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Changes in Nuclear Dry Mass, Area and Structure in Living Onion Epidermal Cells During Observation

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Exposure of onion bulb scales to the ambient atmosphere for 48 hr by removing outer bulb scales, resulted in an increase in nuclear dry mass (NDM) and nuclear area (NA) in cells of the newly exposed outer epidermis (Courtis 1972). The increase in NDM was attributed to an increase in nuclear RNA and protein; DNA content remained unchanged. Changes in NDM and NA were also observed while epidermal strips were being studied with the interference microscope. Pappelis and Kulfinski (1971) reviewed this work and recommended that these nuclear indices be determined quickly, especially in cells along the margin of epidermal samples. This study documents some NDM, NA, and nuclear morphology changes while cells were being oberved with an interference microscope, following various pretreatments.

Materials and methods

The outermost, dry, and the next two or three turgid bulb scales were removed from white onions (*Allium cepa* L.) to expose a turgid leaf base for sampling or exposure treatment. A sample consisted of a 4×10-mm rectangle, parallel to the long axis of the bulb, cut from the equatorial region of the newly exposed leaf base. The sample, cut with a razor blade, was removed, mounted in distilled water, and covered with a coverslip which was sealed to the slide with parafin oil.

Cells were observed at 500× and optical path differences were measured in selected nuclei using a Leitz transmitted light interference microscope calibrated with 546 nm green light. NA was determined by tracing the image, measuring the area of the tracing using a planimeter, and adjusting for projection magnification (Pappelis and Kulfinski 1971).

Nuclei were observed for a period of 45 min, and the NDM and NA were determined at 15-min intervals starting as soon as possible after mounting (time 0). Usually the time between preparation of the sample and observation of the first nucleus at time 0 was less than 2 min. Measurements were made on 25 nuclei (one per sample) after 0, 24, or 48 hr of exposure. To prevent rapid desication, exposed onions were placed in glass containers covered with two folds of cheese cloth.

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Results

The NDM of cells in tissue processed immediately after removal of the outer leaf scales decreased significantly over a 45-min period in distilled water (294 to 234 pg) (Table 1). The loss in NDM of non-exposed cells in the first 15 min of observation was statistically significant as was the decrease during the last 15 min. There were no statistically significant changes in NDM during the 45 min when tissues from bulbs exposed 24 and 48 hr were studied.

Table 1. Average nuclear dry mass (NDM) and nuclear area (NA) of outer epidermal cells of onion bulb scales sampled immediately, 24 hr, and 48 hr after the removal of two or three outer bulb scales (25 nuclei per treatment, one per onion)

Minutes after mounting	Hours after exposure					
	0		24		48	
	NA	NDM	NA	NDM	NA	NDM
0	488	294	376	263	380	294
15	380	261	286	263	309	299
30	345	267	243	251	274	276
45	289	234	224	270	257	284

These characteristics for each nucleus were measured four times: immediately after mounting in distilled water and 15, 30, and 45 min after the first measurements. NA is expressed in units of μm^2 and NDM in pg.

The NA (Table 1) decreased significantly in cells of epidermal tissue taken from onions at 0, 24, or 48 hr after removal of the outer leaf bases. The decrease was greatest in the newly exposed onions and least in those exposed for 48 hr. In each treatment, the NA decreased most rapidly during the first 15 min. The NA changes were statistically significant in all treatments when compared to those at 0 min. The changes from 30 to 45 min in cells given 48 hr of exposure were not statistically significant. In general, there was high correlation between NDM and NA (Table 2).

Table 2. Correlation coefficients (r values) for nuclear dry mass (NDM) and nuclear area (NA) of outer epidermal cells of onion bulb scales sampled immediately (0 hr), 24 hr, or 48 hr after the removal of two or three outer bulb scales (25 nuclei per treatment, one per onion)

Hours after removal of bulb scales	Minutes after mounting on microscope			
	0	15	30	45
0	0.84	0.79	0.80	0.84
1	0.88	0.96	0.97	0.93
2	0.63	0.80	0.82	0.62

The nuclear characteristics for each nucleus were measured at four times: Immediately after mounting in distilled water, and 15, 30, and 45 min after the first measurements.

