A QUANTITATIVE STUDY OF EPITHELIAL NUCLEI OF THE BODY OF THE UTERUS IN CARCINOMA AND IN NON-MALIGNANT HYPERPLASIA.[1]

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ABSTRACT.

From fifty specimens of human endometrium (formalin-fixed paraffin sections), images of epithelial nuclei were projected (X 1520 diameters), traced and measured. Thickness (from fine adjustment readings) and volume (from the ellipsoid formula) had great observational errors and were replaced by length and breadth. Both these, especially breadth, closely represented volume. Slide thickness did not appreciably influence measurements. Fifty nuclei sufficiently represented a section, since observational errors accounted for the differences between two sets of fifty nuclei from the same section. The main conclusions were:

(a) The average nuclear size was greater in a series of carcinoma specimens than in a series of hyperplasia specimens, when the technique of preparation was the same; (b) The sizes varied more from specimen to specimen in hyperplasia than in carcinoma; (c) The sizes varied more within the specimen in carcinoma than in hyperplasia; (d) Moderate differences in technique (length of time in fixative, method of dehydration and clearing, etc.) appeared to influence nuclear size.

Although these results are concerned with averages, they can be applied to help in the diagnosis of individual specimens, and would be more valuable for that purpose if technique were more standardized.

Shape (ratio of mean breadth to mean length) varied so much from specimen to specimen that neither disease nor technique had any appreciable effect.

Frequency graphs suggested a slightly greater tendency in carcinoma and in hyperplasia towards extremely large rather than very small nuclei.

A simple colorimetric scale was used to classify staining intensity, numerical equivalents being obtained by diluted water colors. No association was detected between staining and disease or technique; but with standardized staining the method would probably have value, because in individual specimens average staining intensity varied directly with thickness and inversely with breadth.

It is argued that differences between carcinoma and hyperplasia nuclei after preparation represent original size differences, and probably indicate metabolic and physico-chemical differences.

[1]A preliminary note on this work was given at the 48th annual session of the American Association of Anatomists, March, 1932 (Anat. Record **52**, no. 1 suppl., p. 67).

INTRODUCTION.

Histology, like other departments of biology, is becoming more and more a quantitative science, and one sign of this is the interest taken in nuclear size. This interest is, however, largely centered on nuclear size as a factor in the nucleoplasmic ratio, and on average nuclear sizes, rather than on the variations exhibited by the different nuclei in the same tissue. In biology the variation is as important as the mean, and, moreover, when the variation is taken into consideration, differences between means may be accountable for by chance. For the application to pathology, also, the nucleoplasmic ratio is frequently not available, for when tissues are prepared by simple methods for diagnosis the cell outlines are often lost.

The object of the present investigations was to study the sizes (including size variations) of the nuclei of the mucosal epithelium of the body of the human uterus, and especially to show what size differences exist between the nuclei in carcinoma and in non-malignant hyperplasia. The tests were carried out in such a way as to show whether they might be used to help in the diagnosis from material prepared by routine methods. The cells in the various specimens were looked upon as individuals in a population, and the nuclear measurements were taken without reference to the size of the cells, as are, sometimes, the cephalic measurements of human individuals, without reference to other dimensions. From the outset it had to be remembered that the measurements were made on the end-products of a series of processes   the processes involved in the fixation, imbedding, staining and mounting of the nuclei. The question to be answered was: When the epithelial nuclei of carcinoma and hyperplasia are subjected to the same processes of preparation, how, in regard to size, do the end products differ from or resemble each other? It will be seen from these introductory remarks that the investigations have been carried out along different lines and with different objects from

the recent work of O'Leary[2] who was concerned with changes in the nucleoplasmic ratio in association with the menstrual cycle. To O'Leary's paper the reader may be referred for some account of the previous very scanty quantitative work on the histology of the uterine epithelium.

MATERIAL.

Fifty specimens, some being sections, the others endometrial scrapings, were investigated, usually one slide from each specimen. The sources were as follows:

From the Pathological Institute, Halifax, N. S.: 21 specimens;
From the Winnipeg General Hospital: 26 specimens;
From the teaching collection of the Department of Obstetrics and Gynecology, Dalhousie University: 3 specimens.

Since it will subsequently be shown that technique influences the nuclear sizes, details regarding it are desirable. Exact information was not available regarding the method of preparation of the specimens in the third group, and therefore the conclusions had chiefly to be based on the first two. The technique in these two groups was as follows:

At the Pathological Institute, Halifax: 4 per cent formaldehyde, 1 hour if warmed to 56°C., overnight if cold; water, 2 hours; 50 per cent alcohol, 6 hours; 90 per cent alcohol, 12 hours; acetone, 1 hour; chloroform, 1 hour; paraffin at 53-55° C., 1 hour.

At Winnipeg General Hospital: 4 per cent formaldehyde, 6-8 hours, cold; 70 per cent alcohol, 11 hours; absolute alcohol, 3 hours; xylol, 2 hours; paraffin at 56-58°C., 1½-2 hours.

The staining in both groups was hematoxylin and eosin. The respective techniques had been used uniformly during the period in which the specimens were collected. Moreover, the technique would be more likely to differ slightly from year to year than from month to month, and there was no evidence to suggest that the size differences could be ascribed to such differences in technique, even over long periods of time.

[2]O'Leary, J. L. *Anat. Record.* **50**, 33, (1931).

METHOD.

General Principles.

A word or two may not be out of place concerning the assumptions underlying the tests[3] applied to prove or disprove the significance of the numerical results. From among the carcinoma specimens in a collection, a number are selected at random, and from each specimen a number of nuclei are measured. The mean or average size (say volume) of these nuclei is determined for each specimen. The average of these averages is found and is compared with the average of similarly determined sizes from specimens of hyperplasia. So that the results may be compared, it is assumed that the means belong to "normal frequency" curves[4]. This is justified because it is comparatively rare to find phenomena (especially

[3] These tests are called statistical, and it is frequently felt that conclusions established with their aid are, as it were, forced. On the contrary, the tests much more frequently throw doubt on the significance of results that the worker might have been willing to accept as fairly conclusive. In all cases the tests enable one to determine the odds that can reasonably be placed on the significance of results, or, in other words, to estimate the number of times one might meet similar results if chance alone were in operation, instead of the factor or agent that the investigator is postulating.

Even those who criticize the rigid methods of statistics and the assumptions, involved, must, in analyzing data, make assumptions based on analogy, previous experience, and so on. The statistical assumptions are merely more explicit and probably more consistent.

[4] An introduction to the idea of the normal frequency curve and to statistical methods in general is well given by the Eldertons (Elderton W. P. and Ethel M., Primer of Statistics, A. and C. Black, London, 1927). The textbooks that are most informative regarding the application of mathematical methods in the testing of results in biological research are those of Fisher[5] and Tippett (Tippett, L. H. C. The Methods of Statistics, Williams and Nazate, London, 1931).

In this paper prolonged discussion would be out of place. The following definitions and notes may, however, be of use. The standard deviation is the commonest expression of the variation in a series of observations. It is

$$\pm \sqrt{\frac{\text{sum of squares of differences between individuals and mean}}{\text{number of individual observations}}}$$

The coefficient of variation is the standard deviation expressed as a percentage of the mean.

