TECHNICAL REPORT
1-75
業 績 報 告 書

PROLIFERATION OF CULTURED HUMAN LEUKOCYTES

ヒトの培養白血球の増殖について

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ABCC JNIH

ATOMIC BOMB CASUALTY COMMISSION

国立予防衛生研究所－原爆傷害調査委員会

JAPANESE NATIONAL INSTITUTE OF HEALTH OF THE MINISTRY OF HEALTH AND WELFARE

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SUMMARY

The proliferative capacity of human leukocytes in cultures was investigated by mitotic index analysis and autoradiography. It was found that the mitotic index in 2-day cultures tended to decrease with increasing age, but was not influenced by other factors, such as sex, radiation dose, and variation in certain clinical biochemical and hematological parameters. Autoradiographic study also demonstrated, in 3-day culture, a tendency towards a decrease with age in the proportion of cells entering their second cell division. These results suggest that the proliferative capacity of leukocytes in culture declines with age. In addition, cells with unstable type chromosome aberrations were found to proliferate in culture at a rate equal to that of the cells with no aberrations.

INTRODUCTION

Since the interpretation of data obtained from studies of the frequency of chromosome aberrations induced by irradiation can be influenced by the number and frequency of cell divisions, it is important to determine whether there are any differences in proliferative capacity of human leukocytes in tissue culture as measured by the frequency and number of cell divisions.[1]

The present study was undertaken to see if there was any significant difference among individuals with respect to (1) mitotic indices in 2-day cultures, (2) the proportion of metaphases in their first and second division in 2- and 3-day cultures, and (3) the frequency of metaphases with normal and structurally abnormal chromosomes in their first and second division in 3-day cultures.

要 約

ヒトの培養白血球の増殖能について分裂指数とオートラジオグラフ法を用いて調査した．2日間培養では分裂指数が年齢の増加とともに減少する傾向がみられた．しかし，性別，被曝線量，臨床生化学および血液学的パラメーターの変異などの要因によって影響されなかった．オートラジオグラフ法による研究では，3日間培養において2回目の分裂に入る細胞の割合が年齢とともに減少する傾向がみられた．これらの結果は培養白血球の増殖能が年齢とともに低下することを示唆している．さらに，不安定型の染色体異常をもつ細胞が異常をもたない細胞と同じ程度に増殖していることが判明した．

緒 言

放射線照者によって誘発された染色体異常の出現頻度についての調査においては，その研究結果の解釈が細胞分裂の回数と頻度とによって左右され得るので，細胞分裂の頻度およびその分裂回数を検討してヒトの培養白血球の増殖能に差があるか否かを確認することは重要である．[1]

本調査においては，次の項目，つまり：1) 2日間培養における分裂指数，2) 2日間および3日間培養において1回目および2回目の分裂にある細胞の割合，3) 3日間培養において1回目および2回目の分裂にある正常および染色体構造異常を有する細胞の出現頻度について個体間に有意な差があるかどうかを検討した．

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MATERIALS AND METHODS

For the present study, 411 individuals were selected from among A-bomb survivors who are members of the ABCC-JNIH Adult Health Study sample (AHS) in Hiroshima for whom dose estimates (T65 dose) have been made.[2] An additional 95 individuals were selected from among the offspring of A-bomb survivors(F_1). Blood specimens from 35 AHS and 9 F_1 participants were also used for autoradiographic study. The characteristics of both samples have been described elsewhere.[3,4]

Routine procedure for microscopic preparations One ml of plasma separated from peripheral blood was suspended in approximately 9 ml of culture medium consisting of 8 ml of TC-199 medium, 1 ml of calf serum, and 0.1 ml of phytohaemagglutinin (Wellcome Res. Lab., England). The cell suspension, incubated at 37 C, with the final 2 hours in the presence of colchicine (4γ/ml), was harvested after 50 hours. Chromosome slides were prepared according to the routine air-dry technique, and stained with Giemsa. The mitotic index (M.I.) is defined as the percentage of cells in mitosis among 500 mononuclear cells on the Giemsa stained slide preparation.

Autoradiography Autoradiographic studies were made of 2- and 3-day cultures as follows. Cells in 2-day cultures were labeled with tritiated thymidine (specific activity 5 c/mM, The Radiochemical Center, Amersham, England) which was added to the cultures at a concentration of 0.1μc/ml 24 hours after incubation began. The labeling was terminated 6 hours later by washing the cells with medium containing nonradioactive thymidine (10μg/ml) and the cells were resuspended in the standard incubation medium supplemented with nonradioactive thymidine (10μg/ml). After a total of 50 hours incubation, the final 2 hours in the presence of colchicine, the cells were harvested and processed for autoradiography. In 3-day cultures, the cells were labeled at 30 hours, washed at 54 hours (24 hours of isotope treatment), and harvested at 74 hours after the initiation of the culture. Other procedures were the same as for 2-day cultures.

Slides were stained with carbol-fuchsin,[5] dipped in Sakura NR-M2 liquid emulsion (Konishiroku Photo. Ind. Co. Ltd., Tokyo), kept for 3 weeks at 4 C in a dark, dry atomosphere, and then developed with Sakura Konidol-X for 4 minutes at 16 C.

The metaphase cells were identified as being either in the first or second division on the basis of the chromosome labeling pattern; at first division both chromatids are labeled, and at second division only one of the two chromatids is labeled. In this

材料と方法

本調査では，広島のABCC－予研成人健康調査対象者中，暫定推定線量（T65線量）の測定されている者411名を選択した.[2] なお，被爆者の子供（F_1）から95名を別に選択した．オートラジオグラフ法による検査に成人健康調査対象者35名およびF_1対象者9名よりの血液標本を使用した．両対象集団の特徴については別に述べている.[3,4]

顕微鏡検査標本のための通常処理方法 TC－199培地8 ml，子牛の血清1 ml，PHA 0.1 ml（英国 Wellcome 研究所）よりなる培地約9 mlに末梢血から分離した血漿1 mlを加えた．この浮游液を37° C，50時間（うち最後の2時間はコルヒチン4 γ／mlを添加）培養した後に細胞を採取した．通常の空気乾燥法によって染色体標本を作製し，Giemsa 染色を行った．分裂指数（M.I.）として，Giemsa 染色スライド上の単核細胞500個当たりの分裂細胞の百分率を用いた．

オートラジオグラフ法 2日間および3日間培養について次のようなオートラジオグラフ法を行った．2日間培養においては tritiated thymidine（比放射能5 c／mM，英国 Amersham の Radiochemical Center）を，培養開始24時間後に0.1 μc／mlの濃度で培地に添加して細胞を標識した．6時間後に非放射性 thymidine（10 μg／ml）を含む培地で細胞を洗浄して標識を止め，非放射性 thymidine（10 μg／ml）を加えた標準培地にて再び細胞を培養した．培養開始後50時間（最後の2時間はコルヒチンを添加）後に細胞を固定し，オートラジオグラフ法検査を行った．3日間培養においては，細胞を30時間目に標識し，54時間目に洗浄して（アイソトープ処理は24時間），培養開始から74時間後に固定した．その他の処理は2日間培養の場合と同じであった．

