Nucleic Acid Content of the Squamous Cancer Cell

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AS A RESULT of the chemical analysis and the particle count of nuclear suspensions derived from the tissues of several mammalian animals, it has been shown (*1, 2*) that the quantity of desoxypentose nucleic acid (DNA) of the nucleus is constant for the interphase somatic (diploid) cells of a particular species, that the amount in diploid nuclei is very similar for different species of mammals, and that this quantity is twice that of haploid nuclei. This concept of the relative constancy of the DNA of the interphase nucleus has been extended to other classes of animals (*3*).

Although exceptions have been noted (*4*), microphotometric methods for the determination of the relative DNA in Feulgen-stained nuclei in the tissues of higher animals confirm this concept (*5–10*) and indicate that some tissues, notably the liver and the pancreas, contain nuclei with one, two, and four times the diploid amount of DNA (*11*). High order polyploidy and correlating DNA values are observed in lower animals (*11*); studies on the cells of plants show either inconstancy (*12*) or constancy (*13*) of the DNA per nucleus; and under other conditions, the DNA per nucleus is observed to be altered spontaneously (*14–16*) or experimentally (*14, 17–19*). A permissible appraisal of the published data is that, although there is often a certain average and modal quantity of DNA per nucleus in a particular type of cell, there is a frequent occurrence of values that are greater and lesser than this modal quantity. Such a dispersion of values may be due to actual variations in the composition of the nucleus and to experimental errors in the methods of measurement.

A number of investigations have dealt with the relative content of DNA and on properties related thereto in normal and neoplastic cells (*5, 6, 20–35*). The DNA content per nucleus as determined chemically is about twice as great in the Ehrlich ascites tumor of mice as in normal somatic cells of this animal (*24, 25, 28*), and this finding is confirmed by the microphotometric analysis of individual cells (*6*). Among the pertinent cytological observations on clinical material are the increases in the nuclear volume (*36*), the chromosome volume (*37–39*), the ultraviolet absorption caused by nucleic acids (*20*), the Feulgen-stained DNA (*34–35*), and the affinity for basic dyestuffs of certain types of cancer cells.

The purpose of the present paper is to determine by microabsorption spectroscopy the quantity of nucleic acid in nuclei of epithelial cells of the mucosa of the human cervix uteri and of squamous cancer cells originating in this tissue. The total quantity of nucleic acid is estimated so that the data pertain to the summation of DNA and of ribose nucleic acid (RNA) and not to a particular type of nucleic acid.

Methods

Cytological material. This consists of cells that are obtained from the pars vaginalis of the cervix uteri by the use of a cotton applicator moistened with physiological saline solution or by means of an aspirating pipette. The applicator is rolled onto slides of optically polished quartz (Corning Vycor No. 791) so as to distribute the cells without compression and distortion. The slides are immediately placed in anhydrous glycerin, in about one third of the cases, or in 10 per cent formalin-saline; all are promptly mounted in glycerin with a quartz cover slip and a paraffin seal and are then available for the selection of cells for microscopic analysis.

Duplicate specimens are taken for staining by the method of Papanicolaou (*40*). The stained preparations are studied, and, with this background of experience and by means of a phase microscope, representative and nondegenerated epithelial cells are selected in the unstained slides and are classified by the morphological criteria of Papanicolaou (*41*) as follows: Class I: negative; epithelial cells, such as squamous and parabasal cells, with no atypical or abnormal features; Class II: negative; atypia, but no abnormal features; Class III: abnormal features, suggestive of, but not conclusive for, cancer cells; Class IV: fairly conclusive for cancer cells; Class V: conclusive for cancer cells.

The material on which this report is based was obtained from twenty patients of whom, as shown by biopsy, eleven are negative for uterine cancer, seven have squamous or epidermoid carcinoma of the cervix, and two have intra-epithelial squamous carcinoma of the cervix. Cells of Class I and Class II were obtained

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from the noncancerous group, and those of III, IV, and V from the cancerous group.