The standard error is an expression for the variation to which the mean is liable if, say, more similar samples are taken. It is

$$\pm \frac{\text{standard deviation}}{\sqrt{\text{no. of observations}}}$$

The standard error is now replacing the probable error (i. e., about 2/3 the standard error), as being more directly calculable.

biological phenomena such as size) in which the means of various groups do not tend to be arranged normally (Fisher[5], p. 99). Even if they are not quite normal, the extent of the error thus introduced is seldom great.

In the present investigations some of the groups contain few individuals (the limitation in the carcinoma groups being imposed by actual scarcity of specimens).   The conclusions derived from them are none the less valid provided the tests devised, for example by Fisher[5], for dealing with small numbers are applied.

It should be mentioned that the term "significant" is, in conformity with the usual convention, employed to indicate that the odds are at least 19 to 1 against the value (e. g., a difference between means) being due to chance.   Such determinations of odds are in general based on the amount of variation between the individual items that make up the average or other value under consideration.

Detailed Technique.

The images of the nuclei were projected at a linear magnification of 1520 on to pasteboard by a microscope used horizontally (with Zeiss ocular K 10X and Zeiss oil immersion objective 90X, n.a. 1.30).   A powerful source of light which can be thoroughly recommended for micro-projection work since it is not subject to the fluctuations of the carbon-arc, was obtained by using the equipment devised by Professor Savage of Winnipeg, to whom the author is indebted for details; viz.: a projection lamp, 5.2 volts, 54 watts, standard base T. 10 C. (Canadian General Electric Co.) used on a circuit of 110 volts a. c., with a resistance of roughly 12 ohms provided by $\frac{3}{4}$-1 lb. of Chromel A resistance wire, no. 14 gauge, the wire being wound on asbestos board[6].   All the apparatus was clamped or screwed in position, including the block of wood against which the pasteboard was held.   On this pasteboard, pencil tracings

[5]Fisher, R. A.   Statistical Methods for Research Workers.   Oliver and Boyd, London, 1930.

[6]Care has to be taken to avoid overheating the microscope lenses.

of the epithelial nuclei were made. Nuclei obliquely placed
with reference to the plane of the screen were avoided, those
only being selected that had their long axes either parallel or
perpendicular to the screen of pasteboard. The nuclei were
focussed so that their outlines could be traced when at their
maximum size. Except where depth measurements, by the
fine adjustment of the microscope, were to be taken, it did not,
of course, matter if a nucleus was not quite complete, provided
only a small slice had been removed in the section-cutting,
leaving the region of greatest length and breadth. For the
most part, however, the nuclei traced were complete.

The amount of material examined corresponded to what
was sufficient for an ordinary diagnosis. To form a thoroughly
representative estimate of nuclear size for each specimen, it
was desirable to have all the nuclei scattered at equal dis-
tances from their neighbors throughout the section. The
nearest practicable approximation to this ideal was obtained
by estimating, under low power, the total area to be examined,
expressed in terms of the number of microscope fields under
the high power oil immersion lens. If there were 500 fields,
and 100 nuclei were to be traced, one nucleus was taken from
each fifth field as the slide was moved by the mechanical stage[7].
It was found useful, especially when scattered scrapings were
to be examined, to outline with ink on the surface of the cover-
glass the area to be investigated.

By screw dividers measurements to the nearest 0.1 mm.
were made, on the tracing of each nucleus, of the greatest length
and of the breadth. (In the taking of the latter measurement,
where the curvature was less convex than that of an ellipse
the breadth was measured not at the widest part, but so as to
approximate to the transverse axis of the corresponding ellipse).
In the specimens where nuclear volume was determined, the
thickness of the nuclei was found by converting the fine ad-
justment reading into mm. at the magnification of the tracings

[7]It should be pointed out that in specimen No. I, instead of the method
described above, eight "oil immersion" fields were taken in different parts
of the section so as to be representative of the different types of structure
and from each field 25 nuclei were traced.

of the other dimensions of the nuclei. The volume of the magnified nucleus was obtained by the formula for an ellipsoid: $4/3 \pi abc$, where a, b, c are the semi-axes. For convenience, the volumes were commonly expressed merely as the product of the three areas, length, breadth and thickness, i. e., the volume of the corresponding rectangular prism.

Influence of Slide-thickness on Measurements Made with a Projection Microscope.

This use of the projection microscope when large numbers of measurements have to be made is becoming more common as a substitute for the employment of the eyepiece micrometer with its attendant eyestrain. It is surprising, therefore, that more attention has not been paid to what is, on theoretical grounds, the most serious disadvantage of the method, namely that if a thicker slide is placed on the stage, the microscope tube must be moved nearer the screen in order to bring the object into focus, and this movement should alter the magnification. In the present series of observations the slide thicknesses varied from 0.686 mm. to 1.487 mm. No correlation was detectable between the slide thickness and the linear dimensions of the nuclei. A more direct test was made as follows. On the thinnest slide available an india ink mark was made covered with transparent mucilage to keep it in place, and then mounted under a coverglass with balsam. Under the conditions of magnification and so on used in the rest of the investigations, two well defined parts of the mark were traced and the distance between them measured. The thickness of the slide was computed from microscope fine adjustment readings on the assumption that the refractive index of the glass and balsam was 1.5. This is a more accurate method than the direct reading of the screw gauge, since the gauge was liable to compress the balsam which held the various glasses together, for, to increase the thickness of the slide, coverglasses were fastened by balsam on to the lower surface of the slide and the tracings and measurements repeated.

The results were as follows:

Slide thickness calculated from fine adjustment readings	Slide thickness from micrometer gauge after tracing	Distance between two points on tracing
mm.	*mm.*	*cm.*
0.6486	0.646	9.215
0.8183	0.931	9.280
0.9588	0.965	9.245
1.1007	1.096	9.285
1.2567	1.238	9.305
1.3989	1.369	9.365
1.5723	1.502	9.225
1.7436	1.667	9.245
1.8621	1.856	9.200
2.0123	1.971	9.260

It will be seen that there is no appreciable influence of slide thickness on the magnification and it is impossible to obtain a correction factor which could be applied to improve the actual readings made on nuclei. Even the variations from the mean that do occur are small (mean distance: 9.2625 mm.; coefficient of variation: 0.5 per cent), compared with the variation from tracing to tracing of the same nucleus on the same slide (see below). Therefore, in projection work of this kind, even extreme variations in slide thickness can be safely neglected, for any effect they produce is obliterated by influence of other agents, e. g., the focussing differences, the personal error, etc. It should be noted that the distance between ocular and screen (about 18 inches) was very great compared with the changes in slide thickness. It is of some interest to contrast with these findings the definite influence of coverglass thickness on magnification when dry lenses are used.[8]

Colorimetric Tests of Nuclear Staining.

