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SOFT X RAYS FOR RADIOBIOLOGICAL STUDIES
放射線生物学的研究のための軟X線

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SOFT X RAYS FOR RADIOBIOLOGICAL STUDIES
放射線生物学的研究のための軟X線

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SUMMARY
Lethal effects and chromosome aberrations induced in cells exposed to low energy (soft) X rays demonstrated that these relatively low energy X rays are just as effective as those of higher energy for radiobiological studies, and even more effective for irradiating cultured mammalian cells than laboratory animals.

INTRODUCTION
Prior to Puck and Fishers' successful cloning of mammalian cells in tissue cultures,[1] studies in cell biology and cytogenetics were possible only when using microorganisms. In radiobiology, there have been numerous studies concerning mutation induction, neoplastic transformation of cells, acceleration of aging, chromosome aberrations, cell membrane damage, intracellular metabolic abnormalities, and damage of intracellular macromolecules (DNA, RNA, proteins) following irradiation. Often used in these studies were high energy X rays produced by apparatus for deep radiation therapy employing relatively high kilovoltage and milliamperage, and gamma rays from ^{60}Co and ^{137}Cs sources. These X rays and gamma rays can penetrate the glass and plastic vessels used in culturing cells, allowing relatively accurate assessments of the cells' absorbed doses. However, high energy X-ray generators and ^{60}Co and ^{137}Cs sources are expensive, and they necessitate using costly protective barriers. Furthermore, X-ray tubes for high energy generators are often in short supply, particularly in Japan.

A soft X-ray generator was recently installed at RERF for radiobiological studies using cultured mammalian cells. The distribution of exposure

要 約
低エネルギー(軟)X線を照射した細胞に致死的影響及び染色体異常が誘発されることは，比較的低エネルギーのX線が，高エネルギーX線と同様，放射線生物学的研究に有効であり，また，実験動物よりも哺乳類培養細胞の照射により一層有効であることを立証した．

緒 言
Puck 及び Fishers[1] が，組織培養における哺乳類細胞クローン化に成功する以前は，細胞生物学及び細胞遺伝学における研究は，微生物を用いた場合にのみ可能であった．放射線生物学では，放射線照射後に認められる突然変異誘発，細胞の腫瘍性形質転換，加齢促進，染色体異常，細胞膜障害，細胞内代射異常及び細胞内高分子(DNA, RNA, 蛋白質)障害などに関して多くの研究が行われてきた．これらの研究においては，比較的高い電圧(kV)及び電流(mA)を用いる深部治療用の放射線装置から発生する高エネルギーX線，並びに ^{60}Co と ^{137}Cs 線源から放出されるガンマ線がしばしば使用された．これらのX線及びガンマ線は，細胞の培養に用いられているガラス容器やプラスチック容器を透過するので，細胞の吸収線量を比較的正確に測定できる．しかし，高エネルギーX線発生装置や ^{60}Co 及び ^{137}Cs 線源は高価であり，また，高価な防護壁を用いる必要性も生じてくる．更に，高エネルギー発生装置用のX線管は，特に日本においては不足がちである．

最近放影研では，哺乳類培養細胞を用いる放射線生物学的研究のための軟X線発生装置が設置された．

dose rates from this apparatus was determined under various conditions.[2] In the present study, the dose-survival responses and dose-chromosome aberration frequencies were determined by irradiating a variety of cells with soft X rays. The results were compared with those obtained after irradiating cells with high energy X rays and ^{60}Co-gamma rays.

MATERIALS AND METHODS

Ionization Radiation

X-ray exposures were incurred using a low energy X-ray generator (Softex, Model CMBW-2, Softex Company, Tokyo). Its X-ray tube (IF-0630) has a beryllium window 0.5 mm thick which absorbs only 3% of the X rays emitted when using 5 mA and 25 kVp. The 2.0 mm thick glass windows of conventional X-ray tubes absorb 99.3% of the X rays produced using similar exposure conditions, thus allowing only 0.7% of the X rays to exit. The results reported here were obtained using X rays which had penetrated a 0.2 mm thick aluminum filter immediately exterior to the soft X-ray tube's window. Cells were exposed to X rays (40 kVp, 5 mA, 0.2 Al filter, and 0.23 mm Al half-value layer, HVL) in a 1 ml cell suspension (1×10^5 cells/ml) on 5 cm diameter plastic petri dishes without a cover. To prevent the shadow effect from dish wall, cell suspension medium was uniformly dispersed within a circle area of 3.0-4.0 cm diameter. In this condition, about 16% of X rays were absorbed by medium. The exposure dose rate, determined with the Nuclear Associates Type 30-330 PTW ionization chamber was 220 rad/min.

