Radiosensitivity of Mammalian Cells, VII. Aggregation and Pseudo-glomerule Formation of Dissociated Kidney Cells from Irradiated New Born Mice

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ABSTRACT

The effects of irradiation on aggregate and pseudo-glomerule forming activity were studied quantitatively using dissociated kidney cells from new born mice. The dissociated cells formed macroscopic aggregates and microscopic inner structures, pseudo-glomerules, on the bottom of plastic dishes.

The inhibitory effects of irradiation on aggregation and pseudo-glomerule formation were detectable with a dose of as low as 10 R. However these abilities to form aggregates or pseudo-glomerules were not completely lost even after irradiation with a dose of 1000 R.

INTRODUCTION

With the development of in vitro colony formation by Puck and Marcus\textsuperscript{1,2} and its application to measurement of reproductive integrity of irradiated single cells, mitotic death and other injuries of proliferative cell populations after irradiation have been quantitated. Although considerable information is available on the survival and colony formation of dividing cell populations after irradiation, little is known on the effect of irradiation on non-dividing somatic cells, especially in vivo.

The mechanisms involved in aggregation and reconstitution of a tissue structure in vitro from dissociated cells of normal and irradiated mice are of major interest in connection with the somatic effects of radiation and the cellular organization of
tissues in organisms.

From many studies on embryonic or mouse cells (Moscona 1952, 1962), it is now known that when embryonic tissue can be dissociated into single cells, the cells can reassociate in culture to form tissue-like structures. Depending on the conditions of cultivation, dissociated embryonic cells in vitro can either be induced to reaggregate, or to grow in a dispersed, “monolayer” state.

Recently, Kuroda reported on the effects of X-irradiation on the HeLa cells for their histoformative aggregate-forming activity and sorting-out activity for embryonic quail liver cells in rotating culture. He observed that HeLa cells which were irradiated with increasing doses of X-rays gradually lost the sorting-out activity.

The present paper deals with the effects of irradiation on the aggregate and pseudo-glomerule forming activity in monolayer state, using dissociated mouse kidney cells in vitro.

MATERIALS AND METHODS

Dissociated kidney cells were prepared from 3 to 6 days old mice (Swiss albino) weighing 1.8 to 2.7 g. Whole-body irradiation was carried out with 60Co Gamma-ray doses of 10, 50, 100, 200, 500 and 1000 R were delivered at a dose rate of 50 R/min.

The kidneys from irradiated mice were removed within 30 min after irradiation. The kidneys from normal and irradiated mice were chopped up with a razor blade or scissors after complete removal of adherent connective tissue.

The minced tissue was placed in a 10 ml centrifuge tube and incubated with about 6 ml of warm 0.5% trypsin (Difco) solution for 30 min at 37°C. The trypsin solution was removed by centrifuging the mixture at 1200 rpm and the tissue was washed three times with basal medium with centrifugations for 3 to 5 min at 1000 rpm. The basal medium (YLE) was prepared as the following formula: 0.5% lactalbumin hydrolysate (Difco) plus 0.1% yeast extract (Difco) dissolved in Earl’s solution and inactivated calf serum in proportion of 90: 10.

The dissociated cells were then suspended in the culture medium (YLE) at a concentration of approximately 5–7×10⁴ cells per ml of medium and distributed to 35 mm plastic dishes in a volume of 1/80 to 1/20 of a whole kidney. The cells were incubated at 37°C under air and without changing medium during the incubation period.

The numbers of aggregates of cells and pseudo-glomerules per 25 or 100 mm² in the plastic dishes were counted after fixation in methanol and staining with Giemsa. As a criterion for scoring radiation damages, the number of aggregates consisting of more than 10 cells was counted. The experiments have been repeated three to four times, and the average count from duplicate dishes was used for each experiment.
Fig. 1. A—I. Photomicrographs of aggregates of dissociated kidney cells of unirradiated mice after culture for 2 to 6 days.
A, 2 days cultivation of cells from 1/60 of the whole kidney.
B, 4 days cultivation of cells from 1/60 of the whole kidney.
C, 6 days cultivation of cells from 1/60 of the whole kidney.
D, 2 days cultivation of cells from 1/40 of the whole kidney.
E, 4 days cultivation of cells from 1/40 of the whole kidney.
F, 6 days cultivation of cells from 1/40 of the whole kidney.
G, 2 days cultivation of cells from 1/20 of the whole kidney.
H, 4 days cultivation of cells from 1/20 of the whole kidney.
I, 6 days cultivation of cells from 1/20 of the whole kidney.
RESULTS

Aggregate formation from unirradiated cells

When a suspension of kidney cells in culture medium was allowed to settle down in a plastic dish for 10 to 24 hours the cells did not remain single but began to gather. After 2 to 3 days they formed flat aggregates of various sizes and shapes at the bottom of the dish (Fig. 1, A—I).

