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Letter to the Editor

Does the cell number 10^9 still really fit one gram of tumor tissue?

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A tumor reaching the size of 1 cm^3 (approximately 1 g wet weight) is commonly assumed to contain 1 x 10^9 cells. This paper comments on the probable origin of this "magic" number and on some possible reasons why it has remained in use until now. However, mostly in epithelial tumors (85% of all human tumors) a cell number one order of magnitude smaller would be more realistic.

In the mid-1970s it was claimed that when solid human tumors are large enough to be clinically detectable (approximately 1 cm^3, or 1 g wet weight) they contain 10^9 cells. This assumption is still taken for granted, and perhaps even considered obvious. The present study started off several years ago from discussions with M.D. and Ph.D. students on the metastatic inefficiency of neoplastic cells, delaying tumor spread through the body. It rapidly became apparent that for a deeper understanding of the pathophysiological problems of neoplastic diseases, the number of tumor cells forming a neoplastic mass of a given size (say, 1 cm^3) had to be quantified more precisely. Having focused on cell size and on advances in medical imaging, it appeared that statements on the putative tumor cell number per unit of volume might be misleading, and sometimes conceptually erratic. A few recent publications[1-3] offered an opportunity to re-examine this point in some detail. The working method used to connect into new concepts separate data (collected from literature or retrieved from personal records) and to provide a new conclusion, was based on principles very similar to those recently referred to as "conceptual" research.[3] The inanimate model devised to estimate numerical differences (per unit of tissue volume) between round and cubic cells derives from information on sphere packings in three dimensions.

As far as I know, the figure of 10^9 cells/cm^3 came to light the first time in a review paper in which the L1210 tumor of the mouse, growing exponentially up to the lethal volume of 10^9 cells (1 cm^3), was examined as a model system to quantify the effects of chemotherapy.[4] The paper claimed that "the average 1-cm^3 tumor nodule in a patient must certainly contain approximately 10^9 cells". This statement was widely accepted and, as far as I know, has never really been questioned. In fact, even the latest editions of textbooks of cell biology and pathology still report it as such.

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10^9 cells/cm^3 matches quite well with tumors containing limited amounts of stroma, and whose cells are small, like transformed fibroblasts.[5] However, such a high number is not consistent with histological pictures showing that the cells of many tumors are much larger than normal cells visible in the same microscopic field (exemplified in Fig. 1).

The diameters of most animal cells range from 10 to 30 μm. If we assume 4 x 10^{-9} cm^3 as the average mammalian cell volume[6] (corresponding to a cube of about 16 μm/side), one cm^3 offers enough space to accommodate not more than 2.5 x 10^8 cells. The diameters of normal parenchymal cells and of normal cell nuclei, measured with callipers in textbook illustrations, were in the range of about 15–25 μm and 5–10 μm respectively. However, nuclei of displastic and cancerous epithelial cells measured in vivo with modern techniques, have larger diameters than normal (up to 20 μm),[7] implying substantially larger cell diameters. Table 1 summarizes the volumes calculated for polyhedric cells (schematised as cubes of increasing size, from 10 to 30 μm/side), and indicates the maximum number of cells of one size or another that will have room in 1 cm^3. However, besides cell shape and size (see later), quantitative analyses must take into account differences in the volume of extracellular structures and spaces (ground substance, fibers, vessels). In desmoplastic tumors even 90% of the tumor mass can be accounted for by stroma.[8] Therefore, tumor cellularity might be as low as 10^7/cm^3.

In ascites tumors, the extracellular space is the ascitic plasma. In Yoshida ascites hepatoma AH130, an anaplastic rat tumor of epithelial origin, tumor cell density at the plateau level is 6 x 10^7/ml (recently calculated by others[2] from two experimental papers I published in the past). I retrieved from my records on this tumor an average cell volume of 5,600 μm^3 (range: 4,800–6,200), calculated by combining with cell counts the volume of the pellet obtained by low-speed centrifugation of the ascites tumor. By applying a correction to these values for the void volume within the pellet (about 20%), hepatoma cells and diploid hepatocytes appear to have similar volumes, nearly half of the average volume (10,600 μm^3)[9] of polyploid hepatocytes. Similar cell volumes can be calculated from published figures of electron microscopy. Figure 1 documents that the tumor cell diameters largely exceed the diameters of leucocytes (A) and erythrocytes (B). In the same figure a primary rat hepatoma induced by feeding ethionine is compared with the surrounding liver (C) and with normal liver (D).

