DETERMINATION OF ABSORBED DOSE OF HeLa S3 CELLS CULTURED ON PLASTIC AND GLASS DISHES FOLLOWING EXPOSURE TO X-RAY

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プラスチック並びにガラスシャーレ上に培養された HeLa 細胞の吸収線量の補正

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単層培養された細胞は培養液と容器との境界面にあり，これをX線で照射すると，シヤーレと培養液との境界でのイオン密度分布が複雑なために，細胞の吸収線量は容易には算定出来ない．ガラスシヤーレ並びにポリスチレンシヤーレに培養された細胞の吸収線量を照射線量から換算するための係数を実験的に求めた．180kVp（HVL 0.35 mmCu）のX線で照射した時のガラスシヤーレ（実効原子番号11.6）およびポリスチレンシヤーレ（実効原子番号 5.7）に培養された HeLa S 3 細胞の吸収線量は照射線量のそれぞれ1.22，0.86倍である．

Introduction

Since the possibility of culturing single mammalian cells in vitro was demonstrated by Puck and Marcus[10], many dose response curves have been reported for normal and malignant cells exposed to X-rays and γ-rays[1)3)12)].

In many of these papers the dose has been expressed as exposure dose in roentgens (R) rather than absorbed dose in rads. As biological effects are dependent on absorbed dose (in rads) and not on exposure dose (in R), it is far more meaningful to express dose in rads than in R especially in comparative studies on the response of cells in various situations, such as in vivo versus in vitro irradiation and to various types of radiation including X-rays and γ-rays and also in the determination of the target size related to cell proliferation on the basis of the dose response curve as demonstrated by Lea[6].

When cells cultured in monolayer on the surface of the culture vessel are exposed to X- or γ-rays, the total absorbed dose is equal to the sum of the dose from the interaction of incident photons with the culture medium and cells and the dose from the interaction with the culture vessel.

The latter is the absorbed dose from secondary electrons produced by the photoelectric effect of the culture vessel. It is therefore expected that it differs depending on the average effective atomic number

(Z_{eff}) of the culture vessel and average photon energy. In the dish culture, if the Z_{eff} of the culture medium and culture vessels differ, the absorbed dose would be different from that when cells are irradiated in suspension in uniform soft tissue equivalent medium.

Since the report of Morkovin and Feldman[8] on this phenomenon, some wokers have experimentally derived the conversion factors to determine the absorbed dose from X-ray exposure dose given to mammalian cells cultured on glass and plastic vessels. Hood and Norris[5] calculated the over-all conversion factors for HeLa cells exposed to 36 kV X-ray on pyrex glass and polyvinyl petri-dishes on the assumption that polystyrene is equivalent to soft tissue. However, as Z_{eff} of polystyrene at 36 kV X-rays differs from that of soft tissue and further the absorbed dose of cells at the interface of the medium and dish is expected to differ depending on the Z_{eff} of the vessel material, their calculation seems untenable.

This report describes the over-all conversion factors in determining the absorbed dose in rads from X-ray exposure dose received by cells at the interface between the medium and glass or poly styrene vessel.

Materials and Method

Cells: In the present study mammalian cells HeLa S3 obtained from Dr. M. Yamada, Department of Pathology, National Institute of Health, Tokyo Japan were used. These were usually cultured in a flat bottle. The composition of the culture medium is 80% Eagle minimum essential medium and 20% bovine serum together with 50 units of penicillin-G and 50 micrograms of streptomycin per milliliter. The serum was inactivated at 56°C for 30 minutes. Cells were planted after treating with 0.1 per cent bacto-trypsin (Difco) made isotonic with calcium and magnesium free phosphate buffered saline[7].

Procedure in cell transplantation: After trypsinization the concentration of the cells was determined with a haemocytometer and dilutions were made with the complete growth medium consisting of 80% Eagle minimum essential medium and 20% calf serum with 50 units of penicillin-G and 50 micrograms of streptomycin per milliliter. The cell suspension was divided into two groups. The first group was irradiated while in suspension in a glass test tube. Enough cells were transplanted on each dish to yield about same number of surviving cells at each dose level as expected in the controls. In the second group cells were transplanted on petri-dishes as described above and after four to six hours when the cells had attached themselves to the surface of culture vessel, they were irradiated.

Irradiation: A full wave rectifier unit (Shimazu Co.) was used at 180 kVp and 20 mA. The filtration at the cell layer was inherent plus 1.0 mm Al, 2 mm pyrex glass (petri- dish cover or glass test tube wall) or 1 mm polystyrene (polystyrene dish) and 2 mm of medium. The radiationquality of this beam was 0.35 mm Cu HVL before glass and medium filtration. The focus to cell distance was 75 cm where the uniform beam was about 20 cm in diameter. The exposure dose was measured by a dose rate meter (Shimazu Co.) under full backscattering conditions (the dose rate meter was corrected by Fricke dosimeter). At the point corresponding to the cell layer, the dose rate was 81.2 R/min in suspension, 76.4 R/min in pyrex glass dish and 89.7 R/min in polystyrene petri-dish.

After irradiation the plates were incubated at 37°C in a CO_2 incubator with the atmosphere controlled for pH and humidity. The medium was not changed during the incubation period of 13 days. Two weeks after irradiation, the colonies were washed by Dulbecco phosphate-buffered saline[7] to prevent serum from precipitation, fixed with 10% neutral formalin in normal saline, and stained with crystal violet.

