Ultra-Violet Damage to Living HeLa Cells as Recorded by Time-Lapse Motion Picture Studies.* (23301)

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In previous communications the authors have described the technics of ultra-violet flying spot television microscopy and of time-lapse motion picture photography for the recording of results(1,2,3). The purpose of this paper will be to report a preliminary study of several types of ultra-violet induced damage occurring in living HeLa cells when subjected to continuous scanning by the ultra-violet cathode ray tube.

Methods. The present cathode ray scanner tube has a peak emission at 2580 Å with a ½ band width of 1000 Å. This spectral emission curve was further modified by inserting at the ocular of the microscope an interference filter. The spot scanning the specimen thus had a peak intensity at 2650 Å with a ½ band width of 75 Å. The spot was made to sweep a 400-line/inch raster every 4 seconds and the resulting radar type monitor tube image was then recorded anew every 4 seconds on a single frame of 16 mm motion picture film. For our purposes, living HeLa cells were carried in continuous culture in a medium consisting of chick embryo extract, human ascitic fluid and Hanks balanced salt solution. During the study the cells were incubated at 37½°C on bare Vycor cover slip preparations which were in turn sealed to Vycor slides by a mixture of vaseline and paraffin. No differences in absorption were noted in cells mounted in balanced salt alone or in cells mounted in stock media. During the entire experiment the cells were continuously scanned and the results continuously recorded photographically.

Results. When the beam current of the scanner tube was adjusted to 10 micro-amps, continuous photographic recording of the cells was possible for 6 to 10 hours with no detectable evidence of damage. Thus, for example, 6 individual cells were observed to pass uninterrupted completely through their mitotic cycle, even though ultra-violet scanning was in some instances begun well before the prophase of mitosis took place. These cells required an average of about 30 minutes to complete mitosis. All of these cells showed typical surface bubbling of mitosis, and these small, rapidly appearing and disappearing bubbles contained cytoplasmic absorbing material. In addition, some of the cells showed 1 or 2 quite large non-absorbing bubbles. The lack of absorbing material in these large, more slowly appearing bubbles was attributed to syneresis. At this beam current typical intermitotic cells were spread out on the Vycor cover slip in an ameboid fashion. They showed continuous pinocytosis at their cytoplasmic borders and gradually increasing cytoplasmic absorption as the perinuclear area was approached. Clustered about the nucleus were small round droplets of lipid which were opaque to ultra-violet. In the undamaged cell these small black granules showed active Brownian motion during the entire course of observation. The nucleus of the intermitotic undamaged cell showed little or no absorption at 2650 Å except for absorption by the nucleoli.

When the beam current of the scanner tube was raised to 15 micro-amps, cells appeared unable to complete mitosis. In this study cells were each observed for 96 minutes. Both cells were in early metaphase at the beginning of the observations. During the period of observation neither cell was capable of reaching the completion of mitosis. Both cells showed a marked diminution in the number of small bubbles containing cytoplasmic absorbing material, and a very marked increase in number of large non-absorbing bubbles. At any one time as many as 10 such non-absorbing bubbles were present in various places on the surface of each cell, and any one cell dis-

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played a total of 30 or more such bubbles during the period of observation. The marked increase in number of large non-absorbing bubbles, the decrease in number of small absorbing bubbles, and the failure of cells to complete mitosis during the time of observation were taken to indicate ultra-violet damage.

When single ameboid intermitotic cells were observed with the beam current of the scanner tube adjusted to 15 micro-amps, they remained visually unaltered for the first 47 minutes. During the next 15 minutes there was a gradual slowing and then a complete cessation of Brownian motion of the lipid droplets. This was accompanied by a gradual cessation of pinocytosis at the cell borders. The cells then remained in this rather fixed state for the remainder of the 20-minute observation period. During this entire period no other visual changes occurred. In particular, there was no change in the absorption pattern of either the nucleus, nuclear membrane or cytoplasm. The cessation of pinocytosis and of Brownian motion of the fat droplets was considered to be due to a gelling of the cytoplasm as a manifestation of ultra-violet damage.

When the beam current of the scanner tube was adjusted to 25 micro-amps, a different and more classical type of irradiation damage was shown by the intermitotic cell. In this study, a field of 14 cells was continuously scanned for 47 minutes. At the end of 10 minutes the first evidence of ultra-violet damage appeared in one cell. This consisted of a very rapid generalized increase in absorption of the cell. Two minutes later the entire cell was completely opaque to the ultra-violet light and at this time it contracted very suddenly into a small round black ball. The surface of this cell then showed some minor bubbling for the following few moments; and thereafter it remained as a small, round, completely opaque black ball. Throughout the remainder of the 37 minutes of observation, 9 other cells showed an identical sequence of events. These tended to occur as single events with one cell after another showing evidence of damage. At the end of 47 minutes of observation there were still 4 intermitotic cells which showed no evidence of ultra-violet damage despite the fact that they were simultaneously scanned.

