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A Simple Method for Premature Chromosome Condensation Induction in Primary Human and Rodent Cells Using Polyethylene Glycol

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Abstract—*Even though polyethylene glycol (PEG) has been shown to be a potent fusogen, it has not been widely exploited as an alternative to the Sendai virus for premature chromosome condensation (PCC) induction. A simple, rapid, and reproducible PEG protocol for primary cells in suspension is presented which allows satisfactory cell fusion and PCC indices, giving at the same time high cell viability and low giant, multinucleated cell formation. Technical details for PEG-mediated fusion and premature chromosome condensation induction in human and rat lymphocytes, rodent spleen cells, and spleen and whole body cells of newborn mice are provided. Further applications of the method are suggested.*

INTRODUCTION

Since many health disorders, including cancer and birth defects, have been related to altered cellular genetic material, emphasis has been given to the development of sensitive methods to visualize and quantify interphase chromatin changes and chromosome malformations (1).

In eukaryotic cells the chromosomes are visible only during mitosis. Therefore, most of the conventional cytogenetic methods require a dividing cell population and the cells are usually analyzed at metaphase. Lately, however, taking advantage of the recently discovered phenomenon of premature chromosome condensation (PCC) induced by cell fusion (2), the chromosomes of noncycling cells and, in general, interphase cells can be also visualized and analyzed (3). Indeed, when interphase cells are fused with mitotic cells by means of Sendai virus, they undergo chromatin condensation

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rapidly followed by dissolution of their nuclear membrane. The interphase chromatin further condenses and assumes a morphology characteristic of the position of the interphase cell in the cell cycle: single chromatid per chromosome for G_1, double chromatid per chromosone for G_2, and pulverized chromosome regions for S (2).

The PCC method has been shown to be a powerful cytokinetic and cytogenetic tool, and it has been successfully used in problems involving cell-cycle analysis (4–6), for diagnostic purposes in human luekemia (7–10), and in the assessment of interphase chromosome malformations resulting from clastogenic treatment with radiation or chemicals (11–15). In particular, the PCC method has proved to be unique in visualizing chromosome damage in cells blocked in G_2 phase (14). It also has been successfully used in determining directly the chromosome repair by allowing a period of time after exposure before fusing the cells (12, 13). Nevertheless, due to the technical difficulties in the preparation and use of Sendai virus, the PCC method is presently applied in only a small number of laboratories.

The preparation of effective Sendai virus samples requires considerable expertise (3). Moreover, the fusion by means of virus requires cells with membranes specially receptive to the virus particles (3). Lymphocytes and lymphoid cell lines, for instance, cannot be satisfactorily fused (16). In addition, cytogenetic laboratories potentially interested in this method are not always willing to handle viruses.

As an alternative to the Sendai virus, the well-known fusogen polyethylene glycol (PEG) (16–20) has been applied to PCC induction procedures (21, 22). However, due to various disadvantages reported in the literature (3, 23), PEG has not been widely used for PCC induction. The conditions for cell fusion and PCC induction have to be adjusted according to the varying cell sensitivities to PEG. Concentration, molecular weight, exposure time, solution for the PEG dilution, and removal time have to be monitored. This makes difficult the use of this chemical on a routine basis.

In this report, a simple and reproducible PEG procedure for PCC induction in primary human and rodent cells in suspension is presented. In particular, technical details are given for PEG fusion and PCC induction of human and rat lymphocytes from peripheral blood. Also, the methodology for PCC induction in rodent spleen cells and in spleen and whole body cells from newborn mice is provided.

MATERIALS AND METHODS

Chemicals, Media, and Buffers. The chemical fusogen polyethylene glycol (PEG) with an approximate molecular weight of 1000 (Sigma Chemi-

cal Co.) and Hanks' buffered salt solution (HBSS, Gibco, No. 310-4170) were used. The PEG solutions were prepared shortly before being used. Melted PEG and HBSS were mixed in the weight/volume PEG concentration required for each experiment.

Other chemical solutions and media required by this method were colcemid (Gibco, No. 120–5211); 20 mM $MgCl_2$; 0.075 M KCl; a Ficoll–Paque gradient (Pharmacia Fine Chemicals); 0.25% trypsin (Gibco); deoxyribonuclease (Sigma No. D-0876); chromosome medium 1A (Gibco, No. 120–1672); culture medium F10 (HAM, Gibco); Acetoorcein stain (Gibco, No. 630-5390), and Pro–Texx mounting medium (S/P, No. M7635-1).

