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Abstract

In cell cultures of Detroit 6, KB, and HeLa cells, treatment with certain amounts of 5-Fluorouracil resulted in the appearance of a strikingly distinct halo or chromophobic area, entirely encircling the compacted or contracted nucleoli, before the ultimate disintegration of the cells.

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THE APPEARANCE OF PERINUCLEOLAR HALOS IN CULTURED CELLS WITH 5-FLUOROURACIL*

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Some of the cellular morphological changes induced in Ehrlich ascites tumor cells by 5-Fluorouracil (FU) have been reported[1,2]. They were enlargement of the cells and after Feulgen staining, irregular distribution of DNA into coarse clumps and strands around vacuoles within the nuclei.

During our studies of the effects of FU on cultured cells, it was observed that the addition of certain amounts of FU to monolayers of cells was followed by, among other changes, the appearance of a strikingly distinct halo or chromophobic area, entirely encircling the compacted or contracted nucleoli, in fixed and stained cells. KOPAC and MATEYKO[3], and GONZÁLEZ-RAMÍREZ[4], have reviewed and postulated concerning perinucleolar localized segmental areas or vacuoles but have not described such condition compelling complete encirclement of nucleoli by chromophobic zones as our material demonstrates.

MATERIALS AND METHODS

The cell strains used were Detroit 6, KB, and HeLa, about 10^5 of these cells, grown at 37°C in Eagle's minimum essential medium[5], supplemented with 15% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml), were inoculated in Cooper dishes[6,7] containing a cover glass and 5 ml of the medium. Five to seven days after inoculation, the cover glasses were transferred to Cooper dishes each containing a definite concentration of FU in the medium. For the controls, the same amonnt of distilled water was added. The cells grown on cover glasses in Cooper dishes were cultured in an atmosphere of 5% carbon dioxide at 37°C. For each concentration of FU, 6 cover glasses were prepared. After 3 days' cultivation in the presence of FU, halves of the cover glasses were taken from Cooper dishes and after being washed with prewarmed (37°C) PBS, they were fixed in cold alcohol-formalin-acetic acid fixative and stained with hematoxylin and eosin. The remaining cover glasses were aimilarly treated after 7 days' cultivation.

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RESULTS AND DISCUSSION

The inhibitory effect of FU on mitosis, as shown in Table 1, was slightly recognized at less than 1 μg/ml; at more than 3 μg/ml the mitotic ratios were

Table 1

cell	control	1 μg/ml	3 μg/ml	10 μg/ml
Detroit 6	1.9	1.2	0.3	$<$ 0.1
KB	2.0	1.9	0.7	$<$ 0.1
HeLa	2.9	2.8	0.6	0.2

The mitotic ratio was counted on three cover glasees by reading the number of mitotic cells in over 5,000 cells for each concentration. They were fixed and stained after 3-day treatment with FU. The value was expressed in per cent.

Table 2

cell	conc. of FU (μg/ml)	3 days	7 days	10 days	14 days
Detroit 6	0	−	−	−	−
	1	−	−	−	−
	3	−	±	+	++
	10	−	±	+	++
	30	+	±	+++	+++
	100	+	+++	+++	+++
KB	0	−	−	−	−
	1	−	−	−	−
	3	−	±	+	++
	10	−	±	++	++
	30	+	++	+++	+++
	100	++	+++	+++	+++
HeLa	0	−	−	−	−
	1	−	−	−	−
	3	−	±	+	++
	10	−	±	++	+++
	30	−	+	+++	+++
	100	+	++	+++	+++

$2-3\times10^5$ cells were inoculated into culture bottles (200 ml) containing 10 ml of medium. After five-day culture the medium was removed and replaced by 15 ml of medium each containing a definite concentration of FU. For the control, the medium containing the same amount of distilled water was added. These bottles were cultured at 37°C and the medium was changed every four days. Cytotoxic changes were observed under microscope.

