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Biological dose estimation by means of radiation induced chromosome aberrations in human blood*

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末梢血の染色体異常による線量推定について

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末梢白血球培養法による染色体異常の頻度と線量との関係およびその照射後の時間的推移についてのデータを文献および著者等の研究室の研究より集め，照射後時間を経ている場合にさかのぼって線量推定が出来るかどうかを検討した．最も可能性のあるのは二動原体染色体の頻度であつて，これによって10年位前の線量の推定が出来る．しかしその頻度については未だ疑問がある．ことに照射後の頻度の変化についての生物学的な基礎づけの研究を更に行うことが極めて大切である．

I. Introduction

It has been well established that chromosome aberration in pheripheral blood of irradiated persons may persist for many years as revealed by Buckton et al. (1), Bender and Gooch (2, 3) and Doida et al. (4). Dose effect relations for some types of chromosome aberrations were established on cells irradiated in vitro by Bender and Gooch (5) and by Norman et al. (6). However, the porportion of persistent aberrations observed in man many years after irradiation did not seem to be compatible with the estimated dose as reviewed below. It is the purpose of this paper to explore the possibility to estimate the dose given many years ago by using data of chromosome aberrations in human blood so far published including some unpublished data in the authors' laboratory. The dose estimation, if available, would be of great value for establishing dose-effect relations for various delayed effects and determining the prognosis of patients.

II. Incidence of chromosome aberrations and radiation dose

Types and rates of X-ray-induced chromosome aberrations in human blood irradiated immediately

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after drawing, has been extensively studied by Bender and Gooch (5). Mitotic figures in the culture colchine-treated at 56hr and fixed at 72hr were analysed. In scoring the chromosome count, the presence of obvious aberrations, and abnormal form of the readily identified chromosomes were recorded. No significant increase in the frequency of chromatid type aberrations occurred in the X-ray-treated samples. Percent aneuploidy did not depend on X-ray dose up to 200R. But the curve drawn for total breakage is the least-squares fit of the data to the expression

$$Y = 0.0023 + 0.002D + 0.7 \times 10^{-5} D^2 \quad (1)$$

where Y is the yield of breaks, 0.0023 the control breakage frequency, D the dose in R of X-rays, 0.002 and 0.7×10^{-5} the coefficients of production for one- and two-break aberrations, respectively. The curve drawn for ring plus dicentrics is the least-squares fit of the data to the expression

$$Y = 0.52 \times 10^{-5} D^2 \quad (2)$$

and for dicentics

$$Y = 0.45 \times 10^{-5} D^2 \quad (3)$$

the classical expression of two-hit aberration yield where their spontaneous frequency is zero.

They suggested that the dose estimate might be calculated from the yield of rings and dicentrics using the expression

$$D = \sqrt{\frac{Y}{0.52 \times 10^{-5}}}$$

where D is the dose in roentgens and Y the observed yield of rings and dicentrics per cell.

A similar experiment but in wider range of radiation dose was carried out by Norman et al (6). The figure from combined data with various radiation and incubation conditions reveals that from a dose of 25 rads to 1,200 rads the yield of dicentrics (Y) is directly proportional to the square of the dose; i.e.

$$Y = (2.7 \pm 0.14) \times 10^{-6} D^2 \quad (5)$$

where D is the dose in rads. Two cases of *in vivo* radiation in which the blood was drawn immediately and 72 hr after irradiation of 300 rads showed the different incidences of dicentrics, but their geometrical mean falled almost exactly on the line representing the *in vitro* data.

Incidence of chromosome aberrations of blood from patients under daily radiotherapy was followed by Doida et al. (4). The data was re-examined as for the incidence of dicentrics and percent aneuploidy, since other types of aberrations appeared to be independent on the dose received. Radiations were given to the supraclavicular region of either side, γ-ray from ^{60}Co, 250R in air daily, 5 days per week, total dose 4500R. Blood samples were taken about 23hr after the exposure, two to three samples per person during the above treatment.