Mitotic CHO Cell Preparation. Chinese hamster ovary (CHO) cells were routinely grown in F10 culture medium supplemented with 10% calf serum, 5% fetal calf serum, and antibiotics. They were maintained as monolayer cultures in 75-cm^2 plastic flasks (Falcon, No. 3024) and were used for the supply of mitotic PCC inducer cells as follows: About 1×10^6 CHO cells were transferred to a flask with 10 ml medium and gassed with 5% CO_2. The flask, tightly closed, was kept in the incubator at 37°C for two days. The medium was then discarded and replaced by 7 ml fresh warm medium and 0.15 ml of colcemide solution. The final concentration was 0.2 μg/ml. The cells were further incubated for 5 h and then about 1.5×10^6 accumulated mitotic cells were harvested by selective detachment and transferred to a culture tube.

The mitotic index, i.e., the percentage of cells in mitosis, was routinely estimated from samples of harvested cells by the following procedure: Using a cytocentrifuge, slides with different cell concentrations were prepared and air dried for 5 min. A drop of acetoorcein was placed on the cells and a 22 × 22-mm No. 1 coverslip was immediately placed on top and sealed with Pro-Texx mounting medium. The mitotic index was calculated with the slide with the most appropriate cell concentration, and it was most often found to be greater than 95%.

Preparation of Human and Rodent Somatic Cells for PEG-Mediated Fusion. Human and rat lymphocytes isolated from whole blood, rat and mouse spleen cells, and whole body and spleen cells from newborn mice were used.

Venous human blood and rat blood freshly withdrawn from the abdominal aorta into 10-ml heparinized syringes was utilized. The lymphocytes were separated according to a standard Ficoll–Hypaque method (24) with some modifications. In a 50-ml glass centrifuge tube, 5 ml whole blood diluted 1:5 in HBSS were layered on the top of a 10 ml Ficoll–Paque gradient and run at 400*g* for 30 min. Using a Pasteur pipet, the lymphocyte-enriched interface fraction was collected, washed once with HBSS, and resuspended in F10 medium with 20% serum.

To isolate spleen cells, a rodent spleen was removed and transferred to a 60 × 15-mm petri dish containing F10 medium. All fatty tissue was discarded. The spleen was either gently teased or rubbed against the frosted edges of two microscope slides. The released cells were repeatedly pipetted and then filtered through gauze to obtain a single-cell suspension. These cells could either be used directly or after their purification from red blood cells by means of the Ficoll–Paque method described above.

The whole body cells from newborn mice were prepared as follows: A newborn mouse was minced, transferred to a tube, washed once in 5 ml 0.25% trypsin, resuspended in 5 ml trypsin, and incubated at 37°C for 40 min. Then 5 ml of 0.025% deoxyribonuclease was added, and the tube was incubated for an additional 10 min. After centrifugation at 200*g* for 5 min, the supernatant was discarded and the pellet was resuspended in 10 ml F10 medium with 20% serum. The cells were repeatedly pipetted and then filtered through gauze.

Fusion of Cells in Suspension. Mitotic cells harvested by selective detachment, as described above, were poured into a round-bottom culture tube (Falcon, No. 3033). Five to seven times as many human or rodent somatic cells prepared for fusion were transferred to a second tube. After centrifugation at 200g for 5 min, supernatants were discarded and each pellet was resuspended in 5 ml of HBSS. The somatic cell suspension was then poured into the mitotic cell suspension tube, and the cell mixture was centrifuged for 5 min. The supernatant was poured off the tube and the pellet was gently resuspended in only 2 ml HBSS so that the cells were kept clustered. After another centrifugation for 5 min, the supernatant was poured off, keeping the tube upside down and blotting it from time to time for 1 min. Then, using a 1-ml syringe, 0.25 ml of 55% PEG was added to the pellet all at once and held for a 1-min exposure. At the same time the pellet was resuspended by gently shaking the tube. For the next 3 min, 2.5 ml of HBSS were added drop by drop, gently shaking the tube after each drop. The cell suspension was then centrifuged at 200*g* for 3 min. The supernatant was discarded, the tube blotted, and the pellet was resuspended in 0.5 ml chromosome medium 1A. Finally, 0.05 ml of colcemid and 0.05 ml of 20 mM $MgCl_2$ were added to the suspension. The tube was tightly closed and then incubated at 37°C for one hour. The cell fusion and induction of PCC was completed by that time.

When peripheral blood lymphocytes from rodents are to be used for in vivo experiments or, generally, when only small amounts of blood are available, the following cell fusion microtechnique was found to be convenient. In a 15-ml tube (Falcon, No. 3033), 0.5 ml whole human blood or an even smaller amount of rodent blood was diluted 1:5 in HBSS, layered on the top of a 3-ml Ficoll–Paque gradient and run at 400*g* for 20 min. Using a Pasteur

pipet, 8×10^5 cells were collected from the interface, washed once with HBSS and fused with 1.5×10^5 mitotic CHO cells following the procedure described above.