スライドは石炭酸フクシン[5]で染色し，「さくらNR－M2」液体乳剤（東京，小西六写真株式会社）に浸し，乾燥した暗所に4 ° Cで3週間保存したのちに，16° Cで4分間さくらコニドールーXで現像した．

分裂細胞は染色体標識像から1回目，または，2回目の分裂にあるものを判定した．すなわち，1回目の分裂では染色分体の双方が標識されており，2回目の分裂では2個の染色分体のうち1個のみが標識されていた．本調

observation, unlabeled metaphases would be those cells which did not begin the DNA synthesis until after H^3-thymidine was removed. Such metaphases would in all likelihood be in their first cell division when harvested, since the generation time of cultured leukocytes is more than 12 hours[6] whereas the time interval between termination of labeling and harvesting was only 20 hours. The frequency of labeled mononuclear cells was also determined in 2- and 3-day cultures.

In three cases from the 3-day culture series, slides stained with carbol-fuchsin were inspected for chromosome aberrations before autoradiography, and all of the cells with definite or suspected structural aberrations detected by direct microscopy were photographed for karyotype analysis. After karyotype analysis, the same preparations were processed for autoradiography so that the number of in vitro divisions was determined for the previously analyzed metaphases on the basis of the chromosome labeling pattern, as described above.

Other laboratory tests such as leukocyte differential counts, blood group, blood sugar, uric acid, and cholesterol were performed in the ABCC Clinical Laboratories using standard methods.

RESULTS

Mitotic index Since a minor technical modification* of blood culture was made in the latter half of this study, the sample was separated into two groups, i.e., Group I (before modification) and Group II (after modification). Group I included 273 cases from the AHS sample; in Group II there were 138 from the AHS and 95 from the F_1 sample. Figures 1 and 2 represent the results of the mitotic index in males and females in both groups of the AHS sample, expressed as a function of the donor's age. In both groups the mitotic indices seem to decline with increasing age, and the correlation coefficients computed are statistically significant (see Table 4). Analyses of the data yielded the following regression equation each with statistically significant regression coefficients ($p<0.05$).

Group I

Male : Mitotic index(%) = 2.168 − 0.0156 (Age)
Female: Mitotic index(%) = 2.744 − 0.0240 (Age)

査においては，非標識分裂細胞は H^3 − thymidine の除去後に DNA 合成を開始した細胞であろう．培養白血球の世代時間は12時間以上であり，[6] 一方，標識終了後細胞の固定までの時間的間隔は20時間にすぎないので，このような非標識分裂細胞は，細胞を固定した時点では１回目の分裂である可能性が強い．標識単核細胞の出現頻度も２日間培養および３日間培養において調査した．

３日間培養のうち３例においては，オートラジオグラフ法を行う前に，石炭酸・フクシンで染色して染色体異常の有無について直接顕微鏡検査し，構造上明確な，または，疑わしい異常を示す細胞のすべてについて写真による核型分析を行った．核型分析後，これらの標本をオートラジオグラフ法で処理し，上に示した染色体標識像に基づいてさきに分析した分裂細胞の試験管内における分裂回数を確認した．

白血球分類像，血液型，尿酸およびコレステロールのような諸臨床検査は，ABCC 臨床検査部において標準方法を用いて実施した．

結　果

分裂指数　本調査の後半に血液培養法にわずかな修正*を加えたので，対象者を二つの群，すなわち，第Ⅰ群（修正前）と第Ⅱ群（修正後）とに分け，第Ⅰ群は成人健康調査対象者273例から成り，第Ⅱ群には成人健康調査の138例と被爆者の子供（F_1）の調査対象者95例が含まれている．この両群における成人健康調査対象者について分裂指数を年齢の関数で表したものを男女別にして図１および図２に示した．両群とも分裂指数は年齢の増加に伴って下降する傾向にあり，計算から得た相関係数は統計的に有意である（表４）．資料の解析からそれぞれに統計的に有意な回帰係数（$P<0.05$）をもつ次の回帰方程式が得られた．

第Ⅰ群

男：分裂指数（%）＝ 2.168 − 0.0156 ×（年齢）
女：分裂指数（%）＝ 2.744 − 0.0240 ×（年齢）

* For the simultaneous initiation of cultures from several blood specimens which were taken at different times and dates, some were kept in the refrigerator at 4 C without any treatment (Group I). For more reliable storage of leukocytes, plasma containing leukocytes was separated from the blood, allowed to stand at 4 C for about 2 hours, and suspended in the culture medium (20% FBS) without PHA. In this procedure the mixture was kept in the refrigerator until the start of incubation (Group II).

異なった日時に採取した血液標本の培養を同時に開始するために，あるものは未処理のまま４°Cで冷蔵庫に保存した（第Ⅰ群）．血液を４°Cで約２時間放置した後に白血球を含んだ血漿を分離し，これを PHA を含まない培地（20% FBS）に入れて，培養開始まで冷蔵庫に保管することが白血球の貯蔵法としてはより優れている．（第Ⅱ群）

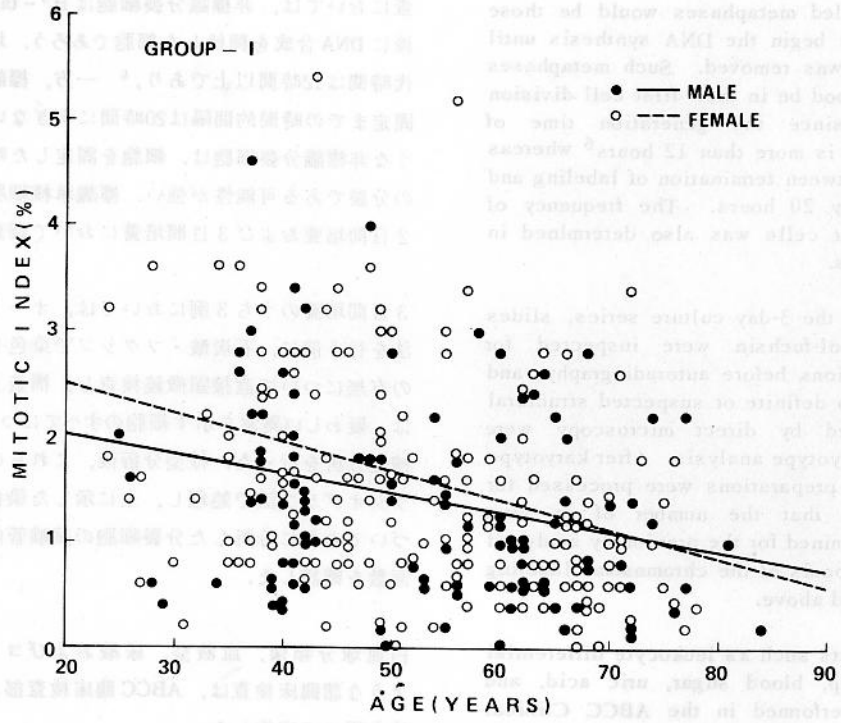

Figure 1 Mitotic index in 2-day cultures from Group I by age and sex.

