How big is a cell?

How big is a cell? Or, how big are any of the parts of a cell as one sees them through a microscope? Scientists today are as far away from the answer to these questions as they were over a century ago because the available technical means are still inadequate to cope with the problem. The questions were asked immediately with the discovery that living systems comprised cells, which were found to be basic units that anyone could see in plants and animals. Everywhere one looked in the plant and animal kingdom were cells of all manner and shape, and in an era that was dominated by the orderliness superposed on the natural world by Newtonian mechanics it seemed natural to want to measure cells.

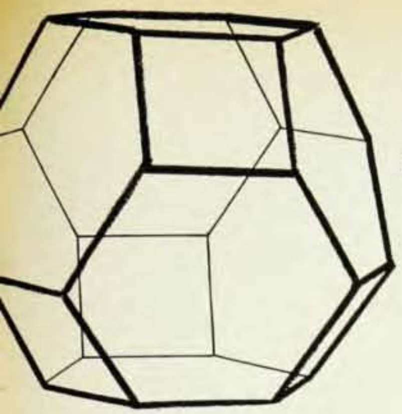
The question was quickly extended to the cancer problem by scientists eager to seize upon any new idea offering hope for its solution. They immediately wanted to know whether human cancer cells might be larger than normal ones, or whether any of the cell parts might be different. The discovery of the cell as the unit of living matter opened completely new vistas of research in cancer and one of the simplest and most obvious of these was to measure cancer cells and compare them with normal cells. We can now look back with the wisdom of hindsight and say, "little did they know what they were getting into", for the technical difficulties in measuring cells in tissues have not been overcome to this day. Yet there is a persistent hope for an answer to the question, "How big is a cell?"—a question which has cropped up recurrently in the long and tedious search for an understanding of malignant growth.

Voices in the Wilderness

Controversy arose from the very moment that the answer to the question "Is the cancer cell or any part of it larger than normal?" was first formulated. From perusing arguments in the literature one might be led to believe that every slice of tissue seen under a microscope was distinctly different from every other slice. At least the cells in each slice seemed to look different to each observer. Flurries of statement and contradiction have persisted across the century to the present times. It was in the late 1840's that the first consistent series of measurements on tissue cells was made by Hermann Lebert. These measurements were first published in Lebert's book in 1851: "Traité Pratique des Maladies Cancereuses et des Affections Curables Confondues avec le Cancer" (Paris, J. B. Baillière).

Lebert seems to have been a scientific genius possessed of great energy. He published numerous handbooks and treatises in German and in French on a wide variety of medical subjects. The above-mentioned treatise on cancer is a remarkable book not only because it was the most important book on cancer before Virchow's epoch-marking works but also because of the author's personal opinions. It reflects the deep despair of his times toward the cancer problem, and also Lebert's impatience with the vague talk about it, as, for example, when he says that the arguments about cancer reminded him of the discourses of the savants of the middle ages who disputed whether angels talked in Greek or in Hebrew. Lebert had measured thousands of cell nuclei and also the nucleoli which are inside the nuclei with a simple eyepiece micrometer on a microscope.

In the late 1830's the German biologists, Schleiden and Schwann, had promulgated their theory that living systems are composed of cells. Lebert picked up the line of thought which is as valid today as it was then: in order to measure the behavior of tissues one should measure the cells constituting the tissues. His conclusions were that cancer cell nuclei were larger than normal, but, more important to us today, he found that the nucleolus inside the nucleus was larger in relation to its nucleus. The latter result has been sporadically confirmed and denied in the subsequent years. The real impetus to Lebert's measurements of cells was his



By Joseph G. Hoffman

Although a knowledge of the dimensions and volumes of living cells is fundamental to a proper understanding of biological processes, science has provided no means for their accurate measurement. Physicists, whose specialty is the measuring of small things, might provide a real contribution, the author suggests, by applying their techniques and skills to the problem.

claim that he could diagnose cancer by this means. Parisian surgeons provided him with plenty of material in the way of human tumor tissues to work on, but the clinical diagnoses were not relied upon for very long.

