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Ultra-Violet Motion Picture Studies of Effects of Ribonuclease and Deoxyribonuclease on Living HeLa Cells.* (24333)

P. O'B. MONTGOMERY AND W. A. BONNER

Dept. of Pathology, University of Texas Southwestern Medical School, Dallas, Texas

The technic of time lapse motion picture photography by means of the ultra-violet flying spot television microscope has been previously described by the authors(1-4). With this technic it is possible to obtain time lapse motion pictures of undamaged living cells as they appear when illuminated with ultra-violet light at 2600 A. The motion pictures thus obtained represent principally the nucleic acid absorption images of the living cells. The typical appearance of the undamaged intermitotic HeLa cell obtained with this technic has been reported(2,4). It seemed of some interest to utilize this technic to record the effect of ribonuclease and deoxyribonuclease on U.V. absorption images of living HeLa cells, and this communication reports the results of this study.

Methods. The time lapse ultra-violet motion pictures were obtained by recording on one film frame the ultra-violet absorption image obtained by exposing the living cell to one complete one second scan of ultra-violet emitting cathode ray scanner tube employed as an illuminating source in this technic. During the following one second scan the scanner tube raster was electronically blanked so that no emission of ultra-violet occurred. This one second interval was utilized for the purpose of pulling the next camera film frame into position. The ultra-violet absorption image of the living cell produced by the following one second sweep of the ultra-violet scanner tube was then recorded on the new film frame following which the entire cycle was repeated. In this way the living cells and the film frame were only exposed to the scanning spot of ultra-violet light on alternate one second sweeps. HeLa cells were studied. They were prepared on quartz flying cover slip preparations in a media consisting of Hank's balanced salt solution, human ascitic fluid and embryo extract. The cultures were studied on either second or third day after sub-culture and all cultures contained vigorous healthy cells when examined by phase microscopy. These cover slip preparations were then prepared for study by mounting them on quartz slides in Hank's balanced salt solution containing ribonuclease or deoxyribonuclease. Three concentrations of ribonuclease were used, ½ mg/ml, 1 mg/ml, and 2 mg/ml. Two concentrations of deoxyribonuclease were used, 1 mg/ml and 4 mg/ml. The quartz slide cover slip preparations were then placed in the ultra-violet flying spot television microscope where they were kept at 37½°C and observed and photographed continuously for 6 hours. Replicate cultures were placed in the identical ribonuclease and deoxyribonuclease solutions and in Hank's balanced salt solution alone and were similarly incubated at 37½°C. Samples of each of these latter cultures were then removed at 1, 2, 4 and 6 hours and fixed in osmic acid and formalin. Following this fixation they were stained with Methyl-green-Pryonin, with Azure Blue B, and with the Fuelgen stain.

Results. When living HeLa cells were incubated in a solution containing ½ mg of

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ribonuclease per ml of Hank's balanced salt solution and then studied for 6 hours by time lapse ultra-violet motion pictures, the following changes were noted. For approximately the first hour the cells tend to shrink slightly in size by simply assuming a more rounded form. During the second hour of incubation the cells rather rapidly expand by flattening themselves out against the cover slip. In this process the nucleus tends to flatten slightly, however, the bulk of the change is a cytoplasmic one. At about the end of the second hour of observation the cells reached their maximum size. At this time they were about 3 times their original diameter. The cytoplasmic absorbing material was now distributed throughout a much thinner area, hence any given area of the cytoplasm showed less absorption than when the cells were in a more rounded state. The cytoplasmic absorption was greatest in the perinuclear area. In the expanded round flattened out cell it virtually completely disappeared before the cell membrane was reached. Clustered about the nucleus in the cytoplasm of the typical HeLa cell were small round absorbing droplets which showed a continuous random movement. The borders of the typical round cell showed active pinocytosis all during the process of contraction and expansion. The cells then remained in essentially this state for the next 3 hours. After approximately 5 hours incubation the cells began to slowly contract again, accompanied by a loss of pinocytosis and by a clumping of the absorbing cytoplasmic droplets. For the next half hour the cells slowly continued to contract into small round balls. In the end they remained as motionless contracted small masses about twice the diameter of the original nuclei. During the entire experiment the nucleoli did not change in number, size, shape, appearance, or absorption, and the nuclei remained similarly unchanged.

When the cells were incubated with a concentration of 1 mg/ml or 2 mg/ml of ribonuclease, the same changes were observed, although they appeared to be somewhat accelerated with increasing concentrations of enzyme.