(−) monolayer intact with no cytological changes; (±) monolayer intact containing thin and enlarged cells; (+) parts of the monolayer replaced by granulated cells and rounded forms; (++) about half of the monolayer replaced by granulated cells and rounded forms; (+++) monolayer disintegrated.

decreased abruptly. On the other hand, the cytotoxicity of FU on the monolayers at more than 3 μg/ml, in which concentrations mitosis was inhibited, appeared within two weeks, as shown in Table 2. This process, however, developed considerably slower.

In the samples fixed and stained, after 3 days treatment with FU, at 10 μg/ml nucleoli became compact and round (Figs. 1b, 2b, 3b); at 30 μg/ml (Figs. 1c, 2c, 3c), this tendency was recognized more evidently and distinct halos encircling around nucleoli were observed in some cells. At 100 μg/ml (Figs. 1d, 2d, 3d), some cells were dying, but most of the remaining cells also showed contracted nucleoli surrounded by chromophobic halos which separated nucleoli from nuclei. After 7 days, these changes were advanced further and most of the remaining cells showed circular halos around nucleoli (Figs. 1e, 1f, 2e, 2f, 3e, 3f).

These morphological changes around nucleolus, among other changes, were observed in all the three cell strains used: Detroit 6, KB, and HeLa. However, by the addition of 5-Fluoro-2′-deoxyuridine (FUDR) to these cells in the same manner, these morphological changes were not clear.

The significance of these morphological changes around nucleoli is unknown. The meaning of the difference between FU and FUDR on these changes is likewise obscure. However, it is suggested that by the addition of FU to cultured cells some serious changes in the nuclear, especially in the nucleolar functions, occur.

More detailed observation on these phenomena is in progress.

SUMMARY

In cell cultures of Detroit 6, KB, and HeLa cells, treatment with certain amounts of 5-Fluorouracil resulted in the appearance of a strikingly distinct halo or chromophobic area, entirely encircling the compacted or contracted nucleoli, before the ultimate disintegration of the cells.