Since irradiated cells may be mixed with circulating blood, equivalent whole body dose D was calculated according to the following formulae:

$$D = d.n.\frac{v}{V} \quad \text{for effects in proportion to the dose} \quad (6)$$

$$D = d\sqrt{n}\sqrt{\frac{v}{V}} \quad \text{for effects in proportion to the square of the dose} \quad (7)$$

where d is the dose to the volume v, V the volume of whole body, and n the number of exposures repeated.

Incidences of dicentrics in the above three experiments are plotted againt dose in Fig. 1 neglecting the difference between rads and roentgens. Dose for radiotherapy patients is claculated according to

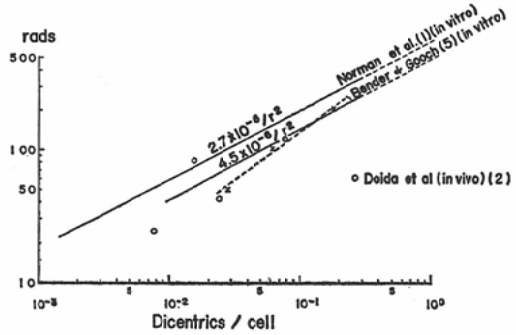

Fig. 1 Dose and dicentrics per cell

the formula (7) since the incidence was shown to be proportional to the square of the dose. The points are scattered but almost in the range between two lines represented by the expression (3) and (5).

In Fig. 2 percent aneuploidy of cells irradiated *in vivo* by Doida et al. (4) including some new data are plotted against doses calculated according to (6) and (7) respectively and the number of exposures and those irradiated *in vitro* against absorbed dose by Bender and Gooch[5]. Contrary to percent aneuploidy of cells irradiated *in vitro*, those *in vivo* showed marked increase at the low doses and an apparent plateau above the eighth or ninth exposure, i.e., about 10 days after the first exposure. This may indicate that cells with chromosome aberrations of this type have an average life span of less than 10 days.

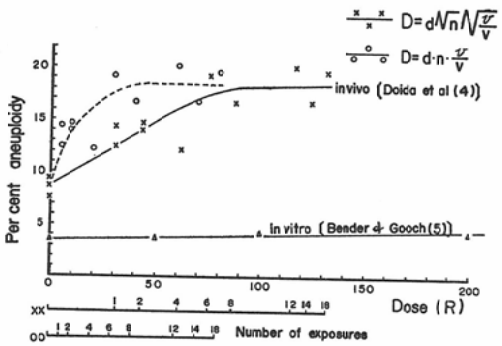

Fig. 2 Accumulated dose and per cent aneuploidy

III. Changes in the incidence after irradiation

Changes in the incidence of various types of chromosome aberrations after X-ray therapy for ankylosing spondylitis were studied by Buckton et al. (1) for about twenty years. Classification of cells was not the same as by other workers. Percent aneuploidy was shown only in cells with no evidence of a structural chromosome abnormality (Type-A cells). The proportion of non modal type-A cells rised to a maximum of about 14% in a few weeks after exposure returning to control levels in 1 to 2 years. They suggested that the aneuploidy was mainly due to loss of chromosome during preparation of the cells, but the possibility of real errors in division was not discounted.

The proportion of cells with chromosomes showing only chromatide type aberrations (type-B-cells) did not change significantly from the non-irradiated control values at any time after exposure. How-

ever there were big changes in proportions of cells with marked structural abnormality (type-C cells). The trends for the numbers of cells containing fragements, dicentric and tricentric chromosomes, or ring chromosomes were similar, reaching maximal levels 1–3 weeks after exposure, and then dropping to a fairly steady level after 5 years. When the proportion of cells with such unstable abnormalities as these are plotted semilogarithmically, they fall in an approximately exponential fashion, about 3.5% of the cells desappearing each year; but the rate may be faster in the earlier and slower in the later period. They obtained the average life of 29 months of these unstable cells. In relation to the result of the proceeding section; the incidence of dicentric and tricentric chromosomes was plotted semilogarithmically as in Fig. 3 from their data. Two approximately straight line are obtained with a half life of about two years and that of about four years respectively. Recently Norman et al.(7) followed the incidence of acentric