A deep X-ray therapy generator (200 kVp, 20 mA, 0.3 Cu + 0.5 Al, 1.2 mm Cu HVL, 60 R/min, Kyoto University) and ^{60}Co-gamma ray energy (1.17 and 1.33 MeV, average 1.25 MeV, 180 rad/min, Research Institute for Nuclear Medicine and Biology, Hiroshima University) were used as reference sources. Cells suspended in 1 ml medium were put into test-tubes and exposed to high energy X rays or ^{60}Co-gamma rays.

Cells

Some of the human lung fibroblasts used in the present study were established elsewhere;[3-7] the remainder were established in our laboratory. The skin cell cultures of patients with ataxia telangiectasia (AT cells), an autosomal recessive hereditary disease, were established by Taylor

この装置を用いた場合の照射線量率の分布が様々な条件下で測定されている.[2] 本研究においては，軟X線を種々の細胞に照射して，線量-生存反応並びに線量-染色体異常頻度を測定した．その結果を，高エネルギーX線照射及び^{60}Co ガンマ線照射の場合と比較した．

材料及び方法

電離放射線

X線照射には，低エネルギーX線発生装置（東京 Softex 社製，Softex，CMBW-2 型）を用いた．本装置のX線管（IF-0630）には厚さ 0.5mm のベリリウムの窓があるが，これは，5 mA，25kVp 下で放出されたX線の3％しか吸収しない．従来のX線管における厚さ 2.0mm のガラス窓は，同様の照射条件下で発生するX線の99.3％を吸収するので，全X線のうち0.7％しか外部に放出されないことになる．ここで報告する結果は，軟X線管の窓のすぐ外側にある厚さ0.2mm のアルミニウム・フィルターを透過したX線を用いて得られたものである．直径 5 cmの無蓋のプラスチック・ペトリ皿に入れた 1 ml の細胞浮遊液（1×10^5 個/ml）にX線を照射した（40kVp，5 mA，0.2Al フィルター，0.23mm の Al 半価層，HVL）．ペトリ皿の壁による陰影効果を防ぐため，細胞浮遊培地を直径3.0～4.0cmの円形領域内に均等に分散させた．この条件下で約16％のX線が培地に吸収された．Nuclear Associates 30-330PTW 型の電離箱で測定した照射線量率は220rad/分であった．

深部治療用X線装置（京都大学．200kVp，20mA，0.3Cu ＋0.5Al，1.2mm の Cu HVL 60R/分）と ^{60}Co ガンマ線エネルギー（広島大学原爆放射能医学研究所．1.17MeV 及び 1.33MeV，平均 1.25MeV，180rad/分）を比較線源として用いた．1 ml の培地に浮遊させた細胞を試験管に加え，高エネルギーX線又は ^{60}Co ガンマ線を照射した．

細　胞

本研究で用いたヒト肺線維芽細胞のうち，幾つかは他の研究室で樹立されたものであるが，[3-7] 残りは当研究室で樹立したものである．常染色体劣性遺伝病である毛細管拡張性運動失調症患者の皮膚培養

et al[8,9] and Arlett,[10] and obtained from Professor Osamu Nikaido (Kanazawa University). Chinese hamster (V79) cells[11] were also obtained through the courtesy of Professor Nikaido. All cells used in the present study were cultured in plastic dishes (Falcon Company, USA, Cat. No. 3002).

