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COMPARISON OF TYPE AND FREQUENCY OF CHROMOSOME ABERRATIONS BY CONVENTIONAL AND G-STAINING METHODS IN HIROSHIMA ATOMIC BOMB SURVIVORS

広島原爆被爆者における染色体異常の種類と頻度の通常法及びG-分染法の併用による比較

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SUMMARY

Somatic chromosomes derived from cultured lymphocytes of 23 atomic bomb survivors of Hiroshima were analyzed to determine the type and frequency of radiation-induced structural aberrations, using in sequence the ordinary staining method (O-method) and the trypsin G-banding method (G-method). Of 896 cells examined, 342 were found to contain induced aberrations, including 31 cells in which the precise identification of the type of aberrations was not possible even by the G-method.

The number of chromosome aberrations observed was 376 in the 311 cells where aberrant precise identification was possible. The majority (288 or 76.6%) were intra- or inter-chromosomal symmetric exchanges due to a two-break event, while only 24 were found to be asymmetric exchanges (dicentrics, rings, and interstitial deletions). Further, there were 28 aberrations showing acentric fragments and terminal deletions, and the remaining 36 were complex intra- and inter-chromosomal exchanges involving three or more breaks which result in insertions and double translocations.

A comparative karyotype analysis of the same metaphases examined by the sequential O- and G-methods was carried out independently on

要 約

広島の原爆被爆者23例の培養リンパ球を用いて，体細胞の放射線誘発性染色体異常の種類と頻度について，通常法とトリプシンG-分染法(G-法)を併用して比較検討した．分析した896個の細胞のうち，染色体異常をもつ細胞は342個であった．この中には，G-法を用いても異常の種類を判別できなかった細胞が31個あった．

G-法で異常の種類の判別が可能な311個の細胞に見られた376個の染色体異常について，詳細に検討した．その大部分(288個，76.6%)は，染色体上の2個の切断に由来する染色体内又は染色体間の相称性交換で占められ，非相称性交換(二動原体染色体，環状染色体，中間部欠失)はわずか24個であった．更に染色体断片や端部欠失が28個あり，そのほかに，3個以上の切断点が関与して形成された挿入や重複転座などの染色体内又は染色体間の複雑な異常が36個識別された．

通常法とG-法の染色体異常の種類と頻度を比較するために，同一細胞について両法を併用して分析した

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361 aberrations, mostly of the symmetric type. It was found that 78 (21.6%) of the 361 were detected only by the G-method; among these were 14 paracentric inversions, 48 reciprocal interchanges of chromosome segments with either equal length (11) or unequal length (37), 14 minor deletions and 2 complex rearrangements, all of which were nevertheless judged to fall within the normal range of variation by the O-method. In contrast, 25 aberrations detected in O-method chromosomes which were over-contracted or twisted, were shown to have normal banding patterns by the G-method.

結果によれば，G-法での相称性異常を主体とする361個の異常のうち，78個（21.6%）はG-法によってのみ識別可能なものであった．その中には14個の偏動原体逆位，同長転座（11個）又は非同長転座（37個）を含む48個の染色体部の相互交換，14個の欠失及び2個の複雑異常があり，これらは，G-法での異常染色体が通常法では形態的に正常範囲内とみなされるものである．これに対して，通常法では染色体の異常な短縮やねじれなどのために異常と判定されたが，G-法では正常と判定されたものが25個みられた．

INTRODUCTION

Radiation-induced chromosome aberrations are known to persist in circulating lymphocytes of Hiroshima and Nagasaki A-bomb survivors for more than three decades after radiation exposure. The frequency of these aberrant cells proved to be proportional to the estimated dose received by each individual.[1,2] Furthermore, symmetric aberrations, such as reciprocal translocations and pericentric inversions, were found to predominate over asymmetric exchanges (dicentrics and rings), and the former aberrations were the major components contributing to the dose-aberration relationship.

Heretofore the usefulness of asymmetric aberrations was considered to be a more sensitive indicator for evaluating the dose-aberration response than symmetric aberrations, because the latter were not easily detectable, and the experience of the microscopist may further influence the observed results.

Recently developed banding methods for identifying individual chromosomes have enabled us to detect more accurately and objectively a variety of radiation-induced structural rearrangements, and some types of symmetric exchanges, previously undetectable by the O-method, can now be identified by these new methods; these include paracentric inversions and intra- and inter-chromosomal symmetric exchanges of chromosomal segments of equal length.

The present report describes radiation-induced chromosome aberrations in the somatic cells of Hiroshima A-bomb survivors, by comparing the results derived from G-method with those from O-method in the same metaphases. Results of comparative analysis of aberrant cells between

緒　言

広島，長崎の原爆被爆者の末梢血リンパ球中には，被爆後30年以上経過した今日もなお，放射線誘発性染色体異常が残存していることが知られている．これらの異常細胞の頻度は，個々の推定被曝線量に比例している．[1,2] 更に染色体異常の中でも，相互転座や挟動原体逆位などの相称性交換が大多数を占めており，非相称性交換（二動原体染色体や環状染色体）は少ない．したがって，前者が線量反応関係の主体となっている．

これまでのところ，線量反応関係を得るためには，相称性交換よりも非相称性交換がより敏感な指標であると考えられている．なぜなら，相称性交換の識別が容易ではなく，しかも検鏡者の経験が観察結果に大きく影響を与えがちである．

近年，開発された分染法によって個々の染色体の同定が可能となり，種々の放射線誘発性の構造異常をより正確に識別できるようになった．つまり，通常法では識別できない相称性交換のあるもの，偏動原体逆位や等長交換による染色体内交換や染色体間交換などが，新しい分染法によって識別が可能となってきた．

本報告では広島の被爆者の体細胞に観察された放射線誘発性染色体異常について，同一分裂中期像に対する通常法並びにG-法による分析を比較検討した結果を述べる．これら2法による異常細胞について

the two methods have already been reported elsewhere,[3] and thus the present report concerns the type and frequency of induced symmetric aberrations, which could not be detectable by the O-method.