It is interesting that the above changes occurred primarily in cells that were more or less round in shape and that displayed active pinocytosis over their entire periphery. Cells which were spindle shaped and showed pinocytosis principally at their ends appeared to be little changed by the action of the enzyme until collapse ensued, which sometimes required as long as over-night incubation.

When the replicate cultures containing all concentrations of the enzyme and the controls were studied histochemically for basophilic material, it was found that the cells exposed to the enzyme for 3 hours or longer contained no cytoplasmic basophilic material. With all concentrations of the enzyme the basophilic material was not completely removed from the nuclei or the nucleoli. In the case of the cells incubated in 2 mg/ml of ribonuclease for 6 hours prior to fixation and staining, the nuclei assumed an appearance quite different from normal. This change was characterized by the appearance in the nucleus of basophilic bars and strands of material which were of varying size and shape. These bars were frequently anastomotic and gave a heavy reticulated appearance to the nuclei. The vast majority of the cells showed this change in these circumstances. Similar changes were rarely if ever encountered in controls or in other preparations containing lesser concenrtations of ribonuclease.

When cells were incubated in 2 mg/ml of ribonuclease and observed for 7 hours with phase contrast microscopy similar cytoplasmic changes to those described before were encountered, but the above described nuclear changes were not observed.

In the case of cells incubated with 1 mg/ml and 4 mg/ml of deoxyribonuclease there were no changes at the end of 6 hours of ultraviolet time lapse motion picture photography. In all cases the size and appearance of the cells, their nuclei and nucleoli, and their ultra-violet absorption remained the same throughout the course of the observations. When replicate cultures and controls were fixed and then subjected to the Fuelgen procedure, it was discovered that no Fuelgen positive material was present in the nucleus at the end of 3 hours of incubation.

Discussion. Ribonuclease is an enzyme which produces intramolecular transphos-

phorylation followed by hydrolysis in the internucleotide bonds of ribonucleic acid. This biochemical reaction prevents the staining affinity of the ribonucleic acids for the basic dyes but does not alter the ultra-violet absorption spectrum of the enzymatically attacked ribonucleoprotein(5). This latter observation was confirmed by this study and extended to include the fact that cell activity continued in a morphologically normal way for as long as 2 hours after enzymatic action was known to have reached a point where the cytoplasmic affinity for basic dye staining was completely prevented. Brachet has reported that living amoebae exposed to concentrations of ribonuclease of the order of 1 mg/ml show a modification in shape of the nucleoli after 15 minutes followed by a swelling of the nucleus and nucleoli at the time that basophilia disappears from the nucleolus(6). In view of these observations measurements of nuclear size and nucleolar size were made throughout our experiments and no significant changes were observed until the cells collapsed and assumed a pyknotic appearance, despite the fact that the cytoplasm was without affinity for the basic dyes for as long as 2 hours prior to this collapse. Chevremont *et al.* have reported that incubation of living cells in ribonuclease induces a pseudo prophase appearance in the nucleus(7). This phenomenon was observed by us only in the case of the fixed and stained preparations and then usually only after 6 hours of incubation in the highest concentrations of ribonuclease. It was not observed by us in similar but living cultures with either phase microscopy or with the use of the ultra-violet flying spot television microscope.

Deoxyribonuclease is considered to depolymerize deoxyribonucleoprotein to oligonucleotides following which the Fuelgen staining procedure becomes negative(8). Kunitz has reported that this procedure is accompanied by an increase, amounting to as much as 30%, in the ultra-violet absorption of DNA at 2600 Å(9). In the studies reported here no change in nuclear appearance or ultra-violet absorption of living HeLa cells was noted for as long as 2 hours after the Fuelgen stain was known to be completely negative in the nucleus, including the area immediately around the nucleolus. In fact, no changes occurred in morphologic appearance of ultra-violet absorption of the cell until it became pyknotic after 24 hours of incubation in the deoxyribonuclease.

Summary. 1) Living HeLa cells were incubated in various concentrations of ribonuclease and deoxyribonuclease and their ultra-violet absorption characteristics were followed for 6 hours by means of time lapse motion pictures obtained with the ultra-violet flying spot television microscope. 2) No change in ultra-violet absorption images of cells was detectable as long as 2 hours after the time that replicate cultures demonstrated a loss of affinity of cytoplasm for basic dyes and the nuclei were negative with Fuelgen stain. 3) Living HeLa cells react to incubation in ribonuclease by first shrinking slightly and then expanding their cytoplasm markedly. 4) Prolonged incubation of HeLa cells in either enzyme eventually produces collapse and pyknosis of all of the cells in the culture.

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