Culture Medium

Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS) was used for subcultivation and maintenance of the fibroblasts derived from human lung. In tests of cell survival rates by the colony formation method, either Eagle's MEM or alpha MEM supplemented with 15% FCS was used. Alpha MEM supplemented with 15% FCS was used for subculture and for all tests of AT cells. Eagle's MEM supplemented with 10% FCS was used for subculture and for all tests of V79 cells.

Chromosome Analysis

V79 cells in the logarithmic growth phase were irradiated and incubated for eight hours at 37°C in a 5% CO_2 - 95% air atmosphere. The chromosome slides were prepared by the usual flame method[7] with hypotonic treatment of the cells using 0.075 M KCl (2 volumes) + 1% Na citrate (1 volume) buffer solution after one hour colchicine treatment.

RESULTS

Comparison of Lethal Effects of Soft X rays and High Energy X rays on Exposed Normal Human Lung Cells

The sensitivity of human fibroblasts to soft X rays and high energy X rays is illustrated in Figure 1. High energy X rays were used to irradiate at a dose rate of 60 R/min.[3] A dose-survival curve with an extrapolation number, n value, of 2.2 and D_0 of 120 R was obtained.[3] Soft X rays (40 kVp, 5 mA, 0.2 Al filter) were used to irradiate at a dose rate of 220 rad/min. Although the cells were lung fibroblasts obtained from autopsy materials of donors of various ages, no difference in radiation sensitivity by age of the cell donor was observed. A dose-survival curve with an n value of 1.8 and D_0 of 125 rad was obtained. Thus, no difference was evident between high energy X rays and soft X rays as to their lethal effects on cultured human cells.

細胞(AT細胞)は Taylor ら[8,9] 及び Arlett[10] により樹立されたもので，二階堂 修教授(金沢大学)から入手した．チャイニーズ・ハムスター(V79)細胞[11]も二階堂教授の御好意により入手した．本研究で使用した細胞はすべてプラスチック皿(米国，Falcon 社，カタログ番号3002)で培養した．

培　地

10％胎牛血清(FCS)を添加した Eagle の最少必須培地(MEM)を，ヒト肺由来線維芽細胞の継代培養及び維持に用いた．コロニー形成法による細胞生存率検査には，15％の FCS を加えた Eagle MEM 又は15％ FCS を添加した αMEM を用いて，AT細胞の継代培養と全検査を行った．また，V79 細胞の継代培養及び全検査には，10％ FCS を加えた Eagle MEM を用いた．

染色体分析

対数増殖期にある V79 細胞を X 線照射し，37°C，CO_2 5％，空気95％の条件下で8時間培養した．染色体標本はコルヒチンで1時間処理後，0.075M KCl (2容量)＋1％ Na citrate (1容量)緩衝液で細胞の低張処理を行い，通常の火焔法[7]で作成した．

結　果

軟X線照射と高エネルギーX線照射が正常ヒト肺細胞に及ぼす致死的影響の比較

ヒト線維芽細胞の軟X線及び高エネルギーX線に対する感受性を図1に示した．高エネルギーX線は60R/分の線量率で照射した．[3] 補外数，すなわち n 値が2.2であり，D_0 が 120R である線量-生存率曲線が得られた．[3] 軟X線(40kVp，5mA，0.2Al フィルター)は，220rad/分の線量率で照射した．使用した細胞は，様々な年齢の供与者の剖検材料から得た肺線維芽細胞であったが，細胞供与者の年齢による放射線感受性の差は認められなかった．n 値1.8，D_0 125rad の線量-生存率曲線が得られた．したがって，ヒト培養細胞に対する致死的影響については，高エネルギーX線と軟X線との間に差はなかった．