MATERIALS AND METHODS

Twenty-three Hiroshima A-bomb survivors, in whom radiation-induced chromosome aberrations were observed in more than 10% of cultured lymphocyte metaphases by previous cytogenetic examination,[1,2] were selected and reexamined cytogenetically using the G-method. Subjects studied here were participants in the RERF Adult Health Study sample who received biennial physical examination,[4] and whose estimated exposure dose was over 100 rad of mixed gamma rays and neutrons.[5] None had received either radiation therapy at any time in the past or any extensive diagnostic irradiation within the year preceding blood drawing.

Peripheral lymphocytes were cultured using the whole blood culture method of Hungerford,[6] with an incubation time of 52 hours, during the last 2 hours of which colchicine was added. Slides were prepared for microscopic examination by hypotonic pretreatment with a solution of 0.075M KCl and 1% sodium citrate, followed by 3:1 alcohol-acetic acid fixation and flame-drying, and finally stained for 20 minutes with 5% Giemsa solution.

Well-spread metaphases were first selected and photographed without mounting the coverslip on the slide. The exact location of each photographed metaphase on the microscopic stage was recorded for the later examination of the same metaphases. After microscopic observations, the slides were soaked with tetrachloroethylene to remove immersion oil from the slide, and then in an acetic acid-methanol mixture to destain the cells, washed by tap water, and dried.

The G-banded preparations were prepared by a minor modification of the trypsin technique of Seabright[7]; the slides were treated in a 0.2% trypsin solution (1:250, Difco) for 1 to 15 sec at room temperature, then washed with running tap water and restained with a 5% Giemsa solution for 10 to 15 minutes.

The banded metaphases, which had been previously photographed, were then relocated,

比較分析した結果は，既に他に報告してあるので，[3] 今回は通常法で識別できない放射線誘発性相称性異常の種類と頻度に限定して報告する．

材料及び方法

これまでの培養リンパ球を用いた細胞遺伝学検査[1,2]に基づいて，10%以上の異常細胞が観察された広島の被爆者23例についてG-法によって再検査を行った．本調査対象者は，放影研成人健康調査集団で2年ごとに医学的検査[4]のために来所しており，推定被曝線量はガンマ線と中性子線とを合わせて100 rad以上であった．[5] 静脈血採血前1年以内の治療用又は多量な診断用放射線被曝例はなかった．

末梢血リンパ球培養はHungerford[6]の方法に従い，全血を52時間培養し，最終の2時間にコルヒチンを添加した．染色体標本は低調処理として，0.075 Mの塩化カリウムと1%のクエン酸ナトリウム溶液の混合低調液による前処理後，メタノール：酢酸が3：1のカルノア液で固定し，火炎乾燥によって作製した．更に5%のギムザ液で約20分間染色した．

よく広がった分裂中期像を選び，カバーグラスを掛けずに検鏡し，顕微鏡写真を撮った．以後に行われる同一細胞に対する分析のために，個々の分裂中期像の顕微鏡上の正確な位置を記録した．検鏡後，スライド上の検鏡オイルをテトラクロールエチレンで洗い落とし，酢酸とメタノールの混合液で脱色後，水道水で洗って乾燥した．

G-分染法はSeabright[7]のトリプシンG-分染法の変法を用いた．染色体標本を，0.2%のトリプシン溶液（1：250，Difco社製）で1～15秒間室温処理し，水道水で洗った後5%のギムザ液で10～15分間再染色した．

あらかじめ通常法で顕微鏡写真を撮った同一分裂

reexamined, and again photographed for karyotype analysis. All of the cells with definite or suspected structural rearrangements detected by either or both the O- and G-methods were karyotyped separately using the printed photographs to determine the type and frequency of radiation-induced chromosome aberrations by both methods.

Chromosome aberrations were classified into the following two groups according to the number of breaks involved in the formation of aberrations: simple aberrations produced by one or two breaks, and complex aberrations, involving three or more breaks.

RESULTS

Of a total of 896 metaphases analyzed by both methods, 342 cells (38.2%) were found to show radiation-induced chromosome aberrations. In 31 cells of these 342, aberrations were so complicated that even the banding method could not specify the types of aberrations (unidentifiable cells). Therefore, an analysis of the type and frequency of chromosome aberrations was restricted to the remaining 311 identifiable aberrant cells. Among these aberrant cells, 376 aberrations were detected, the majority of which were classified as simple aberrations (340 or 90.4%). The remaining 36 (9.6%) were identified as complex aberrations (Table 1).

