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Relationship Between the Radiation Dose and Chromosome Aberrations in Atomic Bomb Survivors of Hiroshima and Nagasaki

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Radiation-induced chromosome aberrations were found to persist in cultured peripheral blood lymphocytes derived from Hiroshima and Nagasaki A-bomb survivors long after their radiation exposure. Earlier observations that the frequency of cells with chromosome aberrations increased in proportion with increasing dose in both cities were confirmed. However, in every dose group, the frequency of aberrant cells was consistently higher in Hiroshima than in Nagasaki. It is suggested that a higher neutron dose in Hiroshima than in Nagasaki may be a major component contributing to the difference in dose response between the two cities.

Among the types of chromosome aberrations so far identified, reciprocal translocations were observed to predominate, and they played an important role in determining the dose-aberration relationship.

INTRODUCTION

In our preliminary data reported previously[1], we have indicated that among atomic bomb survivors of Hiroshima and Nagasaki, radiation-induced chromosome aberrations have persisted *many* years in circulating lymphocytes, and that the frequency of such cells is proportional to the estimated dose received by each individual. The results showed that scoring cells with radiation-induced chromosome aberrations was a useful indicator for the evaluation of late somatic effects of irradiation upon humans.

The present report describes the results of a detailed chromosome analysis of cultured lymphocytes from atomic bomb survivors of Hiroshima and Nagasaki to obtain further information about the late somatic effects of atomic radiation. Special reference is made to clarifying the dose-response relationship of chromosome aberrations together with some factors such as age when exposed, radiation components of the atomic bombs and others, all of which may have some effects on the observed results.

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MATERIALS AND METHODS

Selection of Sample

The sample was selected from among A-bomb survivors who are resident in Hiroshima and Nagasaki for whom tentative dose estimates[2,3] are available, and who are participants in the R. E. R. F.-Adult Health Study[4].

Subjects who reported receiving radiotherapy or radioisotope exposure at any time were excluded. Also excluded were cases with unsuccessful culture as well as those producing poor mitosis of less than 30 scorable cells per case. With the above limitations, cytogenetic examination was made on 649 cases in Hiroshima and 403 cases in Nagasaki.

The controls studied consisted of two groups; those who were not in the cities at the time of bombings (or non-exposed), and those who were exposed distally to the bombings, but whose estimated doses were less than 1 rad. The remainder received an estimated dose of more than 1 rad, and are hereafter referred to as the exposed group. Exposed individuals were further divided into the following six groups according to their estimated doses: 1-99 rad, 100-199 rad, 200-299 rad, 300-399 rad, 400-499 rad, and over 500 rad. Further breakdown in the number of cases and number of cells

Table 1. Distribution of cells with chromosome aberrations by dose—Hiroshima and Nagasaki.

Dose group (rad)	Mean total dose	Gamma	Neutron	No. of cases	No. of cells examined	No. of cells with all aberrations	(%)	No. of cells with exchange aberrations*	(%)
HIROSHIMA									
Control	—	—	—	263	24414	294	(1.20)	210	(0.86)
1- 99	37.3	30.0	7.2	70	6459	175	(2.71)	141	(2.18)
100-199	142.9	112·1	30.7	137	12634	626	(4.95)	528	(4.18)
299-299	243.2	185.7	57.5	72	6484	615	(9.48)	544	(8.39)
300-399	348.4	261.7	86.8	43	3896	489	(12.55)	433	(11.11)
400-499	441.1	334.9	106.2	30	2869	407	(14.19)	371	(12.93)
500+	1062.5	728.9	333.7	34	3222	532	(16.51)	489	(15.18)
NAGASAKI									
Control	—	—	—	156	14748	199	(1.35)	128	(0.87)
1- 99	48.5	48.4	0.1	57	5472	103	(1.88)	77	(1.41)
100-199	147.0	145.6	1.4	62	5727	96	(1.68)	73	(1.27)
200-299	249.9	245.9	3.9	58	5443	150	(2.76)	117	(2.15)
300-399	336.1	330.3	5.8	30	2753	94	(3.41)	83	(3.01)
400-499	437.2	430.0	7.1	24	2312	164	(7.09)	147	(6.36)
500+	765.4	746.1	19.3	16	1566	206	(13.15)	196	(12.52)

* Exchanges include dicentrics, rings, reciprocal translocations and pericentric inversions, see in text.

scored by city is shown in Table 1.

The present study covered the period from January 1968 to November 1969 in Hiroshima and from January 1968 to March 1971 in Nagasaki.

Methods

Peripheral blood was cultured by the method of Moorhead *et al.*[5], with minor modification.