A colony of 50 or more cells was regarded as having originated from a surviving cell and scored.

Results

The results obtained in the present study are shown in Figure 1. Curve A shows the usual survival curve when irradiated in suspension, curve B of cells attached to the glass and curve C of cells attached to the polystyrene petri-dish.

When compared with curve A, curve C shows less radiation injury and curve B shows greater injury, suggesting that the absorbed dose is lower in C and higher in B. Dose correction factor can be computed from Figure 1. This factor is defined as the ratio of irradiation doses of two different types of radiation which give equal per cent survival. The ratio was determined graphically at arbitrary survival rates of 10, 1, 0.1 per cent.

The reason for making the dose correction comparison at constant per cent survival instead of the direct comparison of equal irradiation dose is described in the report of Hood and Norris.[5] In comparison with cells irradiated in suspension the dose correction factor in term of roentgen is 0.92 for cells cultured on polystyrene dish and 1.3 for those on pyrex glass. This ratio was the same for any selected survival level. As the factor in converting roentgens into rads is 0.94[11] for HVL 0.35 mm Cu X-rays in homogenous medium, overall conversion factors for the absorbed dose in rads are 0.86 for cells cultured on polystyrene dish and 1.22 for cells on pyrex glass.

Discussion

Spot checks were made to demonstrate the effects of physiological conditions of the cells by time after trypsinization on the radiosensitivity of cells, but there was no significant difference in the sensitivity of cells one hour after trypsinization and of cells six hours after trypsinization.

As mentioned in the Introduction, the difference between the absorbed dose of cells irradiated in

Fig. 1. Dose-response curves of HeLa S3 cells irradiated in various conditions.

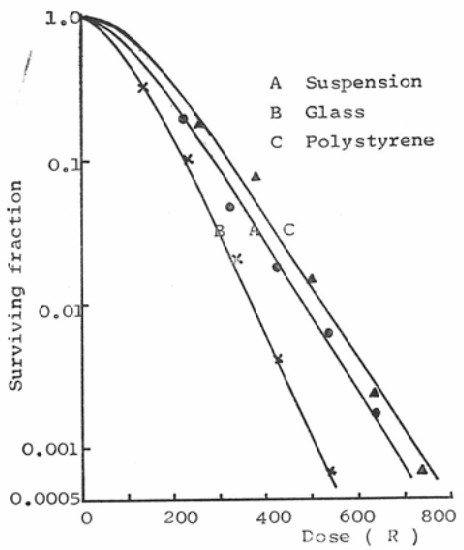

Table 1. Roentgen to rads conversion factors (on glass)

Investigators	HVL	Conv. fact.	Reference
Dewey and Humphrey	1.26mm Cu	1.5	(2)
Feldman and Morkovin	3 mm Al	1.4	(4)
	1.5 mm Cu	1.33	
Hood and Norris	2.6 mm Al	1.42	(5)
Morkovin and Feldman	3 mm Al	1.68	(8)
	1.5 mm Cu	1.44	
	3 mm Cu	1.24	
Muta	1.01mm Cu	1.1	(9)
Yamada and Puck	230KV	1.35	(14)

suspension and that of cells on glass or polystyrene dish is attributable to the difference in the effect of photoelectrons from the medium or vessel which adds to the absorbed dose of the cells. The effect of photoelectrons depends on the quality of radiation (even if HVL and average effective voltage are the same, the effect may differ) and average effective atomic number of the material in question.

Calculated by Walter's model[13], the average effective atomic number with regard to photoelectric effect of HVL 0.35 mm Cu X-rays are 7.4 for soft tissue, 5.7 for polystyrene and 11.6 for pyrex glass.

The factors for various cells at various irradiation conditions as reported by other workers are summarized in Table 1. The same HVL does not give the same overall conversion factor. However, there is a tendency for radiation of softer quality (lower HVL) to have a larger overall conversion factor.

The reason for this difference is considered to be due to irradiation conditions or cell size (this brings about a difference in distance from the vessel surface to the radiosensitive structure) and quality of radiation. Strictly speaking, the overall conversion factor reported in this paper is applicable only to a beam of 180 kVp, HVL 0.35 mm Cu X-rays with 1.0 mm of Al and 2 mm of glass as added filtration and to HeLa S3 grown in 80% Eagle minimum essential medium plus 20% calf serum. This factor may differ slightly for other irradiation conditions or cells. For each irradiation condition a different overall conversion factor should be used for the accurate determination of the absorbed dose in rads of cells. When cells are irradiated in suspension in soft tissue equivalent medium or by beams of HVL exceeding 3 mm of Pb, it is not necessary to experimentally obtain such factor in determining the absorbed dose.[8]

Summary

The factors in converting roentgens into rads for mammalian cells HeLa S3 grown on the non-tissue equivalent materials and exposed to X-ray were determined experimentally.

The results of these experiments indicate that the absorbed dose of radiosensitive structure related to cell replication is 1.22 when irradiated on glass dish (average effective atomic number: 11.6) and 0.86 when irradiated on polystyrene dish (Z_{eff}: 5.7) by HVL 0.35 mm Cu X-rays.

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