Microspectrography. The analytical instrument, referred to elsewhere as system II (*42–43*), consists of an ultraviolet light source, reflecting microscope (numerical aperture, 0.56), and quartz spectrograph. The microscope condenser focuses the continuous spectrum (wavelength, 0.2–0.4 μ) of the source on the object, such as a cell and the adjacent mounting medium, and the reflecting objective images the object on the entrance slit of a spectrograph (*42, 44*). The images are dispersed in the spectrograph and refocused on a photographic plate (Kodak 103-0 UV or Tri X Pan, type B) as a series of overlapping monochromatic images, referred to as a *microspectrogram.* The objective magnification is 50; thus, with an entrance slit 100 μ in width, the light admitted to the spectrograph may be considered as passing through successive square areas of the object that measure 2 μ by 2 μ or 4 μ². By the nature of the microspectrographic procedure, the light transmission is integrated over this object area, the image of which becomes essentially homogeneous.

Standard methods of photographic photometry are used (*45*): plate calibration is carried out by means of a rotating sector, and photographic developing conditions are rigorously controlled. A recording densitometer with an illuminating aperture 50 μ in diameter is used to scan the microspectrogram in one direction in order to relate the absorption with wavelength, and in the other direction to determine the absorption in various morphological components. In this investigation, more than 1000 plates were utilized, and a minimum of 24 microspectrograms were recorded on each cell, with varying conditions of time of exposure, width of slit, and focus. A quantitative analysis of about one third of the accumulated spectra is reported herewith.

Calculations. A graphic computation is made of the extinction $\left(E\lambda = \log_{10}\frac{I_0}{I}\right)$ at various wavelengths of light from 240 to 350 mμ where I_0 is the incident intensity, and I is the transmitted intensity of light with wavelength λ. In nuclear material a broad absorption band occurs in the region 250–280 mμ with an extinction maximum, designated E_{260}, in the region of 260 ± 5 mμ.

The quantity (Q) of nucleic acid per nucleus is given by the expression:

$$Q = C \cdot V,$$

where C is the concentration of nucleic acid, and V is the volume of the nucleus expressed in appropriate units.

The concentration, as given by the Bouguer-Lambert-Beer law, for the absorption of radiation by a solute molecularly dispersed in a nonabsorbing solvent is:

$$C = \frac{E_{260}}{\varepsilon_s l}$$

where C is in g/l, l is the optical path length in cm, and ε_s is the specific extinction coefficient, which for nucleic acid is 20 (the extinction at 260 mμ for a concentration of 1 g/l and an optical path of 1 cm).

The volume of the nucleus when treated as a flattened (oblate) spheroid approaches that of an enclosing elliptical cylinder with surface area $A = \pi ab$, and thickness l, so that

$$V = A \cdot l = \pi ab \cdot l,$$

where a and b are the semimajor and the semiminor axes, respectively, of the nuclear surface, and l is the thickness.

For an ellipsoid, the volume is two thirds that of the enclosing elliptical cylinder, and

$$V = \frac{2}{3} A \cdot l = \frac{2}{3} \pi ab \cdot l.$$

The quantity of nucleic acid per nucleus, for the case of the elliptical cylinder, becomes

$$Q = \frac{E_{260}}{20 \cdot l} \cdot A \cdot l \cdot 10^{-3} = \frac{E_{260} \cdot A}{20} \cdot 10^{-3}\ \text{g}.$$

where A is in cm² and Q is in g. If A is expressed in μ², the equation becomes

$$Q = \frac{E_{260} \cdot A'}{2} \cdot 10^{-12}\ \text{g} = \frac{E_{260} \cdot \pi a'b'}{2} \cdot 10^{-12}\ \text{g},$$

where a' and b' are the nuclear semiaxes in μ, which may be accurately measured from phase photomicrographs (500–1000 times enlargements) of the cell nuclei. The computations of Q values in this paper are made on the basis of both an elliptical cylinder and an ellipsoid, the values in the latter being two thirds that for the cylinder. The uncertainty in the computation resides in the fact that at the time this work was undertaken, there was no method for the exact determination of the three-dimensional spatial configuration of the nuclear volume. Recent observations in our laboratory by means of multiple-beam interference microscopy indicate within an accuracy of 0.02 μ that the optical paths (product of thickness and refractive index) in isolated, optically homogeneous nuclei are those to be derived from a volume that is intermediate between an ellipsoid and an elliptical cylinder.