Heparinized blood was allowed to stand for a few hours at room temperature to separate plasma containing white blood cells. To one ml of the plasma thus separated was added 8 ml of culture medium (TC199, Difco), 1 ml of fetal bovine serum (Microbiological Assoc.), and antibiotics. Just before incubation, 0.1 ml of phytohemagglutinin (PHA, Wellcome) was added to the culture. After 50 hours of incubation, the culture was treated with colchicine (Ciba) at a concentration of 0.4 γ/ml for 2 hours, followed by hypotonic treatment with 1% sodium citrate solution. The cells were fixed with a mixture of methanol and acetic acid (3 : 1), and were then flame dried on slides for spreading. The slides were stained with Giemsa solution.

Culture time in this study was 52 hours including the last two hours of colchicine treatment, so that most of the observed metaphases appeared to be in their first *in vitro* cell division.

All slides were coded and microscopic examinations were carried out without knowledge of individual exposure status. In each case, an attempt was made to analyze 100 metaphases. However, there were a few instances with poor mitoses in which cases 30 metaphases were the minimum accepted number.

The chromosomes were grouped into A to G groups directly under the microscope. All of the cells with either definite or suspected structural rearrangements detected by direct microscopy were photographed for further detailed karyotype analysis. Chromosome aberrations were confirmed in the final analysis by at least two senior cytogeneticists.

Only chromosome-type aberrations were subjected to the present analysis. However, attention was paid to the fact that the chromatid aberrations in their first mitosis would often reappear as "derived" chromosome-type aberrations in the second or subsequent cell divisions[6,7].

The classification of chromosome aberrations used here was essentially the same as that described in the UNSCEAR report[6], and by Lea[8].

Asymmetric exchanges include dicentrics and rings, while symmetric exchanges are reciprocal translocations and pericentric inversions. The aberration defined here as "deletion" was characterized by a loss of acentric material from the complement due perhaps to a result of interstitial or terminal deletion. Interstitial deletions including minute fragements and possible acentric rings were designated as acentrics.

Paracentric inversions, which were the counterpart of acentric rings, could not be detected by the conventional Giemsa staining method. These aberrations undoubtedly must be included in the normal cells.

The observed aberrations thus were classified into the following six types; *dicentrics, rings, reciprocal translocations, pericentric inversions, acentrics,* and *deletions.* The first four types were here referred to as exchange aberrations, while acentrics and deletions were excluded from the exchanges, and were scored separately in the total aberrations.

RESULTS

I. Frequency of cells with chromosome aberrations in relation to dose

Approximately one-fifth of the total cases examined (139 of the 649 in Hiroshima, and 72 of the 403 in Nagasaki) were found to have received diagnostic gastrointestinal fluoroscopy other than routine chest X-ray within a year before cytogenetic examination. A preliminary statistical analysis, performed to compare the frequencies of cells with induced chromosome aberrations between these subjects who received diagnostic fluoroscopy and those who had no such radiation history in the past, failed to demonstrate any appreciable statistical difference between the two groups. Therefore, the data from these two groups were combined in the present analysis.

In both cities, the frequency of cells with radiation-induced chromosome aberrations, mostly exchange types, increased with increasing doses (Table 1). In Hiroshima, an increased frequency of aberrant cells was already noted in the 1-99 rad group. Increase in the frequency of aberrant cells in Nagasaki was not so marked as for Hiroshima, and was rather gradual in the low dose range, increasing sharply above 300 rad.

There was no difference in the control values between the two cities, but in every dose group among the exposed, the frequency of aberrant cells was higher in Hiroshima than in Nagasaki. The shape of the curves, which show the dose-aberration response relation, is also different between the two cities. The curve is almost linear for Hiroshima, but for Nagasaki it appears to be exponetial or dose-squared. This trend was the same in terms of both exchange and total aberrations. Analysis of the relative biological effectiveness (RBE) is in progress, and the results will be presented elsewhere.

The subjects were divided into two age groups those who were less than 30 years of age at the time of bombings, and those who were over 30, to see if there was any age-related differences in exchange aberration frequencies: these two age groups were compared for each dose group (Table 2). The frequency of cells with aberrations in controls appeared to be slightly higher in the older people than in the younger ones in both cities, but the difference was statistically insignificant. For the exposed, there was no difference between the two age groups. Though this comparison may appear rather crude, further breakdown into more restricted age groups leads to insufficient numbers of cases in each age group to permit detailed analysis for evaluation of chromosomal radiosensitivity related to age.

Table 2. Distribution of aberrant cells by age and dose—Hiroshima and Nagasaki—.