For testing cell viability, the trypan blue exclusion test was adopted and 0.4% trypan blue stain was routinely used. For the purpose of PCC induction by fusion, this viability criterion was considered sufficient.

To calculate the fusion index, PEG-treated cell samples were diluted in F10 medium and slides were prepared and stained with acetoorcein following the same procedure described for the mitotic index. The fusion index was then calculated as the percentage of the interphase cells under study found in multinucleated cells either as nuclei or as PCCs. This percentage was aways corrected in order to exclude the background fusion index of untreated cells.

Using the slides prepared to calculate the fusion index, the giant cell formation was estimated as the percentage of multinucleated cells which included more than four primary cells.

Chromosome and Slide Preparation for PCC Analysis. To the fused cells, 13 ml of a hypotonic (0.075 M) KCl solution were added for 10 min at room temperature and then the cells were fixed in methanol–acetic acid (3:1). In order to avoid cell clumping during fixation, especially when the interphase to mitotic CHO cell ratio was greater than three, all of the hypotonic solution was discarded after centrifugation at 200*g* for 5 min except for about 0.5 ml in which the pellet was resuspended. Then, 10 ml of fixative were added, at the beginning drop by drop and afterwards with gentle agitation. Following centrifugation at 200g for an additional 5 min, the cells were washed in 5 ml fixative, dropped on precleaned wet slides, air or flame dried, and stained with 3% Giemsa. The slides were then mounted with coverslips, and the PCC induction was analyzed by means of light microscopy.

The PCC index was determined as the percentage of interphase cells showing prematurely condensed chromosomes. When primary human or rodent cells were to be fused, the PCC index was corrected in order to exclude those PCCs resulting from the fusion of the 5% contaminant CHO interphase cells to 95% mitotic CHO cells. For this purpose, a study was run at the same time as the fusion experiment. A number of mitotic cells equal to the number used in each fusion were also treated as well with PEG and the background number of PCCs was estimated. It was found that, on the average, PCCs had been induced in 7% of the 5% contaminant interphase CHO cells. More than 95% of those PCCs were found to be in S-phase and less than 5% were found to be in G_2 or G_1 phase. Therefore, when calculating the PCC index for peripheral blood lymphocytes, since they are normally expected to give only G_1 PCCs, any S-phase or G_2 PCC was considered as a contaminant and consequently disregarded.

RESULTS

Variables Affecting PCC Induction. To examine the different aspects of the PCC induction by PEG-mediated cell fusion in primary cells, the effects of PEG concentration, PEG molecular weight, PEG exposure time and interphase-to-mitotic ratio on cell viability, fusion index, giant cell formation, and PCC index were thoroughly studied. For these analyses, mitotic CHO cells were fused to human or rat peripheral blood lymphocytes.

The effects of varying PEG concentrations were analyzed in experiments where approximately 1×10^6 mitotic CHO cells were fused with 6.5×10^6 human peripheral blood lymphocytes or with 2.8×10^6 rat lymphocytes. The mean values and ranges from three experiments are given in Tables 1 and 2.

With respect to the PCC indices, it should be mentioned that all lymphocytes in the analyzed slides were taken into account, including dead ones with their characteristic pyknotic nuclei. However, when clumped, dead cells were difficult to count. It may be, therefore, that the PCC indices for 50 and 55% PEG concentrations were overestimated due to a possible underestimation of the total number of lymphocytes.

In order to better contrast the PCC induction for each PEG concentration, the percentages of CHO metaphase cells having lymphocyte-induced PCCs (M-PCC indices) were estimated as an alternative to the PCC indices. Since the viability of the mitotic CHO cells was found to be 95% even at 55% PEG concentration, and the same amount of mitotic cells was used in each

Table 1. Effects of PEG Concentration on PCC Induction in Human Peripheral Blood Lymphocytes[a,b]

	PEG concentration (%)			
	35	45	50	55
Cell viability (%)	98	94	79	72
Fusion index (%)	5 (3.0–6.8)	9 (7.0–12.3)	23 (19.2–25.5)	35 (29.5–38.2)
Giant cell formation (%)	0	0	2.4 (1.3–3.5)	9.1 (7.5–11.2)
PCC index (%)	0.8 (0.5–1.2)	2.6 (1.7–3.2)	7.6 (6.2–9.7)	14.5 (11.5–16.7)
M-PCC index[c] (%)	1.8 (1.1–2.3)	4.6 (3.8–5.1)	8.7 (7.5–10.1)	17.2 (16.1–18.6)

[a] Fusion conditions: PEG molecular weight = 1000, PEG exposure time = 1 min, interphase to mitotic ratio = 6.5.