A test of nuclear staining intensity was applied as follows: On a piece of card about $\frac{3}{4}$ ins. by $\frac{1}{2}$ ins. was laid a wash of dark violet water color of an intensity like that of the darkest hematoxylin-stained nuclei. This card was labelled A. On another similar piece a somewhat lighter colored wash was laid,

[8]Mainland, Donald. *Anat. Record*, **50**, 53, (1931).

and so on, until, on the fifth card, E, the tint was very pale. The five pieces were glued in a row to an index card, so that they could be easily held near each nucleus as the latter was examined. The color intensity was thus recorded without so much liability to subjective error as if it had been classified as "dark", "medium" or "light". The chief drawback to such a method is the presence of tissue near the nucleus under examination, so that white light does not always shine on the scale. There is, however, very often a small clear area some-where in the field, against which the scale can be held.

RESULTS.

Variation Due to Observational Errors.

Before evaluating the significance of differences between specimens it is necessary to know how much of the variation may be due to observational errors, and in choosing dimensions (volume, length or breadth) of greatest use in such a study, it is desirable, other things being equal, to select the one with least observational error. From one specimen of carcinoma (No. I of Tables 2 and 3) two adjacent nuclei were selected, each was traced thirty times in different parts of the field, and the thickness of each was taken thirty times with different parts of the fine adjustment. Nucleus A was lightly stained and more sharply defined than nucleus B, which was not only darker, but more uniformly stained. The results are shown in Table 1.

To be "significant", a difference must be at least twice its standard error, and therefore some of the apparent differences in this table are not of any account. The most striking points are the great variation of the volumes and the similar variation in thickness measurements. The variation in length and breadth is comparatively small. This difference justifies the subsequent avoidance of thickness measurement, in spite of the desirability of volume determination. The weakness of thickness measurement is partly, perhaps, the fault of the fine adjustment, but probably to a large extent the difficulty in focussing. Where the nucleus is not dense, and its membrane

TABLE 1.

Observational Variations: Thirty Sets of Measurements on Each of Two Nuclei
(A and B)

(All dimensions are as magnified)

	Mean	Standard deviation	Coefficient of variation with its standard error *per cent*	Significance of difference of coefficients of variation.
Length A	21.79 mm.	±0.28	1.3±0.17	Difference signifi-
B	13.44 mm.	±0.47	3.5±0.46	cant. (standard error: ±0.5).
Breadth A	9.87 mm.	±0.37	3.8±0.49	Difference not sig-
B	9.94 mm.	±0.27	2.7±0.35	nificant (s. e.: ±0.6).
Thickness A	13.68 mm.	±2.52	18.4±2.45	Difference not sig-
B	14.04 mm.	±2.25	16.0±2.13	nificant.
Volume A	2954 c.mm. (corresponding rect. prism)	±609.4	20.6±2.77	
	1547 c.mm. (ellipsoid)	±319.1		Difference not significant.
B	1875 c.mm. (corresponding rect. prism)	±313.2	16.7±2.24	
	982 c.mm. (ellipsoid)	±164.9		

is well defined, the variability of thickness determinations may be no greater than that of breadth.[9]

The breadth variation is in neither nucleus significantly less than that of length; in nucleus B the breadth variation is significantly greater than that of length. The narrow ends of an ellipse would be expected to give more definite points for measurement than the sides, and the variation from one length measurement to another would therefore not be so great. The less well defined nucleus B gives a greater variation in length than does the better defined nucleus A.

[9]Mainland, Donald. *Trans. Roy. Soc. Can.*, 3rd ser., **25**, sect. V. (1931).

The Number of Nuclei to be Measured in One Specime n.

This question necessarily arises in all such invest igations, where it is only practicable to measure a small sam ple of the available nuclei. The accuracy of a mean of a set of observations in biology increases according to the square root of the number of observations. The mean of $4n$ observations is only twice as accurate as that obtained from n observations. The question of how many readings to take is solved by the physicist by taking a number of the observations and dividing them into two groups. If the two means differ from each other by no more than the step in the measuring instrument, the number is considered sufficient in the circumstances of the experiment (see Campbell[10], pp. 164-5, etc.). In biological investigations it seems possible to extend that method so that measurements can cease when the difference between the means obtained from the two samples is no greater than can be accounted for by the variability of the method itself. In the present instance the preceding section has shown the variability of the method. On the same specimen tests were carried out to determine the necessary number of nuclei. Two hundred nuclei were traced (see footnote to Table 3), and the volumes calculated, the data for each nucleus being on a separate card. The cards were thoroughly mixed by shuffling and one hundred cards picked out at random. F rom these, again at random, two lots of fifty were taken. T he mean volumes (of the corresponding rectangular prisms) w ere:

Set (1): 1242.38 c.mm.

Set (2): 1184.50 c.mm.

Difference: 57.88 c.mm.

Let the variation be assumed to be the same as that of the test nucleus B (above), i. e., 16.7 per cent or $31 3.2/1875$, i. e., the variation due to the observations on ly. The standard deviation of set (1) would then be $\pm(31 3.2/1875)$ x $1242.38 = \pm207.5$. Therefore the standard err or of the mean 1242.38 would be $= \pm207.5/\sqrt{50}$. Similarly the mean 1184.50 would have a standarderror of $\pm197.8\sqrt{50}$.

[10]Campbell, N. R. An Account of the Prin ci pies of Measurement and Calculation. Longmans, Green and Co., London. 1928.

The standard error of the **difference** is—

$$\pm\sqrt{(\text{standard error of set } (1))^2 + (\text{standard error of set } (2))^2}$$

$$= \pm\sqrt{\left(\frac{207.5}{\sqrt{50}}\right)^2 + \left(\frac{197.8}{\sqrt{50}}\right)^2} = \pm 40.5$$

The difference is 57.88, i. e., much less than twice its standard error. Therefore the difference would not be significant even if it were due only to observational errors. The sets of fifty nuclei were thus sufficient to give a representative mean. In some specimens it was not possible to obtain so many nuclei well defined and suitably placed with reference to the plane of the screen. A smaller number was then taken. In each case the number of observations is given in Table 3.

Volumes.

At the outset of the investigations the volumes were calculated for five specimens, as shown in Table 2.

TABLE 2.

Volumes of Nuclei

(All dimensions are as magnified)

Specimen No.	Condition	No. of nuclei	Mean vol. (corresponding rect. prism) *c. mm.*	Standard deviation of vol.	Coefficient of variation of vol. *per cent*	Mean length *mm.*	Mean breadth *mm.*
I	carcinoma	200	1276.93	±683.7	53.5	15.48	9.04
IV	carcinoma	95	13 52.2	±587.2	43	13.72	8.84
VII	hyperplasia	118	813.17	±266.8	33	11.77	7.23
III	healthy	99	411.16	±174.9	43	8.77	4.56
11	healthy (premenstrual)	84	724.07	±223.6	31	10.68	6.72

The results suggested that in carcinoma the mean nuclear volume was greater than in hyperplasia, and that in hyperplasia the volume was greater than in the healthy mucosa. It will be noticed that the actual volumes vary from about 700 c. mm. (or 200 cubic microns unmagnified) to about 200 c. mm. (or 60 cubic microns unmagnified). There were not, of course, sufficient specimens prepared by the different techniques to make conclusions from them justifiable, and, rather than pursue farther the volume estimation, it was thought desirable to make use of some criterion of size that would have less observational variation than volume and would be quicker and easier to determine. Such criteria were the length and breadth. In the carcinoma first examined (No. I of Table 3) the coefficient of correlation between length and volume (200 nuclei) was $+.49$; standard error: ±0.054, while the coefficient of correlation between breadth and volume was $+.63$; standard error: ±0.043. These coefficients were not only significant themselves, but differed from each other, for the z test (Fisher[5], p. 170) showed that the correlation between breadth and volume was greater than between length and volume. In the main body of the investigation, therefore, breadth and, secondly, length received attention to the exclusion of thickness and volume.