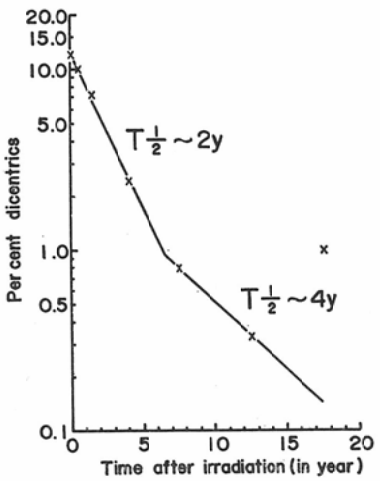

Fig. 3 Decay of dicentrics (and tricentrics)
Data from Buckton et al (1962)

frangment in peripheral lymphocytes of patients irradiated for cervical carcinoma. Linear regression analysis of the relation between acentric chromosome fragments and time after radiation therapy showed that the percentage of lymphocytes y with acentric fragments was related to the number of days x, after therapy by equation

$$y=(16.5\pm1.2)\exp-(0.00189\pm0.00023)x$$

From this relation they obtained the estimate of an average life of about 18 ± 2 months, a half life being calculated to be about 366 days. The estimates have some ambiguity as will be discussed later.

Bender and Gooch (2, 3) studied chromosome aberrations of the same patients two years and three and one-half years after irradiation by a nuclear excursion. Changes in percent aneuploidy, percentage of cells with ring and dicentric chromosomes are tabulated in Table 1 from their data. Ring chromosomes was observed only in high dose level group and disappeared within 3.5 years. As far as percent aneuploidy and dicentrics are concerned, neither consistent relations between the percentage and dose nor definite change for 1.5 years are observed.

Table 1. Changes in the incidence of chromosome aberrations in man irradiated by a nuclear accident (Bender & Gooch 2,3))

Case	Estimated dose	Percent aneuploidy		Percent rings		Percent dicentrics	
		2y	3.5y	2y	3.5y	2y	3.5y
A	365	14	14	0	0	1	4
C	339	23.2	8	3.2	0	13.4	2
D	327	14	12.0	0	0	3	2
B	270	20	16	0	0	1	2.4
E	236	6.7	14	0	0	1.3	3
F	68.3	14	13	0	0	0	0
G	68.5	8.4	16	0	0	0	0
H	22.8	4	14	0	0	0	1
Control	0	1.7	4.1	0	0	0	0

Doida and Sugahara (8) has studied chromosome aberrations again one year later in some of the atomic bomb survivors as reported previously (Doida et al. (4)). Neither rings nor dicentrics were observed in any of them. But percent aneuploidy appeared to be consistent between 17 years and 7 months and 18 years and 7 months so far studied as shown in Table 2. However a definit relationship between radiation dose estimated from distance and percent aneuploidy could not be demonstrated.

Table 2. Changes in percent aneuploidy in atomic bomb survivors in Hiroshima and Nagasaki (4,8)

Case No.	Age at the first examination (y)	Estimated dose (rads)	Percent aneuploidy 17y7m after nuclear detonation	18y7m
35	69	1,000	6.9	9.9
73	42	400	16.7 (17y9m)	19.5
34	48	100	15.6	—
36	32	100	19.0	—
37	36	100	17.8	15.2
135	20	15	—	5.2
92	42	0.1	11.0 (18y)	—
Control 1, 23—24			3.6	
Control 2, 46—48			8.7	