Among the simple aberrations, the term "acentric fragment" is used when the acentric material was present in the complement, and "terminal deletion" indicates the loss of an acentric part from the broken chromosome in the complement, perhaps being eliminated through the preceding mitoses. There were only 28 (7.4%) aberrations produced by a simple break, consisting of 8 acentric fragments and 20 terminal deletions (Table 1). In the present study, four minute fragments, which also could be acentric rings, were detected and tentatively classified as acentric fragments.

The majority of chromosome aberrations observed were exchanges involving two breaks (312 or 83.0% of total aberrations). There were only 24 asymmetric exchanges; 4 dicentrics, 2 rings, 1 acentric ring, and 17 interstitial deletions (Table 1). The remaining 288 (76.6%) were symmetric exchanges among which were 234 reciprocal translocations (62.2%), 40 pericentric

中期像をG-法で処理した後に再び写真に撮り，核型分析を行った．構造異常を疑われたすべての細胞について，写真による通常法とG-法との核型分析を別個に行い，放射線誘発性染色体異常の種類と頻度について比較検討した．

染色体異常は異常の生成に関与した切断点の数に基づいて，1個又は2個の切断点を有する異常を"単純異常"とし，3個以上の切断点をもつ異常を"複雑異常"と名付けた．

結　果

通常法とG-法の双方で分析可能な細胞は896個あり，342個(38.2%)の細胞に放射線誘発性染色体異常が観察された．この342個の中には，異常が極めて複雑なために分染法を用いても，異常の種類を決めることができない細胞(分析不可能の細胞)が31個認められた．したがって，残りの311個の識別可能な異常細胞に見られた染色体異常の種類と出現頻度について検討した．これら異常細胞中に376個の異常が識別され，その大多数(340個，90.4%)は単純異常に分類されるものであり，残りの36個(9.6%)は複雑異常であった(表1)．

単純異常の中で「染色体断片」という呼称は，細胞内に動原体部位を含まない染色体が存在する場合として用い，「端部欠失」は，切断染色体に由来する染色体断片が，細胞分裂の過程で消失したために観察されない場合と定義した．単一切断に基づく染色体断片8個と端部欠失20個，計28個(7.4%)の異常が観察された(表1)．本観察で，微小染色体断片4個が認められた．環状染色体断片の可能性も考えられるが，ここでは一応染色体断片として分類した．

染色体異常の大部分(312個，83.0%)は2個の断点を含む交換型異常であり，非相称性交換はわずか24個であった．4個の二動原体染色体，2個の環状染色体，1個の環状染色体断片，並びに17個の中間部欠失が観察された(表1)．したがって残りの異常288個(76.6%)は相称性交換であった．その内訳は

TABLE 1 TYPE AND FREQUENCY OF CHROMOSOME ABERRATIONS IDENTIFIED BY G-METHOD IN 311 ABERRANT CELLS* FROM 23 HEAVILY EXPOSED A-BOMB SURVIVORS OF HIROSHIMA

表1 広島の強度原爆被爆者23名におけるG-法によって識別された311個の異常細胞*中の染色体異常の種類と頻度

Type	Number (%)		
Simple aberration:	340 (90.4)		
One break	28 (7.4)		
Acentric fragment		8	(2.1)
Terminal deletion		20	(5.3)
Two breaks	312 (83.0)		
Asymmetric exchange	24 (6.4)		
Dicentric		4	(1.1)
Ring		2	(0.5)
Acentric ring		1	(0.3)
Interstitial deletion		17	(4.5)
Symmetric exchange	288 (76.6)		
Reciprocal translocation		234	(62.2)
Pericentric inversion		40	(10.6)
Paracentric inversion		14	(3.7)
Complex aberration:	36 (9.6)		
Insertion		13	(3.5)
Complex translocation		13	(3.5)
Complex exchange		10	(2.7)
Total	376 (100.0)		

*Excluding 31 unidentifiable cells 異常識別不能の細胞31個は除外した.

inversions (10.6%), and 14 paracentric inversions (3.7%). Of the 234 translocations, 5 were characterized as incomplete exchanges owing to the loss of one of the two chromosome segments participating in the exchange.

Complex aberrations described here were further divided into three groups as shown in Figure 1; 1) either direct or inverted "insertion" within a chromosome or between two chromosomes due to three-break rearrangements (Figure 1A, B), 2) "complex translocations" such as sequential exchanges and double reciprocal translocations due to rearrangements with at least three breaks (Figure 1C), and 3) three- or more-break rearrangements by a combination of a translocation and an insertion (Figure 1D), an inversion and a translocation, and so on, referred to as "complex exchanges".

Of the 36 complex aberrations, 13 insertions, 13 complex translocations, and 10 complex exchanges were observed (Table 1). Partial karyotypes of representative complex aberrations

相互転座が234個(62.2%), 挟動原体逆位が40個(10.6%), 及び偏動原体逆位が14個(3.7%)識別された. 転座234個中の5個は, 2本中の交換染色体部位の一方が消失した, いわゆる不完全型交換であった.