Doge group (rad)	Age group (ATB*)	No. of cases	No. of cells	No. of cells with all aberrations (%)	No. of cells with exchanges (%)
HIROSHIMA					
Control	A	120	10971	118 (1.08)	85 (0.77)
	B	143	13443	176 (1.31)	125 (0.93)
1- 99	A	29	2571	56 (2.18)	44 (1.71)
	B	41	3888	119 (3.06)	97 (2.49)
100-199	A	58	5492	272 (4.95)	235 (4.28)
	B	79	7142	354 (4.96)	293 (4.10)
200-299	A	39	3442	308 (8.95)	267 (7.76)
	B	33	3042	307 (10.09)	277 (9.11)
300-399	A	28	2509	319 (12.71)	280 (11.16)
	B	15	1387	170 (12.26)	153 (11.03)
400-499	A	18	1749	259 (14.81)	237 (13.55)
	B	12	1120	148 (13.21)	134 (11.96)
500+	A	18	1683	252 (14.97)	235 (13.96)
	B	16	1539	280 (18.19)	254 (16.50)
NAGASAKI					
Control	A	103	9946	130 (1.31)	82 (0.82)
	B	53	4802	69 (1.44)	46 (0.96)
1- 99	A	34	3309	61 (1.84)	46 (1.39)
	B	23	2163	42 (1.94)	31 (1.43)
100-199	A	36	3405	57 (1.67)	45 (1.32)
	B	26	2322	39 (1.68)	28 (1.21)
200-299	A	34	3219	95 (2.95)	73 (2.27)
	B	24	2224	55 (2.47)	44 (1.98)
300-399	A	17	1561	54 (3.46)	47 (3.01)
	B	13	1192	40 (3.36)	36 (3.02)
400-499	A	14	1400	112 (8.00)	103 (7.36)
	B	10	912	52 (5.70)	44 (4.82)
500+	A	13	1266	158 (12.48)	149 (11.77)
	B	3	300	48 (16.00)	47 (15.67)

* ATB: age at the time of bombing. A: age under 30 ATB, B: age over 30 ATB.

II. Types and frequency of chromosome aberrations

As described in the preceding section, the majority of chromosome aberrations observed were of the exchange type. In this connection, it seemed worth while to determine the kinds of aberrations predominating in the aberrant cells of the exposed subjects. This section describes the types and frequencies of chromosome aberrations in A-bomb survivors. Since the data from the two cities were similar in general, analysis of the data obtained from Hiroshima is given here.

All of the identifiable chromosome aberrations observed were classified into the six

different types, and the frequencies of abberrations per cell were determined for each aberration category by dose.

Cells containing more than 5 exchange aberrations of unidentifiable nature were observed in both the controls and exposed: 5 in the 24414 cells examined in the controls (0.02%), and 11 in 35564 cells in the exposed (0.03%). These were designated as "cells with multiple aberrations", and were excluded from any of the aberration categories as well as from the total examined cells.

The frequency of aberrations per cell clearly increased with increasing dose in every aberration group (Table 3). Of the six types of aberrations, reciprocal translocations were found to occur predominantly in all dose groups, and constituted the major contribution to the dose-aberration relationship.

In contrast to the predominance of symmetric aberrations, asymmetric exchanges were far less frequent, though a dose-aberration relationship was nonetheless apparent. In order to demonstrate more clearly the strong predominance of symmetric aberrations over the asymmetric ones, the ratio of these two types of aberrations was calculated as shown in Table 4. Approximately 80% of exchanges were the symmetric type in the low dose group (1-99 rad), gradually increasing in frequency with increasing dose,

Table 3. Types and frequencies of chromosome aberrations by dose—Hiroshima.

Dose group (rad)	No. of cells examined*	No. of aberrations scored (per cell)					
		dic	r	ace	t	inv	del
Control	24409	58**	5	59	137	18	35
		(.0024)	(.0002)	(.0024)	(.0056)	(.0007)	(.0014)
1- 99	6458	26	5	17	97	26	29
		(.0040)	(.0008)	(.0026)	(.0150)	(.0040)	(.0045)
100-199	12632	52	12	59	445	64	76
		(.0041)	(.0009)	(.0047)	(.0352)	(.0051)	(.0060)
200-299	6482	38	11	29	501	76	77
		(.0059)	(.0017)	(.0045)	(.0773)	(.0117)	(.0119)
300-399	3896	31	10	14	428	67	76
		(.0080)	(.0026)	(.0036)	(.1099)	(.0172)	(.0195)
400-499	2866	12	5	15	377	49	51
		(.0042)	(.0017)	(.0052)	(.1315)	(.0171)	(.0178)
500+	3219	32***	8	21	521	66	80
		(.0099)	(.0025)	(.0065)	(.1619)	(.0205)	(.0249)
Exposed Total	35553	191	51	155	2369	348	389
		(.0054)	(.0014)	(.0044)	(.0666)	(.0098)	(.0109)

* Cells with multiple aberrations of unidentifiable nature are excluded.