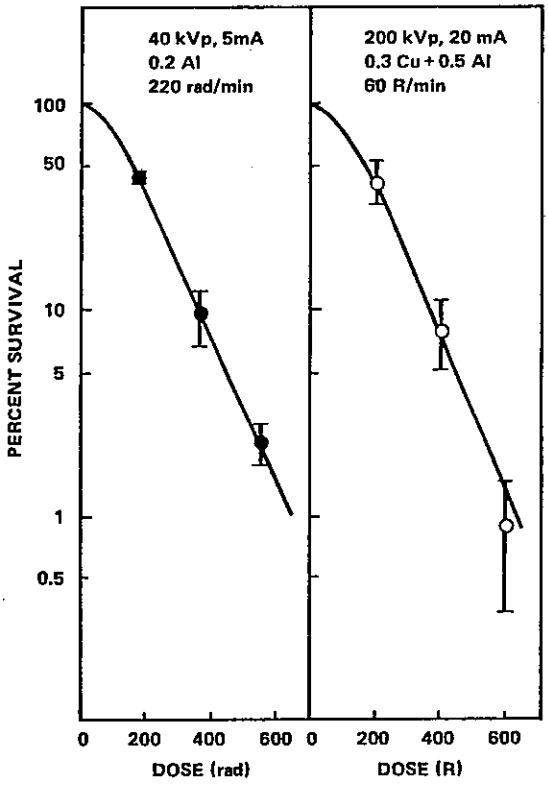


FIGURE 1 DOSE-SURVIVAL RESPONSES OF CULTURED HUMAN LUNG FIBROBLASTS TO SOFT X RAYS (40 kVp, 5 mA, 0.23 mm Al HVL) AND TO DEEP THERAPY X RAYS (200 kVp, 20 mA, 1.2 mm Cu HVL). BARS SHOW STANDARD DEVIATIONS BASED ON FOUR OR MORE EXPERIMENTS

図1 ヒト肺培養線維芽細胞の軟X線(40kVp-5mA, 0.23mm Al HVL)及び深部治療X線(200kVp, 20mA, 1.2mm Cu HVL)に対する線量-生存率反応. 棒印は4回以上の実験に基づく標準偏差を示す

Sensitivity of Ataxia Telangiectasia Cells to Soft X rays

AT (Louis-Bar syndrome), an autosomal recessive hereditary disease, is associated with decreased immune function, occurs in high frequency in some malignancies, such as leukemia and malignant lymphoma, and often develops in young patients. The AT cells of patients with this disease have been shown to be highly sensitive to ionizing radiation.[8-10] The dose-survival curve of AT cells to soft X rays is shown in Figure 2, and it is virtually identical with that reported by Taylor et al[9] using ^{60}Co-gamma radiation. Apparently, AT cells are also highly sensitive to soft X rays.

Radiation-Induced Chromosome Aberrations

The frequency of chromosome aberrations induced in V79 cells after irradiation with soft X rays and ^{60}Co-gamma rays is shown in Figure 3. The doubling time of V79 cells was approximately 12 hours. Chromosome analysis was made on metaphase cells collected 8-9

毛細管拡張性運動失調症(AT)細胞の軟X線に対する感受性

常染色体劣性遺伝病であるAT(Louis-Bar 症候群)は免疫機能低下を伴い，白血病や悪性リンパ腫など，幾つかの悪性腫瘍に高頻度で発生し，この疾患は若年期に現れる場合が多い．本疾患の患者のAT細胞は，電離放射線に対して高い感受性を示すことが報告されている．[8-10] AT細胞の軟X線に対する線量-生存率曲線を図2に示したが，これは，^{60}Coガンマ線を用いてTaylorら[9]が報告したものとほとんど同じである．明らかに，AT細胞も軟X線に対して高い感受性を示す．