[b] Indices in this table refer to peripheral blood lymphocytes and were measured 1 h after PEG treatment.

[c] The M-PCC index is defined as the percentage of CHO metaphase cells having induced PCCs, i.e., (metaphases with PCCs/total no. of scored metaphases) × 100.

Table 2. Effects of PEG Concentration on PCC Induction in Rat Peripheral Blood Lymphocytes[a,b]

	PEG concentration (%)			
	35	45	50	55
Cell viability (%)	95	93	77	70
Fusion index (%)	3.5 (2.1–4.9)	14.8 (11.5–16.9)	30.4 (25.0–35.1)	53.1 (47.3–57.2)
Giant cell formation (%)	0	0	4.3 (2.9–5.5)	13.2 (10.4–15.3)
PCC index (%)	1.8 (1.1–2.4)	9.8 (7.5–12.3)	14.7 (12.8–16.1)	20.6 (17.4–24.6)
M-PCC index[c] (%)	0.6 (0.5–0.7)	2.5 (1.8–3.1)	4.3 (3.3–5.2)	7.0 (5.9–8.3)

[a] Fusion conditions: PEG molecular weight = 1000, exposure time = 1 min, interphase to mitotic ratio = 2.8.
[b] Indices in this table refer to peripheral blood lymphocytes and were measured 1 h after PEG treatment.
[c] The M-PCC index is defined as the percentage of CHO metaphase cells having induced PCCs, i.e., (metaphases with PCCs/total no. of scored metaphases) × 100.

fusion, this new index gives a better approximation to the total amount of PCCs obtained in each fusion experiment.

The effects of different PEG molecular weights on cell viability and PCC index were analyzed in experiments using 55% PEG to fuse 5.6×10^5 mitotic CHO cells to 2×10^6 human peripheral blood lymphocytes. Average values from two experiments are given in Table 3.

To study the effects of PEG exposure time on cell viability and PCC index, approximately 1.2×10^6 mitotic CHO cells were fused to 4.2×10^6 human peripheral blood lymphocytes. For the cell fusion procedure, 0.25 ml of 45% PEG was chosen in order to have very good cell viability at 1 min

Table 3. Effects of PEG Molecular Weight on Cell Viability and PCC Index in Human Peripheral Blood Lymphocytes[a,b]

	PEG (manufacturer, mol wt)			
	Sigma, 1000	BDH,[c] 1500	Sigma, 8000	Baker, 8000
Cell viability (%)	70	73	68	70
PCC index (%)	18.1 (15.3–20.9)	16.0 (14.1–17.9)	17.4 (16.3–18.5)	15.5 (13.6–17.4)

[a] Fusion conditions: PEG concentration = 55%, PEG exposure time = 1 min, interphase to mitotic ratio = 3.6.
[b] As in Table 2.
[c] BDH Chemicals Ltd.

Table 4. Effects of PEG Exposure Time on Cell Viability and PCC Index in Human Peripheral Blood Lymphocytes[a,b]

	Time of PEG treatment (min)			
	1	3	5	7
Cell viability (%)	98	93	85	78
PCC index (%)	5.5 (3.9–6.8)	4.7 (3.2–6.1)	8.8 (6.5–10.5)	7.9 (6.3–8.8)

[a] Fusion conditions: PEG molecular weight = 1000, PEG concentration = 45%, interphase to mitotic ratio = 3.5.
[b] Lymphocyte viability measured 1 h after PEG treatment.

exposure and so that the exposure time could be increased to up to 7 min. The average values and ranges from three experiments are given in Table 4.

The effects of interphase-to-mitotic ratio on PCC index were analyzed in experiments using 0.25 ml 50% PEG to fuse 1.5×10^6 mitotic CHO to 1.5, 3.0, 7.5, and 10.5×10^6 lymphocytes so that the ratios were 1, 2, 5, and 7, respectively. For ratios 10 and 16, 5×10^5 mitotic CHO cells were fused to 5 and 8×10^6 lymphocytes, respectively. Mean figures and ranges from three experiments are given in Table 5.

Required Time for Cell Fusion and PCC Induction. The fusion process resulting from this protocol was closely monitored using a hemacytometer. It was observed that the fusion begins only after the PEG-treated cells are resuspended in 0.5 ml chromosome medium 1A and transferred to the incubator. No fusion was observed in cell samples drawn just before transferring the cells to the incubator. However, the cells were seen to be in pairs or small clusters and, after about 2 min at room temperature, they were observed to start fusing. From this point on, the whole fusion process, even at room temperature, could be followed on the hemacytometer and photographed step by step.