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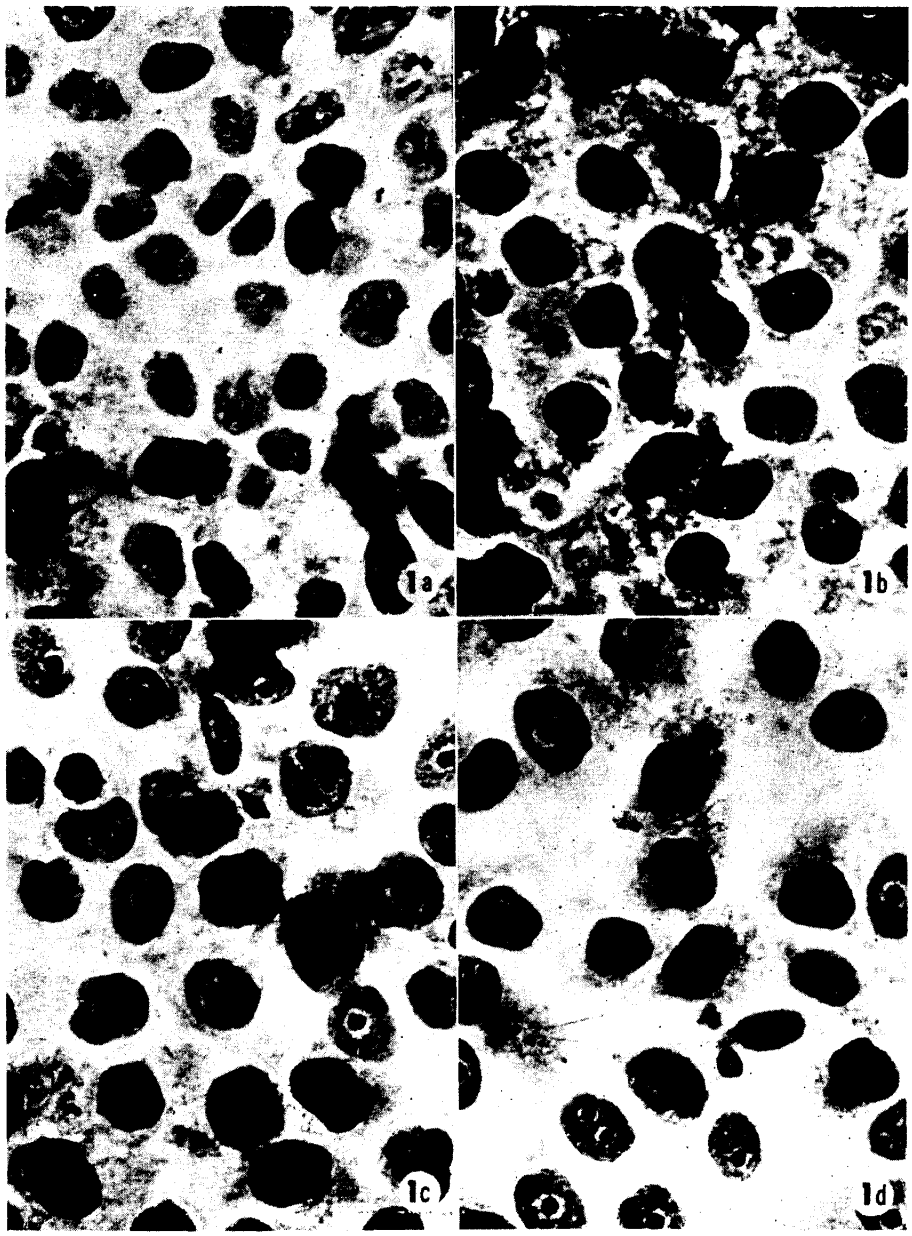
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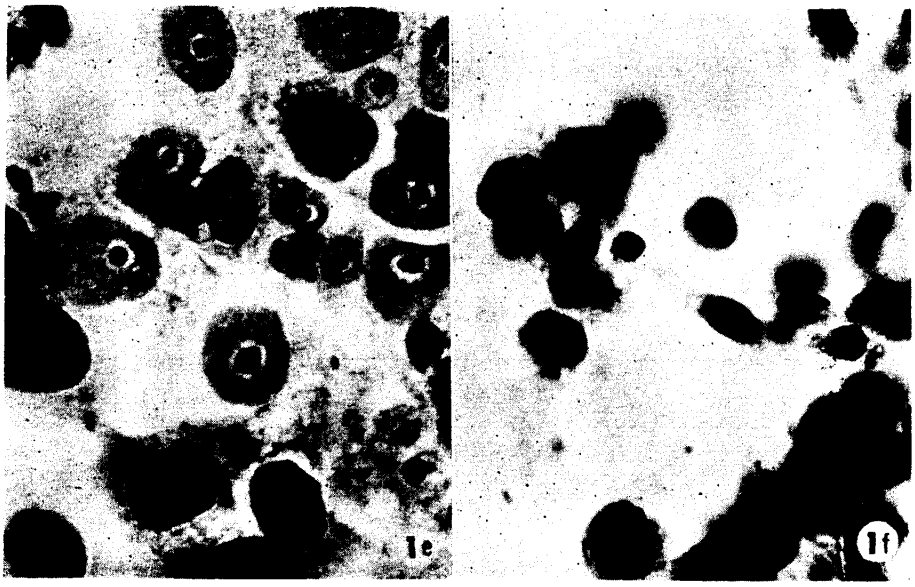

Fig. 1a Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in control medium 3 days
Fig. 1b Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 10μg/ml of FU medium 3 days
Fig. 1c Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30μg/ml of FU medium 3 days
Fig. 1d Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100μg/ml of FU medium 3 days
Fig. 1e Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30μg/ml of FU medium 7 days
Fig. 1f Detroit 6 cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100μg/ml of FU medium 7 days