Relationship of Length and Breadth.

The method employed was such that the axis called "length" in some nuclei was not the same as the axis given the same name in others, and it might be thought that the results would depend more on the position of the nuclei than on anything else. It has been shown, however, that breadth and volume are fairly highly correlated, and, to a less extent, length and volume are correlated. The relationship of length to breadth throws further light on this question. In the Winnipeg carcinomata there was a significant correlation between length and breadth $(+.6834)$. This shows that not only is the average length greater than the average breadth in each specimen, as it must be by definition, but, in the series as a

whole, the greater the length the greater the breadth. In the four groups: Winnipeg carcinoma, Halifax carcinoma, Winnipeg hyperplasia and Halifax hyperplasia, the correlation between the group mean length and the group mean breadth was very high (+.9938) and significant in spite of the small number of groups (Fisher[5], p. 176). These correlations are explicable by the fact that both mean length and mean breadth are indications of mean volume. In a series of this size or larger, therefore, a very adequate indication of volume can be obtained without actual volume determination.

Comparison of Groups by Linear Measurements.

Table 3 shows the data for this comparison.

Comparison of Mean Breadths:[11]

Winnipeg carcinoma (15 specimens): 9.202 mm.; standard error: ±0.238.

Winnipeg hyperplasia (10 specimens): 8.270 mm.; standard error: ±0.326.

Difference: 0.932 mm.; standard error: ± 0.404.

Since one of the means is based on less than fifteen observations, it is necessary to apply special tests of significance. The t test (Fisher[5], p. 107) is similar in its principle to the standard error test. The t test gives the result in this instance: $t = 2.27$; $n = 23$, and the tables of t show that the difference must therefore be significant. There are between 20 and 50 chances to 1 against so large a difference being due to chance variations.

Halifax carcinoma (5 specimens): 8.512 mm.; standard error: ±0.341.

Halifax hyperplasia (14 specimens): 6.371 mm.; standard error ±0.191.

Difference: 2.141 mm.; standard error: ±0.391.

The t test showed the high significance of this difference $(t = 5.33; n = 17; P < .01)$.

[11]All the sizes are given as magnified. For conversion to the approximate actual size in microns, it is sufficient to take two-thirds of the magnified size in mm. Thus the actual mean breadth for the Winnipeg carcinoma group is approximately 6.1μ.

The mean breadth, therefore, forms a definite criterion of distinction between the carcinoma group and the hyperplasia group in both the Halifax and Winnipeg specimens. By means of a larger number of specimens it would be possible to plot with greater and greater accuracy the limits of the two classes, and to discover the probabilities of an undiagnosed or doubtful specimen lying in one or other of the classes. Considerable interest attached to specimen No. XXI (Halifax). The pathologist's report on this was: "A very profound adenomatous hyperplasia. While not frankly positive for malignancy, I regard the indications sufficiently marked to warrant the case being regarded and treated as such."

The mean breadth is 7.56 mm., and it lies therefore between the hyperplasia and the carcinoma means, but nearer the latter.

Difference between Winnipeg carcinoma and Halifax carcinoma: 0.690 mm.; standard error: ±0.349.

The difference is not quite significant, for it is hardly twice its standard error.

Difference between Winnipeg hyperplasia and Halifax hyperplasia: 1.899 mm.; standard error ±0.378.
The t test showed the high significance of this difference.

The importance of similarity of technique cannot be too strongly emphasised where histological measurements are to be made.

These results concerning mean breadths can be summarized as follows: The mean breadth of the nuclei in each of a series of specimens has been obtained, the specimens have been grouped according to disease and technique, and the mean for each group has been determined. When the technique remains the same, the mean nuclear breadth in carcinoma is greater than in hyperplasia. For hyperplasia the mean nuclear breadth is greater in Winnipeg specimens than in Halifax specimens. For carcinoma the Winnipeg mean is again apparently the greater, but the difference is not quite up to the conventional level of significance.

Comparison of Mean Lengths:
Winnipeg carcinoma (15 specimens): 14.320 mm.; standard error: ±0.355.

Winnipeg hyperplasia (10 specimens): 13.446 mm.; standard
    error: ±0.452.
            Difference: 0.874 mm.; standard error: ±0.575.
Halifax carcinoma (5 specimens): 13.652 mm.; standard error:
    ±0.684.
Halifax hyperplasia (14 specimens): 10.381 mm.; standard
    error: ±0.311.
            Difference: 3.271 mm.; standard error: ±0.751.
Specimen No. XXI, referred to above, had a mean of 11.11 mm.
and therefore lay between the Halifax the hyperplasia mean and
the Halifax carcinoma mean. In the Halifax specimens the dif-
ference between the means was highly significant. In the Winni-
peg specimens, although the carcinoma means appeared greater,
the variation between the items that formed the means was
so great that the difference was not significant.

Difference between Winnipeg carcinoma and Halifax car-
    cinoma: 0.668 mm.; standard error: ±0.771; difference
    not significant.
Difference between Winnipeg hyperplasia and Halifax hyper-
    plasia: 3.065; standard error: ±0.549.

The t test confirmed the supposition that the mean length in
the Winnipeg series of hyperplasia specimens was definitely
greater than that in the Halifax series.

The mean lengths, therefore, in no instance disagreed
with the conclusions reached by a study of the mean breadths,
but length, probably on account of its variability, was appar-
ently not so good a criterion as breadth. In this connection
it should be remembered that the breadth is much more closely
correlated with volume than is length.

The actual differences, although significant, may not
appear great until it is noted that they range from 6 per cent
to over 25 per cent, and, if represented as volumes (the cube
of the linear measurements), the differences would become
much more striking.

Comparison of Variations: Two classes of variation come
under consideration: the variation from specimen to specimen

and the variation within the specimens.   In each of these
classes the variation must be considered in two ways: as
absolute variation and as variation in proportion to the mean
value, about which the variation takes place.