図1　第Ⅰ群の2日間培養における分裂指数：年齢・性別

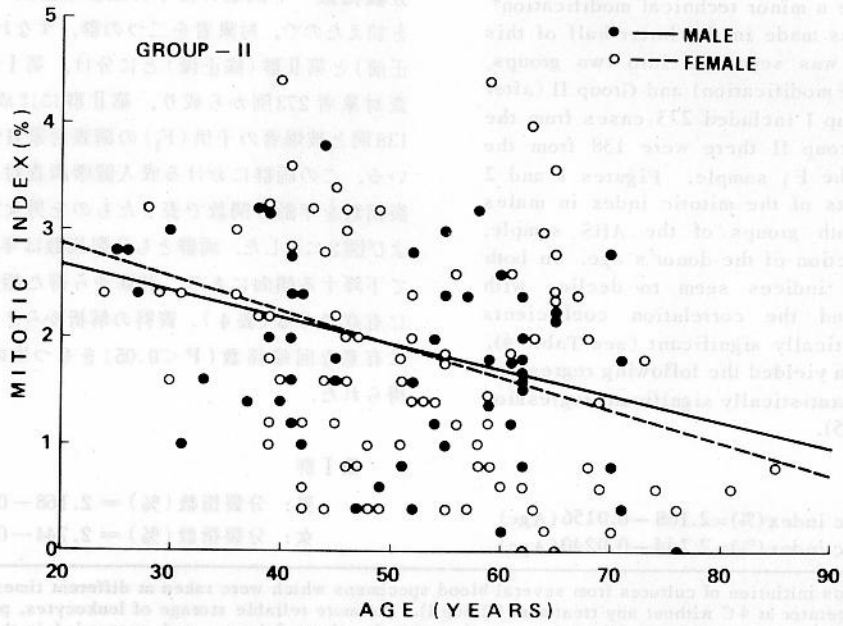

Figure 2 Mitotic index in 2-day cultures from Group II (excluding F_1) by age and sex.

図2　第Ⅱ群（F_1を除く）の2日間培養における分裂指数：年齢・性別

TABLE 1 MITOTIC INDEX (M.I.) IN 2-DAY CULTURES OF PERIPHERAL BLOOD FROM THE AHS AND F_1 SAMPLES BY SEX AND AGE

表1 成人健康調査対象者および被爆者の子供(F_1)の末梢血2日間培養における分裂指数(M. I.): 性・年齢別

	Sample	Age (yrs.)	Group I		Group II	
			Cases	M.I.(%)	Cases	M.I.(%)
Male	F_1	12-22	-	-	54	2.46
	AHS	24-39	18	1.70	10	2.34
	AHS	40-49	27	1.52	13	1.77
	AHS	50-59	19	1.23	15	1.84
	AHS	60-69	30	1.20	13	1.69
	AHS	70+	13	0.95	5	1.16
Female	F_1	12-22	-	-	41	2.84
	AHS	24-39	29	1.85	13	2.23
	AHS	40-49	46	1.79	25	1.92
	AHS	50-59	34	1.45	18	1.51
	AHS	60-69	38	1.05	20	1.68
	AHS	70+	19	1.07	6	0.73

Group II (excluding the F_1 subjects)

Male : Mitotic index(%)=2.895−0.0211(Age)
Female: Mitotic index(%)=3.147−0.0269(Age)

This tendency is shown more clearly in Table 1 where the AHS subjects were subdivided into five groups according to age.

For either sex in the F_1 sample, the average values of the mitotic indices were higher than those of the youngest groups in the AHS sample who were older than any of the F_1 sample (Table 1). This finding is further support for the observation that the mitotic index decreases with increasing age.

The declination of the regression line for the mitotic index vs age in both sexes was almost identical for Groups I and II (Figures 1 and 2), and no significant difference between males and females was seen in the mitotic index.

Comparison of the mitotic index by estimated (T65) radiation dose showed no significant difference between the less than 1 rad and 1 rad or more group in either group (Table 2). Furthermore when the 1 rad or more group was subdivided according to exposure dose, no significant correlation between mitotic index and exposure dose was seen (Tables 2 and 4). Further, it was found that the mitotic index had no significant relationship to acute radiation symptoms (Table 2). Since the occurrence of acute radiation symptoms is closely related to radiation dose, this finding may be adduced as supporting an

第Ⅱ群(F_1 対象者は除く)

男: 分裂指数(%)＝2.895−0.0211×(年齢)
女: 分裂指数(%)＝3.147−0.0269×(年齢)

表1に示すように，この傾向は成人健康調査(AHS)対象者を年齢によって5群に細分類するとより明らかになる．

第1世代集団においては男・女いずれの場合も分裂指数の平均値は，年上である成人健康調査対象者中の最若年者の分裂指数よりも高値であった(表1)．この結果は，年齢の増加につれて分裂指数が減少することをさらに裏付けるものである．

分裂指数と年齢との間の回帰直線の下降は第Ⅰ群と第Ⅱ群において男・女双方ともほとんど同じであって(図1，図2)，男女間に有意な差は認められなかった．

推定線量(T 65 D)別分裂指数の比較では，いずれの群においても1 rad以下と1 rad以上との間に有意の差は認められなかった(表2)．さらに，線量1 rad以上の群を被曝線量別に細分した場合，分裂指数と被曝線量との間に有意な相関関係は認められなかった(表2および表4)．また，分裂指数と急性放射線症状との間にも有意な関係はなかった(表2)．急性放射線症状の発生は放射線量と密接な関係があることから，この結果は被曝線量と分裂指数との間に有意な相関関係のないことを裏付けるもの

TABLE 2 MITOTIC INDEX (M.I.) IN 2-DAY CULTURES OF PERIPHERAL BLOOD FROM THE AHS SAMPLE BY RADIATION DOSE AND ACUTE RADIATION SYMPTOMS

表2 成人健康調査対象者の末梢血2日間培養における分裂指数(M. I.): 被曝線量・急性放射線症状別

	Group I		Group II	
	Cases	M.I.(%)	Cases	M.I.(%)
Radiation dose				
< 1 rad	139	1.44	51	1.68
≥1	134	1.41	87	1.82
1-99 rad	2	2.00	3	3.13
100-199	61	1.53	34	1.72
200-299	30	1.09	21	1.93
300-399	15	1.43	12	2.00
400+	26	1.43	17	1.50
Acute radiation symptoms*				
None	163	1.43	64	1.74
One	32	1.68	22	1.70
Two	37	1.15	26	1.64
All	40	1.40	25	2.01

* Epilation, bleeding, and oropharyngeal lesions.

