ULTRAVIOLET MICROBEAM IRRADIATION IN LIVING CELL MEMBRanes

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In previous communications the authors have described the cellular and subcellular responses to ultraviolet irradiation directed to whole Chang liver cells and to portions of Chang liver cells (3-6). Two sources of ultraviolet have been utilized in these studies. One source was a mercury arc lamp the output of which was passed through a grating monochromator, and the second source was the ultraviolet flying spot scanner tube. A modification of this latter technique has been described by Bonner (1), and further modifications by Cook have yielded the present instrumentation (Fig. 1).

One reaction of living cells to ultraviolet light is the phenomenon of zeiosis. This term was originated by Costero and Pomerat to describe a type of bubbling which occurs on cell surfaces in some circumstances (2). Brief or continuous exposure of cells to varying amounts of ultraviolet light has been demonstrated by the authors to produce zeiosis of the peripheral cytoplasmic membrane, irrespective of whether the ultraviolet source was the monochromator or the flying spot scanner tube and whether the ultraviolet was delivered to the whole cell or to the cytoplasm alone (5-7).

Utilizing the improved equipment depicted in the block diagram in Fig. 1, it is possible to generate an ultraviolet microbeam of any desired shape or dimension and to bring it to focus on the specimen by means of a 0.72 NA Polaroid Grey Reflecting objective. The ultraviolet-emitting scanner tube used as a light source in these experiments had a peak emission at 2680 A, with emission from 2,400 to 3,500 A. In Fig. 1, MPT refers to multiplier phototube. In order to test the response of the peripheral cytoplasmic membrane to ultraviolet irradiation, a microbeam of ultraviolet was shaped to fit the entire peripheral membrane of a single Chang liver cell maintained in a tissue culture chamber. This shaped beam followed the irregular outline of the cytoplasm and was 0.5 micron in diameter with an energy of $0.18 \times 10^{-7}$ ergs/sec/cm$^2$. The ultraviolet microbeam was continuously applied to the outer edge of the cytoplasmic membrane while the results were recorded by visible light phase-contrast time-lapse motion pictures. The ultraviolet irradiation was applied in this fashion to 40 single cells, and the experiment was considered at an end when cell death occurred. In these circumstances, this required an average of 30 hours of irradiation per cell.

An analysis of the visible light phase-contrast time-lapse motion picture films demonstrated that cell death from this form of ultraviolet irradiation differed from the cell deaths occurring when the entire cell or the cytoplasm alone was exposed to ultraviolet light. In the present experiment, the cells did not show any of the previously noted membrane responses to ultraviolet irradiation. They did not show a gradual cessation of pinocytosis, nor did they manifest either generalized or localized zeiosis. At the time of death these cells simply rounded up without other morphologic evidence of damage. This collapse and cell death was not preceded by alterations in the behavior of the rest of the cell or its organelles, insofar as such alterations might be detected in the motion picture images. Cessation of pinocytosis, rate of cytoplasmic particulate motion, mitochondrial swelling, change in the size and shape of the nucleus or nucleolus are all examples of cellular alterations which may be observed with this technique. After
collapse, these cells did not develop the large membranous bubbles due to syneresis, which were observed on the surface of cells very shortly after their death from total cell exposure to ultraviolet irradiation, and which were similar to those reported by Zirkle and Uretz after high intensity ultraviolet microbeam irradiation of a portion of a living cell membrane (8).

Several possibilities might be considered to explain the mechanism of cell death in these experiments. Cell membranes are known to change their permeability when exposed to ultraviolet light, and such a change might produce irreversible damage (9). Some small portion of the cytoplasm of the cell was inevitably included in the microbeam shaped to fit the membrane, and it is conceivable that UV damage to this material may have resulted in cell death. In the same way that some cytoplasm was irradiated by the beam, a small portion of the medium was simultaneously irradiated. Dendy and Smith were able to influence cell metabolism by UV irradiation of the adjacent medium (10). A fourth possibility is that the absorbed energy may be conveyed elsewhere in the cell and produce its effect at some unknown site.

Irradiation of the peripheral portion of the membrane does not induce the membranous manifestations of ultraviolet irradiation seen when the whole cell, or its cytoplasm alone, is irradiated. The site or sites of ultraviolet light damage within the cell which do produce the cytoplasmic membrane phenomena of zeiosis and of cessation of pinocytosis remain unknown. The unmasking of such a site or sites would greatly aid our understanding of the basic mechanisms of the cellular control of the form and function of the cytoplasmic membrane.

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