(a)   *Varitaion from Specimen to Specimen*:   The absolute
variation is expressed as the standard deviation, and the
variation in proportion to the mean is expressed as the co-
efficient of variation.   These values were:

Group	No. of Specimens	Breadths		Lengths	
		Standard deviation	Coeffi- cient of variation *per cent*	Standard deviation	Coeffi- cient of variation *per cent*
Winnipeg carcinoma	15	0.9202	10.00	1.375	9.60
Winnipeg hyperplasia	10	1.0296	12.45	1.429	10.63
Halifax carcinoma	5	0.7635	8.97	1.530	11.21
Halifax hyperplasia	14	0.7160	11.24	1.174	11.31

Since the number of specimens was small, the z test
(Fisher[5], p. 195) was applied to determine the significance of
the differences between the standard deviations.   The con-
clusions with regard to length and breadth were (1) that there
was no difference between the absolute variations from speci-
men to specimen in the carcinoma groups as compared with
the same variations in the hyperplasia groups, and (2) that no
significant difference could be ascribed to technique.   But, if
the actual variation is the same in carcinoma as in hyperplasia,
and, as has been shown, the mean breadth is greater in car-
cinoma, then the variation considered in terms of the mean
(i. e., the coefficient of variation) should be greater in hyper-
plasia.

In regard to both length and breadth this appears, from
the data just recorded, to be correct.   To prove whether the
effects might be due to chance the t test was applied in the
following manner:

Difference between coefficients of variation (breadth)—
    Winnipeg specimens: 2.45;   Halifax specimens: 2.27.
       Mean of the two differences: 2.360.

t(Fisher[5], p. 105) $= 26.2$; $n = 1$; $.05 > P > .02$. This result shows that the mean difference is significant, and therefore the variation of breadth considered in relation to the mean breadth is greater in carcinoma than in hyperplasia. In the cases of length the differences are not sufficiently alike in Halifax and Winnipeg to justify the conclusion, but this does not detract from the importance of the results derived from breadth. The exact meaning of these results deserves consideration, and a clearer conception of this meaning may be obtained if it is recalled that roughly three times the standard deviation on either side of the mean marks, in the normal frequency curve, the upper and lower limits of the series of observations that form the curve. For a given technique the standard deviation is the same for hyperplasia as for caricnoma, and therefore the upper and lower limits of the series must be as far away from the mean in hyperplasia as in carcinoma, and, relative to the size of the mean, the hyperplasia collections are less uniform than are the corresponding carcinoma collections.

There was no definite association between technique and these coefficients of variation.

(b) *Variations within Specimens.* The standard deviations of each specimen are shown in Table 3, and instead of the thorough, but rather complicated method of analysis of variance, the means of these standard deviations were studied. Similarly, the coefficients of variation give for each specimen the variation in proportion to the mean of that specimen, and the means of these coefficients show the relation between variation and mean for an average specimen of the group concerned, say carcinoma.

Group of No. specimens		Breadths		Lengths	
		Mean of standard devia- tions	Mean of coeffi- cients of variation *per cent*	Mean of standard devia- tions	Mean of coeffi- cients of variation *per cent*
Winnipeg carcinoma	15	±1.837	20.00	±2.799	19.53
Winnipeg hyperplasia	10	±1.762	21.50	±2.245	17.00
Halifax carlnoma	5	±2.340	27.20	±2.984	21.80
Halifax hyperplasia	14	±1.316	20.86	±2.053	19.07

Where there was doubt regarding the significance of the differences, the *t* test was applied, and the conclusions regarding absolute variation in breadth were as follows.   There was no significant difference in mean standard deviation between Winnipeg carcinoma and Winnipeg hyperplasia.   The average Halifax carcinoma, however, varied more within the specimen than did the average Halifax hyperplasia specimen, and more also, than the average Winnipeg carcinoma.   The standard deviations of length gave more conclusive information.   The average length variation was definitely greater in carcinoma than in hyperplasia, both in the Winnipeg and Halifax groups. Technique had no uniform effect.

When the variation within the specimens was considered in relation to the mean, by use of the coefficient of variation, the breadth variation in the Halifax carcinoma group was again shown to be greater than in the Halifax hyperplasia group.   For the lengths the differences in the two groups (Winnipeg and Halifax) were combined, giving a mean difference between carcinoma and hyperplasia of 2.63, the carcinoma being the greater.   The *t* test showed that this difference was significant, and therefore it can be concluded that there is greater variation in length in the average carcinoma specimen, even in proportion to its mean length, than in the average hyperplasia.   In a similar way it was shown that the average Halifax specimen varied more in itself in regard to length, even in proportion to its mean length, than did the average Winnipeg specimen.

The conclusions to be drawn from sections (*a*) and (*b*) may be summarized by stating that the nuclear size varies more from specimen to specimen in hyperplasia than in carcinoma, but varies more within the specimen in carcinoma.   Technique seems to have to have no very definite influence on the variation from specimen to specimen, but the Halifax technique is associated with a greater variation in nuclear length within specimens than is the Winnipeg technique.

(*c*)   *Correlation of Breadth and Standard Deviation of Breadth*:   The question to which the preceding two sections

naturally lead is: Are the greater mean breadths associated with greater variations, and the smaller mean breadths with smaller variations?   The coefficients of correlation between mean breadth and standard deviation were:

Winnipeg carcinoma (15 specimens): +.557; significant.
Winnipeg hyperplasia (10 specimens) : +.144; not significant.
Halifax Carcinoma)5 specimens) : +.941; significant.
Halifax hyperplasia (14 specimens) : +.041; not significant.

In both groups of carcinoma there is a definite positive correlation: the variation tends to vary in the same direction as the mean breadth.   In hyperplasia a high mean may be associated with either a high or low variation, and the same applies to a low mean.

Shape of Nuclei.

The impression obtained from a preliminary survey of the specimens was that in carcinoma the nuclei tended to be broader ellipsoids than in hyperplasia.   In seven specimens the ratio of breadth to length was determined for each nucleus separately.   In all the specimens there was calculated an index: (mean nuclear breadth x 100)/(means nuclear length).

The results set down show a mean nuclear length very close correspondence between the results of the two methods.   The "mean index" shows, in the form of a percentage, the results of the first method, the "index of means" shows those of the second.

Specimen No.		No. of Nuclei	Mean Index	Index of Means
I	(carcinoma)	200	60.8	58.39
II	(healthy pre-menstrual)	84	65.0	69.92
III	(healthy)	99	53.8	52.00
V	(carcinoma)	100	60.4	59.76
VI	(hyperplasia)	102	53.9	49.22
VII	(hyperplasia)	118	63.3	61.43
IX	(hyperplasia)	69	71.7	69.80

The coefficient of correlation between the mean index and the index formed by the means was +.9900, and this, in spite of the smallness of the numbers compared was highly signi-

ficant.   Therefore, one is perfectly justified in substituting the shorter method of estimating average shape for the longer method, whereby the ratio for each nucleus is found.

Table 3 gives for each specimen the index formed by the mean breadth and mean length.   The mean values derived from these are:

Winnipeg carcinoma (15 specimens): 64,453 per cent.
Winnipeg hyperplasia (10 specimens): 61.592 percent.
Halifax carcinoma (5 specimens): 62.514 per cent.
Halifax hyperplasia (14 specimens): 61-881 per cent.

The standard error and t tests showed that there was no significant difference attributable to disease or to technique.   The average shape of the nuclear image is, therefore, so variable from specimen to specimen, that in the series examined neither disease nor technique had any appreciable effect.

Frequency Distributions.