The changes in NA often were accompanied by the formation of one or more perinuclear vacuoles (Fig. 1). These usually appeared within the first 15 to 30 min.

Occasionally, they receded with pycnosis or appeared to break off and move into the cytoplasm as round vacuoles. Exposure of the onions for 24 and 48 hr resulted in more perinuclear vacuoles appearing at 0 min and a rapid disappearance of these over the 45 min of observation (Table 3). In some cases, pycnosis occurred without perinuclear vacuole formation.

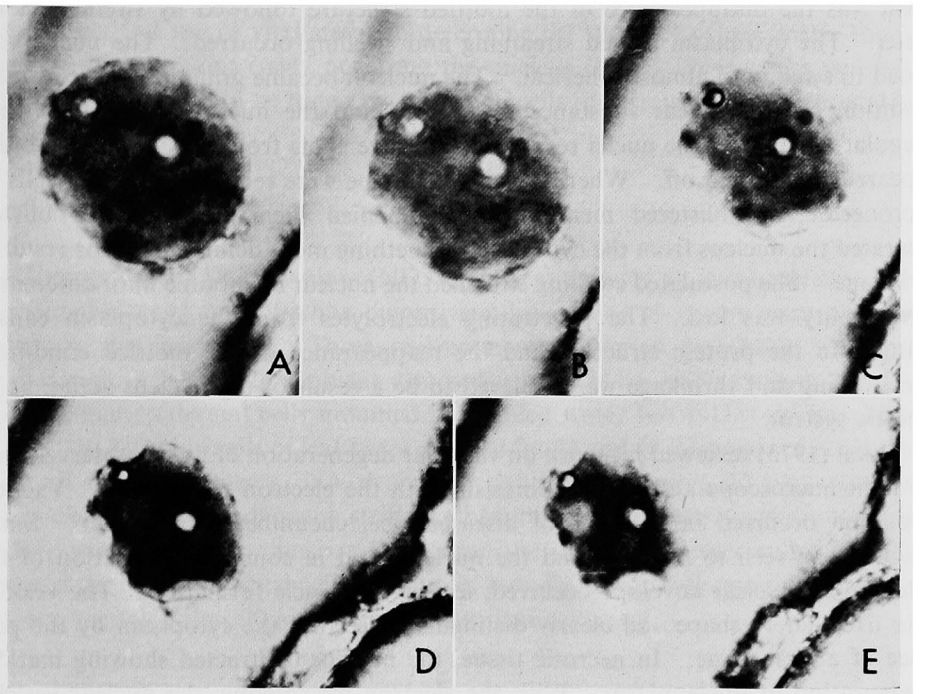

Fig. 1. Nucleus of an outer epidermal cell of a turgid bulb scale (*Allium cepa*) after obtaining the tissue from a non-exposed leaf base. A through E show a decrease in nuclear area and dry mass (pycnosis) as well as an increase in the number of perinuclear vacuoles during the 45 min observation period. The times after the beginning of observation were: A, 0 min; B, 10 min; C, 15 min; D, 30 min; E, 45 min.

Table 3. Percent of nuclei in outer epidermal cells of onion bulb scales with perinuclear vacuoles sampled immediately (0 hr), 24 hr, and 48 hr after removal of two or three outer bulb scales (25 nuclei per treatment, one per onion)

Minutes after mounting	Percent perinuclear vacuoles at various hours after exposure		
	0	24	48
0	8	12	20
15	40	40	40
30	44	36	24
45	40	24	8

Perinuclear vacuoles were noted at four times of study: immediately after mounting in distilled water and 15, 30, and 45 min after the first observations.