放射線誘発染色体異常

軟X線及び^{60}Coガンマ線の照射後，V79細胞に誘発された染色体異常の頻度を図3に示した．V79細胞の倍加時間は約12時間であった．放射線照射後8～

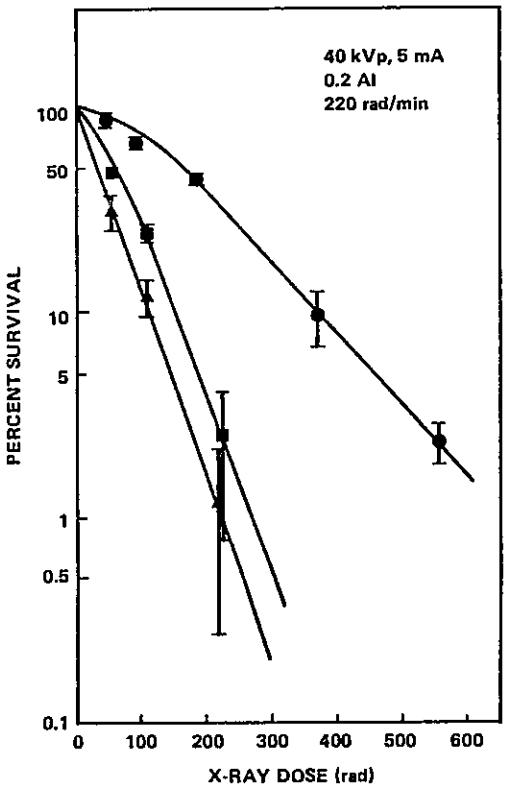


FIGURE 2 DOSE-SURVIVAL RESPONSES OF ATAXIA TELANGIECTASIA PATIENTS CELLS [AT3BI (▲), AT5BI (■)] AND NORMAL CELLS (FIGURE 1) TO SOFT X RAYS. BARS ARE STANDARD DEVIATIONS BASED ON FOUR OR MORE EXPERIMENTS

図 2 毛細管拡張性運動失調症患者の細胞〔AT3BI(▲), AT5BI(■)〕と正常細胞(図1)の軟X線に対する線量-生存率反応. 棒印は4回以上の実験に基づく標準偏差を示す

hours after irradiation. The purpose was to study chromosome aberrations in cells irradiated in the presynthetic stage of DNA, the G_1, in the cell cycle. Accordingly, more than 500 metaphase cells for each dose were examined to analyze the relationship between dose and chromosome aberrations, using dicentric and ring chromosomes as indexes. It was apparent that the yield of dicentrics and rings per cell is proportional to radiation dose, with no difference between soft X rays and ^{60}Co-gamma rays.

DISCUSSION

X rays are a kind of electromagnetic wave. X rays of short wavelength are usually referred to as "hard" X rays; those of long wavelength, as soft X rays. X rays travel in straight lines and penetrate substances to varying degrees. As they penetrate, they are absorbed at a rate proportional to the density and thickness of the object, the degree of penetration being greater in the case of short wavelength hard X rays. However, in examining soft and thin materials,

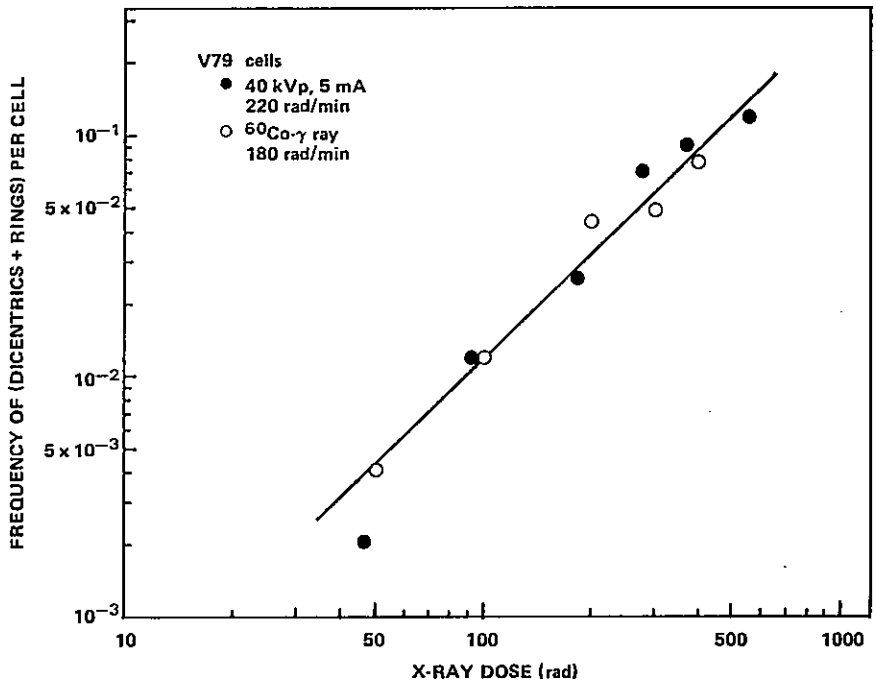
9時間で採取した分裂中期細胞について染色体分析を行った. これは, 細胞周期におけるDNA合成前期, すなわち G_1 期において放射線照射を受けた細胞に発生する染色体異常を調べるためである. したがって, 各線量に対して500個以上の分裂中期細胞を調べ, 二動原体及び環状染色体を指標として線量と染色体異常との関係を分析した. 細胞1個当たりの二動原体及び環状染色体の頻度は放射線量に比例し, 軟X線と ^{60}Co ガンマ線との間に差がないことは明らかであった.