Virchow was studying cell sizes too, and in 1847 came out emphatically against the idea that the size of a cancer cell or any of its parts was a distinctive feature by which it could be recognized as being cancerous. Thus Lebert's measurements were deemed worthless before they were published in 1851. But even though they were worthless for clinical diagnosis they are considered as being remarkably good because, strictly speaking, his conclusions and his measuring techniques have not been significantly improved upon in all the century since his time.

The fate which befell Lebert's data has been pretty much the fate of all subsequent measurements of sizes of tissue nuclei. To bring the story up to modern times we cite the measurements of cells made by the Scandinavian, Heiberg. His measurements made in the first decade of the 1900's were praised by Dr. Max Borst, an outstanding German cancer authority, at the International Conference on Cancer held in Paris in 1910. Yet similar measurements published by Heiberg in 1931 on the sizes of cells during division brought upon him scathing denunciation. For instance, an American reviewer says among other things: "Like the generations of cancer workers, who have occupied themselves with this question, Heiberg is empty-handed. Indeed, it may be said that there has been no more futile field of cancer research than this one."

Thus the cancer biophysicist approaching the subject of cell size measurement must realize that it is a well-worn subject shrouded in more than a century of despair and frustration, and that there are violent opinions about it. There has been no apparent progress in this type of measurement and much less is there agreement as to what the measurements might mean. Let us examine the record as to what has been measured.

What Have People Been Measuring?

The cells of normal and malignant human tissue offer widely varying appearances when seen under the optical microscope. As geometrical objects for measurement the physicist might well consider them as being very messy. In normal cells there are, broadly speaking, three important parts that stand out as the salient features for the expert and novice alike. These are the cell wall, and within the cell wall the nucleus, and within the nucleus the nucleolus. Sometimes the nucleus seems to fill the entire space within the cell wall leaving little room for cytoplasm. In certain cells, no longer capable of division, there is no nucleus. In other cells one sees two or more nuclei. Similarly in the case of the nucleoli, sometimes there are none visible, while occasionally there are two. But generally it is true that cell, nucleus, and nucleolus follow one another with decreasing size in this order. In the case of malignant tissues the order of size is maintained but the numbers of nuclei and/or nucleoli seen are sometimes bizarre. As many as seventeen nuclei have been counted in such cells. And the sizes of malignant cells and nuclei sometimes are fantastic in magnitude and shape when compared to the normal.

An examination of the voluminous literature on the measurements of tissue cells over the century beginning with and including Lebert's treatise of 1851 reveals a curious phenomenon in scientific thinking. The measurements have dealt exclusively with the nucleus and nucleolus, and not at all with the cell as a whole. The reason for this is not hard to find, for it turns out that the nucleus and nu-

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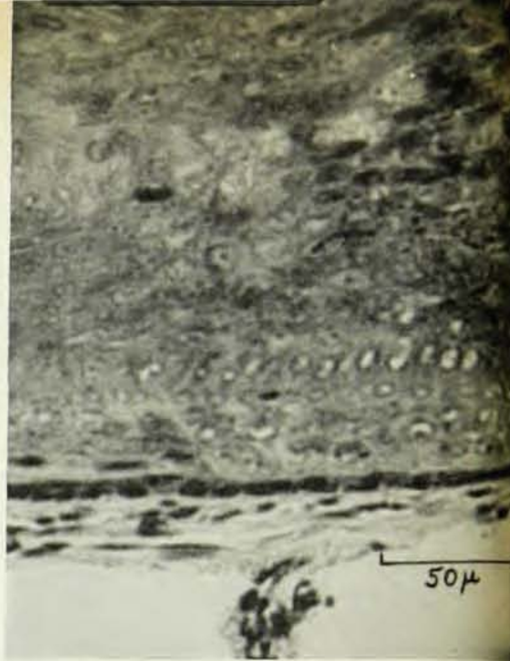
cleolus are easily measurable because they are seen as elliptical or sometimes round shapes. The nuclei, moreover, stain readily to give a good contrast against the cytoplasmic medium in which they are set; the nucleolus likewise has strong staining properties that help it stand out clearly.