Results

Nuclear extinction. The average values of the extinction at 260 mμ, E_{260}, are given in Table 1 for the nuclei of epithelial cells of the cervix uteri. As previously stated, the extinction as determined from a microspectrogram is the integrated value for a central 4 μ² area of the nucleus. Cells of Class I and Class II combined have extinctions of the order of 0.24 and 0.33, whereas those of Class III and of IV and V combined have values of 0.51 and 0.54, respectively. The differences between the negative Class I and Class II combined and the abnormal Class III, or between the negative classes and the cancer classes, IV and V, combined, are statistically significant and have, by chance alone, a probability of occurrence of the order of one in a few thousand to one in several thousands.

TABLE 1

NUCLEAR EXTINCTION AT 260 Mμ*
(Epithelial Cells of the Cervix Uteri)

Cell class or type	No. cells	E_{260}	SD	Comparison			
				Classes	Δ	SE Δ	$\frac{\Delta}{SE\,\Delta}$
I and II (Sq)	23	.24	.10	Sq and Pb	.09	.04	2.2
I and II (Pb)	21	.33	.15	(Sq and Pb) and (III and IV and V)	.25	.04	6.2
III	20	.51	.22	III and Pb	.18	.05	3.5
IV and V	31	.54	.23	(IV and V) and Pb	.21	.05	4.0

* Average nuclear extinction at 260 mμ, E_{260}; standard deviation, SD, of average; difference, Δ, between classes; standard error, SEΔ, of this difference; and ratio, $\frac{\Delta}{SE\Delta}$, which for values greater than 3 indicates an extremely remote probability of occurrence of the difference by chance alone. Symbols: Sq, squamous; Pb, parabasal; I–V, classification of Papanicolaou.

Nuclear dimensions. The nuclear semiaxes (the largest and the smallest), the nuclear areas computed as ellipses, and the volumes calculated as ellipsoids are given in Table 2 for a random sample of epithelial cells of the cervix uteri. The average area of the nucleus is 62 μ^2 for I and II combined and 112 μ^2 for Class III and IV and V combined, and the average volumes are 335 μ^3 and 760 μ^3, respectively; that is, the nuclear volume of the category of abnormal and cancer cells is approximately twice that of the class of normal and atypical cells.

Nucleic acid content. The nucleic acid content per nucleus in resting (interphase) epithelial cells of the cervix uteri is given in Table 3. The values have been computed from the extinction at 260 mμ as measured for individual nuclei and for two representations of

TABLE 2

NUCLEAR SEMIAXES, AREAS, AND VOLUMES*
(Epithelial Cells of the Cervix Uteri)

Cell class or type	No. cells	Semimajor		Semiminor		Area $\pi ab\,(\mu^2)$	Vol $\frac{4}{3}\pi ab^2(\mu^3)$
		$a(\mu)$	SD	$b(\mu)$	SD		
I and II (Sq)	58	4.8	1.3	3.8	0.8	57	290
I and II (Pb)	52	5.1	1.0	4.3	0.7	69	395
I and II (total)	110	4.9	1.2	4.0	1.4	62	335
III	27	6.7	1.9	5.0	1.1	105	698
IV and V	27	7.2	1.8	5.3	1.7	120	845
III and IV and V	54	7.0	1.9	5.1	1.5	112	760

* Average nuclear semimajor axis, a, and semiminor axis, b, in μ; standard deviation, SD, of these averages; average nuclear area, in μ^2 and volume (as an ellipsoid) in μ^3. Symbols as in Table 1.

