリンパ球のHPRT遺伝子座突然変異の誘発が広範な分布を示すことがSandersonら[22]により報告された．11名の健常人について幾つかの線量での線量反応曲線が平均値±SEによって示されているにすぎないので，分布の正確な推定は不可能である．健常人における特定遺伝子座の突然変異誘発に，放射線感受性の差が存在するとの結論

same individuals and experiments comparing the results of different kinds of cells to conclude the existence of the variability of radiation sensitivity for the induction of specific locus mutations among normal individuals.

On the other hand, it has been reported that the variability of the induction of chromosome aberrations in T lymphocytes by ionizing radiations is small among individuals, although differences of the induction rate at the same doses between laboratories have existed.[23] Because a control study examining X-irradiated cells provided by one experiment in different laboratories showed very small variances between laboratories,[24] variability among normal individuals of the induction rate of chromosome aberrations by ionizing radiations must be small.

Some resolution might be provided for explaining the tendency observed in this study if a direct measurement of the radiation dose of each individual is possible. Electron spin resonance (ESR) spectroscopy can measure the long-lived $CO_3^{3-}$ radicals produced by radiation in carbonate-containing materials. ESR signals have been shown by in vitro experiments[25,26] to increase linearly as the radiation dose increases, and the fading of the signal with lapse of time after irradiation is negligible. Measurements of ESR signals of tooth enamel have been conducted on some survivors.[25] It has been shown that the radiation dose estimated by this method decreases with increasing distance from the bomb and correlates with T65D. However, in a few survivors, large differences between ESR dose and T65D have been observed and the collection of many more samples from the survivors is continuing.

Although the results obtained here have not been fully explained, the present study suggests the importance of measuring multiple biological endpoints in the same individual to evaluate the genetic effects of environmental mutagens on somatic cells. Other than the method used here, two additional valid methods have been reported for detecting somatic mutations in vivo; one detects the loss of MN antigens on erythrocytes[27] and the other detects the loss of HLA Class I antigens on T cells.[28] Spontaneous mutant frequencies measured by these methods are of the order of $10^{-5}-10^{-6}$, which are similar to the reported values for HPRT mutations. Besides increasing the number of available methods

のためには，同一被検者のリンパ球を用いる反復実験と，各種類の細胞について得た結果を比較する実験など，広範な研究が必要である．

他方，電離放射線によるTリンパ球染色体異常誘発頻度については，同一被曝線量においても研究所間で誘発頻度の差異が存在すると認められているものの，個人差は少ないと報告されている．[23] 一つの実験から得られたX線照射細胞を数か所の研究所で調べた対照研究では，成績における研究所間の分散は極めて小さかったので，[24] 電離放射線による染色体異常誘発率の健常人における変動は少ないものと思われる．

各対象者の放射線量が直接測定できれば，本研究で認められた傾向を説明する上で何らかの解決が得られるであろう．電子スピン共鳴（ESR）分光測定により，炭酸塩含有物質内に放射線により産生された長命の $CO_3^{3-}$ ラジカルを調べることができる．試験管内実験[25,26] によれば，ESR の信号は放射線量の増加と共に直線的に増加することが示され，しかも，放射線被曝後の時間の経過に伴う信号の消退はわずかである．歯のエナメル質の ESR 信号測定が一部の被爆者について行われた．[25] この方法によって推定された放射線量は爆心からの距離に伴い減少し，T65D と相関関係にあることが示された．しかし，少数の被爆者においては，ESR 線量と T65D との差が大きく，被爆者からのより多くの試料の収集が継続されている．

今回得られた結果は完全に説明されてはいないが，本研究により，環境内突然変異原が体細胞に及ぼす遺伝的影響を評価する上で，同一対象者における複数の生物学的指標を測定することの重要性が示唆された．今回用いた方法以外に，体細胞突然変異の生体内検出に適当なものとして二つの方法が報告されている．すなわち，その一つは赤血球における MN 抗原欠損を検出する方法[27] と，一方は，T細胞における HLA Class 1 抗原欠損を検出する方法[28] である．これらの方法により測定された自然突然変異頻度は $10^{-5}$～$10^{-6}$ の範囲であり，HPRT 突然変異について報告された値と類似している．生体内で生じた突然変異体頻度を測定できるもっと多くの

for measuring in vivo mutant frequencies, it will also be important to analyze the isolated mutants at the molecular level as has been reported for several mutagens in in vitro experiments.[10,29–31] Molecular analysis of HPRT⁻ T cells obtained from A-bomb survivors at the DNA level with Southern blotting techniques is being conducted in our laboratory.

方法を利用することのほかに，試験管内実験[10,29-31]で幾つかの突然変異原について報告されているように，得られた突然変異体の分子レベルでの解析も重要であろう．原爆被爆者から得たHPRT⁻T細胞のDNAレベルでの遺伝子解析が，Southern blotting法を用いて当研究室で進行中である．

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