複雑異常を, 図1に示すように以下の3群, つまり1) 3個の断点からなる再配列で, 1本又は2本の染色体が関与する直接方向又は逆位の「挿入」(図1A, B), 2) 少なくとも3個の断点を含む連続的交換や重複相互転座などの「複雑な転座」(図1C), 及び3) 転座と挿入(図1D), 逆位と転座などが相互に組み合わされた少なくとも3個の断点を含む異常を「複雑な交換」, に分けた.

36個の複雑異常の内訳は, 挿入が13個, 複雑な転座が13個及び複雑な交換が10個であった(表1). 代表的な複雑異常の核型分析の一部を図2に示す. 2個の

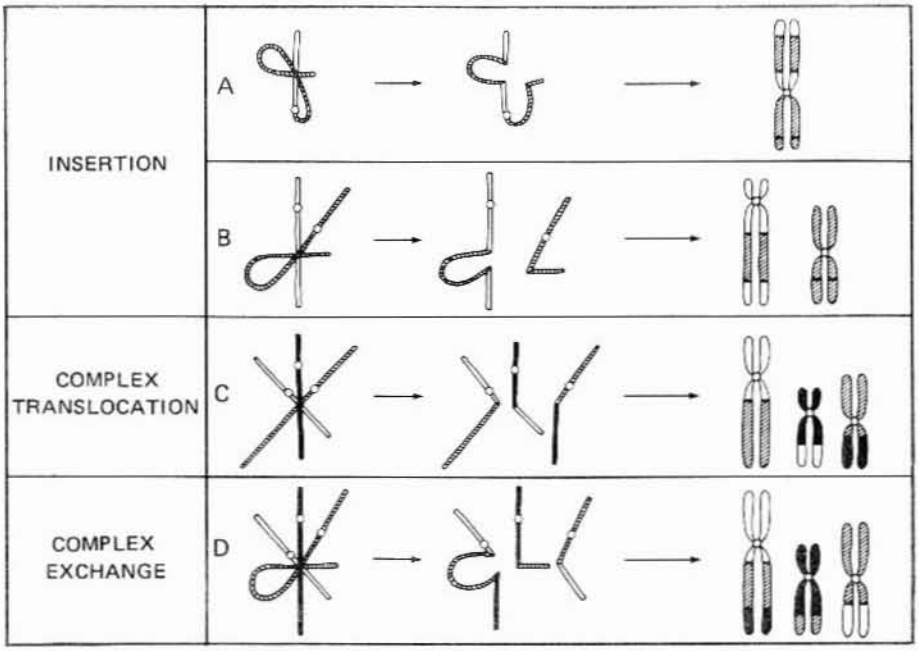

Figure 1 Diagramatic representation of complex chromosome aberrations: A–insertion within a chromosome; B–insertion between two chromosomes; C–complex translocation involving three chromosomes; and D–complex exchanges produced from a combination of insertion and translocation.

図1　複雑な染色体異常の模式図．A－1本の染色体内の挿入，B－2本の染色体間の挿入，C－3本の染色体が関与した複雑な転座，及びD－挿入と転座を合併した複雑な交換異常．

are shown in Figure 2. In two complex exchanges, partial deficiency of a chromosome segment was observed, whereas in the remaining 34 complex aberrations, neither deficiency nor duplication of a chromosome segment could be seen.

Excluding 15 asymmetric aberrations from the total of 376 aberrations observed, the remaining 361 were classified into the following three groups; 1) aberrations identical by both methods, 2) aberrations not identical though detectable by both methods, and 3) aberrations detected only by the G-method. Of the 361 aberrations, 188 (52.1%) were classified into the first group: 153 translocations, 23 inversions, and 12 deletions (Table 2). The second group included 95 (26.3%) aberrations: 41 translocations, 9 inversions, 11 deletions, and 34 complex aberrations. The majority of reciprocal translocations in this group were identified as pericentric inversions or terminal deletions by the O-method, since the abnormal counterpart could not be identified by this method. For the same reason, complex aberrations identified by the G-method were

複雑な交換に染色体の部分的欠失が観察されたが，残りの34個の複雑異常には，欠失も重複も認められなかった．

総異常数376個のうち，15個の非相称性異常を除いた361個の異常は次の3群，すなわち1)通常法とG-法で異常の種類が同じもの，2)両法ともに異常が識別されても異常の種類が異なるもの，3)通常法では識別されずG-法にのみ識別された異常，に分類した．第1群には188個（52.1%）が属し，153個の転座，23個の逆位，12個の欠失が含まれる（表2）．第2群には95個（26.3%）が含まれ，41個の転座，9個の逆位，11個の欠失及び34個の複雑異常があった．このうちの多くの相互転座では，異常に関与する2本の染色体のうちの片方が通常法では識別できなかったため，挟動原体逆位や端部欠失として判定されていた．同様な理由によりG-法で識別された複雑な