Not so, however, for the cell wall. There have been no measurements of tissue cells if one considers such cells as being defined by their walls. The reason for this is that cell walls have complex geometries having eleven, twelve, or fourteen sides, or mere lobulations. But more important is the fact that cell walls do not stain strongly and with a consistency which is conducive to elaborate measurements. The delineation of cell walls is poor, so much so that an inexperienced observer has difficulty in telling where the walls might be, even though the nuclei stand out clearly and sharply. On the other hand, it must be noted that if the walls were stained too heavily they would obscure vision through the cell, which happens to cause one of the difficulties in measuring the densely staining nuclei. The fundamental problem in measuring cell walls lies in staining them by some means or other in order to make them clearly discernible and at the same time have them sufficiently transparent to permit one to see the three-dimensional structure by looking through the cell. Requirements for solving this problem have not yet been met, and even when they are met the biophysicist is still a long way from being able to achieve a mensuration of the complex volume of the cell.

The data one finds in the literature have been acquired as follows. On looking down a microscope tube the observer sees a cross section of a nucleus. This cross section is in a plane at right angles to the direction of vision. The only measurements that can be made are the dimensions of the cross section seen. Since nuclei are generally ellipsoidal the cross section is usually an elliptically shaped affair which is far from being a regular ellipse. But one measures the largest and smallest diameters. If the idea of applying the formula for the area of a regular ellipse to these highly irregular ellipses is too repulsive, one can resort to the device of following Epantchin by making a graph in which the abscissae are the longest diameters and the ordinates are the shortest diameters. This gives a graph which tells something about the diameters of cross sections of nuclei seen and can be constructed for normal and malignant cells. It does not involve directly the volumes of the nuclei.

The difficulty of dealing with ellipsoidal nuclei can be circumvented by choosing to measure "only

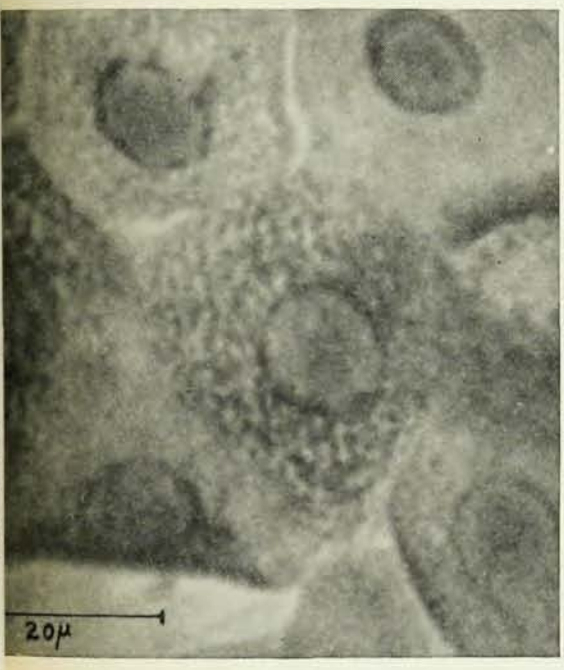


tissues which have round nuclei". This has been attempted by Ehrich and collaborators on an extensive scale. The theory is that if nuclei are spheres, their volumes are proportional to the cube of the diameters which one can measure. In practice the theory suffers violent misapplication because nuclei are rarely perfectly round. The less round cells must be disregarded completely; and if they are not disregarded, a considerable error in the computed volume results. The published data probably include many cells which are definitely not round. At any rate, in the literature on "tissues having round nuclei" the question of non-roundness is left undiscussed. On the basis of nuclear volumes computed from the cube of the diameters Ehrich states that certain forms of malignancy can be diagnosed because the distribution curve of volumes is different from the normal tissue case.