Discussion. Ultra-violet light of 2600 Å wave length has long been known to be extremely damaging to living material. The excellent studies of Bovie(4) in this regard demonstrated that marked changes in the ultra-violet absorption image of living amoebae occurred after only 15 seconds of irradiation. These changes consisted of a pronounced diminution of cytoplasmic absorption with an increase in nuclear absorption. He interpreted these findings to indicate a loss of the many colloidal interfaces in the cell such that formerly immiscible areas were rendered miscible with a resulting change in the absorption image. The changes described by Bovie are quite similar to the changes recorded in this experiment with the higher levels of irradiation.

Brumberg and Larionow(5) reported that the undamaged living cell showed a lack of nuclear absorption when photographed at 2600 Å. This observation was in marked contrast to the previous observations of Caspersson(6). Walker and Davies(7) were unable to confirm the results of Brumberg and Larionow, and concluded that the undamaged living cell did show nuclear absorption when photographed at 2600 Å. In some cases this nuclear absorption at 2600 Å exceeded the cytoplasmic absorption. Our own results indicate that the undamaged intermitotic HeLa cell nucleus, exclusive of the nucleoli, shows little or no ultra-violet absorption at 2650 Å. They further indicate that this condition obtains even after significant ultra-violet induced damage can be observed elsewhere in the cell upon examination of the motion picture film. This damage consists of a gelling of the cytoplasm with a cessation of Brownian motion of the lipid droplets and a cessation of pinocytosis.

Summary. 1. When living HeLa cells are continuously irradiated by ultra-violet emitting cathode ray scanner tube, no ultra-violet damage can be detected when low scanner tube beam currents are used. 2. When the beam current is increased, dividing cells show (a) inability to complete mitosis, (b) in-

creased syneresis during mitosis, and (c) decreased cytoplasmic bubbling during mitosis. 3. With a similar beam current, intermitotic cells show (a) cessation of pinocytosis, and (b) cessation of Brownian motion of cytoplasmic lipid droplets, even though no change occurs in the absorption image or the structural image of the cell. 4. When the beam current is further increased, intermitotic cells show a sudden generalized increase in absorption followed immediately by a marked contraction of the cell into a small round intensely absorbing mass.

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A Stable Preparation of Antigen-Sensitized Erythrocytes. (23302)

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In work reported elsewhere(1), the hemagglutination technic devised by Boyden(2) was used to assay the antibody-forming capacity of spleen cultures from rabbits given either bovine serum albumin (BSA) or bovine γ-globulin (BGG). This method, however, has the disadvantage of not making a stable preparation of antigen-sensitized erythrocytes, requiring preparation of freshly sensitized cells for each day's titrations. The present paper describes a method by which sensitized cells may be prepared which remain stable in the frozen or lyophilized state.

Methods. A 50% saline suspension of washed sheep red blood cells was treated with an equal volume of a 37% formaldehyde solution containing 0.9% NaCl for 2-3 days at 4°C. Since formaldehyde causes clumping of the cells, it was usually necessary to disperse these clumps by homogenization in a Waring blendor for 1 hour at 2-5°C. After centrifuging, only the heavy cell layer was saved. No attempt was made to save the lighter material which separates to the surface as a pellet. The formaldehyde was removed by 7-8 washings in 5-6 volumes of saline over a ten-day period, followed by treatment of a 25% cell suspension with 5% $NaHSO_3$ at 4°C overnight. The formaldehyde-bisulfite complex was then removed by dialysis against running tap water for 24 hours. Eegriwe's chromotropic acid test for formaldehyde(3) was used to detect residual formaldehyde. Unless all formaldehyde is removed, spontaneous agglutination occurs. A 50% suspension of the formalinized cells in saline was stable at 4°C for at least 5 months. The sensitization of the cells to the protein antigens after formalinization was essentially the same as the Boyden technic(2). However, when several liters of cells were prepared at one time, the times of treatment with tannic acid and antigens were extended to 30 minutes and one hour respectively. The sensitization was carried out as follows. One volume of a 2.5% suspension of formalinized cells was incubated at 37°C for 30 minutes with 1 volume of a 1:20,000 dilution of tannic acid (Merck, reagent grade) in 0.9% NaCl. The cells were washed once with 0.15 M phosphate buffered saline (PBS) at pH 7.3, centrifuged at 1500 rpm for 5 minutes and resuspended to volume in saline. One volume of these cells was incubated at room temperature for 1 hour