Excluding two unknown cases.

absence of any significant correlation between exposure dose and mitotic index.

The relation of mitotic indices to the number of white blood cells, differential count of lymphocytes (%), blood level of uric acid, sugar and cholesterol, and blood type (ABO) is presented in Table 3. With one exception, there is no significant correlation between mitotic index and these parameters, judging from the absence of significant correlation coefficients estimated for each of the comparison (Table 4). The single exception, number of white blood cells in Group I, is marginal and probably fortuitous. Therefore, those factors do not seem to exert any appreciable influence on the mitotic index.

Cell division. 2-day Culture: Table 5 shows the percentage of metaphase cells in their first and second division, and the percent of labeled mononuclear cells in 2-day cultures. In the younger group (F_1) no second division was observed, while in the older group (AHS) six metaphases (2.0%) were found to be in their second division. The proportion of the labeled metaphases in their first division and of the labeled mononuclear cells in the older group were also slightly higher than those of the younger group. The difference of these values between the younger and older group, however, was not statistically significant ($p>0.05$). These

と考えられる.

表3に, 白血球数, リンパ球の百分比(%), 血中尿酸, 糖およびコレステロール値ならびに血液型(A, B, O)と分裂指数との関係を示す. 各比較項目について有意な相関係数が欠如していたことから判断して, 分裂指数とこれらのパラメーターとの間には, 一つの例外を除き有意な相関関係は認められない(表4). 唯一の例外とは, 第Ⅰ群における白血球数で, 有意性の限界に近い値を示し, おそらく偶然の結果と思われる. 従って, これらの要因は分裂指数に対し影響を及ぼしていないようである.

細胞分裂 1) 2日間培養: 2日間培養における1回目および2回目の分裂細胞ならびに標識単核細胞の百分率を表5に示す. 若年者群(F_1調査対象者)において, 2回目の分裂にあるものは観察されなかったが, 高齢者群(成人健康調査対象者)においては, 2回目の分裂にあった細胞が6つ(2.0%)認められた. 高齢者群における1回目の分裂にある標識細胞および標識単核細胞の割合は, ともに若年者群よりもわずかに高かった. しかしながら, 若年者群と高齢者群との間の差は統計的に有意ではな

TABLE 3 MITOTIC INDEX (M.I.) IN 2-DAY CULTURES OF PERIPHERAL BLOOD FROM THE AHS SAMPLE BY HEMATOLOGICAL, BIOCHEMICAL AND SEROLOGICAL PARAMETERS

表3　成人健康調査対象者の末梢血2日間培養における分裂指数(M. I.):
血液学的，生化学的および血清学的パラメーター別

	Group I		Group II	
	Cases	M.I.(%)	Cases	M.I.(%)
White blood cell				
- 44.9($\times 10^2$/cmm)	31	1.79	21	1.80
45.0 - 54.9	64	1.40	35	1.94
55.0 - 64.9	72	1.40	33	1.55
65.0 - 74.9	50	1.40	23	1.80
75.0 +	56	1.30	26	1.76
Lymphocyte				
- 24 (%)	70	1.24	27	2.05
25 - 29	48	1.30	19	1.66
30 - 34	48	1.56	32	2.03
35 - 39	49	1.55	25	1.48
40 +	58	1.52	35	1.57
Uric acid				
- 3.4 (mg/dl)	55	1.43	20	2.20
3.5 - 4.4	83	1.50	45	1.56
4.5 - 5.4	69	1.40	33	1.84
5.5 - 6.4	35	1.42	24	1.72
6.5 +	31	1.26	16	1.71
Blood sugar				
- 99 (mg/dl)	29	1.89	11	1.80
100 - 119	39	1.21	16	1.97
120 - 139	38	1.43	22	1.59
140 - 159	22	1.24	13	1.48
160 +	47	1.22	15	1.71
Cholesterol				
- 159 (mg/dl)	61	1.31	28	2.01
160 - 179	51	1.40	38	1.86
180 - 199	58	1.48	29	1.66
200 - 219	45	1.64	22	1.63
220 - 239	33	1.37	15	1.69
240 +	25	1.30	6	1.30
Blood type				
O	59	1.30		
A	68	1.47		
B	33	1.48		
AB	17	1.47		

findings indicate that there is in all likelihood no time difference in the initiation of the DNA synthesis of leukocytes in cultures between young and old persons.

3-day Culture: The percentage of the first and second or subsequent cell divisions, and of labeled mononuclear cells in 3-day cultures were compared by age (Table 6 and Figure 3). It can be seen that the proportion of cells at second division tended to decline with increasing age, showing a significantly negative linear regression ($p<0.01$). On the other hand, the frequency of unlabeled metaphases in their

かった($P>0.05$)．このことは培養白血球におけるDNA合成の開始に，若年者と高齢者との間におそらく時間的な差のないことを示している可能性が強い．

2) 3日間培養：　1回目，2回目およびそれ以降の細胞分裂の百分率と標識単核細胞のそれとを年齢別に比較した(表6，図3)．2回目の分裂にある細胞の割合は年齢の増加につれて減少する傾向にあり，有意な負の回帰直線を示した($P<0.01$)．他方，1回目の分裂にある非

TABLE 4 SUMMARY OF STATISTICAL TESTS FOR CORRELATION BETWEEN MITOTIC INDEX AND AGE, RADIATION DOSE, AND SEVERAL PARAMETERS FROM LABORATORY TESTS IN THE AHS SAMPLE

表4　成人健康調査対象者における分裂指数と年齢，被曝線量および数種の臨床検査パラメーターとの相関関係についての統計学的検定の要約

Parameter Tested	Group I		Group II	
	r	Test	r	Test
Age				
Male	−0.231	*	−0.306	*
Female	−0.313	*	−0.314	*
Total	−0.283	*	−0.311	*
Radiation dose†	−0.029	NS	−0.071	NS
Laboratory tests				
White blood cells	−0.119	*	−0.035	NS
Lymphocytes	0.112	NS	−0.117	NS
Uric acid	−0.061	NS	−0.062	NS
Blood sugar	−0.110	NS	−0.041	NS
Cholesterol	−0.004	NS	−0.125	NS

r: Correlation coefficients; computed only for the AHS sample.

test: Test whether or not correlation coefficients are significantly different from zero.