Discussion

Plowe (1931) described the nuclei of *Allium cepa* in normal cells and during cell death. In normal cells, nuclei were usually flattened and roughly disc-shaped. Many nuclei showed deep, transparent grooves which occasionally passed completely through the nucleus. Each had two nucleoli. The nuclear substance presented a finely mottled, apparently alveolar structure in living cells. The first sign of cell death was the disappearance of the mottled structure followed by swelling of the nuclei. The cytoplasm ceased streaming and swelling occurred. The nuclei continued to swell until almost spherical. The nucleoli became granular. The mottled condition of the nuclear substance reappeared and the nucleus began to shrink. Irregular collapse of the nuclei resulted in concave spots from which the membrane appeared to be lifted off. When nuclei of this type were removed from the cells by microneedle, the blistered membrane accompanied them. The nuclear blisters separated the nucleus from the cytoplasm, something more definite than the result of shrinkage. She postulated swelling stretched the nuclear membrane until differential permeability was lost. The penetrating electrolytes from the cytoplasm caused changes in the protein structure and the reappearance of the mottled condition. The swelling and shrinkage were believed to be a result of the nucleus acting as an osmotic system.

Akai (1973) reviewed his work on vacuolar degeneration of nuclei observed with the light microscope and that of Shiraishi with the electron microscope. Vacuole formation occurred in the cells of diseased rice, cucumber, and tomato. Small vesicles were seen to form around the nucleus, and in some cases, dilation of the space in the nuclear envelope occurred, leading to vesicle formation. The vesicles were irregular in shape and clearly distinguishable from the cytoplasm by the presence of a membrane. In necrotic tissue, the nucleus contracted showing marked condensation of nucleoplasm. With the electron microscope, the nuclear vesicles (perinuclear vacuoles) appeared to be caused by the expansion of space between the inner and outer membranes of the nuclear envelope.

The loss of NDM and pycnosis are indicators of the onset of cell death (Pappelis and Kulfinski 1971). During pycnosis of onion epidermal nuclei, perinuclear vacuoles may form. Death of non-exposed epidermal cells within 15 min (as evidenced by pycnosis and perinuclear vacuole formation) and the prevention of cell death in epidermal samples from exposed tissue (as evidenced by NDM retention, slow decrease in NA and recovery from perinuclear vacuole formation) may be related to the quiescent status of non-exposed cells as compared to the activated metabolic status of exposed cells. Nuclei in exposed cells have more RNA and protein (Courtis 1972) and possibly increased numbers of cytoplasmic organelles like those demonstrated by Israel and Steward (1966) who reported that, when quiescent carrot cells were stimulated to become active, changes in the cytoplasm included great increases in the number of ribosomes and mitochondria. It may be that the nuclear membranes of activated cells are changed to allow reversible formation of perinuclear vacuoles. Since membrane stretching and contraction are affected by polyanions and polycations (Ambrose and Forester 1968, Wolpert and Gingell 1968), pycnosis and peri-nuclear vacuole formation may be related to cytoplasmic-

nucleo-plasmic ionic balance and ionic concentration. We conclude that the same mechanism is responsible for the high correlation between NDM and NA for all treatments and time intervals of study. The formation of perinuclear vacuoles and vesicles aie important steps in the sequence of events leading to cell death and should be studied further using both quantitative cytochemistry and electron and interference microscopy. We propose that future studies of this phenomenon include the use of various mounting media to test the hypothesis of nuclei acting as osmometers and use of vital stains to determine the moment of cell death, the time when these vital stains freely penetrate the nucleus (Bessis 1964). The concept of activation of cells could be tested by storage of cells in various atmospheres and temperatures.

Summary

Changes in nuclear dry mass (NDM), nuclear area (NA), and nuclear structure in outer epidermal cells of white onion were determined at 15-min intervals during 45 min in distilled water. Three pretreatments of onion leaf bases were used: no exposure, exposure to ambient atmosphere for 24 hr, and exposure for 48 hr. Non-exposed epidermal cells mounted in distilled water lost NDM during the first 15 min, but NDM in cells of leaf bases exposed for 24 and 48 hr remained unchanged. With all three treatments, NA decreased significantly during 45 min; moreover, the nuclear outline underwent striking changes involving the formation of perinuclear vacuoles and pycnosis. Prior exposure of a leaf base resulted in an increase in the number of perinuclear vacuoles initially and a more rapid disappearance of these vacuoles after 30 and 45 min in distilled water.

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