NUCLEAR-NUCLEOLAR VOLUME RATIO IN CANCER

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In the study of tumor cytology, attention has been drawn in recent years to the importance of the size of the nucleolus. The diagnostic significance of nucleolar size in relation to the nucleus of cancer has been championed for several years by W. C. MacCarty, who with Haumeder in 1934 published a statistical study on the size of the nucleolus in malignant growths and normal tissues. He concluded that a great difference exists between the size of the nucleoli of cancer cells and of regenerative forms, such as fibroblasts, endothelioblasts, and other immature cells. The present study was begun in the early part of 1933, before the appearance of MacCarty's statistics, in an attempt to determine by means of direct measurement whether or not a significant difference exists between the N/n [1] ratio of malignant tissues and that of non-malignant cells.

Little is known of the physiological activity of the nucleolus. From a study of the lower animals it appears probable that the nucleoli are concerned in cell metabolism. Beams and Wu (1929) and Wu (1930) in a study of the spinning glands of certain insects found that the nucleolus plays an important rôle in secretion. Gardiner (1927) concluded from a study of the cells of the crab that the nucleoli have an important function in yolk formation and also that they are probably concerned with phosphorus metabolism. Benoit (1921) studied the epididymis of the higher mammals, and Ludford (1925), who investigated the same organ in the mouse, concluded that the nucleoli played an important rôle in secretion of the cell. Increase in the size of nucleoli was seen by Sayles during regeneration of the hypoderm of Lumbriculus. Ludford (1922), on the basis of a large series of experiments, was inclined to regard the size of nucleoli as an indication of the degree of metabolism existing in the cell; the greater the metabolic activity the larger the total volume of nucleolar material present in the nucleus.

For the present study tissues were fixed in Zenker's fluid and embedded in paraffin. Sections were cut 15 microns in thickness. This was done to permit the measurement of the maximal length and thickness of the nuclei. The sections were stained with Pappenheim's methyl green-pyronin. With this stain the nuclei are dark green in color and the nucleoli bright red, with well defined sharp borders. No

[1] For convenience, the nuclear-nucleolar volume ratio will be expressed by the abbreviation N/n.

advantage was seen in the use of fresh frozen sections as advocated by MacCarty, since all tissues were uniformly treated and one could safely assume that the reaction of the component parts of the cell to the various reagents was alike in all instances. In addition, the cells could be studied over a long period without running the risk of cytological changes which may occur in freshly stained frozen tissues during the course of study. The nuclei and nucleoli were measured by means of a micrometer eye-piece. The oculars were so adjusted that the smallest division on the vernier corresponded to 1/10th micron in the oil immersion field. The magnification was 1700 times. The camera lucida projection method used by MacCarty in his studies involves a large error, since it is difficult accurately to reproduce the size and shape of an object as minute as the nucleolus.

TABLE I: *Nuclear-Nucleolar Volume Ratio of Malignant and Non-malignant Cells*

Type of Tissue:	Number of cells	Mean	Probable Error	Standard Deviation	Maximum	Minimum
Malignant epithelial tumors of skin	450	31.44	1.69	11.95	10.70	0.55
Malignant glandular tumors	800	32.67	2.37	16.75	125.2	6.1
Benign epithelial tumors	300	26.20	2.07	14.50	98.6	4.2
Malignant melanoma	150	24.54	1.27	8.98	70.1	8.1
Benign pigmented mole	150	36.75	2.19	15.50	20.6	2.6
Sarcoma	200	24.12	1.99	14.07	70.7	5.4
Fibrosarcoma	100	33.41	2.85	20.10	121.5	6.0
Ewing's tumor	50	37.25	2.90	20.40	107.7	11.1
Fibroma	50	28.22	2.11	14.95	80.5	12.6
Hyperplasia	250	26.20	2.07	14.50	98.6	4.2
Normal tissues	500	28.61	2.33	16.52	110.2	3.1

Nuclei were picked at random in fields in which the maximum length of the cells occurred parallel to the surface of the slide. The greatest transverse and longitudinal dimensions were determined. Fifty cells of each tumor were measured. In calculating the volume of the nucleoli and nuclei the third diameter was assumed to be equal to that of the shortest or transverse diameter, a justifiable assumption since these structures are fairly well formed ellipsoids or spheres. Only those nuclei were accepted in which the greatest axis was demonstrable by focusing through the thickness of the section.

A total of sixty specimens were examined. These included thirty-five malignant growths derived from skin, breast, uterus, bladder, rectum, bone, and subcutaneous tissue; ten benign growths from similar sites; five tissues showing simple hyperplasia, and ten normal tissues. The N/n volume ratio in each instance was obtained by dividing the average volume of 50 nuclei by the average volume of their nucleoli. The quotient is expressed by a whole number. To facilitate comparison, the standard deviation and probable error were determined for each specimen and given with each ratio.