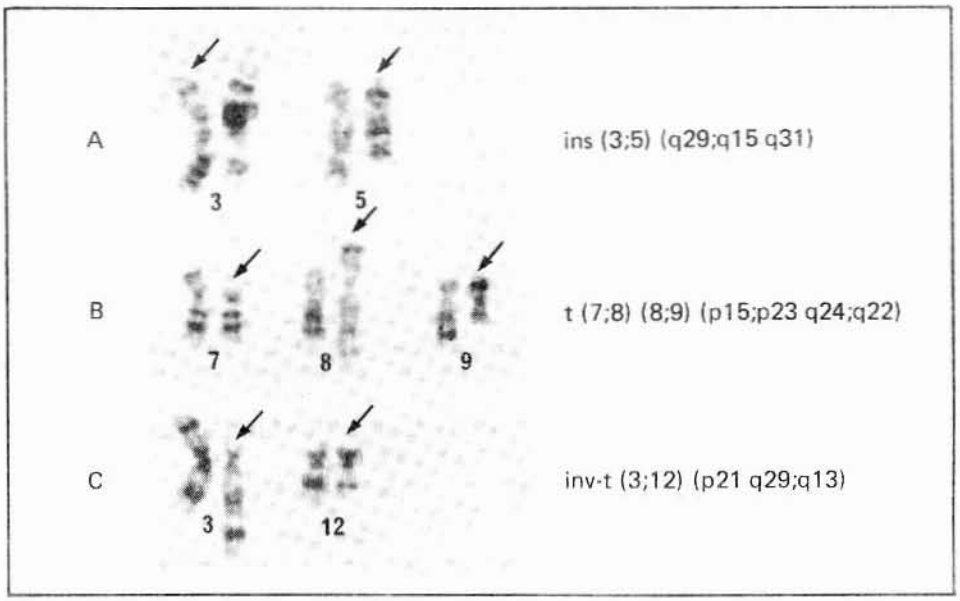

Figure 2 Partial karyotypes of three representative complex aberrations: A—insertion between chromosomes 3 and 5; B—double reciprocal translocations involving chromosomes 7,8, and 9; C—complex exchanges derived from translocation between chromosomes 1·2 and 3 also carrying a pericentric inversion.

図2　3個の代表的な複雑異常の部分的核型分析図．A－第3染色体と第5染色体間の挿入，B－第7，第8，第9染色体を含む重複相互転座，C－抗動原体逆位を有する第3染色体と，第12染色体との転座が関与する複雑な交換異常．

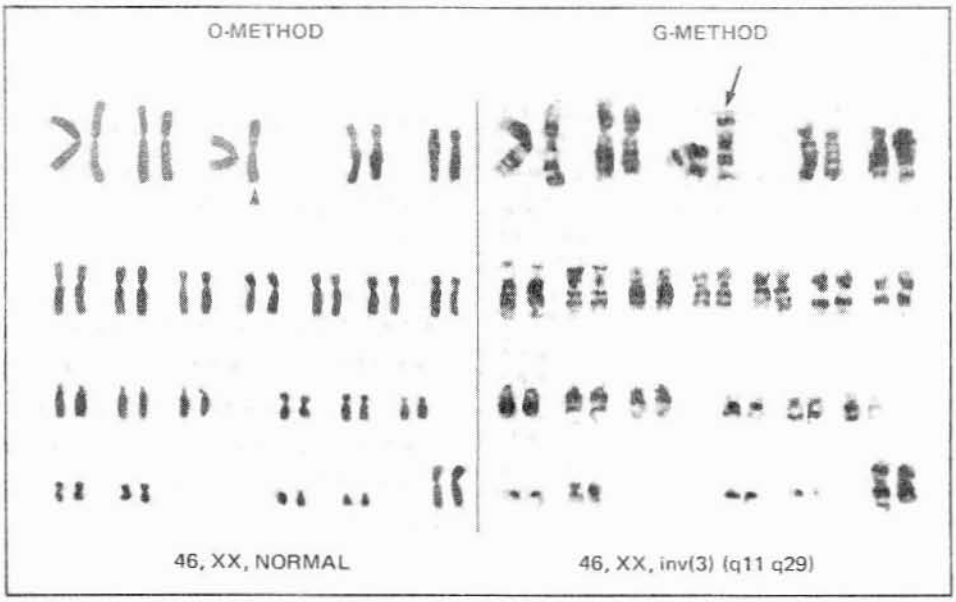

Figure 3 Parallel karyotype analyses of the same metaphase obtained by O-method and G-method. Paracentric inversion of a chromosome 3 identified by the G-method (arrow), is undetected by the O-method (arrow).

図3　通常法とG-法より得られた同一中期分裂像の核型の比較，G-法により識別される第3染色体の偏動原体逆位（矢印）は，通常法（矢印）では判別できない．

TABLE 2 COMPARISON OF CHROMOSOME ABERRATIONS* IN THE SAME METAPHASE BETWEEN O-METHOD AND G-METHOD

表2 同一中期分裂像における通常法とG-法による染色体異常*の比較検討

Classification			Aberrations by G-method					
O-method	G-method	O:G	t	inv	del	complex	Total	%
Abnormal :	Abnormal	Identical	153	23	12	0	188	52.1
Abnormal :	Abnormal	Not identical	41	9	11	34	95	26.3
Normal :	Abnormal	Not identical	40	22	14	2	78	21.6
Total			234	54	37	36	361	100.0

*Excluding 15 asymmetric aberrations (8 acentric fragments, 4 dicentrics, 2 rings, and 1 acentric ring).
15個の非相称性異常（8個の染色体断片，4個の二動原体染色体，2個の環状染色体及び1個の環状染色体断片）を除外した．

TABLE 3 CLASSIFICATION OF 78 ABERRATIONS DETECTED EXCLUSIVELY BY G-METHOD

表3 G-法によってのみ識別された78個の染色体異常の分類

Type	Aberration					
	t	inv	del	complex	Total	%
Paracentric inversion	-	14	-	-	14	17.9
Equal length exchange	11	0	-	0	11	14.1
Unequal length exchange	29	8	-	2	39	50.0
Minor deletion	-	-	14	-	14	17.9
Total	40	22	14	2	78	99.9

classified as simple type aberrations such as translocations, inversions, or deletions by the O-method.