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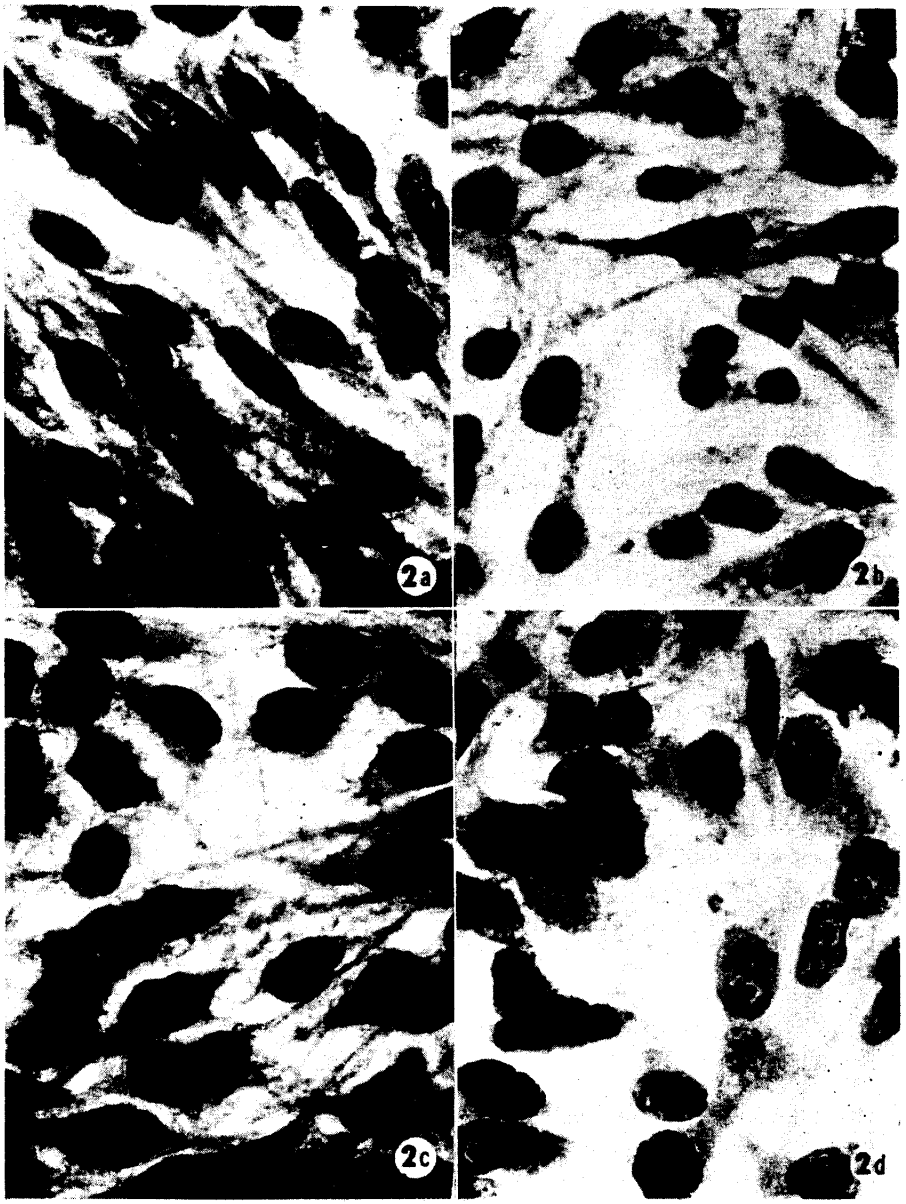
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2b