† Excluding low dose group (1-99 rad), because insufficient cases available.

* Significant ($p < 0.05$).

NS: Not Significant ($p > 0.05$)

TABLE 5 LABELING FREQUENCY (%) OF METAPHASES AND MONONUCLEAR CELLS IN 2-DAY CULTURES IN THE YOUNGER (F_1) AND THE OLDER (AHS) GROUP

表5　若年者群（F_1）および高齢者群（AHS）の2日間培養における分裂細胞と単核細胞の標識頻度

Sample	Age in Years (mean)	Cases	Metaphases				Mononuclear Cells		
			Total	1st Division		2nd Division	Total	Unlabeled	Labeled
				Unlabeled	Labeled	Labeled			
F_1	12-22 (17.6)	5	250	99.6	0.4	0.0	1000	99.3	0.7
AHS	24-85 (58.0)	6	300	97.0	1.0	2.0	1200	97.3	2.7
Total	12-85 (39.6)	11	550	98.2	0.7	1.1	2200	98.2	1.8

first division and of unlabeled mononuclear cells was found to increase with age and showed significantly positive linear regressions ($p<0.01$) with age. The regression equations describing these three characteristics are as follows: Second division (%) $=96.39-0.543$(Age); Unlabeled first division(%)$=-0.21+0.560$ (Age); and Unlabeled mononuclear cells(%)$=31.89+0.423$(Age). The proportion of the labeled metaphases in their first division did not vary significantly with age; the correlation coefficient describing this relationship was not statistically significant ($p>0.05$).

標識分裂細胞の頻度と非標識単核細胞のそれとは，年齢とともに増加しており，年齢とともに有意な正の回帰直線（$P<0.01$）を示した．これら三つの特徴を表わす回帰方程式は次のとおりである：第2回目分裂（%）$=96.39-0.543\times$（年齢）；非標識第1回目分裂（%）$=-0.21+0.560\times$（年齢）；非標識単核細胞（%）$=31.89+0.423\times$（年齢）．1回目の分裂にある標識細胞の割合は年齢とともに有意に変化せず，その，相関係数は統計的に有意ではなかった（$P>0.05$）．

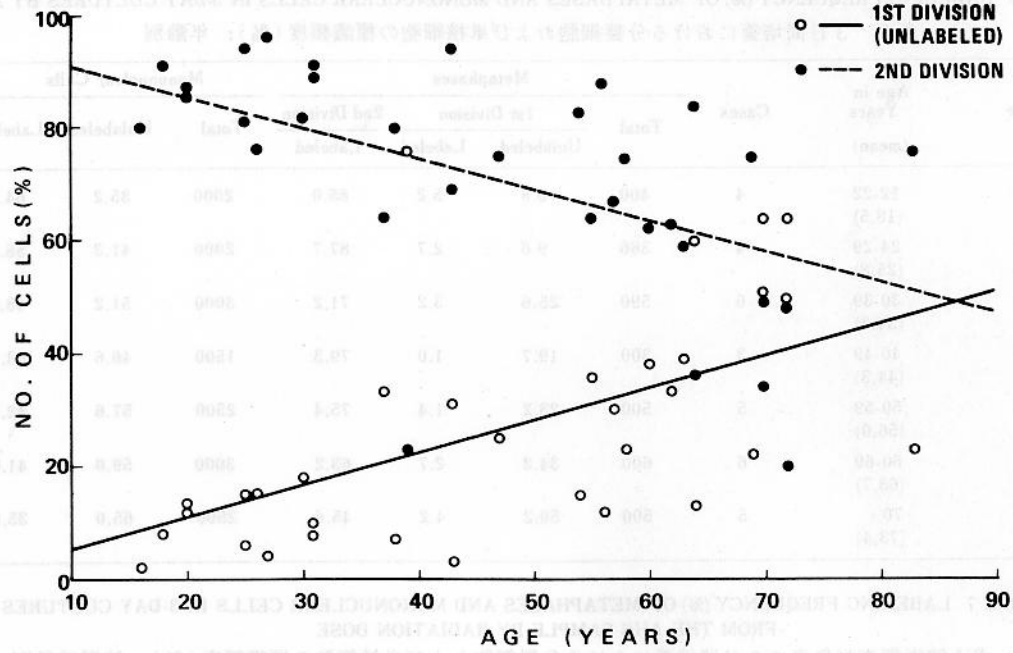

Figure 3 Percentages of metaphases in their first (unlabeled only) and second or subsequent division in 3-day cultures by age.

図3　3日間培養における1回目(非標識)および2回目またはそれ以降の細胞分裂の百分率：年齢別

The labeling frequencies of metaphases and mononuclear cells in 3-day cultures were analyzed by radiation exposure dose (Table 7). There was no remarkable difference in proportion of cells at second division between the low and the high dose groups, and these values were similar to that of the not in city group. Although the value in the less than 1 rad group was lower than that of the other groups, this difference was not statistically significant. A similar situation was found for the percentage of mononuclear cells. These findings indicate that radiation dose apparently has no significant effect to the proliferative capacity of leukocytes in cultures.

No remarkable difference between males and females was observed in frequency of cells in the first and the second division.

Cell division and chromosome aberrations. The proportion of metaphases with normal and abnormal chromosomes in their first and second or subsequent division in 3-day cultures from three cases, is shown in Table 8. It has been demonstrated that the chromatid type aberrations may be produced by H^3-thymidine treatment,[7] and some of these aberrations may in turn give rise to acentric fragments and deletions in subsequent cell division in culture. Therefore, chromosome abnormalities dealt with

3日間培養における標識分裂細胞および単核細胞の頻度を被曝放射線量別に解析した(表7)．低線量群と高線量群との間には2回目の分裂細胞の割合に著しい差はなく，これらの数値は市内にいなかった者の群の場合と類似していた．1 rad以下の対象群における数値は他の群の場合よりも低かったが，この差は統計的に有意ではなかった．単核細胞の百分率についても同じ状態が認められた．これらの結果は，放射線量が培養白血球の増殖能に有意な影響を及ぼしていないことを示している．

1回目および2回目の分裂にある細胞の頻度については，男・女間に著しい差は認められなかった．

細胞分裂および染色体異常　表8は，3例の3日間培養における1回目，2回目ないしその後の分裂にある正常および異常染色体を有する分裂細胞の割合を示す．H^3－thymidine 処理[7]によって染色分体型異常が起り得るということが明らかにされており，これらの異常の一部は培養中の細胞分裂で染色体切断や欠失を起こすことがある．したがって，ここで取り上げる染色体異常は，2動

