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Nuclear Dry Mass and Area of Free and Crowded Human Buccal Cells Observed in Smears

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INTRODUCTION

Cellular and nuclear dry mass and shape changes have been related to human buccal cell development, maturation, senescence, and death (1, 3, 5, 8). Parabasal cells (Pb) and their nuclei increase in dry mass and size as they enlarge to form intermediate cells with round nuclei (I-R). These degenerate to form cells in the following seqence: intermediate cells with oval nuclei (I-O); intermediate cells with rod-pyknotic nuclei (I-RP); cells with pyknotic nuclei (P); and, anucleate cells. Typical averages for nuclear dry mass in these cell types are as follow (3): Pb, 43 pg; I-R, 69 pg; I-O, 51 pg; I-RP, 48 pg; and P, 35 pg. Typical averages for nuclear areas in these cell types are as follow (3): Pb, 70×10^{-8} cm^2; I-R, 92×10^{-8} cm^2; I-O, 86×10^{-8} cm^2; I-RP, 51×10^{-8} cm^2; and P, 23×10^{-8} cm^2. Similar trends were reported for cells of the oral mucosa of the upper lip. These studies formed the basis of the Cell Development Index proposed for use in the study of oral exfoliation studies (5).

Bhattacharya *et al.* (1) used the Cell Development Index to select cells in buccal smears for study of eight nuclear traits: total nucleic acids, DNA, RNA, total protein, histone, non-histone protein, protein-bound lysine, and protein-bound arginine. All of these nuclear indices (except total nucleic acid and RNA) increased significantly as Pb cells became I-R cells. All of the nuclear indices decreased drastically as I-R cells degenerated through the sequence I-O, I-RP, and P. Their data supported the sequence of cellular and nuclear changes described in the Cell Development Index.

One problem was common to both quantitative interference microscopy (3, 5, 6, 7, 8) and quantitative cytofluorescence microscopy (1) when buccal cells were studied in smears: finding sufficient number of flat, free cells for measurement. This is especially difficult when both cellular and nuclear traits are to be studied. Members of this laboratory working with two types of true interference micro-

scopes, Nomarski interference microscopy, and phase contrast-epifluorescence microscopy have observed all of the cell types described for oral cells in smears (5) and variations like those reported in vaginal and cervical smears (4, 9, 10). In addition to free buccal cells, many have folded or curled margins or are exfoliated in sheets or clumps. Many are damaged during collection or in preparation of smears. It would be advantageous to use any cell within a type whether free (with or without folds, curls, and overlap) or in sheets and clumps if the object to be studied is clearly visible (especially the nuclei). These traits have been used as cytological aids to monitor hormone levels in the blood that influences vaginal and cervical cell development, maturation, senescence, and exfoliation (9, 10) using phase contrast microscopy to identify cells. The comments by Hammond (2) regarding the naming and use of these indices (Folded- and Crowded-Cell Indices) in gynecologic cytology is not our concern in this study. Since we can identify these variations in cells in buccal smears with interference microscopy, we thought that some concern should be given to them.

The purpose of this study was to compare the nuclear dry mass (NDM) and nuclear area (NA) means for I-R cells in smears from a male donor showing the following traits: free cells that are flat compared to free cells that have folded or curled margins; and, free cells that are flat compared to cells in sheets or clumps. We also observed the cells from male and female donors for the above traits and the presence or absence of nucleoli in cell types used in the Cell Development Index.

MATERIAL AND METHODS

Buccal cells were obtained by gently drawing a finger across the mucosa to the corner of the lips. These cells were transferred by touching the finger to a glass slide. The cells were suspended in one drop of distilled water and covered with a cover glass which was then sealed to the slide with paraffin oil. NDM and NA of I-R cells were determined using a Leitz interference microscope as described in earlier studies of buccal cells (3, 5, 6, 7, 8). The same donor was used throughout (male, 10 years old). One smear per day was prepared for study. The Lietz interference microscope (Horn: Mach-Zender) was equipped with 50x A 0.85/∞/0.17 objectives and 16x Periplan GF oculars (field diameter 0.3 mm, 800x total magnification) and Orthomat camera (10x ocular). Both fringe and even field modes were used with polychromatic or monochromatic (546 nm) light to study cells and their inclusions. The quantitative determinations of optical path difference (between cytoplasm and nucleus) was made with the even field mode and monochromatic light. Nuclear area was determined by projecting negatives, tracing outlines, and planimetry of tracings (compared to stage micrometer negatives projected at the same distance).

Two quantitative studies were conducted. In the first, NDM and NA of I-R cells were compared using five flat (without folded or curled margins) cells and five cells in sheets or clumps (more than three cells in each) per slide in each of six slides (replications). In the second, NDM and NA of I-R cells were compared using five flat cells and five cells with either folded or curled margins per slide in each of six slides (replications). All cells were free of others except as described (sheets, clumps). Cells in sheets and clumps have been defined as crowded cells (9, 10).