Table 5. Interphase to Mitotic Ratio and PCC Index in Human Peripheral Blood Lymphocytes[a]

	Interphase–mitotic ratio					
	1	2	5	7	10	16
PCC index[b] (%)	16.2 (12.7–19.0)	14.1 (11.5–16.8)	8.7 (5.7–11.1)	5.8 (3.8–6.9)	3.2 (2.4–3.9)	1.3 (0.8–2.1)
M-PCC index[c] (%)	1.9 (1.2–2.7)	3.0 (2.3–3.9)	7.0 (5.8–7.8)	8.1 (7.2–8.9)	8.5 (7.0–9.6)	6.0 (4.1–7.3)

[a] Fusion conditions: PEG molecular weight = 1000, PEG concentration = 50%, PEG exposure time = 1 min.
[b,c] As in Table 2.

Figure 1 shows a photograph taken from a PEG-treated CHO cell sample that was incubated for 5 min. Five successive stages of the fusion process are pointed out. In samples incubated for 10 min, up to 70% of the total number of cells fused in 1 h was observed.

With respect to the time required for PCC induction, human and rat lymphocytes were prepared and fused with mitotic CHO cells, as described in Materials and Methods, and incubated for varying times after the PEG treatment. A sample of fused human lymphocytes was harvested for chromosome preparation after only 15 min incubation. It was observed that PCC was already induced, but the interphase chromosomes had a decondensed appearance (Fig. 2A) in contrast with those observed after 1 h incubation (Fig. 2B). Chromosome preparations were also made from samples of fused rat lymphocytes incubated for 20, 30, 45, 60, and 90 min after PEG treatment, in order to observe the gradual condensation of the interphase chromosomes. Figures 3A and 3B are representative of the condensation stage at 30 and 60 min, respectively. After 1½ h of incubation, even more condensed chromosomes were observed.

PCC Induction in Primary Newborn Mouse Cells. Following the procedures described in Materials and Methods, spleen and whole body newborn mouse cells were successfully fused with mitotic CHO cells and prepared for PCC analysis. Figure 4A shows G_1 PCCs induced in a newborn mouse spleen

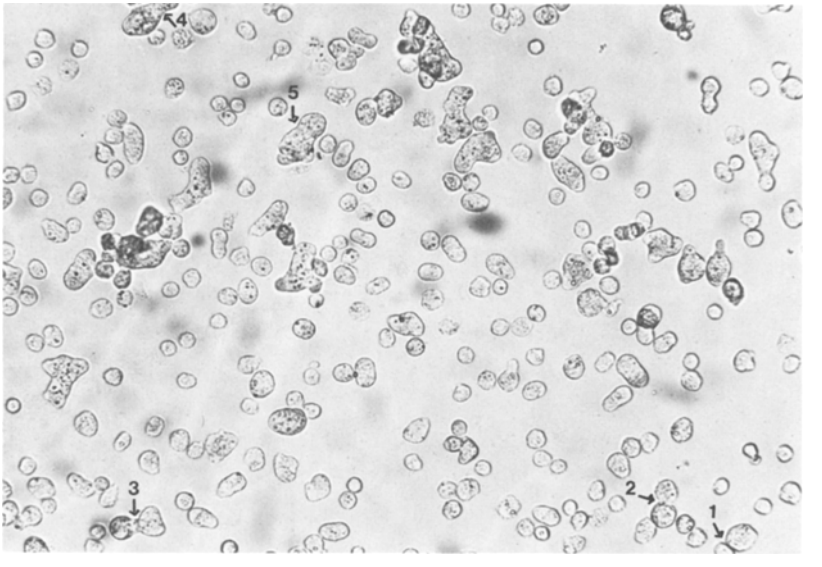

Fig. 1. Fusion of cells in suspension. Five successive stages of the fusion process are shown for CHO cells incubated for only 5 min after the PEG treatment.