TABLE 6 LABELING FREQUENCY (%) OF METAPHASES AND MONONUCLEAR CELLS IN 3-DAY CULTURES BY AGE

表6　3日間培養における分裂細胞および単核細胞の標識頻度(%): 年齢別

Sample	Age in Years (mean)	Cases	Metaphases				Mononuclear Cells		
			Total	1st Division		2nd Division	Total	Unlabeled	Labeled
				Unlabeled	Labeled	Labeled			
F_1	12-22 (18.5)	4	400	8.8	5.2	86.0	2000	35.2	64.8
AHS	24-29 (25.8)	4	366	9.6	2.7	87.7	2000	41.3	58.7
AHS	30-39 (34.3)	6	590	25.6	3.2	71.2	3000	51.2	48.8
AHS	40-49 (44.3)	3	300	19.7	1.0	79.3	1500	46.6	53.4
AHS	50-59 (56.0)	5	500	23.2	1.4	75.4	2500	57.6	42.4
AHS	60-69 (63.7)	6	600	34.2	2.7	63.2	3000	59.0	41.0
AHS	70+ (73.4)	5	500	50.2	4.2	45.6	2500	65.0	35.0

TABLE 7 LABELING FREQUENCY (%) OF METAPHASES AND MONONUCLEAR CELLS IN 3-DAY CULTURES FROM THE AHS SAMPLE BY RADIATION DOSE

表7　成人健康調査対象者の3日間培養における分裂細胞および単核細胞の標識頻度(%): 被曝線量別

Dose in rad (mean)	Cases	Metaphases				Mononuclear Cells		
		Total	1st Division		2nd Division	Total	Unlabeled	Labeled
			Unlabeled	Labeled	Labeled			
NIC*	4	400	22.8	1.5	75.8	2000	53.4	46.6
<1	11	1090	37.5	3.9	58.5	5500	60.1	39.9
1-299 (146.3)	8	800	23.4	2.1	74.5	4000	50.9	49.1
300+ (519.0)	6	566	23.0	1.8	75.3	3000	49.5	50.5

* NIC: Not in city at the time of the bomb. Their estimated dose was zero.

TABLE 8 FREQUENCY OF METAPHASES WITH NORMAL AND ABNORMAL CHROMOSOMES IN THEIR FIRST AND SECOND DIVISION IN 3-DAY CULTURES FROM THREE CASES IN THE AHS SAMPLE

表8　成人健康調査対象者3例の3日間培養における1回目および2回目の分裂にある正常・異常染色体を伴う分裂細胞の出現頻度

Case No.	Sex	Age	Dose in rad	Chromosome Condition	Cells in		
					1st Division	2nd Division	Total
H0574	F	72	<1	Normal	68(57.1)	51(42.9)	119
				Abnormal*	3(16.7)	15(83.3)	18
				Total	71(51.8)	66(48.2)	137
H0833	F	30	445	Normal	8(11.6)	61(88.4)	69
				Abnormal*	1(4.3)	22(95.7)	23
				Total	9(9.8)	83(90.2)	92
H0866	M	62	612	Normal	13(21.7)	47(78.3)	60
				Abnormal*	1(6.3)	15(93.7)	16
				Total	14(18.4)	62(81.6)	76

* Only cells with exchange aberrations (dicentrics, rings, translocations, and inversions) were included. Parentheses indicate percentages.

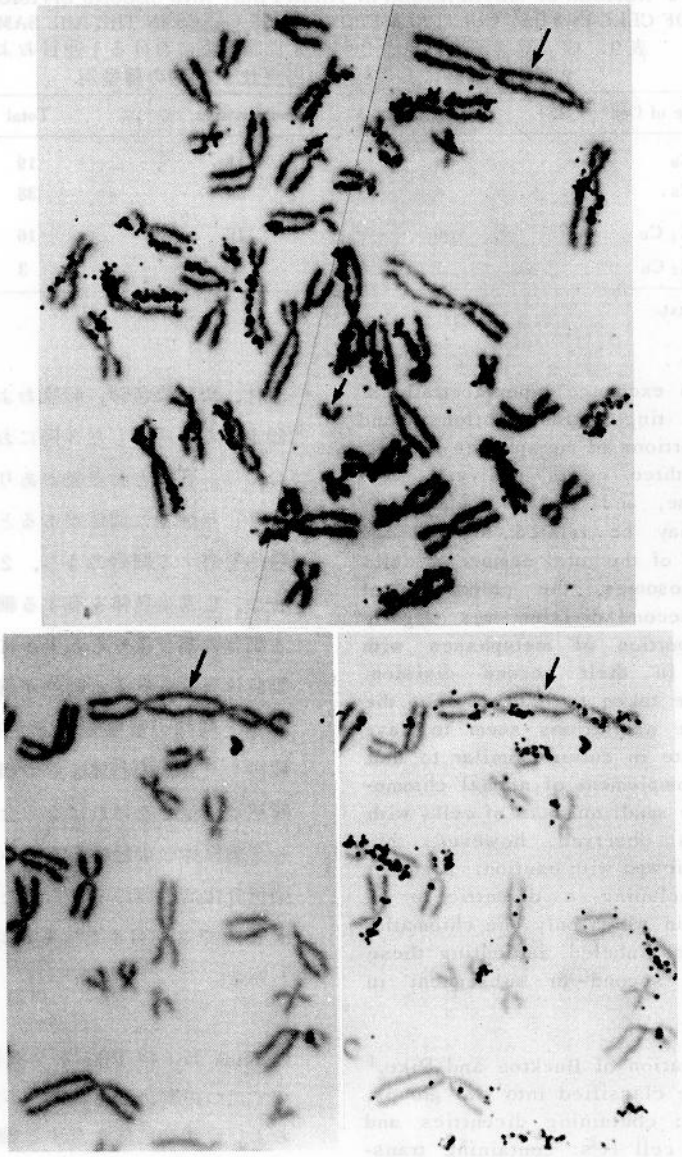

Figure 4 Metaphases observed in 3-day cultures. Upper: Metaphases after autoradiography, having a dicentric and an acentric fragment (arrows). Lower: Partial metaphase before (left) and after (right) autoradiography, having a tricentric chromosome (arrow). Note that grains are distributed primarily over one chromatid of the chromosomes, indicating these metaphases are in the second or subsequent cell division in culture.

