USE OF THE M-9 IMAGE CONVERTER TO STUDY THE ACTION OF ULTRAVIOLET RAYS ON TISSUE CULTURE CELLS

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By means of ultraviolet (uv) microscopy a high-contrast image may be obtained of living unstained cells, and the degree of contrast depends on the selective absorption of the uv rays by the cell components containing nucleic acids and proteins.

However, the uv image cannot be observed directly in the microscope. To convert it into a visible image several methods have been used: photography on nonsensitized photographic materials, fluorescent screens made of uranium glass, and the use of image converters and television methods.

The image converter method is the most promising, for it has certain advantages over the others.

When photomicrography is carried out in uv rays using a nonachromatic optical system difficulties arise in focusing. Furthermore, the photographic process is prolonged and the image cannot be observed at once. On a uranium glass fluorescent screen a bright enough image cannot be obtained. Television methods are much too complicated and cumbersome.

Only brief accounts of the use of image converters with electrostatic focusing for visual uv microscopy are to be found in the literature [3, 4]. However, although image converters with electrostatic focusing are small in size and can yield a bright image with good resolution in the center, they have not been widely used in uv microscopy. The reason presumably is that they provide an image on a comparatively small screen, and the quality of the image falls off sharply as the distance from the center of the screen increases. Accordingly these converters are used mainly for focusing and for choosing the area to be photographed, and not for the actual study of the fine structure of the object [4].

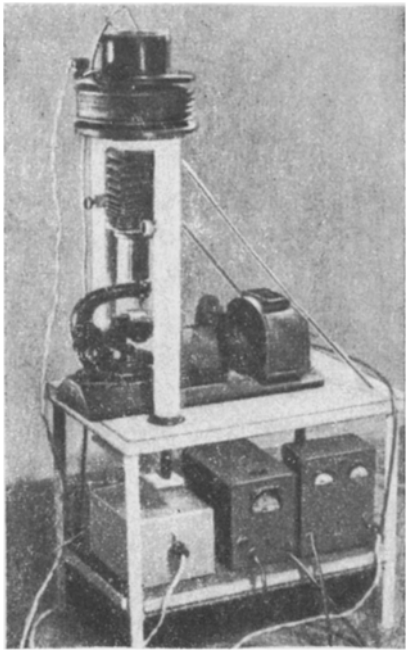

Fig. 1. General view of the uv microscope with the M-9 image converter.

The object of this investigation was to study the possibility for using image converters with magnetic focusing (M-9) and an uviol window in conjunction with a type MUF-3 uv microscope for the visual study of the changes arising in tissue culture cells under the influence of uv irradiation.

The image converter with magnetic focusing used in the present investigation has important advantages over converters with electrostatic focusing. It can produce an image on a screen 45 cm in diameter with good resolution in the center and over the field of the screen (from 40-50 to 100 lines/mm).

The general appearance of the instrument is shown in Fig. 1. A more detailed account of the apparatus used is given elsewhere [2].

A strain of human amnion cells (A_1) was cultivated on quartz slides in medium No. 199 with 10% bovine serum and

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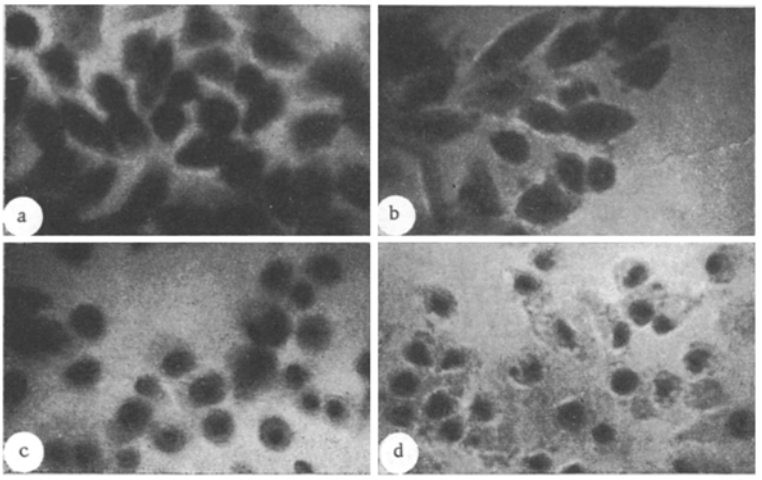

Fig. 2. Tissue culture cells (strain of human amnion cells). a — Unirradiated b — irradiated with uv light for 2 min; c — for 3 min; d — for 5 min. Photographed from the converter screen by a reproduction attachment with "Industar-50" objective to a scale of 1 : 1 on RF-3 film. MUF-3 microscope, objective 58 × 0.8 (water immersion), ocular 8. Exposure 2-4 sec.

antibiotics for 3-7 days. The slides were then taken from the medium and irradiated with unfiltered uv rays from a type PRK-4 mercury-quartz burner. The radiation from this mercury-quartz burner has a maximum at 253 mμ.

In the first experiments one half of the quartz slide with the culture was covered by an ordinary glass cover slip and the other half with a quartz cover slip. In this way half the cells of the culture were protected from the uv radiation. The duration of irradiation was 1 and 3 min and the distance from the lamp to the preparation 20 cm. In subsequent experiments, in order to eliminate any action of heat on the cells, the distance from the lamp to the preparation was increased to 40 cm and the irradiation time was also increased to 2 and 5 min. In the next experiment two quartz slides were irradiated simultaneously: one was covered with a quartz slip and the other with an ordinary glass slip.

After irradiation (in sterile conditions) the quartz slides with the cultures were replaced for 18-24 h in the same nutrient medium as that in which they had been cultivated before irradiation. The slides were then removed, covered with a quartz cover slip, and quickly examined by means of the MUF-3 microscope in uv light: with UFS-1 filters and a chlorine + bromine gas mixture, a 58 × 0.8 uv objective (water immersion 0.8), and a quartz ocular 8×.

For visual uv microscopy, the M-9 image converter was placed above the MUF-3 microscope. The invisible uv image obtained in the microscope was projected by the ocular of the microscope on to the photocathode of the converter, where it was converted into a stream of electrons which was accelerated by an electric field, focused by a magnetic field, and fell on a fluorescent screen, where a visible image corresponding to the uv image was obtained.

This image may also be photographed from the screen of the converter by means of a special film reproduction attachment.

The investigations showed that by the use of the M-9 image converter with magnetic focusing in conjunction with the MUF-3 uv microscope, it is possible to observe a sufficiently bright visible image of tissue culture cells, obtained in the uv region of the spectrum (250-285 mμ), on the fluorescent screen of the converter 45 mm in diameter. However, the image of the tissue culture cells was of lower contrast than the image of unstained, fixed sections at the same magnification.

Comparison of tissue culture cells exposed and not exposed to uv irradiation showed that as a result of this procedure the volume of the nuclei was reduced and the degree to which they absorbed uv rays was increased. The nuclei were more clearly outlined. The cells were rounded and reduced in size, and their cytoplasm was more transparent (Fig. 2).

With an increase in the time of irradiation the degenerative changes in the cells were intensified.

In the control preparations most cells were elongated and only a few were round. Their nuclei were less clearly outlined and their cytoplasm was denser. The contrast between nucleus and cytoplasm was less marked.

Similar results were obtained by photomicrography in uv light [1].

On visual observation the structure of the cells could be discerned more clearly than on photographs taken from the screen of the image converter.

These investigations show that the use of image converters with magnetic focusing, enabling a sufficiently clear and bright image to be obtained on the screen, will extend the scope of uv microscopy as a method of studying the processes taking place in living cells.

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