Structural differences between epithelial tumors, in which the bulk is represented by rather large polyhedric cells (shown as cubes without intercellular substance), and tumors in which small, spherical cells prevail (simulated here by sphere packings) are important. Gross comparisons of these two different groups of tumors must consider at least the ratio of the volume of the sphere to the cube (0.52359), and the void space within sphere packings. This space varies from 25.95% for hard spheres tightly packed in a face-centered cubic (fcc) lattice to 40–45% for calibrated hard spheres randomly packed in ample or narrow spaces (unpublished experiments). However, in living tissues cell deformation and variability of extracellular spaces modify the density of cell packings. Furthermore, any tumor contains, in variable proportions, macrophages, lymphoid cells, etc. Thus, any tabulation of definite tumor cell numbers per unit of tumor tissue volume is likely to be misleading unless all these limitations are kept in mind. However, from the above results and observations (volumetric data exemplified in Table 1 and in Figure 1, coupled with the various cell measurements mentioned above), it can be inferred that for tumors epithelial in origin (85% of all human tumors), 10^8 cells/cm^3 is a more suitable reference than 10^9.

How can we explain why 10^9 is still accepted and used as a magic number? First, it is common knowledge that 1 cm^3 of human blood contains as many as five billion erythrocytes and that blood cells, including leucocytes, account for less than half of the blood volume. Second, as long as small cells are the prevailing component of tumors in which the extracellular volume is scarce, 10^9 cells/cm^3 is reliable. Third, the literature provides scant detail, barely sufficient for thorough analysis. Thus, unless one goes deep into details, everybody still can

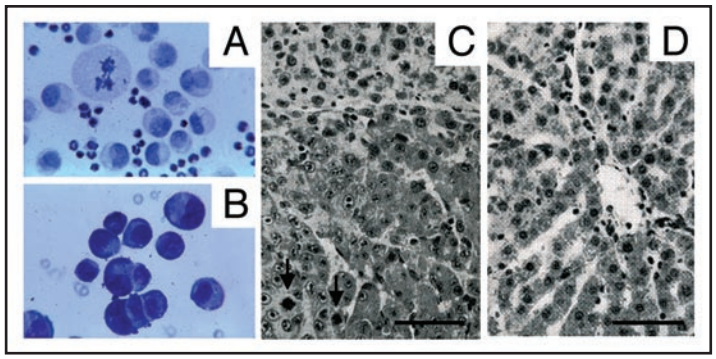

Figure 1. Rat hepatomas compared for cell size with blood cells and with normal liver. Allowance should be made for cell deformations due to techniques and histological reagents. Smears of ascites hepatoma AH130 (A and B) show that the variability in size and shape of the tumor cells largely exceeds the size of rat blood cells. Leucocytes are numerous the day of tumor transplantation (A); however, when tumor growth attains the plateau level, there are only a few erythrocytes (B). HE-stained histological sections show: in (C) a primary ethionine-induced hepatoma and, at the top of the figure, the surrounding liver; the tumor cells are large, closely packed and pleomorphic, and mitotic activity can be seen (arrows). Normal rat liver is shown in (D). Bars: 100 μm.

take it for granted that palpable human tumors contain around 1 x 10^9 tumor cells.

Before concluding, let us consider that the concept of metastatic inefficiency—if nothing else—is based in part on the size of the tumor and on the number of tumor cells released into the circulation, and that very small tumors may not have enough tumor cells to shad and produce successful metastases.

Summing up: "The past decade has seen significant advances in medical imaging"[1] to the extent that we can now detect tumors that are still very small. The evidence summarized here indicates that in most cases conventional reference numbers ten times smaller than 10^9 tumor cells/cm^3 provide everybody with more realistic information, most importantly students and young oncologists who may still have "limited knowledge of the integrative pathophysiology of tumors".[10]

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Table 1 **Side, volume and maximum number of cells virtually cubic in shape, present in 1 cm^3 tumors (clinically detectable)***

Side (μm)	Cubic cells Volume (μm^3)	Number/cm^3
10	1,000	1 x 10^9
16	4,096	2.44 x 10^8
22	10,648	9.39 x 10^7
30	27,000	3.70 x 10^7

*Assuming that "all cells remain cycling and no cells are lost", the increase of cell side from 10 μm to 30 μm theoretically requires 25 instead of 30 population doublings to form a tumor clinically detectable.