図4　3日間培養において観察された分裂細胞．上：オートラジオグラフ法処理後の分裂細胞で，2動原体および染色体断片（矢印）が認められる．下：オートラジオグラフ法処理前（左）および処理後（右）の部分的分裂細胞で，3動原体染色体（矢印）が認められる．粒子は主として1個の染色分体上に分布しており，これらの分裂細胞が培養中に2回目またはそれ以降の分裂にあることを示している．

TABLE 9 NUMBER OF METAPHASES IN THEIR FIRST AND SECOND DIVISION BY TYPE OF CELL IN 3-DAY CULTURES FROM THREE CASES IN THE AHS SAMPLE

表9　成人健康調査対象者3例の3日間培養における1回目および2回目の分裂にある細胞の出現数：細胞の種型別

Type of Cell*	1st Division	2nd Division	Total
Cu	1	18	19
Cs	4	34	38
X_1 Cu	0	16	16
X_2 Cu	1	2	3

* See text.

here were confined to exchange type aberrations, such as dicentrics, rings, translocations, and inversions. The proportions of metaphases in their second division in three cases analyzed were different in each case, and the differences, as already described, may be related to the age difference. Generally, of the total number of cells with abnormal chromosomes, the proportion of metaphases in their second division was slightly higher than the proportion of metaphases with normal chromosomes in their second division. These findings may be taken to indicate that the cells with chromosome aberrations seem to have a capacity to proliferate in culture similar to that of cells with a full complement of normal chromosomes. Because of the small numbers of cells with abnormal chromosomes observed, however, this interpretation must be viewed with caution. Figure 4 shows metaphases including a dicentric or a tricentric chromosome, in which only one chromatid of the chromosomes was labeled, indicating these cells were in their second or subsequent in vitro division.

Following the classification of Buckton and Pike,[1] all abnormal cells were classified into two groups, the unstable cell (Cu: containing dicentrics and rings) and the stable cell (Cs: containing translocations and inversions), and the proportions of these two types of cells in their second division were compared (Table 9). The Cu cells were found to be present in a slightly higher proportion at second division than the Cs cells. Further, according to Buckton and Pike,[1] Cu cells may be classified into two subgroups, X_1Cu cells which are in their first division and X_2Cu cells which are probably in their second or later division after exposure to radiation. The X_1Cu cells contain one "nonidentical" acentric fragment, whereas the X_2Cu cells are either without acentric fragments or with "two equal-sized" fragments. All cells defined as X_1Cu in the 3-day cultures were in fact found to be in their second or subsequent divisions (Table 9), suggesting that the fragments in those cells divided equally into

原体，環状染色体，転座および逆位などの交換型異常に限定した．解析した3例における2回目の分裂細胞の割合には，各例とも差異があり，この差異はすでに述べたように年齢差に関係があると思われる．一般に，異常染色体を有する細胞のうち，2回目の分裂にある細胞の割合は，正常染色体を有する細胞のうち2回目の分裂にある細胞の割合よりもわずかに高かった．これらの所見は，染色体異常を有する細胞が培養中には正常染色体を示す細胞と同様の増殖能を示すものと解釈できる．しかし，観察した異常染色体をもつ細胞が少数であるので，この解釈は慎重でなければならない．図4は，2動原体ないし3動原体の染色体を有する分裂細胞を示し，うち標識染色分体は1個のみであったことからこれらの細胞は試験管内で2回目またはそれ以降の分裂期にあることがわかる．

Buckton および Pike の分類に従って，[1] 異常細胞をすべて二つの群，すなわち，不安定型細胞（Cu: 2動原体および環状染色体を有する細胞）と安定型細胞（Cs: 転座および逆位を有する細胞）に分類し，2回目の分裂にあったこの二つの型の細胞の割合を比較した（表9）．Cu細胞における2回目の分裂の割合はCs細胞よりもわずかに高かった．さらに，Buckton および Pike[1] によれば，Cu細胞は放射線照射後の1回目の分裂にあるX_1Cu細胞と2回目ないしはそれ以降の分裂にあるX_2Cu細胞との二つに細分類できる．X_1Cu細胞は1個の“不等”染色体断片を有するが，X_2Cu細胞は染色体断片を有しないか，または“二つの等長”の断片を有する．3日間培養ではX_1Cuと定義される全細胞は，事実上，2回目ないしそれ以降の分裂にあることが認められ（表9），これはこれらの細胞中の断片が培養中に娘細胞に相等しく分離したことを

daughter cells in culture. Out of three X_2Cu cells, one was in its first division in culture, indicating that this cell probably had already gone through at least two cell divisions, once in vivo, the other in vitro, while the two other cells in their second division, suggesting that those cells passed through at least two cell divisions in vitro. These findings suggest that even the cells with unstable type aberrations could divide at least once after the initiation of culture and survive as well as the cells with nomal chromosome complement or those with the stable type aberration.

DISCUSSION

From the present results, it was found that the proportion of mitotic cells in 2-day cultures of peripheral leukocytes stimulated by PHA tended to decrease with increasing age, but was not correlated with other factors, such as sex, radiation dose, and values from several laboratory tests. Pisciotta et al[8] reported that mitotic index and percentage blast transformation were frequently lower in elderly individuals than in younger subjects, findings with which the results of the present study agree.

The correlation between mitotic index and age was supported by the results obtained from autoradiographic study, in which the proportion of mononuclear cells labeled with H^3-thymidine in 3-day cultures tended to decrease with increasing age. Recently, Weksler and Hütteroth[9] reported similar results indicating that leukocytes from elderly individuals incorporated significantly less H^3-thymidine when cultured with plant mitogens and allogeneic cells compared with leukocytes from young persons treated the same way.

Furthermore, our laboratory demonstrated that the proportion of cells in their second division in 3-day cultures tended to decrease with increasing age. The lower percentage of second division cells in 3-day cultures from older individuals suggests that the initiation of DNA synthesis might be delayed in comparison with younger individuals. However, this possibility was ruled out by the results from 2-day cultures where approximately the same percentage of labeled cells (metaphases and mononuclear cells) was found in both the older and younger groups. Since the frequency of labeled metaphases in their first division in 3-day cultures was almost identical in the two age groups, the difference in the frequency of cells in second division between younger and older subjects may not be due to the longer duration of the cell-cycle of cells in culture from the older persons. Thus,

示唆する．3個のX_2Cu細胞のうち，1個は培養中において1回目の分裂にあり，これは少くとも二度にわたる細胞分裂を，すなわち一度は生体内に，もう一度は試験管内において，すでに経過したことを示している．他方，2回目の分裂にあった残り2個の細胞は少くとも試験管内で2度の細胞分裂を経たことを示唆している．これらの所見は，不安定型染色体異常を有する細胞でも培養開始後少くとも一度は分裂し，正常染色体または安定型染色体異常を有する細胞と同様に生存することを示唆している．

考　察

本調査の結果よりPHAによって刺激された末梢白血球の2日間培養における有糸分裂細胞の割合は年齢の増加とともに減少する傾向がみられたが，性，被曝線量および数種の臨床検査の測定値などのような要因とは相関関係がないことが認められた．Pisciotta らは，[8] 分裂指数と芽球化細胞の百分率は若年者よりも高齢者の方がしばしば低いことを報告したが，本調査の結果もこれと一致した．