Nucleolar observations were made in four buccal smears from males (ages 19 through 45) and four females (ages 18 through 28). Cells in these smears were ex-

amined to determine whether intermediate cells existed in the conditions described above (flat: folded or curled margins; free: in sheets or clumps of three or more cells).

RESULTS AND DISCUSSION

The grand means for the six replicates (30 cell means) for free cells (without folded or curled margins) and cells in sheets or clumps were: free cells, 69.2 pg and 92.1 × 10^{-5} cm^2; and, cells in sheets or clumps, 65.0 pg and 98.0 × 10^{-5} cm^2. The ranges for NDM and NA for the 30 free cells were: NDM, 44.1 to 118.0 pg; and, NA, 56.6 to 152.9 × 10^{-5} cm^2. The ranges for NDM and NA for the 30 cells in sheets or clumps were: NDM, 35.1 to 126.2 pg; and, NA, 62.2 to 134.7 × 10^{-5} cm^2. When the six replicate means (five cells of each type per replicate) for both NDM and NA were compared by analysis of variance, no significant differences were found between the two cell types (5% level).

The grand means for the six replicates (30 cell means) for free cells (without folded or curled margins) and free cells with either folded or curled margins were: free cells (no folds or curls), 69.8 pg and 98.3 × 10^{-5} cm^2; and, free cells with folded or curled margins, 70.5 pg and 103.0 × 10^{-5} cm^2. The ranges for NDM and NA for the 30 cells without folded or curled margins were: NDM, 44.2 to 90.3 pg; and, NA, 69.5 to 139.5 × 10^{-5} cm^2. The ranges for NDM and NA for 30 cells with folded or curled margins were: 46.8 to 108.7 pg; and, NA, 46.8 to 146.0 × 10^{-5} cm^2. When the six replicate means (five cells of each type per replicate) were compared using analysis of variance, no significant differences were found between the two cell types (5% level).

The results of the two studies described above were like those reported earlier (means and ranges) for I-R cells (3). Because there were no significant differences between flat, free cells, free cells with folded or curled margins, or cells in sheets or clumps, we conclude that any of these cell types are acceptable for study of these and/or other nuclear traits in I-R cells. We believe that this will also apply to cells of other types described in the Cell Development Index. The replicate means for NA and NDM for all treatments in the present study fall near the regression line presented by Pappelis *et al* (8). Similarly, the correlation coefficient using these means for NA and NDM was high ($r = 0.80$) and compares favorably with that reported earlier (8); $r = 0.93$.

The exfoliation pattern in vaginal tissue and the morphology of the cells is affected by blood hormone levels (4, 9, 10). Our earlier observations that both male and female buccal smears show cells of this type (used in folded and crowded cell indices) were confirmed in the present study of four male and four female donors. What this means in terms of hormonal influences on the oral mucosa is not known. Care must be taken in any interpretation of this data since these cytological indices have received criticism (2) as they were applied in gynecologic studies.

All of the cell types were observed in the four male and four female oral smears. All of the Pb cells from the eight donors contained nucleoli. The number of nucleoli in intermediate cells varied from donor to donor but their overall means were similar: male, 24% of all intermediate cells contained nuclei; and, female, 26% of all intermediate cells contained nucleoli. Nucleoli were not observed in P cells. We had no difficulty in differentiating between nucleoli and Barr bodies in cells of buccal smears from female donors. We believe that there is a difference in size of nucleoli in the cell types (nucleoli in Pb cells appear to be larger than those in in-

termediate cells) and that the size may be related to cell development, maturation, and senescence. This must be confirmed using specific nucleolar stains to varify that the objects called nucleoli in degenerating cells observed in this study with interference microscopy are not chromocenters.

ABSTRACT

The nuclear dry mass (NDM) and area (NA) means for free, flat intermediate cells with round nuclei (I-R) (NDM, 69.8 pg; NA, 98.3 $\times$ 10^{-8} cm^2) were not statistically different from those of flat I-R cells with folded or curled margins (NDM, 70.5 pg; NA, 103.0 $\times$ 10^{-8} cm^2). Similarly, the NDM and NA means for free, flat I-R cells (NDM, 69.2 pg; NA, 92.1 $\times$ 10^{-8} cm^2) were not statistically different from I-R cells exfoliated in sheets or clumps containing more than three cells (NDM, 65.0 pg; NA, 98 $\times$ 10^{-8} cm^2). We conclude that any I-R cells (and infer that any other cell types) found free with or without folds or curls in margins or in sheets or clumps in buccal smears can be used for nuclear studies (by cell type) related to buccal cell development, maturation, senescence, and death. All parabasal cells appear to contain nucleoli while only 25% of intermediate cells and none of the pyknotic cells contain nucleoli. It may be that nucleolar size and dry mass is an important trait to include in future studies of buccal cell development, maturation, and senescence.

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