The most refined measurements on nuclear volumes were published by Cowdry and co-workers in 1940. These data give what is probably the closest approximation to nuclear volumes ever attained. The method of computing volumes is of interest because the formula relating the volume of the prolate spheroid of the nucleus to the measurable items, cross-sectional area and the smaller diameter, were provided by two physicists, Drs. Rojanski and Scott. This seems to have been the first and only instance in which physicists have been involved in the mensuration of cells or of cell parts. Cowdry and coworkers measured carefully the area of cross section of the elliptical nuclei. This, along with the smaller diameter, provided the measure of the volume of the nucleus according to the Rojanski-Scott formula. The result is a kind of average volume to

At left is shown section through a hair follicle.

The dark granulated cells (below) are immediate precursors of the cornified cells found in the outer surface of epidermis. These cells are thin polygonal sheets.



which no accuracy can be assigned because there are no means for estimating the actual volumes. Thus, although the Cowdry data are the best available they still must be considered to provide less than a first order approximation for nuclear volumes.

The difficulties in measuring cell nuclei are encountered to the same degree in the measurement of nucleoli with the added feature that nucleoli frequently have diameters of about one micron. This small diameter begins to entail considerations of the resolving power of the microscope, which can be charitably assumed to be as small as two-tenths of a micron. But in spite of the errors of measurement the nucleolus has played an important role in the literature on cell measurements. The absolute size of the nucleolus has not provided a consistent criterion for the distinction between normal and malignant cells any more than has the size of the nucleus. But the *ratio* of volumes of nucleus and nucleolus has provided a number which is smaller for cancer cells than it is for normal cells. A qualitative statement summarizing the consensus of opinion would be that the nucleolus is relatively much larger in malignant cells. But this is not a unanimously accepted opinion among cancer researchers. Curiously enough, however, it was one of the conclusions arrived at by Lebert in the first measurements ever made over a century ago.

Before concluding this brief sketch of what people have been measuring it must be noted that the tissues measured have all been subjected to a chemical fixing process, followed by various chemical treatments which accompany staining procedures. Moreover, the nucleus and nucleolus have been stained. Some workers claim that the dimensions of these cellular parts are not affected by the drastic chemical treatments, and have thus dimensions not different from the untreated specimens. In view of the basic difficulties of measurement indicated above one must leave this as an open question. Perhaps a clarification of the problem can be found by the use of the ultra-violet or the phase contrast microscope in examining fresh tissues. The point in question is the degree of accuracy required in computing volumes from measured radii as in the case of spheres. The radius is not a sensitive measure of the volume of a spherical nucleus. If, for example, a nucleus has a volume of 1000 cubic microns and shrinks 20% to a volume of 800 cubic microns, the radius has changed from 6.22 to 5.78 microns, or about 7 percent. The same argument applies to ellipsoidal figures. Such accuracy in the measurement of radii in the optical microscope might be obtainable but only in case the nuclei are perfect spheres.

Looking Down a Tube

The foregoing account indicates that measurements have been made only on those parts of cells which are readily discernible, namely the nucleus and the nucleolus. One can detect in the discussions in the literature a kind of rationalization by which the data on nuclei are interpreted as applying to the cell in its entirety. Since a cell is governed by its nucleus it seems natural to use the two words interchangeably. For example, Ehrich in presenting his elaborate series of measurements on round nuclei claims to have varified Heidenhain's law of the growth of cells. He uses the terms cell and nucleus as freely interchangeable throughout his discussion although there are no measurements of cell walls.

It might seem to a physicist in the year 1951 that the measurement of cell size should be a relatively simple matter, especially since scientists have been looking at cells in the light of Schleiden and Schwann's theory for well over a century. Part of the trouble is that the optical microscope by itself is woefully inadequate as an instrument with which to measure the three dimensions of a single cell. One can easily see two dimensions in a single plane, the plane of focus. One can see "from side to side". And by means of a camera lucida device one can make a faithful drawing of this cross section. The fundamental catch comes in trying to get the third dimension, "the up and down distances", as one looks down into the microscope. Qualitative pictures of entire cells in tissue masses have been sketched for many years, and especially since the development of the binocular microscope which gives the observer a stereoscopic view. But there yet remains to be devised a means for showing the quantitative dimensions of a cell which is accurate to one-half of a micron. Looking down a microscope is literally like looking down a tube and a narrow tube at that. The observer can see the object of study, in this case the cell, from one direction only; he cannot get around it to see how it looks from at least two sides. He is thus something like a horse with side blinders on. But it would ill become us to be merely critical, for to do so would only add to the shouting that has prevailed over this problem in the past century .