Seventy-eight (21.6%) aberrations in the third group were detected exclusively by the G-method, and they were anticipated a priori to be either paracentric inversions or reciprocal translocations of chromosome segments of equal length. In fact, only 14 (17.9%) were identified as paracentric inversions (Figure 3), and 11 (14.1%) as translocations of chromosome segments of equal length (Table 3). Among the remaining 53 aberrations, 39 (50.5%) were intra- and inter-changes of chromosome segments of unequal length (Figure 4), and 14 (17.9%) were deletions with a small segment at the distal end which were judged to be within the normal limits of variation by the O-method.

As shown in Figure 5, 39 exchanges between chromosome segments of unequal length in the third group were further divided into two subgroups: a) both abnormal chromosomes were normal in appearance as judged by the O-method, and b) both excess and deficiency of the

異常は，通常法では転座や逆位又は欠失などの単純な異常と判定していた．

第3群に入る78個（21.6%）の異常はG-法によってのみ識別された．これらは，偏動原体逆位や等長染色体部位の交換による相互転座が予想されていたが，実際には，偏動原体逆位がわずか14個（17.9%）しか認められなかった（図3）．交換部分の等しい相互転座は11個（14.1%）であった（表3）．残りの異常53個中，39個（50.0%）は交換部位に長短が認められる染色体内又は染色体間交換であり（図4），14個（17.9%）の異常は染色体末端部の部分的欠失であったが，そのいずれもが通常法では正常範囲内であると判定されるものであった．

第5図に示すように，第3群中の交換部分の長さの異なる異常39個は，更に，a）2本の異常染色体が通常法ではそれぞれ正常染色体の範囲内にあると判定される場合と，b）長短の生じた異常染色体が

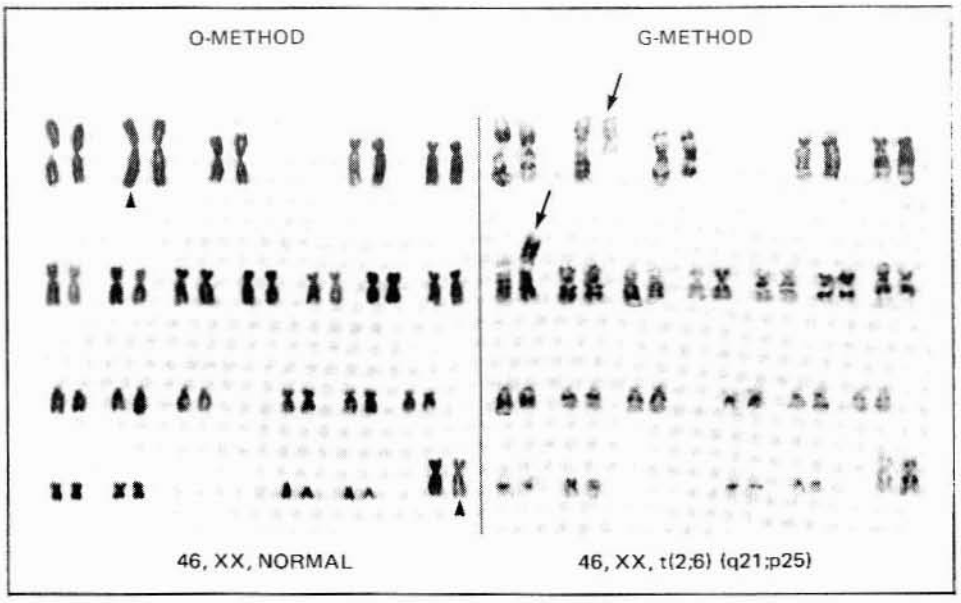

Figure 4 Parallel karyotype analyses of the same metaphase obtained by O-method and G-method. No karyotypic abnormality is seen in the O-method (arrows) while a reciprocal translocation between chromosomes 2 and 6 (arrows) is identified by the G-method.

図4　同一分裂中期像に対する通常法とG-法による核型分析の比較．通常法（矢印）では何ら異常が認められないが，G-法では第2染色体と第6染色体の相互転座（矢印）が識別される．