2c
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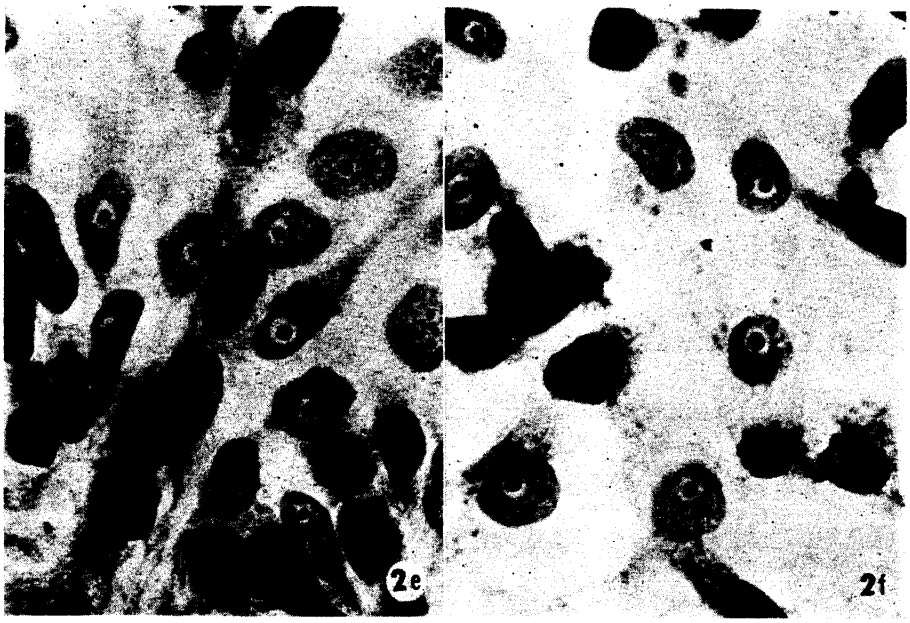
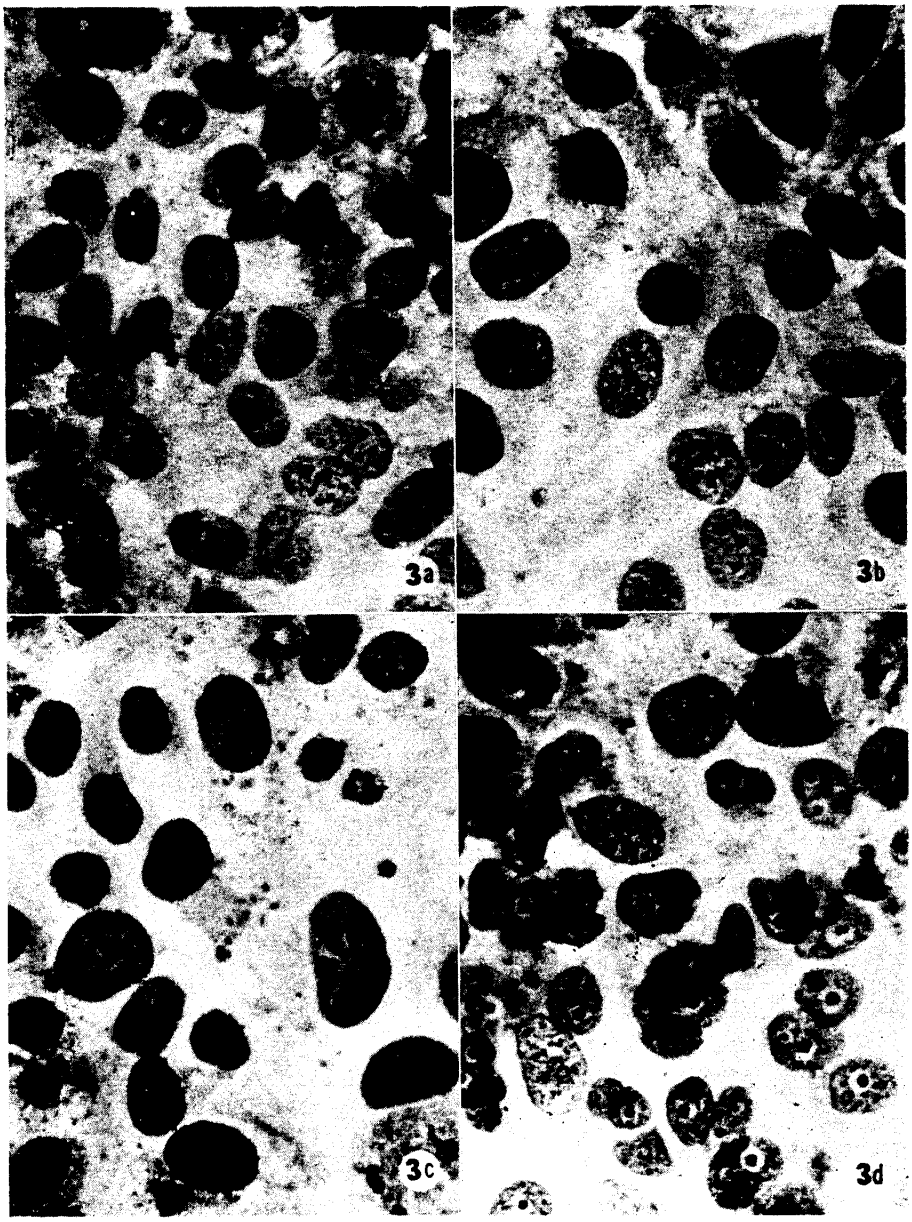