** Including a tricentric as two acentrics.

*** Including two dicentric-rings in two cells.

dic: dicentrics, r: rings, ace: acentrics, t: translocations, inv: pericentric inversions, del: deletions

and reaching a maximum of around 95% in the group of 400 rad or more.

Another interesting feature in relation to the dose was that the number of aberrations per aberrant cell increased with increasing dose (Table 5). This would suggest that the higher the dose administered, the more complex the aberrations induced in a cell.

Radiation-induced chromosome breaks and subsequent rejoining to form exchange aberrations seemed to occur in a random fashion. Therefore, it may be assumed that the probability of a given chromosome being involved in an exchange aberration would be proportional to its relative length. On the basis of this assumption, the following analysis was undertaken: all of the identifiable chromosomes involved in the aberrations were classified according to the seven chromosome groups A to G. The observed values in each chromosome group were thus determined by the frequency of the chromosomes involved in each type of aberrations. Expected values were derived from the relative lengths of metaphase chromosomes described in the Chicago Conference report (1966)[9].

Table 4. Number of asymmetric and symmetric exchanges by dose—Hiroshima.

Dose group (rad)	No. of exchanges			No. of sym. /Total
	Asym.	Sym.	Totol	
Control	63	155	218	0.711
1 -99	31	123	154	0.799
100-199	64	509	573	0.888
200-299	49	577	626	0.922
300-399	41	495	536	0.924
400-499	17	426	443	0.962
500+	40	587	627	0.936

Asym.=asymmetric: dicentrics and rings.
Sym.=symmetric: translocations and inversions.

Table 5. Number of aberrations per aberrant cell by dose—Hiroshima.

Dose Group (rad)	No. of cells* with aberrations	No. of aberrations	No. of aberrations per aberrant cell
Control	289	312	1.08
1- 99	174	200	1.15
100-199	624	708	1.13
200-299	613	732	1.19
300-399	489	626	1.28
400-499	404	509	1.26
500+	529	728	1.38
Exposed Total	(2833)	(3503)	(1.24)

* Cells carrying more than 5 exchange aberrations of unidentifiable nature are excluded.

A chi-square test (d. f.=6) was then performed by a comparison of the observed versus expected values for each type of chromosome aberrations. In this analysis, only the exposed subjects were selected, and the data were further divided according to sex.

As shown in Table 6, the observed frequency in the yield of dicentrics was not statistically different from the expected values; there was no significant difference at 30% level in the female, and the difference was suggestive at 5% level in the male.

Table 6. Frequency of chromosomes involved in the formation of exchange aberrations—Hiroshima.

	Chromosome groups							Total	χ^2(d. f.=6)	
	A	B	C	D	E	F	G			
Males										
Translocations										
Observed	496	290	602	291	217	61	82	2039	86.569	$p<.01$
Expected	482.6	248.6	737.6	207.2	179.4	95.2	88.1	2039.0		
Dicentrics										
Observed	27	27	60	13	18	3	8	156	10.885	$.05<p<.10$
Expected	36.9	19.0	56.4	15.8	13.7	7.3	6.7	155.8		
Inversions										
Observed	95	25	22	2	2	1	1	148		
Expected	35.0	18.1	53.5	15.0	13.0	6.9	6.4	147.9	155.855	$p<.01$
Expected*	59.5	19.1	51.4	5.6	7.3	3.4	1.8	148.0	48.032	$p<.01$
Rings										
Observed	4	3	8	1	1	0	0	17		
Expected	4.0	2.1	6.2	1.7	1.5	0.8	0.7	17.0	2.863	$.80<p<.90$
Expected*	6.8	2.2	5.9	0.6	0.8	0.4	0.2	16.9	3.108	$.70<p<.80$
Females										
Translocations										
Observed	611	369	823	392	272	97	102	2666	120.652	$p<.01$
Expected	619.6	319.5	1020.0	265.8	230.3	122.2	88.5	2665.9		
Dicentrics										
Observed	49	24	97	22	22	7	3	224	5.993	$.30<p<.50$
Expected	52.1	26.8	85·7	22.3	19.4	10.3	7.4	224.0		
Inversions										
Observed	124	29	38	6	1	2	0	200		
Expected	46.5	24.0	76.5	19.9	17.3	9.2	6.6	200.0	186.887	$p<.01$
Expected*	78.0	25.0	73.6	7.4	9.6	4.6	1.8	200.0	56.227	$p<.01$
Rings										
Observed	9	2	17	5	0	0	0	33		
Expected	7.7	4.0	12.6	3.3	2.9	1.5	1.1	33.1	9.132	$.10<p<.20$
Expected*	12.9	4.1	12.1	1.2	1.6	0.8	0.3	33.0	18.972	$p<.01$

Expected values are based on the relative length of the metaphase chromosomes from Chicago Conference (1966).