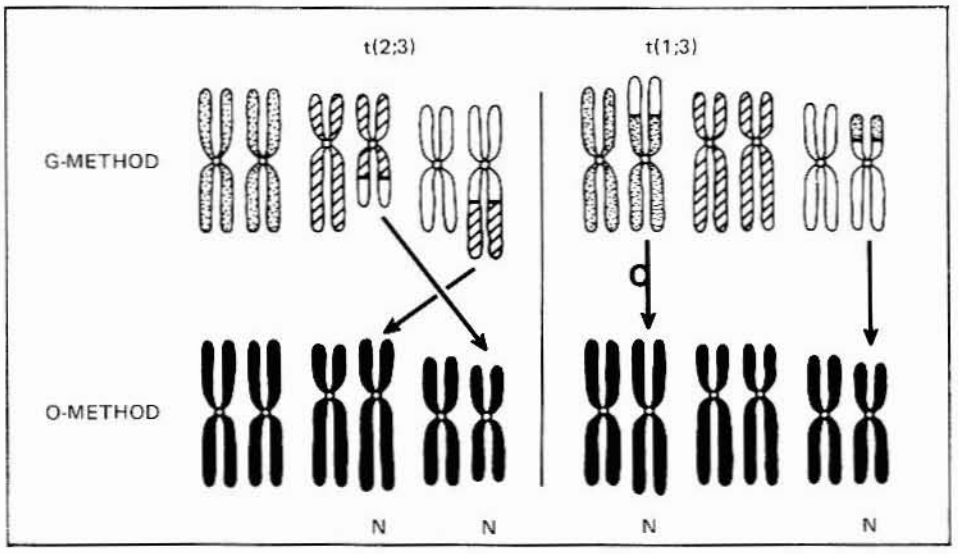

Figure 5 Diagramatic representation of reciprocal translocations between chromosome segments of unequal lengths which are undetectable by the O-method. Left–two abnormal chromosomes are in fact misaligned in their positions between each other, thus leading to a normal chromosome constitution, and right–excess and deficiency in the chromosome material of the two abnormal chromosomes are so subtle that the chromosome constitution is judged to be in the normal limits of vairiation by the O-method.

図5　通常法では判別不能の染色体の交換部分に長短のある相互転座の模式図．左図－通常法では2本の異常染色体が並べ換えによって正常と判定されたもの．右図－2本の異常染色体における染色体物質量の増減の程度が極めて微細なため，通常法では正常範囲内と判定されたもの．

chromosome material due to exchanges of the segments with unequal length were so subtle that there was no apparent morphological change in the chromosome constitution as observed by the O-method. The former comprised 17 translocations and 5 inversions while the latter included 12 translocations, 3 inversions, and 2 complex aberrations.

There were 25 aberrations detected only by the O-method among which 21 aberrant chromosomes were found to be either overcontracted or unusually twisted, while by the G-method the banding patterns proved to be normal. In the remaining four aberrations observed, exchanges (or breaks) could conceivably have occurred at the negative regions of the distal part of the chromosomes. This indicates that even the G-method fails to detect structural aberrations if they occur at the negative bands of the chromosome.

DISCUSSION

The present study of the somatic chromosomes of 23 Hiroshima survivors using the G-method has confirmed our previous findings[1,2] in which asymmetric exchanges were found to be less frequent than symmetric aberrations (6% vs 77% of the total aberrations observed). This suggests that the symmetric exchanges may be the more useful indicators for evaluating the relationship between chromosome aberrations and radiation dose, particularly for those who were exposed many years before the cytogenetic examination.

Until banding techniques for identifying individual chromosomes were developed, the identification of symmetric exchanges by the conventional staining method was technically limited, since only those showing either an unusual shift in the position of the centromere or abnormal arm length were recognizable as abnormal monocentrics and thus constitute only a small proportion of the true symmetric exchanges. Paracentric inversions and some of the reciprocal translocations where the exchanged segments are equal in length are undetectable by the O-method, and it is estimated that the efficiency of scoring symmetrical rearrangements in cultured human lymphocytes following irradiation may be as low as 20%.[8-10]

In the present study, it was found that 22% of G-method symmetric exchanges (62 of 288)

互いに置き換えられた結果，通常法では正常の核型と判定せざるを得ない場合，の二つのサブグループに分けられる．前者には17個の転座，5個の逆位があり，後者には12個の転座，3個の逆位，2個の複雑異常が含まれていた．

通常法でのみ識別された異常が25個観察された．これらの異常に含まれる21本の異常染色体はG-法によると，染色体が極端に縮んだり，ねじれているもので，Gバンドパターンは正常であった．残りの4個の異常は，染色体端部のGバンドのよく染まらない部分に生じた交換（切断）であった．このことは，G-法を用いても，染色体の淡バンド部で生じた構造異常が識別されにくいことを示している．

考　察

G-法を用いた23例の広島原爆被爆者の体細胞染色体分析に基づく本研究においても，非相称性異常が相称性異常よりも低頻度（異常総数の6％対77％）であるという，これまでの研究結果[1,2]が確認された．このことは，細胞遺伝学調査の何年も前に被爆した人々においては，染色体異常と被曝線量との相関関係を評価するために，相称性交換が特に有力な指標になり得ることを示唆している．

分染法を用いて個々の染色体を識別できるようになるまでは，通常法による相称性交換の識別には，技術的に限界があった．つまり染色体の着糸点部位の移動，あるいは染色体の腕の長さの異常だけが異常染色体判別の尺度になっているためであり，それは相称性交換全体のうちの一部を占めていると考えられている．偏動原体逆位と等長交換部位による相互転座のあるものは，通常法では判別できないし，放射線照射後におけるヒト培養リンパ球に見られる相称性交換異常のうちの20％程度しか識別されないのではないかと推定されている．[8-10]

今回の研究では，G-法で識別した相称性交換の22％（288個中62個）が通常法では判別できなかった．言い

were undetectable by the O-method, in other words, about 78% of the symmetric exchanges identified by the G-method were also detected by the O-method. This efficiency of scoring symmetric exchanges by the O-method is higher than expected, assuming that all the symmetric exchanges are detectable by G-method analysis.