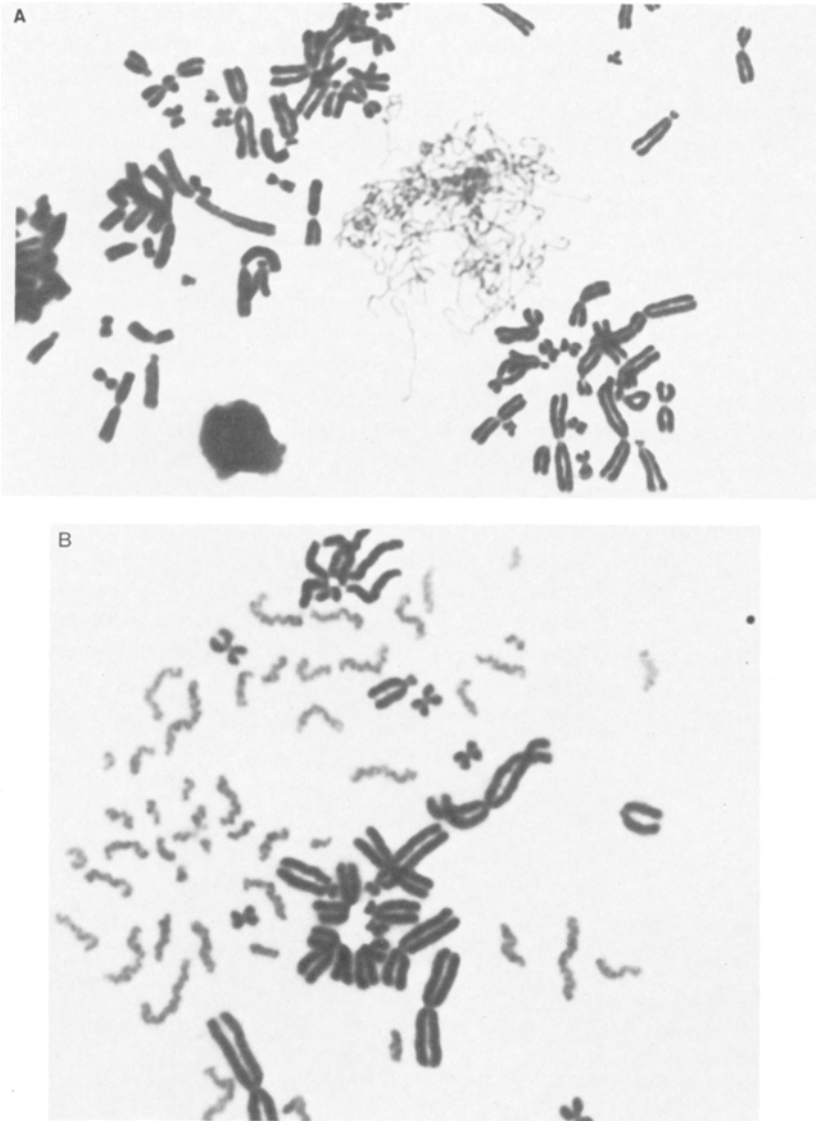

Fig. 2. Premature chromosome condensation induced by PEG-mediated fusion in human peripheral blood lymphocytes fused with mitotic CHO cells. (A) Decondensed appearance of the interphase chromosomes when fused lymphocytes were incubated for only 15 min after PEG treatment. (B) Notice 46 prematurely condensed chromosomes when fused lymphocytes were incubated for 1 h after PEG treatment.