考 察

X線は電磁波の一種である. 波長の短いX線を通常〝硬〟X線と呼び, 波長の長いものを軟X線と呼ぶ. X線は直線的に進み, 様々な度合いで物質を透過する. X線は, 透過する際, 物質の密度と厚さに比例して吸収されるが, 透過の程度は短波長の硬X線の方が大きい. しかし, 医学的研究及び薬学的研究

FIGURE 3 ABILITY OF ^{60}Co-GAMMA RAYS (○) AND SOFT X RAYS (●) TO INDUCE CHROMOSOME ABERRATIONS IN CHINESE HAMSTER V79 CELLS

図3 ^{60}Co ガンマ線(○)と軟X線(●)がチャイニーズ・ハムスター V79 細胞に染色体異常を誘発する能力



small animals, plants, and seeds used in medical and pharmacological studies, and in the nondestructive testing of light metals, resins, and chemical products, it is often advantageous, in the interests of greater accuracy, to employ soft X rays of relatively long wavelength in the radiological technique. The feasibility of applying soft X rays to radiation cell biological studies was examined by using the Softex, which is intended for nondestructive radiological testing. The shortest wavelength of X rays generated by the Softex is approximately 0.246 Å at 50 kVp. Hence, the wavelength of soft X rays used here is considerably longer than those used for deep radiation therapy, whose shortest wavelength is 0.06 Å at 200 kVp.

Cultured cells were exposed to soft X rays. A curvilinear relationship with an n value of 2.2 and D_0 of 120 R was obtained for survival response following exposure to X rays for deep therapy.[3] A similar relationship, with an n value of 1.8 and D_0 of 125 rad for survival response following soft X-ray exposure (Figure 1) was

において用いられる軟らかく薄い材料，小動物，植物並びに種子を調べる際には，また，軽金属，樹脂及び化学製品に対する非破壊検査においては，より高い精度を期するため，放射線学的技法に比較的長い波長の軟X線を用いることがしばしば有用である．非破壊的な放射線学的検査用に作られた Softex を用いて，軟X線を放射線細胞生物学的研究に適用できるかどうかを調べた．Softex によって発生するX線の最短波長は，50kVp で約0.246Å である．したがって，今回用いた軟X線の波長は，深部放射線治療で用いられる波長（最短波長は 200kVp で 0.06Å）よりもかなり長い．

培養細胞に軟X線を照射した．深部治療のX線照射後の生存反応に関しては，n 値2.2，D_0 120R を示す曲線関係が得られた．[3] 今回の実験では軟X線照射（図1）後の生存反応について，n 値1.8，D_0 125rad

obtained in the present experiments. Both types of X rays may be regarded as having nearly the same lethal effect on human cultured cells. When the AT cells (AT3B1 and AT5B1) were exposed to soft X rays, the D_0 values ranged from 40 to 50 R. These doses agreed well with the D_0 values of approximately 50 rad from ^{60}Co-gamma ray exposure reported by Taylor et al[8] and with those reported by Lavin et al[12] (30.5 rad). Thus, it appears that AT cells have a genetically deficient repair mechanism for gamma ray-induced DNA damage, suggesting they are also deficient in their ability to repair soft X-ray-induced DNA damage.[13] It seems that DNA damage induced by gamma rays and by soft X rays closely resemble each other, or that they are identical. Therefore, soft X rays should be useful in studying mechanisms of repair processes at the DNA molecular level.