After 3 days the number of aggregates was linearly related to the initial amount of cell suspension (volume of kidney) (Fig. 2). When cell suspension from 1/20 of a whole kidney were incubated, the number of aggregates increased rapidly during the first 2 to 3 days of incubation, reaching a plateau with 24 aggregates per 25 mm² of dish (Figs. 3 and 4). The size of aggregates increased slightly after 4 days incubation. After 6 days incubation, the aggregates begun to cohere with each other. Cultures after 3 days' incubation had an average of 22 aggregates per 25 mm², ranging from 0.2 to 0.8 mm in diameter.

The mitotic index of the 3 days culture was 0.01±0.005 % and that of the 6 days culture was 0.02±0.005 %.

Effects of irradiation on aggregation

High doses of irradiation reduced both the number and size of aggregates formed after 3 days of cultivation (Fig. 5, A—C). The number of aggregates decreased with increasing the radiation dose (Fig. 6).

The effect of low doses of irradiation (10 to 200 R) on the aggregate formation of dissociated cells was clearly seen as a decrease in the number of aggregates. The dose-response curve suggested that cells were made up of two types, radiosensitive and radioresistant. The number, size, and shape of aggregates from irradiated specimens were different from those of unirradiated controls.

Pseudo-glomerule formation in unirradiated controls

The aggregation of cells mainly occurred within 3 days and before the resumption of significant mitotic activity. The flame-like cells in the initial clumps of cells become sorted out according to their original identities and associated in a manner compatible with histotypic deve-
Fig. 3. Development of aggregates in plastic dishes seeded with dissociated cells of 1/20 of a kidney after incubation for 5 hours (5 h), 1 day (1 d), 3 days (3 d), 4 days (4 d) and 6 days (6 d) in culture medium.

Fig. 4. Increase in aggregation of dissociated kidney cells on incubation. Cells from 1/20 of a whole kidney were seeded. Number of aggregates was counted in 25 mm² areas.
To study the histoformative capacities of isolated cells, the inner structures of the aggregates were examined. A suspension of kidney cells contains several kinds of cells. When the suspension was incubated, the isolated cells grouped together randomly within 24 to 48 hours. But after incubation for 3 to 4 days some of the cells regrouped in a manner conductive to tissue formation. Flame-like cells in the aggregates began to construct characteristic histologically identifiable structures (Fig. 7, A and B). These structures formed by the flame-like cells were so similar to glomerules in vivo that they were named "pseudo-glomerules".

Effects of irradiation on pseudo-glomerule formation

On incubation of unirradiated development.

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Effects of irradiation on pseudo-glomerule formation

On incubation of unirradiated
cells, no pseudo-glomerules were seen during the first 2 days of cultivation and the cells in the aggregates were uniform in appearance with an oval nucleus and single nucleolus. Pseudo-glomerule formation started after 3 days of incubation and the cells in these pseudo-glomerules looked like flame cells, originated from epicytes, visceral and parietal epithelial cells of glomerules.

Fig. 7. A–F. Pseudo-glomerules formed in aggregates of dissociated cells.
A, Unirradiated control. B, Unirradiated control. C, Irradiated with 50 R.
D, Irradiated with 50 R. E, Irradiated with 200 R. F, Irradiated with 200 R. Each scale in all photograms shows 50 μ.
The effect of irradiation on pseudo-glomerule formation was examined after 4 days of cultivation (Fig. 7, A—F). Pseudo-glomerule formation was inhibited by irradiation and the number of pseudo-glomerules decreased with increasing γ-ray dose.

Dissociated cells obtained from new born mice after exposure to a single dose of 500 and 1000 R, formed pseudo-glomerules which were similar in size and shape to those of controls, but fewer in number (Fig. 8).

At lower doses of irradiation, pseudo-glomerule formation based on the number was also depressed as shown in Fig. 9.

**D₅₀ values for aggregation and pseudo-glomerule formation**

The dose response curves for aggregation and pseudo-glomerule formation by dissociated cells from irradiated mice are shown in Fig. 9. Each point is the average value from duplicate plates and the individual values of the duplicate plates were ranged within the limits of error of the sampling procedure. All but one of the points shown on the curve are the means of values determined in a series of 3 to 5 separate experiments carried out over a period of several months.