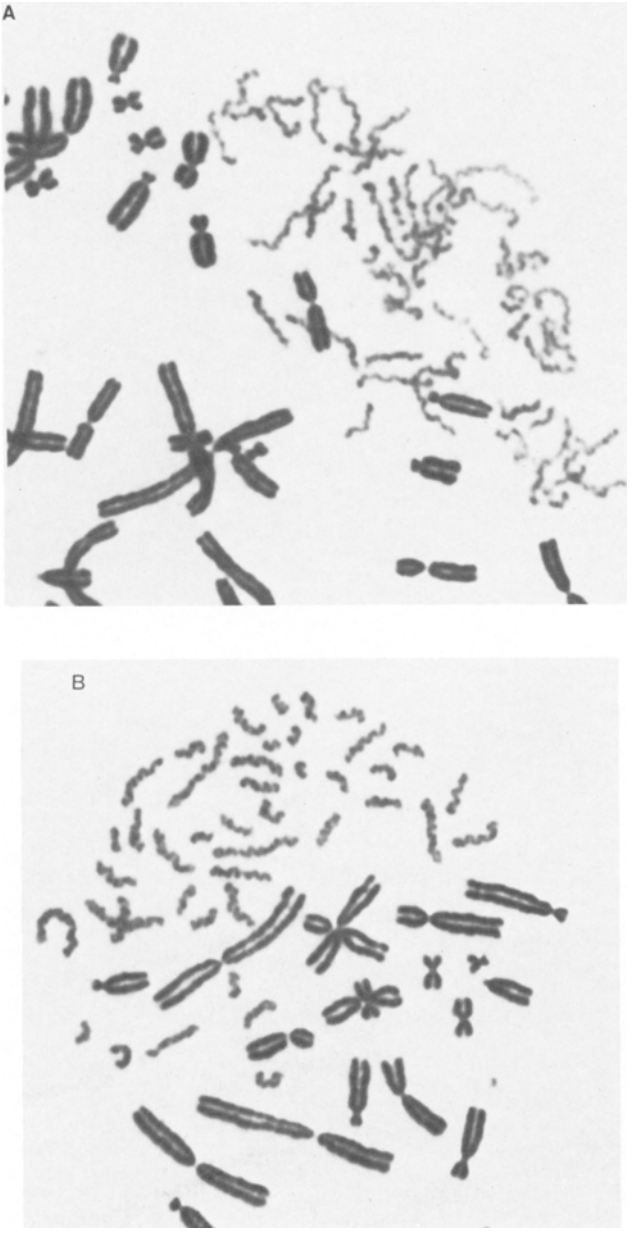

Fig. 3. Premature chromosome condensation induced by PEG-mediated fusion in rat peripheral blood lymphocytes fused with mitotic CHO cells. Condensation of the interphase chromosomes is shown for fused lymphocytes incubated for 30 min (A) and 60 min (B) after PEG treatment. Notice 42 prematurely condensed chromosomes in (B).

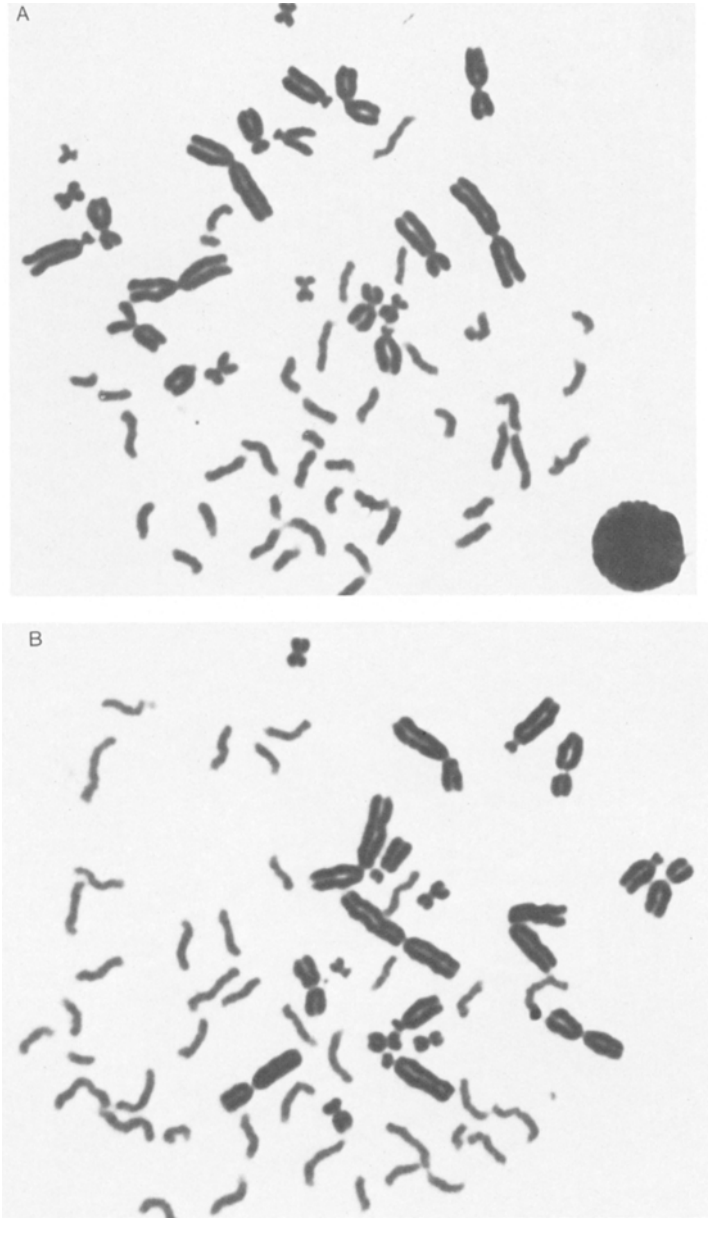

Fig. 4. Premature chromosome condensation induced by PEG-mediated fusion in newborn mouse somatic cells. (A) Spleen fused cells and (B, C) whole body fused cells were incubated for 75 min after the PEG treatment. Notice in (A) and (B) 40 prematurely condensed chromosomes exhibiting a single chromatid per chromosome (G_1 PCC) and, in (C), G_2 PCC exhibiting two lightly stained chromatids per chromosome.