V79 cells were used in tests for radiation-induced chromosome aberrations for several reasons. Since the doubling time of V79 cells is only 12 hours in our culture conditions, it was easy to obtain cells in the mitotic phase. The synchronization hardly occurred during subcultivation because of a very short lag time after cell planting. The main reason was that the majority of V79 cells maintain diploidy with the modal chromosome number of 22. More than 90% of the cells have 20-22 chromosomes; approximately 4% of the cells show tetraploidy. Though gaps and breaks were not studied extensively in this observation, chromatid type abnormalities of rings and dicentrics were observed with gamma ray doses or soft X-ray doses higher than 50 rad, as shown in Figure 3. No difference was evident between ^{60}Co-gamma rays and soft X rays for the induction of chromosome aberrations in V79 cells.

Special attention should be given to the quality and quantity of culture medium and buffer solution used to maintain the cells for irradiation by soft X rays. When irradiated at 5 mA and 40 kVp or 50 kVp, using Eagle's MEM, the radiation dose was reduced by approximately 60% at a depth of 1 cm. Precautions should be taken to assess the dose accurately when the cells to be irradiated are contained in a large volume of culture medium or in whole blood.

RERF's mission is the study of effects of radiation on man. Knowledge of radiation effects

を示す上記と同様の関係が得られた．両種のX線は，ヒト培養細胞に対してほぼ同じ致死的効果をもつと考えてよいであろう．AT 細胞(AT3 B1 及び AT5 B1)に軟X線を照射した場合，D_0値は40から 50R であった．これらの線量は，Taylor ら[8] が報告した ^{60}Co ガンマ線照射による約50rad の D_0値，並びに Lavin ら[12] が報告した D_0値(30.5rad)とよく一致した．AT 細胞のガンマ線誘発 DNA 障害の修復機構は遺伝的に欠損しているのであるが，[13] AT 細胞には軟X線誘発 DNA 障害の修復能も欠損していることが示唆される．ガンマ線と軟X線によって誘発される DNA 障害は互いに酷似しているか，又は，同一のものであるように思われる．故に，修復過程の機構を DNA 分子レベルで研究する際にも，軟X線が有用であるはずである．

放射線誘発染色体異常の検査に V79 細胞を用いたのは幾つかの理由による．我々が用いた培養条件下では，V79 細胞の倍加時間は12時間にすぎないので，分裂期の細胞を得ることは容易であった．細胞播種後の遅滞期は極めて短いため，継代培養期間中の同調はほとんど生じない．主な理由は，V79 細胞の大多数が二倍性を維持し，染色体のモード数が22であった．この細胞の90％以上が20－22個の染色体を有し，約４％が四倍性を示している．本研究では，裂孔や切断については広範囲に検討しなかったが，図３に示すように，環状染色体や二動原体のような染色分体の異常について，50rad 以上のガンマ線又は軟X線を用いて観察した．V79 細胞における染色体異常の誘発に関しては，^{60}Co ガンマ線と軟X線との間に差は認められなかった．

軟X線照射用の細胞を維持するために用いる培地及び緩衝液の質と量には特別な注意を払うべきである．5 mA，40kVp 又は 50kVp の条件下で，Eagle MEM を用いてX線照射を行った場合は，放射線量は１cmの深度で約60％減少した．照射対象の細胞が大量の培地又は全血の中に含まれているときに，あらかじめ線量を正確に測定しておかなければならない．

放影研の使命は，放射線が人体に及ぼす影響を研究

on living organisms is of great importance in elucidating the mechanisms of carcinogenesis and mutation, aging, heredity, and other damage. Results of such experimental studies are useful in estimating radiation effects on man. The present study suggests that soft X rays used primarily for nondestructive testing are also useful in studying radiobiological systems, including cultured mammalian cells, chromosomes, and DNA molecules.

することである．生物に対する放射線の影響を知ることは，発癌及び突然変異，加齢，遺伝並びにその他の障害の機構を解明する上で極めて重要である．このような実験研究の結果は，人体に及ぼす放射線の影響を推定する上で有益である．本研究は，主として非破壊検査に用いられる軟X線が，哺乳類培養細胞，染色体及びDNA分子を含む放射線生物学的システムを研究する上でも有益であることを示唆している．

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