Alternative expected values (*) are also based on the assumption that the participation of chromosomes in the formation of centric rings and inversions is proportional to $p_iq_i/\sum p_iq_i$, where p_i and q_i are the length of long and short arms of individual chromosome.

For reciprocal translocations, the counterpart of dicentrics, there was a statistical difference for both sexes between the observed and expected values. The difference was due to the increase in the observed frequency of chromosomes in B, D, and E groups, and to the decrease in those in C and F.

In the case of pericentric inversions, the observed frequencies for longer chromosomes such as those in the A and B groups were markedly higher than expected, while values for median or smaller chromosomes such as those in the C to G groups were extremely low.

The participation of chromosomes in the formation of inter-arm exchanges, such as rings and inversions, can be expressed as a simple function of the length of the long and short arms of the participating chromosome, i.e., $p \times q$. Alternatively expected values may be calculated by the following formula:

$$p_i q_i / \Sigma p_i q_i,$$

where p_i and q_i are the length of the long and short arms of individual chromosome, respectively. As seen in Table 6, in the row indicated by asterisks, the difference between observed and expected frequencies in the formation of inversions was thus considerably reduced using this formulation, though statistically significant differences were still demonstrable. This finding may suggest that the longer the chromosome, the higher the probability of its being induced to form an inter-arm intrachange. No decisive conclusion can be reached for centric rings, the counterpart of pericentric inversions, using either method of comparison, since the number of rings was extremely low.

III. Cell divisions and chromosome aberrations

Asymmetric chromosome aberrations were considered to cause mitotic disturbances because structural defects at anaphase and telophase, in turn would lead to eventual cell death[6,10]. Therefore, scoring the cells that carry an unbalanced chromosome constitution as a result of unequal segregation of chromosomal material through mitosis

Table 7. Frequency of X_1 and X_2 cells by dose—Hiroshima

Dose group (rad)	No. of cells with dicentric and ring				Total
	X_1	with one acentric	X_2	with two identical acentrics	without acentric	
1- 99	24	(82.8± 7.0)	3	2	29
100-199	50	(92.6± 3.6)	1	3	54
200-299	37	(90.2± 4.6)	0	4	41
300-399	24	(75.0± 7.6)	2	6	32
400-499	9	(75.0±12.5)	1	2	12
500+	23	(85.2± 6.8)	0	4	27
Total	167	(85.6± 2.5)	7	21	195

can be regarded as a good index for determining the frequency of cells which have undergone at least one or more *in vivo* mitoses after the induction of exchange aberrations.

The classification by Buckton and Pike[10] divided cells with dicentric and/or rings into the two groups; (1) those with an accompanying acentric were designated as X_1 cells, and (2) those either without any acentric or with two identical acentrics, probably of common origin, were designated as X_2 cells. The former (X_1) were considered to have undergone no cell division after aberration induction, while the latter (X_2) passed through at least one or more post-irradiation mitoses resulting in an unequal segregation of acentrics. A comparison of the distribution of X_1 and X_2 cells among cells with asymmetric exchanges (Table 7) showed that for every dose group 75 to 90% of the observed cells were identified as X_1 cells.

DISCUSSION

It has been well established by many *in vitro* and *in vivo* experiments of human and mammalian materials that the yield of chromosome aberrations induced in somatic as well as germ cells by ionizing radiation is closely related to the dose administered[6,11]. Atomic bomb survivors of Hiroshima and Nagasaki are unique in being large human populations that were exposed to large doses of acute whole-body exposure consisting of a mixture of neutron and gamma rays. Recent evidence has amply shown that exchange chromosome aberrations have persisted for more than twenty years in their somatic cells[1,14,23,24]. Moreover, these data suggest the existence of a radiation dose-chromosome aberration relationship[1,14]. This relationship has been confirmed in the present study for all of the known types of radiation-induced chromosome aberrations, and as the dose increased, more complex aberrations were observed in an aberrant cell.