IV. Chromosome aberrations in radiation personnel

Percent aneuploidy and percent dicentrics were studied in six radiation personnel who were supposed to be exposed to fairly large doses of radiation for long time till eight years ago. A part of the study was reported previously (Doida et al. (4)). Their radiation and hematological history are summarized in Table 3 including the results of chromosome study. Low blood cell counts in 1951 and 1954 and insufficient shielding against radiation source at working area before 1954 may indicate that they were exposed to at least a few hundreds milliroentgens per day during their work till 1955. Tolerance dose before 1950 was 0.1 R per day in the United States and 0.2 R in other countries. Minimum estimate of the does at that period may be 50R per year. Actual dose in those cases might be much higher than this. The dose appeared to be reduced below the maximum permissible dose level after pocket chamber

Table 3 Radiation and hematological history of radiation personnel

Case No.	Age (1963)	Radiation work since	1951 June W.B.C.	1954Dec. W.B.C. R.B.C.	Pocket chamber 1955 Nov. W.B.C. R.B.C. Dose(/M)	Film badge 1960 Dec. W.B.C. R.B.C. Dose(/M)	1962 March W.B.C. R.B.C. Dose(/M)	1963 Oct. W.B.C. R.B.C. Dose(/M)	Dec. Per cent aneuploidy	Dec. Per cent dicentrics
115	35	1949	4,300	5,200 285×10⁴	6,125 305×10⁴ 443mr	5,350 435×10⁴ 102.5mr	6,100 460×10⁴ 3.3mr	5,420 379×10⁴ 0mr	13.4	0
117	48	1948	5,700 4,200	8,300 350×10⁴	5,800 405×10⁴ 41mr	6,550 415×10⁴ 32.5mr	10,600 540×10⁴ —	—	8.0	0
118	43	1952	—	4,950 365×10⁴	3,300 485×10⁴ —	6,300 505×10⁴ —	—	9,240 442×10⁴ —	4.2	0
119	40	1942 (except 1944—46)	2,400 4,000	—	8,225 470×10⁴ 410mr	7,000 480×10⁴ 15mr	5,500 550×10⁴ 0mr	19,000 463×10⁴ 1.1mr	3.5	2.3
120	30	1952	—	6,300 325×10⁴	6,550 420×10⁴ 92.5mr	5,500 485×10⁴ 25mr	6,900 490×10⁴ 6.6mr	9,000 440×10⁴ 7.7	6.8	0
121	41	1949	—	7,900 350×10⁴	5,970 480×10⁴ —	3,350 470×10⁴ —	5,200 570×10⁴ —	—	13.7	0

and/or film badge had been used for radiation control. Thus doses after 1955 may be negligible as compared with those before 1955.

Estimates of dose level according to the above assumption do not seem to be related to percent aneuploidy. Dicentric chromosomes were found only in one case of the longest working time. The incidence per cell being 2.3 per cent (0.62–6.2% for 90% confidence limit). Decay for ten years is read as 1/20 from Fig. 3. The dose is tentatively estimated from Fig. 1 using Norman's line as 400R (200–900R). Accumulated dose for 12 years, assuming 50R per year, is estimated to be 500R. The fact that dicentrics have not been observed in other cases may be due to the lower doses received (estimated to be 200 to 400 R less on the same assumption), to random fluctuation in detecting the abnormality, or to some unknown factors modifying the decay process. The difference in dose between case 119 and other personnel may be greater than that estimated on 50R per year basis since the dose received per year might be higher in 1940's because of lower protection shielding than in 1950's.

V. Discussion

In order to estimate the dose of radiation using biological changes induced by radiation, a dose-effect relationship and a pattern of changes of the effect after irradiation should be known as quantitatively as possible. In addition for the estimation for the radiations given many years ago, the biological effect in issue should persist for years. Some of chromosome aberrations induced by radiation may be evaluated quantitatively and their persistence has been demonstrated recently by Buckton et al. (1), Bender and Gooch (2, 3), and Doida et al. (4). Dose-effect relationships for chromosome aberrations which may be established in the data reviewed above are represented in Fig. 1 and 2.

Among them the incidence of dicentrics seems to be most reliable. Dicentrics are rather readily detectable without karyotype analysis, and have never been observed in normal nonirradiated samples. Percent aneuploidy is also readily calculated from the data by gross observation under a microscope. But further work would be required to confirm the dose effect relationship. Especially, the assumption

for the calculation of effective dose should be tested with different types of radiation conditions.