Fig. 2a KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in control medium 3 days
Fig. 2b KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 10 μg/ml of FU medium 3 days
Fig. 2c KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30 μg/ml of FU medium 3 days
Fig. 2d KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100 μg/ml of FU medium 3 days
Fig. 2e KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30 μg/ml of FU medium 7 days
Fig. 2f KB cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100 μg/ml of FU medium 7 days


3a
3b
3c
3d

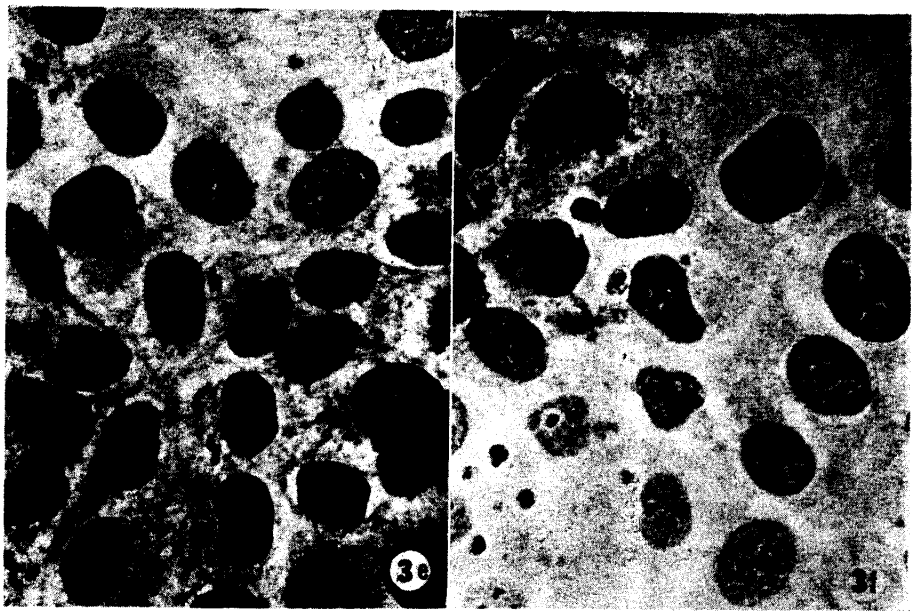


Fig. 3a HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in control medium 3 days

Fig. 3b HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 10 μg/ml of FU medium 3 days

Fig. 3c HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30 μg/ml of FU medium 3 days

Fig. 3d HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100 μg/ml of FU medium 3 days

Fig. 3e HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 30 μg/ml of FU medium 7 days

Fig. 3f HeLa cell monolayer, Hematoxylin-Eosin stain. ×720
Incubated in 100 μg/ml of FU medium 7 days