Some Ideas and the Messy Details

The matter of getting around a cell to see it from at least two sides might be solved by cutting tissues into thin strips about fifty microns thick and one hundred microns wide. This strip could be rotated about its long axis under the microscope and the uncut cells measured from different angles. Tissue, however, is soft and friable, hence there might be trouble in trying to rotate such a strip. Another idea might be to cut serial sections of a tissue in one micron sections. The series would have to be copied on paper by camera lucida and the cells reconstructed from the cross sectional slices seen. Here one has a tedious job, and the distortion due to the multiple slicing might require special care. There is also the not inconsiderable difficulty of making sure that each slice in the series is of a known thickness. Instead of slicing a tissue into a series of sections there is the alternative of making a series of optical sections by means of the ultraviolet microscope as has been suggested by Lucas. In this method the focal plane is moved up and down through the cell and a series of microphotographs taken from which the cell is reconstructed on a macroscopic scale. Here there is difficulty in getting well-defined photomicrographs because of large variations in refractive index. This difficulty is readily seen when one tries to do such a series of optical sections with an oil immersion lens on a standard optical microscope: at boundaries across which the refractive index changes there is a pronounced distortion of the image.

A very promising possibility may be the use of microstereogrammetry. In this method there are two main alternatives. The first is to make a stereoscopic pair of photomicrographs and, just as had been done in the measurement of the depth of the craters of the moon, proceed to measure the relative positions of the corners and boundaries of the cell. Lightly stained cells are sufficiently transparent to permit visualization of the edges. Here again the cell is reconstructed and its volume computed. The other stereogrammetric method is to use the principle of the camera plastic, as it was known before World War I. Since that time it has become known as multiplex aerophotography because it was extensively developed in military aviation mapping of terrain. In this procedure two stereoscopic images from a binocular microscope are projected on a screen and made to superpose one on another. Since the images are real and stereoscopic there is reconstructed in space a real image of the cell. The relative positions of the parts of the cells are found by moving the screen back and forth through the spatial image of the cell. In this case too, the cell has to be reconstructed in a model and its volume computed. In an entirely new and unexplored direction is the method of photography by reconstructed wave fronts as first described by Gabor. In his first ex-

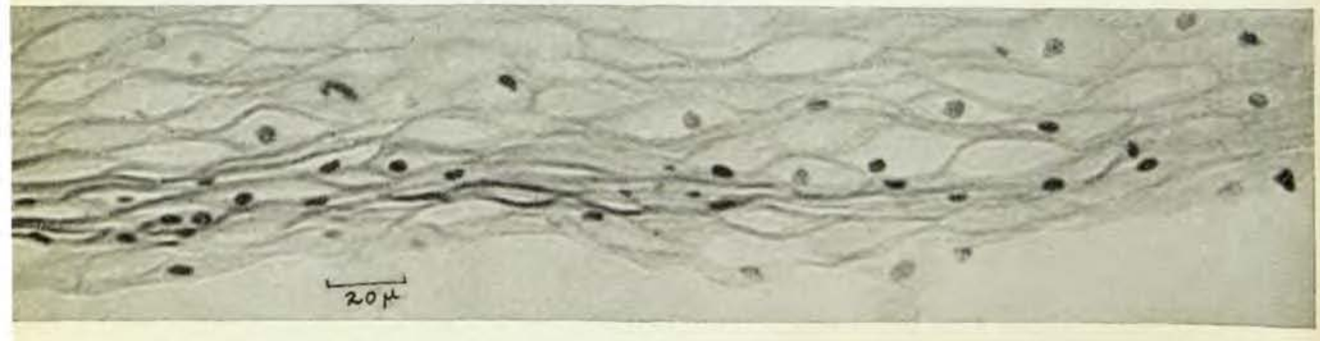


Plate-like cells from uterine cervical epithelium.

perimental results he was impressed by the great depth of focus attainable in his photographs which he called holograms. This depth of focus may offer another possible means for getting at the third dimension of cells.