The D₅₀ values are the doses of irradiation needed to reduce numbers of aggregates and pseudo-glomerules to 50 % of the control values. The D₅₀ value for aggregate formation was 160 R and that for pseudo-glomerule formation was 60 to 70 R.

**DISCUSSION**

For the purpose of obtaining some important information for understanding the radiation effects on cellular functions of somatic cells without proliferation, the present study was carried out with dissociated kidney cells composed of slowly or non-proliferating cells in stationary culture from new born mice with or without irradiation. From the fact that dissociated kidney cells have histoformative activity, such as aggregate and pseudo-glomerule forming activity, they may provide some interesting informations on a mechanism of cellular lesion by radiation in kidney in vivo.

The present results show that there are two radiosensitive processes in the reconstitution of dis-
sociated cells: aggregation and pseudo-glomerule formation. The ability of dissociated cells to form macroscopic aggregates was affected by a dose as low as 10 R. The D_{50} for this aggregate-forming activity was in the neighborhood of 160 R, but the ability was not completely lost even after irradiation with 1000 R. The pseudo-glomerules forming activity was also affected by irradiation as little as 10 R and the D_{50} value was 60 to 70 R which were less than that for aggregation. When the dose response curves of aggregation and pseudo-glomerule formation were replotted in the semi-logarithmic scale, the curves were biphasic. The D_{37} doses of a radiosensitive and a radioresistant of aggregation were 110 R and 1380 R, while the D_{37} doses of a radiosensitive component and a radioresistant component of pseudo-glomerule formation were 80 R and 1320 R, respectively.

In the mixed population of HeLa and embryonic quail liver cells in rotation culture, Kuroda found that irradiation on HeLa cells resulted in a loss of sorting-out activity and that the loss of the sorting-out activity seemed to be more radio-

Fig. 9. Dose-response curves for aggregation and pseudo-glomerule formation by dissociated kidney cells from mice irradiated with low doses (10 to 200 R).
resistant than their ability to form aggregates as expressed by their average diameter.\textsuperscript{5,7,8}) Although aggregation, pseudo-glomerule formation and sorting-out ability were likely to be a reflection of radiation damages on cell membrane, it is very difficult to compare the present experiment with Kuroda's experiment because of different parameters used for expression of radiation damages, number of aggregates vs. average diameter of aggregates and formation of pseudo-glomerules vs. percent of quail liver cells in HeLa aggregates, because of different cell types, mouse kidney vs. HeLa cells and because of different manners of cultivation, settling-down vs. rotation culture. Only thing can be said is that the aggregate formation and pseudo-glomerule formation of nonproliferating cells of mouse kidney were more radiosensitive than aggregate size and sorting-out ability of proliferating HeLa cells.

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**REFERENCES**

1. Puck, T. T. and Marcus, P. I. (1956) Action of X-rays on mammalian cells. J. Exp. Med., 103:653-666.
2. Sinclair, W. K. (1968) Cyclic X-ray responses in mammalian cells \textit{in vitro}. Rad. Res., 33:620-643.
3. Moscona, A. (1952) Cell suspensions from organ rudiments of chick embryos. Exp. Cell Res., 3:535-539.
4. Moscona, A. (1962) Analysis of cell recombinations in experimental synthesis of tissues \textit{in vitro}. J. Cell. Comp. Physiol., 60, suppl., 1:65-80.
5. Kuroda, Y. (1964) Studies on cartilage cells \textit{in vitro} I. Morphology and growth of cartilage cells in monolayer culture. Exp. Cell Res., 35:326-336.
6. Kuroda, Y. (1970) Radiation-dependent loss of sorting-out activity of HeLa cells. Ann. Rep. Natl. Inst. Genet. Japan, 20:73-74.
7. Kuroda, Y. (1970) Effects of X-irradiation on sorting-out property of animal cells in rotation culture. Japan J. Develop. Biol., 24:67-70.
8. Kuroda, Y. (1971) Effects of X-irradiation on tissue formative activity and sorting-out activity of HeLa cells in rotation culture. Rad. Res., 48:565-577.
9. Kobayashi, J. (1962) The cytological effect of chemicals on tumors, XIV. Effect of 1-thia-3-azazulan-2-one on HeLa cells and two animal tumors. J. Fac. Sci. Hokkaido Univ. Ser., VI. Zool., 15:9-17.