TABLE 3

NUCLEIC ACID CONTENT PER NUCLEUS*
(Epithelial Cells of the Cervix Uteri)

Cell class	No. cells	Nucleic acid per nucleus, 10^{-12} g					
		Determined by $Q=\frac{E_{260}\cdot A}{2}$			Theoretical, $Q=K2^n$		
		Elliptical cylinder		Ellipsoid			
		Av	SD	Av	$K=4.5$	$K=3$	n
I and II	24	9.3	5.0	6.2	9	6	1
III, and IV and V	34	35		23			
Subgroup 1	17	17	4.4	11	18	12	2
" 2	8	38	2.5	25	36	24	3
" 3	9	67	9.8	45	72	48	4

* Average nucleic acid content per nucleus determined by microspectroscopy of individual nuclei and computed for a nuclear volume that is an elliptical cylinder and an ellipsoid; and theoretical, geometrical progression of values estimated by the expression $Q=K2^n$, where K is a constant for a species and n is an integer.

the nuclear volume, namely, as an elliptical cylinder and an ellipsoid (two thirds the volume of the elliptical cylinder). As calculated for an elliptical cylinder, the values are 9.3×10^{-12} g nucleic acid per nucleus in I and II combined, and 35×10^{-12} g in Class III and IV and V combined. There is, however, a trimodal distribution of values in the class of abnormal and cancer cells, as shown in Fig. 1, and the averages for the subgroups are 17, 38, and 67×10^{-12} g, respectively. A hypothetical envelope of points is given in Fig. 1 by normal frequency-distribution

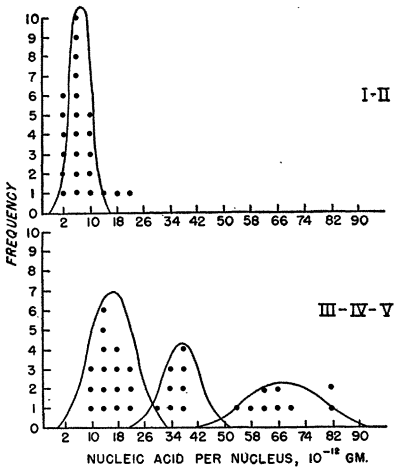

FIG. 1. Frequency of occurrence (ordinate) of certain quantities of nucleic acid per nucleus (abscissa) for cells of Class I and Class II combined (upper graph) and of Class III and Class IV and Class V combined (lower graph). Values computed for the nucleus as an elliptical cylinder. Each dot signifies an observation. Smooth curves have maxima at the average value for the class or the subgroup.

curves, which have maxima in the neighborhood of 9×10^{-12} g for Class I and Class II combined and at 18, 36, and 72×10^{-12} g, respectively, for the subgroups of Class III and Class IV and Class V combined. It can be computed from the data in Table 3 that the averages for values enclosed by a given curve in Fig. 1 differ from those of the adjacent distribution by five or more times the standard error of the difference. Thus, the distributions are statistically distinct.

When computed for an ellipsoid, the values are 6.2×10^{-12} g per nucleus in Class I and Class II combined, and 11, 25, and 45×10^{-12} g, respectively, for the subgroups of the abnormal and cancer cells. The frequency distribution curves for the envelope of points then have maxima in the neighborhood of 6, 12, 24, and 48×10^{-12} g, respectively.

If there is a geometrical progression in the average quantity, Q, of nucleic acid per nucleus in one cell-class as compared with another, the value, Q, may be represented by the expression

$$Q = K2^n,$$

where K is a constant and n is an integer. The relation and the agreement between the Q values, both as determined by microspectroscopy and as computed by the foregoing expression, are given in Table 3, where $n = 1$ for interphase normal epithelial cells and $n = 2$, 3, 4 for interphase squamous cancer cells. Despite the agreement, however, it must be emphasized in view of the askewness of the distribution of the points in Fig. 1 and because of the errors in cytometry and microspectroscopy that this may represent an oversimplification in the interpretation of the data.