One of the most important features of the present study was the difference between Hiroshima and Nagasaki in the frequency of aberrant cells. While the frequencies of aberrant cells in the controls were similar in both cities, the values in every dose group were markedly higher in Hiroshima than in Nagasaki (Table 1). The shape of the curve derived from the observed frequencies against the exposure dose appeared to be linear for Hiroshima, but tended to be dose-squared for Nagasaki. This difference may be ascribable to the difference in the radiation spectra between the two cities. The neutron component was significantly larger in Hiroshima than in Nagasaki (Table 1): in the latter the gamma component was the major constituent of the total air dose. A large body of experimental evidence indicates that the dose-aberration response curve for neutron is linear, while for gamma rays the relationship approximates dose-square curve[6,7]. Furthermore, the rate of chromosome aberration induction is higher for neutron than for gamma or X-rays[6].

In cells which have been exposed to ionizing radiation just before initiation of culture, asymmetric exchange aberrations are considered to be a sensitive indicator of evaluating the dose-aberration response[6]. This is also true for persons exposed to whole-body irradiation if the blood sample for culture is taken immediately after radi-

ation exposure. Buckton *et al.*[12] reported that in human volunteers who received whole-body exposure of 2 MeV X-ray radiation at low doses between 17 and 50 rads, a fairly good relation between the dose administered and the yield of asymmetric exchanges was observed, although there were differences from individual to individual. They further showed that there was in general good agreement between *in vivo* and *in vitro* irrradiation data even at low dose levels. Ishihara *et al.*[13] obtained similar results from their study of irradiated persons accidentally exposed to iridium-192 gamma rays, and they attempted to estimate individual absorbed doses by scoring cells with dicentrics and rings according to the formula proposed by Sasaki[15].

In contrast to the usefulness of asymmetric exchanges, symmetric aberrations such as reciprocal translocations and pericentric inversions are considered to be rather unreliable for the evalution of the dose-aberration response, because of the difficulty in detecting such aberrations; moreover, different criterion for distinguishing the aberrations as well as different experiences with microscopy may further influence the observer who is attempting to detect these types of exchanges. However, a strong predominance of cells with symmetric over those with asymmetric exchanges in A-bomb survivors examined more than twenty years after exposure suggests that cells with asymmetric exchanges have been eliminated from the *in vivo* lymphocyte population by mitotic events after irradiation, assuming that both symmetric and asymmetric aberrations had an equal probability of being induced by irradiation[16], and that the lymphocytes with symmetric exchanges have a greater advantage than those with asymmetric ones to underge normal mitoses. Although we have no direct evidence demonstrating that cells with asymmetric exchanges are eliminated from the *in vivo* lymphocyte population with the passage of time, there is some experimental evidence to support this in man[17], and in swine[18].

It thus appears that radiation-induced symmetric aberrations may be more sensitive indicators of the dose-response relationship than are the asymmetric exchanges, particularly in those who received radiation *many* years before cytogenetic examination; it should be emphasized that a system using standardized criteria for identifying symmetric chromosome aberrations must be established.

In spite of the difficulty of scoring cells with symmetric aberrations, as well as the decrease in the proportion of cells with asymmetric exchanges observed long after exposure to irradiation, Sasaki and Miyata[14], and Sasaki[15] showed that both types of radiation-induced chromosome aberrations can serve as sensitive indicators for the evaluation of absorbed doses in Hiroshima A-bomb survivors, and estimated individual doses by extrapolating the observed values of chromosome aberrations in a formula derived from *in vitro* radiation experiments using high energy X-rays.

Age difference may be considered another important factor which influences the radiosensitivity of cells in the induction of exchange chromosome aberrations. Curtis [19,20] found by *in vivo* radiation experiments on mouse regenerating liver cells that the frequency of induced chromosome aberrations was higher in aged than in younger mice. Sasaki and Tonomura[21] reported that in human lymphocytes irradiated *in vitro*,

chromosomal radiosensitivity in terms of the induction of exchange aberrations was elevated in neonates and then fell with age to a stabilized level within the first 1 or 2 years.

In the present study, a comparison of the frequency of cells with exchange aberrations was made between those who were less than 30 and those over 30 at the time of exposure to the bomb. No statistically significant difference was noted between the two age groups (Table 2), but we feel it is premature to conclude that there is no age effect on the chromosomal radiosensitivity in view of the rather crude age divisions used. Smaller groupings by more restricted age divisions was precluded by the relatively small size of the population sample. Another factor that deserves consideration, but for which there are no data, is whether or not the elimination of cells with aberrant chromosomes from the *in vivo* lymphocyte population is constant at all ages.