Graphs were drawn for nearly all the specimens, to show the numbers of nuclei in the different length and breadth classes —frequency distribution graphs.   These graphs presented very varying appearances, some being practically symmetrical, some skewed to the left, others to the right.   It was thought that in specimens where the variation from the mean was great this variation might be accounted for by the presence, along with adult cells, of a fair number of young cells, with small nuclei; but the graphs showed that it was not possible to account for the differences in variation in any one way.   Composite graphs were constructed, to eliminate minor peculiarities due probably to insufficiency of the numbers of observations in individual specimens.   Two of these graphs are shown in Fig. I.

For comparison, the various specimens of carcinoma (Winnipeg and Halifax) have been supposed to contribute equally to the total 100 nuclei, and each of the hyperplasia specimens (Winnipeg and Halifax) has been supposed to con-
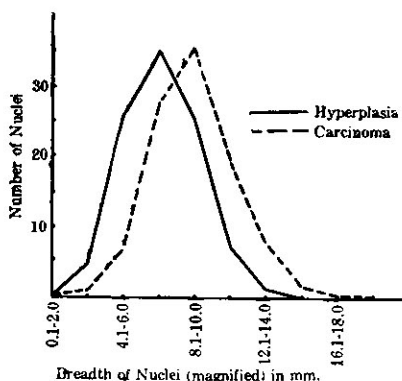
Fig. 1. Frequency Distribution Graphs of Nuclear Breadth. Each is a percentage graph, and is composite—hyperplasia 22 specimens; carcinoma 20 specimens.

tribute equally to the total 100 hyperplasia nuclei. The graph, therefore, is a composite frequency distribution graph of breadths arranged on a percentage basis. It shows that the mode (region of greatest frequency) is greater in carcinoma (8.1-10 mm.) than in hyperplasia (6.1-8 mm.). Each curve is fairly symmetrical, but there is a tendency for a more gradual slope down to the right than to the left. This was seen also in graphs made from Winnipeg and Halifax specimens separately, and, although no statistical tests were applied, it appears probable that the feature has some significance, as indicating a greater tendency for the occurrence of extremely large nuclei than for the occurrence of extremely small ones.

Staining Intensity.

Colorimetric study of ordinary histological material has not been developed to any extent, and yet crude quantitative judgments are frequently heard, wherein it is stated that one specimen or disease shows more darkly stained nuclei than another. The records given here show how little trust may be placed on such statements. It has been mentioned in the section on methods that the nuclei were classified according to a scale of purple tints, varying from A (very dark) to E (very light). Table 3 shows that the specimens varied much from each other in the proportions of nuclei found in the different classes, and tests were applied to ascertain whether these differences were associated with differences in technique or disease.

In the first place the specimens were compared by the χ^2 test (Fisher[5], p. 75) according to the intensity (A, B, C, etc.)

of the chief number of their nuclei. There was no significant difference between the different groups, either in association with disease or technique.

A second test was made by calculating for each specimen the percentage of the total nuclei classified as dark (classes A, B and C)—

	Mean percentage of dark nuclei	Standard error
Winnipeg carcinoma (15 specimens):	68.61	±6.4
Winnipeg hyperplasia (10 specimens):	86.68	±4.8
Halifax carcinoma (5 specimens):	88.04	±4.7
Halifax hyperplasia (14 specimens):	74.99	±6.8

It appears as if in the one group the hyperplasia specimens and in the other the carcinoma specimens had the preponderance of darkly stained nuclei. There was no significance as high as the conventional level of 19 to 1 in any of the differences. The nearest to such was the difference between Winnipeg carcinoma and Winnipeg hyperplasia ($t = 1.91$; $n = 23$; $P < .1 > .05$, but nearer the latter).

It is true that the scheme devised for the test was somewhat crude, but if so crude a test indicates nothing it is questionable if one that was finer would show any more significant difference. The variation from specimen to specimen is so great that it obliterates differences attributable to disease if such exist. The conclusion is that, as routine staining is at present conducted, it cannot form a useful basis for a colorimetric comparison of tissues. The question that therefore arises is whether it is worth while to standardize staining methods in respect of strength of solution, duration, dehydration and mounting. (Probably, of course, the section thickness would have to be standardized). Investigations of individual specimens have indicated that such standardization might well be productive.

To ascertain whether there was in any one specimen an association between size of nucleus and staining intensity, it was desirable to express this intensity numerically instead of by letters. In order to do this, a dark mixture was made of water and the water-color paint that had been used to make

the colorimetric scale. The mixture was placed in a test tube in front of a white paper and compared with the colors on the scale. The mixture was diluted until it matched color A. Then it was further diluted, to a known degree, until it matched color B. This process was continued until the faintest color (E) was matched. The experiment was carried out three times, and the following figures were derived:

$$E = 1$$
$$D = 1.3$$
$$C = 2.5$$
$$B = 4.7$$
$$A = 11.3$$

Although there was a good deal of variation between the three experiments, it was considered that these figures expressed, as closely as so simple a test could do, the relative intensities, and it was certainly a more accurate method than the method of "ranking", whereby the numbers 1, 2, 3, 4, 5 would replace the letters—a method commonly employed where more exact numerical equivalents cannot be obtained (Tippett[12], p. 168). With the values obtained, the following correlations were calculated for specimen No. I (Winnipeg carcinoma; 200 nuclei).

Coefficient of correlation between breadth and staining: —.277; standard error ±0.065.
Coefficient of correlation between thickness and staining: +.352; standard error ±0.062.
Coefficient of correlation between breadth and thickness: —.013; standard error ±0.071.

To be significant, a coefficient of correlation must be at least twice its standard error, and these results therefore indicate that, where the breadth of the nucleus is greater, the staining intensity tends to be less. The possible range of the coefficient is from +1 (perfect positive correlation) through 0 (no correlation) to —1 (perfect negative correlation), and therefore the association here is not very great, but it is none the less definite. Secondly, where the thickness is greater, the staining intensity tends to be greater. The thicker the nucleus,

[12]Tippett, L. H. C. The Methods of Statistics. Williams and Norgate, London, 1931.

the greater will be the impediment to the rays passing through it, provided its composition is similar in both cases.   It might be thought that the inverse relationship between the staining and breadth could be explained if a greater breadth were associated with a less thickness.   There was, however, as shown above, no significant correlation between breadth and thickness, and therefore the breadth-staining relationship must be explained independently.   The broader the nucleus, the more widely spread out is its staining material, and therefore the paler does it appear.

Although similar correlation tests were not applied throughout the series, other observations showed that this correlation existed.   For each specimen the mean breadth and mean length of the nuclei were calculated for the different classes A, B. C, D and E.   Examples are:

	A	B	C	D	E
Specimen No. I					
Mean breadth	—	7.4	8.9	9.9	9.2
Mean length	—	11.2	15.3	17.2	15.1
Specimen No. XXVIII					
Mean breadth	4.9	5.8	6.0	6.8	—
Mean length	7.7	7.8	8.2	9.1	—

In all except a few instances there was an increase in the mean breadth and mean length at each step from the darker nuclei to the lighter.   The staining, therefore, is partly a function of size, and this suggests that there is a similar amount of basiphil chromatin in all the nuclei of a given specimen. The larger the nucleus, the less the amount of stained material in the given volume, and the less the intensity of staining.   It has been shown above that nuclear size is associated with disease, and, now, that, for any given specimen, nuclear staining is associated with size.   It appears therefore that staining intensity could be shown to be associated with disease if the staining technique were uniform for all the various specimens.