In malignant epithelial tumors of the skin the mean N/n ratio is

31.44; in malignant epithelial tumors of glandular origin it is 32.7. Malignant melanoma and sarcoma show slightly lower figures, the ratios averaging 24.54 and 24.10 respectively. Ewing's tumor shows the highest ratio, 37.25, indicating a proportionately larger amount of nuclear as compared with nucleolar substance. No significant difference is seen between the ratio of malignant epithelial tumors, benign hyperplasia, benign tumors, and normal tissues.

It appears from Table I that extreme variations are present in the N/n ratios of individual cells, as expressed by the high standard deviation and wide range between maximal and minimal measurements. A study of the distribution of N/n ratios of malignant tumors, benign tumors, and normal tissues, showed no significant difference in these three groups.

TABLE II: *Volume of Nucleolus of Malignant and Non-Malignant Cells*

Type of Tissue:	Number of Cells	Mean	Probable Error	Standard Deviation	Maximum	Minimum
Malignant epithelial tumors of skin	450	21.12	2.02	14.16	141.15	3.5
Malignant glandular tumors	800	22.95	0.95	6.75	118.0	1.7
Benign epithelial tumors	300	24.06	1.65	11.6	90.1	3.1
Malignant melanoma	150	23.35	0.65	4.56	79.4	4.3
Benign pigmented mole	150	9.67	0.36	2.53	102.4	11.8
Sarcoma	200	24.82	1.24	8.78	134.4	4.2
Fibrosarcoma	100	10.30	0.72	5.08	30.5	2.3
Ewing's tumor	50	8.8	0.70	4.97	55.2	2.6
Fibroma	50	9.2	0.72	5.08	19.6	4.4
Hyperplasia	250	12.93	0.38	0.72	49.2	11.5
Normal tissues	500	8.49	0.47	3.47	18.6	1.4

In the measurement and comparison of the nucleolar volume, independent of the nuclei of the various tumors and tissues examined, significant differences were observed (Table II). The average nucleolar volume of malignant epithelial tumors is 25.1 cubic microns as compared to 12.93 cubic microns for simple hyperplasia and 8.4 cubic microns for normal tissue. However, little difference in volume is seen in the nucleoli of benign and malignant epithelial tumors. On the other hand, a comparison of benign and melanotic tumors indicates distinct differences in the volumes of their nucleoli. In the malignant melanotic tumors the nucleoli are distinctly larger than in benign tumors of the same origin. Ewing's tumor contains small nucleoli which are not larger in average volume than those seen in normal cells. In sarcomas nucleoli are observed which are equal in volume to those in malignant epithelial tumors. Wide variation in the size of nucleoli occurs in malignant tumors; in normal tissue and benign hyperplasia the variation is less. However, considerable overlapping of the various groups is noted. Whereas in the majority of benign tumors and normal tissues the average nucleolar volume is less than 20 cubic microns, that in malignant tumors exceeds this figure.

Discussion

In the report of MacCarty and Haumeder (1934) a statement is made, in quotation of a previous article, that "the cancer cell may not always be distinguished from a normal regenerating cell, but this can be done frequently because there is a difference in the volume-relationship between nucleolus, nucleus and the whole cell in reparative regenerative cells and malignant regenerative cells." However, in the computations given, no mention is made of the volume relationship but only of area relationship. It is readily seen that in the computation of area relationship of three dimensional objects, a very different result is obtained than if the volumes are considered, since much greater values are given to the sums of the areas of multiple nucleoli of small dimension than to the aggregate of their volume. Consequently, much lower N/n ratios are obtained by this method than by measurement of the volume relationship.

The hazard of venturing diagnosis on the cytological appearance of an isolated tumor cell is great, since, as shown, great variation may occur in both malignant and non-malignant cells in respect to the size of their nucleoli and no significant difference is seen in their respective nuclear-nucleolar ratios. In other words, with increase in size of the nuclei there is corresponding increase in the size of the nucleoli. Nucleoli of simple hyperplastic tissues frequently may be larger than nucleoli of malignant tissues containing enlarged nuclei. Moreover, in many malignant tumors the nucleoli may be as small as in normal cells.

The authors therefore feel that in the diagnosis of tumors little is gained by the estimation of the size of the nucleoli. Contrary to MacCarty's view, well prepared thin paraffin sections obtained from various parts of the tumor are much to be preferred to fresh frozen tissues, which at the best give poor cellular differentiation and a limited field for study of the important criteria of malignancy, namely cellular differentiation and the relationship of cells to their environment.

Summary

1. The nuclear-nucleolar volume ratios of normal tissue, hyperplastic tissue, benign and malignant tumors show no essential quantitative differences.

2. The volume of nucleoli of normal tissue is significantly smaller than the volume of nucleoli of hyperplastic tissue and of benign and malignant tumors. There is no significant difference between the nucleolar volume of benign tumors and malignant tumors.

3. The determination of the nuclear-nucleolar volume ratio offers little aid in diagnosis of malignancy. The nucleolar volume may be used only as an adjunct in the differentiation between malignant and non-malignant tissue. Because of the marked variation in the nucleolar volume of malignant neoplasms, benign tumors, and hyperplastic tissue, this criterion cannot be used as a decisive factor in determining whether or not a tumor cell is malignant.

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