Juding from our culture sampling time, the majority of observable metaphases are believed to be in the first *in vitro* cell division (X_1 cells). There were, however, a small proportion of cells in the second or subsequent cell division *in vitro*, or X_2 cells. Some of these X_2 cells would have been produced by successive mitoses *in vitro*[22]. An unknown proportion of the remaining cells would have already divided *in vivo*.

A possible explanation for the presence of X_2 cells *in vivo* is that in A-bomb survivors who received acute whole-body irradiation at the time of the bombings, repopulation of blood cells by active mitoses would have occurred in the recovery phase after acute radiation symptoms. The majority of cells with asymmetric aberrations would thus have undergone mitoses with unequal segregation of chromosomal material, leading to either cell death or production of X_2 cells *in vivo*. The presence of *in vivo* clones of lymphocytes with induced chromosome aberrations in those heavily exposed to the A-bomb[1,14,23], or to fallout from nuclear tests[24,25] would seem to be evidence in support of the *in vivo* production of X_2 cells.

The majority of cells with asymmetric exchanges were identified as X_1 cells (Table 7), which probably have persisted in a dormant state for many years in the lymphocyte population, without passing through mitoses, as originally hypothesized by Fitzgerald[26].

The present data provide some examples of biologically interesting phenomena, one of which is that the number of exchange aberrations produced is in proportion to the relative length of the participating chromosome ; this applies particularly to dicentrics[27]. As stated earlier, the elimination of cells with dicentrics or rings from the *in vivo* lymphocyte population appears to increase almost in proportion to increasing dose but our data indicate that, nonetheless, the elimination of such cells probably occurred in a random fashion, so that there is a rondom distribution of chromosomes that participate in the formation of dicentrics.

There is also a tendency, with respect to reciprocal translocations, that the longer the chromosomes the more frequent they are involved in the formation of exchanges. In these types of abnormalities, however, there is a considerably lower frequency of C group chromosomes involved in exchange formations when compared with the expected

value. This observation is an indication of the difficulty in identifying individual chromosomes in this group, and is a major contribution to the statistical difference between observed and expected values.

Aberrations designated here as "deletions" are considered to be produced as a result of either an incomplete exchange or a terminal and/or interstitial deletion where the deleted acentric part is lost. Since there was a positive relation between dose and the frequency of these aberrations, it seems that the deletions result from an exchange of incomplete form; a deleted part of an acentric is likely to be lost from the cell through subsequent mitosis.

Finally, recently developed techniques such as Q-, G- and C-band methods for identifying individual chromosomes have enabled us to detect more accurrately a variety of radiation-induced structural rearrangements, so that the abnormality, hitherto undetected by the conventional staining method currently in use, can thus be identified by these new methods. For example, in addition to those detected by the current method, by the use of a trypsin G-band technique[28] in cultured lymphocytes from heavily exposed A-bomb survivors of Hiroshima, it has become possible to identify a variety of abnormalities, such as paracentric inversions, with an exchange at the equidistant points from the centromere, and reciprocal exchanges of equal lengths of chromosomal material between the two chromosomes. Furthermore, exchange aberrations identified as simple reciprocal translocations by the current method are in fact more complex in nature involving more than two chromosomes (Ohtaki and Shimba, unpublished). Therefore, the frequency of cells with symmetric exchanges observed in the present study is in all likelihood understimated, in light of the above observations.

These new techniques, alone or in combination, make possible further detailed analysis of structural chromosome aberrations to determine the biological and clinical significance of cells with radiation-induced chromosome abnormalities, particularly in relation to the possible etiology of malignant neoplasms developing in man as late somatic effect of radiation exposure.

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REFERENCES

1. A. A. Awa, S. Neriishi, T. Honda, M. C. Yoshida, T. Sofuni and T. Matsui (1971) Chromosome-aberration frequency in cultured blood-cells in relation to radiation dose of A-bomb survivors. *Lancet*, 2: 903-905.