DISCUSSION.

The smallness of the number of specimens used in these investigations may give rise to doubt regarding the validity

of the conclusions, and therefore it is desirable to note the principles upon which the conclusions are based.   Each set of specimens (carcinoma and hyperplasia) has been divided into two groups according to technique (Winnipeg and Halifax). Where the Halifax specimens corroborate the Winnipeg specimens regarding differences associated with disease, and the differences are proved significant by the finest statistical tests, confidence in the results is felt to be justified; and similarly where carcinoma specimens corroborate hyperplasia specimens regarding differences associated with technique.

Four main size differences between carcinoma and hyperplasia nuclei have emerged—the greater average size in groups of carcinoma specimens; the greater variation within carcinoma specimens; the greater variation from specimen to specimen in hyperplasia; and the positive correlation between size and variation in carcinoma, in contrast to the lack of correlation in hyperplasia.   Before these results can be finally interpreted, a large amount of experimental work must be carried out, but the possible lines of the interpretation can already be indicated. That differences of some kind, morphological or physico-chemical, between the two groups of nuclei have been disclosed will surely be admitted even by those who are most ready to explain histological findings as artifacts.

It must first be asked whether the size differences denote an original size difference between carcinoma and hyperplasia nuclei, or whether these nuclei may have started with the same average size and size range, and then, under the influence of the fixation and other processes, become differentiated.   Little of detail is established regarding the influence of such treatment on nuclear size (cf. Tarkhan[13]), but it seems reasonable to suppose that, where shrinkage occurs owing to dehydration, clearing and imbedding, the amount of shrinkage will be proportional, more or less, to the original bulk of the nucleus, the larger losing more than the smaller.   If, in a collection of nuclei, say one fifth of their volume were taken off each, i. e., from those of average size, the smallest and the largest, then

[13]Tarkhan, A. A.   *J. Royal Microsop. Soc.*, **51**,387 (1931).

the absolute size range would lessen, but the proportion of the range to the mean would not alter, i. e., the coefficient of variation would remain the same as before.   Now the mean of a series of hyperplasia specimens is less than the corresponding carcinoma mean, and yet the standard deviation (and therefore the absolute range of variation) is the same in hyperplasia as in carcinoma, and the coefficient of variation is correspondingly greater in hyperplasia.   It seems therefore very unlikely that the hyperplasia has merely been diminished from the carcinoma size in the preservation processes.   It seems much more reasonable to suppose that the size differences in the fixed specimens represent, to some extent at least, original size differences.

The next question that might be asked is whether the size differences represent a difference in the amount of primary nuclear material, say chromatin, or whether they indicate a difference of activity and a corresponding difference of the physico-chemical state of the nucleus (which would also probably give a differential response to technique).   It is true that the results suggest that a series of hyperplasia specimens might, as regards nuclear size, be converted into a series of carcinoma specimens by adding a constant amount of nuclear material to each nucleus.   The mean size would be increased, the absolute range would remain the same, and the coefficient of variation would therefore be less, just as in the actual carcinoma series.   It appears much safer, however, to suppose that the metabolic activity could, if sufficiently known, account for the phenomena.   If this view is adopted and applied to the interpretation of the other facts noted, it can be stated that the hyperplasia specimens differ from each other more widely than the carcinoma specimens in the average activity or physico-chemical state of their nuclei, and also differ widely from each other in the range of these phenomena found in the same specimen; but that on the average, carcinoma nuclei, within the small area of an ordinary section, differ more from each other than do hyperplasia nuclei.

It appears, therefore, as if, by study of more specimens, and of individual alveoli and cell clumps, light may be thrown

on the intimate structure and activity of tissues, but at the same time the possibility of the application of the method to differential diagnosis should not be overlooked. Routine quantitative methods in pathology are now largely confined to fluids and to cells (especially blood cells) suspended in fluid. There is already a chemical pathology, but there seems no reason why there should not be a ""physical pathology", concerned with the measurement of solid tissues in section.[14] If this were shown to be a useful technique, the labor of the measurements could be rendered very small, especially by electrical devices connected with the screw dividers. All that would be necessary beyond this would be the standardization of the technique of fixation, imbedding, staining, etc. The present results have shown, for instance, that the Halifax technique is associated, for some reason or other, with greater variation in nuclear length within the specimen than is the Winnipeg technique. There is a strong suggestion that nuclear sizes are larger with the Winnipeg technique, and it seems, also, as if the Halifax method gives a greater difference between carcinoma nuclear sizes and those of hyperplasia, but this may well be due to differences in the specimens rather than in the techniques. The two methods differ from each other in various respects, and it would be necessary to isolate these differences experimentally and to standardize the technique which best brought out measurable differences. It is true that the diagnostic value of the method has not yet been proved and there is much overlapping of the two series, carcinoma, and hyperplasia, when arranged according to size, as may be seen by comparing the various sections of Table 3, but this overlapping is probably in part due to small differences in technique from specimen to specimen, and the part so caused would be eliminated by standardization of technique.

[14]The possibilities of this kind of test are shown in the pioneer work of Savage, (Savage, A., Williams, W., and Fowler, N. M., *Trans. Roy. Soc. Can.* 3rd Ser. **21**, Sect. v, 425 (1927), *Can. J. Research* **3**, 327 (1930)) and his collaborators, who have shown that low fertility of bulls and horsesis indicated by a high coefficient of variability of the head length of their spermatozoa.

It might be that in this particular problem no sufficiently fine differentiation could ever be reached, but, whatever may be the final outcome of work along these lines, there is little doubt that there exists in such quantitative procedures a method which, because of its simplicity, can be used in differential diagnosis, and, because of its objective accuracy, can be used to disclose the details of structure and metabolism of tissues.

TABLE 3.