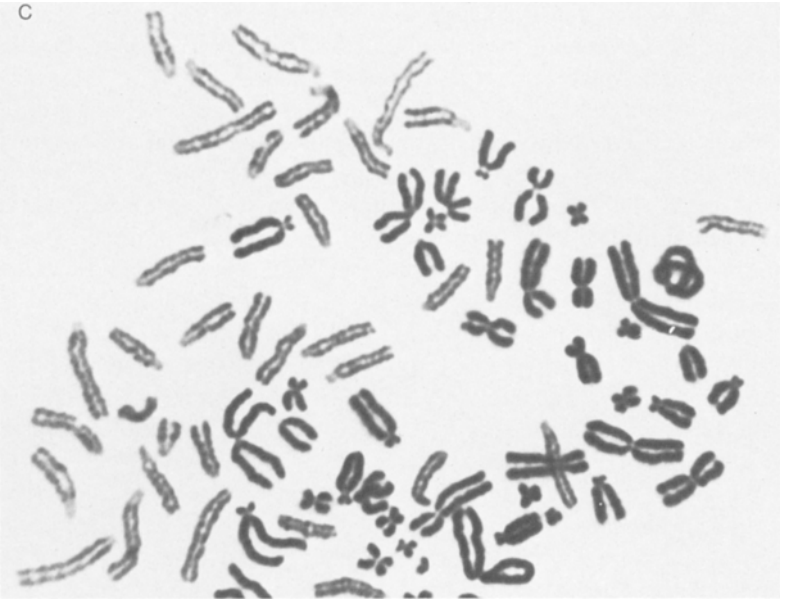

Fig. 4. Continued

cell. Figures 4B and 4C show G_1 and G_2 PCCs, respectively, induced in whole body newborn mouse cells.

DISCUSSION

The PCC phenomenon induced by cell fusion is known to be a powerful cytokinetic and cytogenetic tool. It has been already successfully used for diagnostic purposes in human leukemia by means of the visualization and quantification of interphase chromatin changes and chromosome malformations.

In this work a simple method for the induction of PCC on primary cells in suspension using the potent fusogen polyethylene glycol is presented. With this method, the PCC technique could be more widely applied in the study of various cellular phenomena and as an assay to detect and evaluate DNA and chromosome damaging agents. In particular, the fact that this method can be applied to 0.5 ml or even smaller amounts of blood makes it especially suited for in vivo studies using rodents and also in clinical applications for diagnostic purposes.

The experiments described in the previous section were designed in order to study PCC induction in lymphocytes under different cell fusion conditions. The results suggest that the main variables influencing PCC induction are the

PEG concentration and the interphase-to-mitotic cell ratio. The PEG treatment time plays a role in the cell viability without affecting significantly the PCC induction. With respect to the PEG molecular weight, it seems that molecular weights varying from 1000 to 8000 can be successfully used.

To obtain satisfactory cell fusion and PCC indices, and at the same time keeping high cell viability with low giant cell formation, a 50% PEG concentration and an interphase-to-mitotic ratio of five are adequate. However, for quantitative PCC studies requiring the analysis of large amounts of PCCs, a 55% PEG concentration and a 6–7 interphase-to-mitotic cell ratio could be more appropriate. The M-PCC indices in Tables 1 and 5 clearly support these cell fusion conditions. For a 50% PEG concentration, an average of 8.7 PCCs were scored for every 100 CHO metaphases, whereas double the amount of PCCs were scored when 55% PEG concentration was used. As to the interphase-to-mitotic cell ratio, Table 5 shows that even though a ratio of 1 gives the highest PCC index, the number of PCCs scored per 100 CHO metaphases (M-PCC index) is the lowest. In contrast, ratios of 6–7 give lower PCC indices but considerably larger amounts of PCCs. The difference in the results obtained for human and rat peripheral blood lymphocytes in Tables 1–3 reflects the different ratios of interphase to mitotic CHO cells used in the experiments. Indeed, for human lymphocytes, the PCC indices were 14.5 and 18.1 for ratios 6.5 and 3.6, respectively. The PCC index further increased to 20.6 when a 2.8 ratio was used for rat lymphocytes.

Obviously, the conclusions drawn for the interphase-to-mitotic cell ratio apply only to lymphocytes and other cells of comparable size. When larger cells are to be fused, similar experiments should be carried out to determine the most appropriate cell fusion conditions. Our experience with interphase CHO, V79, and mouse mammary tumor cells suggests the use of ratios up to 3.

With respect to the time required for the cell fusion to take place, this work confirms previous observations that most of the cell fusions are completed in 15 min following the PEG treatment (25). In addition, it was observed that in about the same time dissolution of the nuclear membrane of the interphase fused cells and PCC induction had also occurred (Fig. 2A).

In our laboratory, the method presented here for PCC induction in primary cells by PEG-mediated fusion is being used for applied biological dosimetry. Human and rat lymphocytes, rodent spleen cells, spleen and whole body newborn mouse cells are routinely used for this purpose. Chromosomal damage in cells exposed to clastogens is analyzed as breaks, gaps, or exchanges in G_1 or G_2 PCCs. The experiments are always reproducible, but the amount of PCCs obtained varies from experiment to experiment, depending mainly on the interphase-to-mitotic cell ratio used. The sensitivity of the method has been seen to be extremely good.

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