One-third (25 of 78) of the aberrations by G-method showed paracentric inversions and reciprocal translocations of the chromosome segments with equal length. No pericentric inversion due to breaks at equidistant points from the centromere and subsequent rejoining were observed in the present study.

Nevertheless some of the unequal length exchanges could not be detected by the O-method. One possible explanation for this is that both of the abnormal chromosomes thus produced fell within the normal range of variation for the corresponding chromosome groups in terms of the length and the position of centromere, as shown in Figure 5 (37 of the 78 undetectable-type aberrations belonged to this category). Further, 14 minor terminal deletions and 2 complex aberrations were also undetected by the O-method.

It is worth noting that complex exchange aberrations involving three or more breaks observed by the G-method were present in the lymphocytes of A-bomb survivors with a frequency of about 10% of total aberrations so far detected.

Seabright[11] reported, from in vitro irradiation experiments of human lymphocytes using the G-method, that 8 complex exchanges, including double reciprocal translocations between three chromosomes and simultaneous production of one dicentric and one translocation chromosome, were observed in a total of 131 aberrations. Complex exchanges were also identified by Buckton[12] in X-irradiated peripheral lymphocytes in vitro, in which 8 translocations between three chromosomes and 3 dicentrics with the acentric fragments translocated onto another chromosome were detected by sequential R- and G-methods.

In the present examination, however, there were no complex aberrations involving dicentrics or rings, suggesting that cells with unstable complex aberrations would have been eliminated from the lymphocyte population due to mitotic distur-

換えれば，G-法で識別した相称性交換の約78%は通常法でもまた判別された．G-法によってすべての相称性交換が識別されると仮定した場合，本研究における通常法による相称性交換の検出率は，予測されるものよりも高いものであった．

G-法によってのみ識別された異常の¼（78個中25個）は，偏動原体逆位及び同じ長さの染色体部分が交換した相互転座であった．今回の研究では，着糸点部位から等しい距離に切断点を有する挟動原体逆位は観察されなかった．

染色体の交換部分の長さが異なるにもかかわらず，交換異常の中には通常法で識別できないものがあった．それを説明するものとして，通常法では染色体の長さと着糸点部位の位置によってのみ判別されるために，図5で示すように，通常法ではそれぞれの長さと腕比が正常範囲内にあると判定されるためである（これで説明される異常は，78個のうち37個であった）．更に，通常法で判別できなかった異常には，染色体端部微小部分の欠失が14個と複雑異常2個があった．

特記すべきことは，G-法による観察から切断点を3個以上も含む複雑な交換異常が，原爆被爆者のリンパ球中に現在もなお総異常の約10%の頻度で観察されたことである．

G-法を用いた Seabright[11] の in vitro におけるヒトの放射線照射実験において，131個の総異常の中で8個の複雑な交換異常を観察した．それらは，3本の染色体に関与した重複相互転座や，二動原体染色体と転座染色体が同時に見られるものであった．また複雑な交換異常は Buckton[12] のヒトの末梢血リンパ球X線照射実験でも観察され，それらは，3本の染色体が関与した複雑な転座が8個，染色体断片が他の染色体に転座している複雑な二動原体が3例見られ，R-バンド，G-バンドの連続染色法によって識別されている．

今回の観察では二動原体染色体や環状染色体を含む複雑異常は観察されなかった．これは不安定型の複雑異常をもつ細胞が，被爆後の時間の経過とともに細胞の分裂障害などにより，リンパ球集団から消失

bance in the lapse of time that has occurred after in vivo exposure to A-bomb irradiation.

Of the 342 aberrant cells detected, there were 31 unidentifiable cells in which a total of 145 chromosomes were found to show abnormal banding patterns, and thus considered to have participated in exchange formations. There were already 36 cells with complex but identifiable exchanges, yielding a total of 67 cells with complicated structural rearrangements of chromosomes which have persisted to date in the circulating lymphocytes of A-bomb survivors.

In the present study, no attempt was made to analyze the chromosome aberration frequency by estimated radiation dose for individual survivors because of the paucity in the number of cases as well as in the number of cells per case.

Since G-method analysis is time-consuming requiring laborious works, our current studies using the G-method have been restricted to selected subjects, such as heavily exposed A-bomb survivors who were identified by the previous examination as having clones of cells with radiation-induced chromosome aberrations.

したためであろう．

342個の異常細胞の中でも，31個の異常細胞は識別不能の異常分染パターンを示したが，この31個の中には交換異常の生成に関与していると思われる145本の異常染色体が観察された．既にG-法により複雑な異常をもつ36細胞と合わせて，67細胞が原爆被爆者の末梢血リンパ球中に，今なお残存していることが観察された．

今回の研究では例数も少なく，しかも1例当たりの分析細胞数も少なかったために，個々の被爆者の推定被曝線量に基づく染色体異常の頻度に関する分析は行わなかった．

G-法による染色体分析は極めて多くの時間を費すために，既に確認されている放射線誘発性染色体異常をもつ細胞のクローン例など，強度原爆被爆者群のみに限定して，G-法による染色体検査を集中的に行っている．

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