2. J.A. Auxier, J.S. Cheka, F.F. Haywood, T.D. Jones and J.H. Thorngate (1966) Free-field radiation-dose distributions from the Hiroshima and Nagasaki bombings. *Health Phys.*, **12**: 425-429.
3. R.C. Milton and T. Shohoji (1968) Tentative 1965 radiation dose (T65D) estimation for atomic bomb survivors, Hiroshima-Nagasaki. *ABCC Technical Report* 1-68.
4. G.W. Beebe and M. Usagawa (1968) The major ABCC samples. *ABCC Technical Report* 12-68.
5. P.S. Moorhead, P.C. Nowell, W.J. Mellman, D.M. Battips and D.A. Hungerford (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.*, **20**: 613-616.
6. United Nations (1969) *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation.* General Assembly Document, 24th Session, Annex C. Radiation-induced chromosome aberrations in human cells. Suppl. No. **13(A/7613)**. New York, United Nations.
7. J.H. Evans (1974) Effects of ionizing radiation on mammalian chromosomes. *Chromosomes and Cancer (Ed. J. German)*. New York, John Wiley and Sons. 191-237.
8. D.E. Lea (1956) Chap. VI. The Production of Chromosome Structural Changes by Radiation. Chap. VII. The Mechanism of Induction of Chromosome Structural Changes. *Actions of Radiations on Living Cells.* Cambridge University Press, London. 2nd ed., pp. 189-281.
9. Chicago Conference (1966) Standardization in human cytogenetics. *Birth Defects*: Original Article Series, Vol. **II**, No. 2. The National Foundation, New York.
10. K.E. Buckton and M.C. Pike (1964) Time in culture—an important variable in studying *in vivo* radiation-induced chromosome damage in man. *Int. J. Rad. Biol*, **8**: 439-452.
11. United Nations (1972) *Report of the United Nations Scientific Committee on the Effects of Atomic Radiation.* General Assembly Document, 24th Session. Ionizing radiation: Levels and Effects. Suppl. No. **25(A/8725)**. New York, United Nations.
12. K.E. Buckton, A.O. Langland, P.G. Smith, G.E. Woodcock, P.C. Looby and J. McLelland (1971) Further studies on chromosome aberration production after whole-body irradiation in man. *Int. J. Rad. Biol.*, **19**: 369-378.
13. T. Ishihara, S. Kohno, K. Hirashima, T. Kumatori, H. Sugiyama and A. Kurisu (1973) Chromosome aberrations in persons accidentally exposed to ^{192}Ir gamma-rays. *J. Radiat. Res.*, **14**: 328-335.
14. M.S. Sasaki and H. Miyata (1968) Biological dosimetry in atomic bomb survivors. *Nature*, **220**: 1189-1193.
15. M.S. Sasaki (1971) Radiation-induced chromosome aberrations in lymphocytes: possible biological dosimeter in man. *Biological Aspects of Radiation Protection (Eds. T. Sugahara and O. Hug)*. Igaku-Shoin, Tokyo. pp. 81-91.
16. J.A. Heddle (1965) Randomness in the formation of radiation-induced chromosome aberrations. *Genetics*, **52**: 1329-1334.
17. A. Norman, M.S. Sasaki, R.E. Ottoman and A.G. Fingerhut (1966) Elimination of chromosome aberrations from human lymphocytes. *Blood*, **27**: 706-714.
18. A.F. McFee, M.W. Banner, M.W. Sherrill and J.B. Hailhes (1972) Disappearance rates of radiation-induced chromosome aberrations from swine leukocytes. *Mutation Res.*, **15**: 325-330.
19. H.J. Curtis (1963) Biological mechanisms underlying the aging process. *Science*, **141**: 686-694.
20. H.J. Curtis (1967) Recovery of mammalian chromosomes from radiation injury. *Recovery and Repair Mechanisms in Radiobiology.* Brookhaven Symposia in Biology. No. **20**. pp. 223-240.
21. M.S. Sasaki and A. Tonomura (1969) Chromosomal radiosensitivity in Down's syndrome. *Jap. J. Human Genet.*, **14**: 81-92.
22. T. Sofuni, K. Tanabe, T. Matsui and A.A. Awa (1975) Proliferation of cultured human leukocytes. *ABCC Technical Report* 1-75.

23. A. D. Bloom, S. Neriishi, A. A. Awa, T. Honda and P. G. Archer (1967) Chromosome aberrations in leucocytes of older survivors of the atomic bombings of Hiroshima and Nagasaki. *Lancet*, **2**: 802-805.
24. T. Ishihara and T. Kumatori (1965) Chromosome aberrations in human leukocytes irradiated *in vivo* and *in vitro*. *Acta Haem. Jap.*, **28**: 291-307.
25. T. Ishihara and T. Kumatori (1969) Cytogenetic studies on fishermen exposed to fallout radiation in 1954. *Jap. J. Genet.*, **44** (Suppl. 1) : 242-251.
26. P. H. Fitzgerald (1964) The immunological role and long lifespan of small lymphocytes. *J. Theor. Biol.*, **6**: 13-25.
27. A. Norman and M. S. Sasaki (1966) Chromosome-exchange aberrations in human lymphocytes. *Int. J. Rad. Biol.*, 11: 321-328.
28. M. Seabright (1971) A rapid banding technique for human chromosomes. *Lancet*, **2**: 971-972.