分裂指数と年齢との相関関係は，オートラジオグラフ法検査から得られた結果によって裏付けられた．すなわち，3日間培養におけるH^3－thymidine 標識単核細胞の割合は年齢増加とともに減少する傾向があった．最近，Weksler および Hütteroth[9] は，同じような結果を報告している．すなわち，白血球を植物分裂誘起剤や異質細胞と共に培養した場合，同じ方法で処理したものでも高齢者のものは若年者のそれと比べてH^3－thymidine の取り込みが著しく低いという．

さらに，当研究室では，3日間培養において2回目の分裂にある細胞の割合が年齢の増加とともに減少する傾向のあることを立証した．高齢者の3日間培養における2回目の分裂にある細胞の百分率が低いのは，DNA合成の開始が若年者に比べて遅延していたことを示唆している．しかし，2日間培養の結果，すなわち，標識細胞（分裂中期および単核細胞）の百分率が若年者および高齢者両群ともほぼ同じであったことからこの可能性はない．3日間培養における1回目の分裂にある標識細胞の頻度が両年齢群ともほとんど同一であったことから，若年者と高齢者間に認められる2回目の分裂細胞の頻度差は，高齢者の培養における細胞周期が長いことによるものではないという可能性を示している．したがって，若年者

it is conceivable that the higher frequency of the second division cells in younger individuals might be due to a greater number of cells having begun DNA synthesis in the younger group compared to the older group. In other words, the older individuals seem to have fewer cells that respond rapidly to PHA and therefore seem to need a longer premitotic stage than do the cells of the younger subjects in vitro.

It has been reported that the frequency of the cells with such radiation-induced chromosome aberrations as dicentrics and rings decreases with increasing culture time.[1] The mechanism for this decrease is presumed to be the inability of these aberrant cells to survive multiple cell divisions. However, this has not been noted among Japanese fishermen exposed to test bomb fallout and A-bomb survivors.[10,11] From the present findings, it is conceivable that if the subjects studied were older individuals, rapid increase of the second division cells in the 3-day culture might not be expected, because of the probably prolonged duration of the premitotic stage. Consequently, a decrease of cells with unstable type chromosome aberrations would not necessarily be evident with prolonged culture time in the older persons.

Generally, it is accepted that cells with unstable type aberrations do not survive through the repeated cell divisions. Our findings, however, demonstrate that some of those cells are apparently able to divide successfully without suffering mechanical disturbance during cell division and survive in vitro to the next cell division. These results support the observations made in 3-day cultures from the exposed fishermen and A-bomb survivors, since both normal and abnormal cells appear equally able to divide and survive in vitro yielding the same proportion of aberrant cells in 2- and 3-day cultures.

If in vitro cultured cells with chromosome aberrations are in the premitotic stage for a longer period than chromosomally normal cells, one might assume that the probability would be higher of finding aberrant cells in 3-day than in 2-day cultures. However, this seems unlikely, from our observations where almost all aberrant cells found in 3-day cultures were in their second division, indicating that the premitotic stage of both normal and aberrant cells was of similar duration.

The mitotic effect of PHA is generally considered to be a kind of immunological reaction, being either a ubiquitous antigen or one capable of stimulating a wide range of otherwise specific immunological clones.[12-14] Therefore, the decreased mitotic activity of lymphocytes from older individuals may

において2回目の分裂細胞の頻度が高いのは，DNA合成を開始した細胞数が高齢者と比べて多いことによるものと考えられる．換言すれば，試験管内でPHAに対して急速に反応する細胞数が高齢者では少なく，大部分の細胞の細胞分裂前期の期間が若年者の細胞よりも長いであろうと考えられる．

2動原体および環状染色体のような放射線誘発性染色体異常を伴う細胞の出現頻度は，培養時間の増加とともに減少することが報告されてきている．[1] この減少の機序は，異常細胞が多くの細胞分裂を経過することができないことに原因があると思われる．しかし，核実験の降下物に被曝した日本人漁夫および原爆被爆者からはこれを証明することはできなかった．[10,11] 本調査所見から，被検者が高齢者ならば，分裂前期の期間の長い細胞が多いと考えられるので，3日間培養で2回目の分裂細胞が急速に増加するとは考えられない．したがって，高齢者において培養時間が長い場合，不安定型染色体異常を有する細胞の減少は必ずしも認められないことになる．

不安定型染色体異常を有する細胞は，幾度も分裂を繰り返すことができないことは一般に認められている．しかし，われわれの研究結果は，試験管内においてこれらの細胞のあるものは細胞分裂中に機械的な障害を受けずに分裂し，次の細胞分裂まで生存でき得ることを証明した．試験管内において正常細胞と異常細胞が同じように分裂して生存するなら，2日間培養と3日間培養において異常細胞が同じ割合で現われることになるので，これらの結果は，被曝漁夫および原爆被爆者についての3日間培養で得たことを裏付けるものである．

もし試験管内で染色体異常を有する細胞の細胞分裂前期の期間が染色体の正常な細胞よりも長いならば，2日間培養よりも3日間培養において異常細胞を検出する可能性が高くなると考えられる．しかし，3日間培養において検出した異常細胞のほとんど全部が2回目の分裂にあり，正常細胞と異常細胞の細胞分裂前期の期間が同じ程度の長さであったということから，上述の可能性はあり得ないことと思われる．

PHAの有糸分裂への効果は，免疫学的反応であると一般に考えられており普遍的な抗原か，さもなくば特定の免疫的クローンを広範に刺激できる抗原のいずれかである．[12-14] 従って，高齢者のリンパ球有糸分裂能の減退

be a consequence of reduced immunological reactivity of leukocytes to PHA. Recently, Weksler and Hütteroth[9] reported that the percentage of thymus-derived lymphocytes responding to foreign histocompatibility antigens was identical in the blood of old and young subjects, and suggested that the reduced reactivity of lymphocytes from older subjects probably resulted from a deficiency of a cofactor necessary for the expression of cellular immunity in thymus-derived lymphocytes. They also mentioned that a low level of thymosin in the blood might be related to the impaired reactivity of lymphocytes from old subjects, since the concentration of thymosin had been found to decline with age.[15]

は，PHAに対する白血球の免疫学的反応の減少の結果であるかも知れない．最近 Weksler および Hütteroth は，[9] 異質の組織適合性抗原に反応する胸腺由来白血球の百分率は高齢者および若年者の血液においても同じであったと報告しており，胸腺由来の白血球における細胞免疫性の発現に必要な補助因子の欠如によって，高齢者における白血球の反応に減退が恐らく生じたものであろうと示唆した．かれらは，また血中 thymosin 濃度が年齢と共に減少しているので，[15] 高齢者における血中 thymosin 値の低下はリンパ球反応の障害と関係があるだろうと述べている．

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