An analogous expression for the average quantity, Q_{DNA}, of DNA per nucleus is given by

$$Q_{DNA} = K_{DNA}\, 2^n,$$

where K_{DNA} is a constant for the cells of a species and n is an integer. The value of K_{DNA} as derived from the literature is of the order of 3×10^{-12} g for mammalian cells (*1, 2*), and n has the value $n = 0$ for haploid (germ) cells; $n = 1$ for interphase diploid (somatic) cells; $n = 2$ for dividing diploid cells; and $n = 2, 3 \ldots$ for various orders of polyploidy. If a geometrical progression in the average quantity of DNA per nucleus does occur, it is quite possible that the frequency distribution of cells (both resting and dividing) with respect to the n values will be a characteristic expression of the pattern of growth of a tissue. The envelope of points for the tissue will be the integral, with respect to the n values, of the frequency-distribution curves of the component cells.

The geometrical progression in the total nucleic acid content of the interphase nucleus of the squamous cancer cell is consistent with the general concept of growth by reduplication and division (*46–49*). Since nucleic acids are prominent constituents of chromosomes (*20, 50–52*) and of nucleoli (*20*), the increase in the nucleic acid content of the single nucleus of the interphase cancer cell—or of any other cell—is conceivably related to the following mechanisms of growth: an acquisition of an even integral multiple of the fundamental number of chromosomes (polyploidy); a reduplication of the mass of individual chromosomes without division (endomitosis and polyteny); and a geometrical increment in the heterochromatin or in the nucleolar (RNA) apparatus. It is of interest in this regard that certain cancer cells of man are found to have, relative to normal cells, a two and four times greater volume of the nucleus (*36*), the mass of which has long been held to be related to the number of chromosomes (*46*); a two to four times greater volume of the individual chromosomes (*38*); an even multiple of the basic number of nucleoli (*38*); even, odd, and nonintegral multiples of the fundamental chromosome number (*29, 30, 53–55*); and many morphological abnormalities of the chromosomes (*29–30, 53–55*). It is intriguing to think that the nucleic acids themselves may perpetuate these alterations in the cell. Aside from such considerations, the observations reported herewith have tangible bearing upon the cytological diagnosis of cancer.

Upon the completion of the foregoing study, it was apparent that there were two conditions which ideally

should be, but were not, fulfilled in this or in contemporary microspectroscopic work of a similar kind: (*a*) the nuclei should be optically homogeneous, and (*b*) the nuclei should be randomly studied. Utilizing mechanical procedures for the homogenization and the conversion of tissues into suspensions of nuclei and with controlled conditions of mounting, it has been possible in recent work to fulfill conditions (*a*) and (*b*). The nuclei are optically homogeneous as shown by phase and interference microscopy which measure differences in optical path of the order of 0.02 μ. A preliminary comparison of nuclei so derived from normal cells and cancer cells of certain tissues of the mouse indicates a difference in nucleic acid content substantially similar to that reported herewith for a tissue of the human being.

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News and Notes

Wildlife Disease Association

IN conjunction with the seventeenth North American Wildlife Conference, held in Miami, Fla., March 17–19, a number of investigators met informally and officially organized the Wildlife Disease Association. During the previous year a preliminary draft of a statement of scope and purpose had been prepared by Drs. Bell, Cheatum, and Jellison. This was circulated among other members of a committee, and their suggestions were incorporated into the statement where it seemed justified during discussion at the Miami meeting. The revised statement is as follows:

The practice of wildlife management in North America has attained the status of a recognized profession. As professions go, it is a youthful one, but the responsibilities with which it is charged are heavy. It is asked to manage desirable populations of wildlife species so that they may be at least maintained and, in many instances, increased beyond their current numbers. On the other hand, the profession is charged with the control of nuisance species whose populations are believed to conflict so excessively with human health and economy, or with the welfare of more desirable species of wildlife, that their reduction appears warranted.

Much more knowledge than is currently available is required for judicious handling of many of the problems incident to managing wildlife populations. There are natural limitations to the abundance of all species, limitations that are affected by climate, food, and natural enemies.

Among the natural enemies, diseases are of great im-