Data on Nuclear Sizes and Staining Intensities
(All sizes are as magnified)

Specimen No.	Staining intensity— no. of nuclei							Breadth			Length			Index:— mean L × 100) ÷ mean Br	Section or scraping	Path. report
	A	B	C	D	E	Un-class-ified	Total	Mean mm.	Stand-ard devia-tion	Coeff't of variation *per cent*	Mean mm.	Stand-ard devia-tion	Coeff't of variation *per cent*			
Winnipeg carcinoma																
I	—	18	118	61	3	—	200	9.04	±2.24	25	15.48	±4.15	27	58.39	sect.	spher.; sev.
IV	—	2	45	40	7	1	95	8.84	±1.90	22	13.72	±2.63	19	64.43	sect.	spher.; sev.
XVI	—	5	40	43	3	—	91	8.38	±1.39	17	12.36	±1.92	16	67.80	sect.	adeno-; mod
XXII	2	8	21	13	1	—	45	8.87	±1.82	22	18.88	±3.12	23	60.52	sect.	adeno-; mod
XXIII	—	3	34	11	1	—	49	10.38	±2.00	19	14.93	±2.41	16	69.52	sect.	adeno-; mod
XXV	—	1	23	7	—	—	31	10.05	±1.32	13	13.98	±2.29	16	71.89	sect.	adeno-; mod
XXVII	—	1	6	14	4	—	25	9.84	±2.14	22	15.77	±3.47	22	62.40	sect.	adeno-; sev.
XXX	2	17	36	7	—	—	62	9.11	±2.09	23	14.28	±2.86	20	63.79	sect.	adeno-; sev.
XXXI	2	25	17	5	—	—	49	11.66	±2.70	23	17.38	±3.48	20	67.09	scrap.	spher.; sev.
XXXIV	11	21	16	1	—	—	49	8.33	±2.07	25	13.90	±2.51	18	59.93	sect.	spher.; sev.
XXXV	—	14	26	8	1	—	49	9.33	±1.66	18	13.69	±2.23	17	68.65	scrap.	spher.; sev.
XXXVI	—	2	24	8	1	—	35	8.41	±1.64	19	15.34	±2.85	19	54.82	sect.	adeno-; mod
XXXVII	1	10	29	9	—	—	49	9.27	±1.71	18	13.69	±2.96	22	68.21	sect.	adeno-; sev.
XXXIX	—	2	18	29	—	—	49	8.86	±1.41	16	15.11	±3.08	20	58.64	scrap.	adeno-; sev.
XLVI	5	22	19	3	—	—	49	8.16	±1.47	18	11.54	±2.03	18	70.71	sect.	spher.; sev.
Halifax carcinoma																
V	3	22	42	29	4	—	100	9.49	±2.78	29	15.88	±3.39	21	59.76	sect.	adeno-; sev.
X	11	29	19	5	—	—	64	8.08	±2.11	26	12.95	±3.05	24	62.39	sect.	adeno-; sev.
XII	3	48	42	7	—	—	100	7.50	±1.51	20	11.52	±2.18	19	65.10	scrap.	spher.; sev.
XIV	14	60	23	5	—	—	102	9.31	±3.06	33	14.83	±2.99	20	62.78	scrap.	sev.
XV	—	24	67	7	—	—	98	8.18	±2.29	28	13.08	±3.31	25	62.54	scrap.	adeno-; sev.
Winnipeg Hyperplasia																
XXXVII	6	31	12	1	—	—	50	8.55	±1.45	17	12.86	±2.08	16	66.49	scrap.	sev.
XL	—	5	20	24	1	—	50	8.51	±1.34	16	13.42	±1.82	14	63.41	sect.	sev.
XLI	1	7	29	13	—	—	50	8.43	±2.56	30	14.13	±1.55	11	59.66	scrap.	mod.
XLII*	18	2	—	—	—	—	20	5.58	±1.48	27	10.20	±2.37	23	54.71	scrap.	mod.
XLVII*	—	8	2	—	—	—	10								scrap.	mod.
XLIII	—	21	29	—	—	—	50	9.51	±1.55	16	14.21	±1.93	13	66.92	scrap.	sev.
XLIV	1	12	20	—	2	—	33	8.08	±2.16	27	12.36	±2.63	21	65.87	scrap.	mild
XLIX	—	27	21	2	—	—	50	9.29	±1.67	17	14.00	±2.54	18	66.86	scrap.	mod.
XLV	—	4	22	6	—	—	32	8.21	±1.49	18	13.00	±2.96	23	63.15	scrap.	mod.
XLVIII	1	18	14	4	2	—	34	8.81	±2.23	25	14.41	±2.68	19	61.14	scrap.	mod.
L	2	9	35	8	—	1	55	7.73	±1.69	22	15.87	±1.94	12	48.71	scrap.	mod.
Halifax Hyperplasia																
VI	2	29	49	19	1	2	102	5.67	±1.42	25	11.52	±3.58	31	49.22	scrap.	mod.
VII	1	12	57	42	3	3	118	7.23	±1.49	21	11.77	±2.14	18	61.43	scrap.	sev.
IX	—	2	32	30	5	—	69	6.68	±1.50	22	9.57	±2.57	27	69.80	sect.	mild
XIII	—	10	43	16	1	—	70	5.78	±1.51	26	9.88	±2.38	24	58.50	scrap.	mild
XVII	—	7	26	5	—	—	38	6.00	±1.08	16	9.81	±1.94	20	61.16	scrap.	mod.
XVIII	—	24	37	7	1	—	69	5.81	±1.07	20	8.65	±1.50	17	61.39	scrap.	mild
XIX	1	45	26	6	—	—	78	6.41	±1.51	24	10.37	±2.21	21	61.81	sect.	sev.
XX	—	1	18	6	—	—	25	7.86	±1.08	14	11.72	±1.70	15	67.06	sect.	very mild
XXIV	—	15	48	1	—	—	64	7.09	±1.55	22	12.16	±2.43	20	58.81	scrap.	sev.
XXVI	—	5	25	20	2	—	52	5.69	±1.34	24	9.87	±2.51	25	57.65	sect.	sev.
XXVIII	2	22	23	3	—	—	50	5.88	±0.93	16	8.05	±1.02	13	73.04	scrap.	sev.
XXIX	—	15	54	7	—	2	78	7.04	±1.19	17	10.00	±1.33	13	70.40	scrap.	mild
XXXII	—	3	33	13	—	—	49	6.64	±1.36	21	10.72	±1.70	16	61.94	scrap.	mod.
XXXIII	—	31	17	1	—	—	49	5.92	±1.39	24	11.25	±1.78	16	52.62	scrap.	mod.
Miscellaneous																
XXI	—	18	44	2	—	—	64	7.56	±1.80	24	11.11	±2.07	19	68.05	scrap.	(see p. 91)
III	4	65	26	4	—	—	99	4.56	±1.24	27	8.77	±1.48	17	52.00	sect.	healthy
II	1	15	51	16	1	—	84	6.72	±1.26	19	10.68	±2.12	20	62.92	sect.	healthy premenst.
XI	4	31	34	6	—	—	75	8.19	±1.55	19	11.28	±1.97	17	72.61	sect.	healthy posmenst.
VIII	1	12	50	30	4	—	97	7.51	±1.76	23	12.20	±2.48	20	61.56	sect.	hyperplasia

In the two specimens marked with asterisks (**) relatively few nuclei were suitable for tracing, owing partly to the intensity of staining and to the closeness with which the nuclei were packed together. Both had a rather low mean breadth, and, after careful consideration, in order to avoid bias the two were combined and in all the calculations were treated as one.

All the specimens examined have been included in the table, but the healthy ones were too few for comparison with the others. The last three specimens belonged to the teaching collection and were not accompanied by sufficient data regarding technique.

For the Pathological Reports the author is specially indebted to Doctor MacKeen of the Pathological Institute, Halifax. It will be noted that there is no very close relationship between type or degree of severity and nuclear size.

Abreviations: adeno-, adenocarcinoma; spher., spheroidal celled cancer; mod., moderate; sev., severe.