The changes after irradiation appear to be rather confusing. Approximately linear decreases in semilogarithmic scale are observed for unstable abnormalities by Buckton et al.(1) and for acentric chromosomes by Norman et al.(5). The fact that neither rings nor dicentrics were observed in total 765 cells from the persons irradiated with nuclear detonation more than seventeen years ago may be explained on the basis of the decay curve mentioned above. In radiation personnel exposed to a nuclear accident, though per cent rings showed a decrease during one and half a year, a consistent trend in the change in percent dicentrics could not be demonstrated during the same time period.

It was suggested by Norman et al.(6) that the decay of accentric chromosomes corresponded to the decay of the lymphocyte. Slower decay of unstable chromosome aberration by Buckton et al.(1) was assumed to be due to higher probability that the decentrics and rings would survive one or more cell division. But exponential survival curve of the cells should be resulted from random elimination of the cells at mitosis if the cells have a definite lifetime and the elimination occurs only at mitosis. In other words, some of the cells should survive mitosis for the exponential survival curve. If the elimination of dicentrics occurs at mitosis on random basis, the probability for the cells to survive is 1/2. Thus the half-life of the curve corresponds to the lifetime of the cells. If the probability is much lower, the lifetime of the cells would be much longer. Results of the present analysis in Fig. 3 may indicate that about 28% of dicentrics induced have the lifetime about double as the others or the probability to survive mitosis about double. However, saturation of percent aneuploidy within ten days in Fig, 2 can not be explained on such a long life span of the cells. It is possible that there are two or more types of cells, i.e., cells with a short life span of several days and those with a long life span of about two years or more. It is generally assumed that cells in mitosis by the method culturing peripheral blood cells for 54 to 72 hours are lymphocytes. The life span of lymphocytes has been believed to be several days while the data of chromosome aberration suggested much longer one as shown above. Longer life span of the cells may favor the dose estimation by surveying the aberrations in them. The relationship between lifetime of cells and decay of the cells with chromosome aberrations will be discussed in detail elsewhere.

Percent aneuploidy and dicentrics in radiation personnel after life time exposure was study by Sasaki et al.(9). No correlation between accumulated doses indicated by film badge and the incidence of these abnormalities has been found though the abnormalities appeared in significantly higher frequencies than in a roughly comparable control group. Similar results was obtained by the studies by Doida et al. (4) as cited above.

Estimation of the dose received ten years ago was tried as an example of the method to be developed. Estimate from the incidence of dicentrics is roughly in the range of that from other data.

In conclusion, the available data at present for estimating the dose received many years ago are represented in Fig. 1 and 3. Percent of cells with dicentrics in peripheral blood at the time of irradiation will be given by extrapolating the percentage at the time of examination along the lines in Fig. 3. The dose will be estimated from Fig. 1 which shows the relationship between the incidence of dicentrics and radiation dose.

However, the accuracy of the above estimate is quite questionable, since a deviation from the decay curve in Fig. 3 may be probable in various cases as indicated in the case of a nuclear accident. Furthermore, the method can be applied to atomic bomb survivors whose dose estimations are quite desirable only

in limited cases exposed heavily since it is supposed that most of the decentrics have been lost already. Percent aneuploidy seems to be more promising in this respect since most of hypo- or hyper-diploid cells may survive many divisions. Follow up study on various irradiated persons would be required for applying the percentage to the dose estimation.

Summary

The possibility to estimate the dose given to man many years prior to examination was explored by reviewing the literatures on the incidence of radiation-induced chromosome aberrations in man including some unpulished data in the authors' laboratory. A dose-effect relationship and a decay curve of the effect were presented for the incidence of dicentric chromosomes in blood cells. These data are availabe for the estimation, but the accuracy in the estimate is still questionable due to possible fluctuation in a decay. Percent aneuploidy was studied for the same purpose. Much more work may be required before reaching definite conclusions on this point.

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