These are speculations about the possible approaches which could be considered in handling this difficult technical problem. They are not inspiring approaches, but rather seem tedious and fraught with possibilities for error.

Why Measure Cell Sizes?

From the first times that cells were known to be the units of living systems there has been an urge to measure them. The many diverse functions performed in tissues are carried out by the cells. There is an intellectual or even aesthetic sense of satisfaction in being able to achieve a precise measure of these basic units of living matter. In Lebert's time the motive underlying the measurements was to exorcise the scourge of cancer, and this has remained the motive to this day. But as has happened so many times in cancer research the means have not been adequate. There is in the modern repertoire of physical techniques no method by which it can be said that cell parts can be measured accurately; but so many histologists have repeatedly asserted that the cells in cancerous tissue are different that one cannot doubt that they are measurably different from normal cells. Thus it would be worthwhile from the practical angle of cancer research for the biophysicist to perfect a means for measuring cell parts.

The reasons for perfecting such a means have a broader basis in cancer research than the mere characterization of cancer cells as being bigger or smaller. Up to now this has been the sole interpretation placed on cell measurements. The broader basis lies in the measurement of growth of cells both in the normal and malignant states. In the process of carrying out their normal functions cells undergo changes in shape and in size. In many tissues cells are produced and sloughed away or destroyed. For example, in the formation of hair the cells in the hair bulb undergo drastic changes in shape from the time at which they are well defined cells in the bulb until they are long, thin, inert fibers in the hair shaft. Another example is found in the human epidermis, which is never more than two-tenths of a millimeter thick. Cells are produced in this layer of tissue and made to die in order to form the horny outer layer of cells—the cornified cells which are sloughed away by mechanical rubbing. These cells undergo a remarkable series of shape changes before ending up as cornified cells. Such instances of normal tissues are examples in which cells undergo a sequence of differentiation. On the other hand, in tumors such as the experimental tumors which are transplantable in mice, there is no demonstrable sequence of changes in the rapidly growing state. The cells stay at the site of inoculation and, as a consequence of binary fission, their number increases exponentially with time. Here the frequency distribution of volumes would provide a new method for the measurement of growth rate. It would provide a method for measuring the growth rates of the individual cells. The volumes of the whole tumors have been measured for growth, but such tumor measurements give no information about the growth of individual cells unless one assumes that all the cells which are present grow, and do so by binary fission. The frequency distribution of volumes of the cells would give a direct measure of the length of time during which a cell has a given volume.

Another basic reason for measuring cell parts is that cells reflect the activities of the molecular system which comprises the genes. It may be that some day in the future the electron microscopist will be able to measure chromosomal growth. According to present day notions the fundamental measure of growth is the rate at which the gene system is reduplicating itself in preparation for the next binary fission of the cell. But this reduplication is occurring at a macromolecular level and it may not be measurable for some time to come. In the meantime a good approximation to this basic *rate* of growth can be had in the measurement of cell parts. It may be that the measure of cell parts has some advantage over the measure of gene molecules because in many normal cells the gene system is deliberately destroyed or is being destroyed. Examples of this are found in the granular cells of human epidermis or in the cells at the neck of the hair shaft just above the bulb. In these instances, among others, the gene system is gone, leaving a highly specialized cell which is no longer capable of reproducing itself and which carries out certain special predestined functions.

Thus there are both very practical and philosophic reasons for wanting to measure cell parts. The wonders of modern science do not yet provide an obvious means for making the measurements. But physicists are known as a group to be specialists in measuring small things. They should be urged to take on the prefix "bio" and become biophysicists willing to meet the challenge of this problem.