The Fine Cytology of the Walker Tumor*

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SUMMARY

An electron microscope study of thin sections of the Walker ascites tumor reveals that the cells possess the essential organelles found in most cells, such as nucleus, mitochondria, etc.; but they differ from most normally differentiated cells in two respects: First, the dense cytoplasmic particles usually associated with protein synthesis are mainly free and not connected to an intracellular membrane reticulum. Second, the highly convoluted cell surfaces with numerous projecting villi, together with the frequent intercellular gaps, are consistent with the loss of mutual adhesiveness characteristic of many other types of tumor cells.

The possible significance of these features is discussed.

No virus-like particles, such as those found in Ehrlich ascites tumor cells, were noted.

The Walker tumor 256 is widely used as a test object in the screening of compounds for carcinostatic properties. It is therefore desirable to have a detailed description of its typical cytology to form a background against which to assess the nature of changes produced by chemical agents. It is also a convenient material for an electron microscope study of a well-characterized anaplastic tumor concerning which a great deal of ancillary information has been accumulated.

The original tumor was found by Dr. George Walker in 1928, and its early history has been recorded by Earle (8). The tumor apparently originated in the breast of a rat and was diagnosed as a carcinoma, with a typical adenocarcinomatous structure. Since 1945 the tumor has been carried in Chester Beatty rats, being transplanted approximately every 7 days. For the production of the ascites tumor, animals are given inoculations intraperitoneally of a suspension of cells derived from suspended solid tumor. It may form compact masses, small islands attached to the mesenteries, or assume an ascitic form either as small islands or as single cells. To facilitate the problem of fixation and processing, we have used small mesenteric nodules and a suspension of ascites cells in these experiments. The results indicate that the cells are identical in the various forms.

No detailed account has been previously given of the fine cytology of this tumor, although numerous similar tumors have been examined and other ascitic tumors have proved favored objects for electron microscopy (7, 10, 14, 20, 26, 31, 32). In this Institute, Mr. M. S. C. Birbeck has examined a “solid” subcutaneous specimen of this tumor, and we are grateful for the opportunity to study his micrographs.

MATERIALS AND METHODS

Rats bearing tumors inoculated 5 days previously were killed by breaking their necks, and the abdomen was opened. Drops (0.5 ml.) of the cell-bearing ascitic fluid were added without delay to 10 ml. of the standard buffered (pH 7.3) solution of osmium tetroxide (1 per cent) and allowed to fix for 2 hours. During this time the cell suspension usually clumped, and thus subsequent handling was facilitated. For the solid form small islands (0.1–0.5 cu. mm.) of cells attached to the mesenteries were cut off and dropped immediately into the fixative.

After fixation (2 hr.) the osmium solution was poured off, and the cellular material was washed

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rapidly with several changes of tap water (5–10 min.) and then dehydrated in an ethanol sequence of increasing strength. The specimen was left in absolute alcohol for 1 hour and then transferred to xylene.

The embedding material was the “Araldite” mixture, described by Glaue et al. (12), which in this laboratory has proved an excellent medium for mounting biological material (4). After 1 hour in xylene the specimens were transferred first to a mixture of xylene and the “Araldite” (50/50) monomer and finally to pure “Araldite.” The resin was hardened by heating for 48 hours at 60°C.

Sections for light microscopy and electron microscopy were cut by means of a modified Cambridge microtome (16) and were mounted in the conventional way on copper grids covered with a film of Formvar. The sections were examined and photographed in a Siemens Elmiskop 1.

RESULTS

The cytoplasm.—The cytoplasm was not densely populated with inclusions and, as was to be expected in such an anaplastic cell, was devoid of specialized differentiated objects, such as granules or fibrils. Mitochondria (m, Figs. 1–3) were small, often elongate, and sparsely distributed, with no preferred sites of concentration. Their internal structure of membranes and cristae usually appeared normal, but abnormal forms (m', Fig. 4) were not uncommon.

There were only very few small vesicles (R, Figs. 1–4), either flattened or rounded, whose surfaces were covered with small, dense particles and which could be regarded as representative of the “endoplasmic reticulum,” the typical organelle of the differentiated cell engaged in protein secretion. On the other hand, the entire cytoplasmic volume appeared rich with the small dense particles themselves, which appeared either single or associated in small clusters (P, Figs. 1–3).

Clusters of small vesicles enclosed by smooth membranes were to be seen in various regions of the cells. These, when associated in considerable numbers, may form the Golgi clusters recognizable by light microscopy. They were particularly common in the nuclear indentations when these were observed. A distinct type of inclusion consisting of a single smooth membrane enclosing a number of smaller vacuoles (S, Figs. 2, 4) was often noted. This type of object has been recognized in a number of cells but has not yet been named or associated with a function. Occasional vesicles containing the characteristic pattern of concentric circles given by phospholipides (29) were also seen (L, Fig. 4). Other vacuoles enclosing ill-defined amorphous material were noted but not identified (A, Fig. 1). Fat droplets, identified by their density and crenated edges (F, Figs. 2, 3), were occasionally present.

Except for an occasional doubtful instance, no small vesicles containing dense bodies (often described as “virus particles”) were found. These are particularly common in Ehrlich ascites cells (26) and in numbers of other tumors (2) and would have been readily recognized in our preparations had they been common.

The plasma membrane and intercellular contacts.—The plasma membrane appeared as a dense line (<100 A thick), which, both in free cells and in cells clustered to form islands, was extremely convoluted (M, Figs. 1–4). This irregularity of the cell surface was in fact the most striking characteristic of the material examined. Two types of surface protrusion may be distinguished: the first, the less regular, consisted of large-scale convolutions (or sections of pseudopods) which appeared to have no particular size or structural peculiarity (W, Fig. 2). The second may be more accurately described as a type of villus, since it is an elongated cylindrical protrusion of rather regular diameter (~0.1 μ) (V, Figs. 1–3), which recalls the more regular villi which characterize the surface of many differentiated cells (11). The protrusions are of variable length, unlike those found on the normal cells, which often have well defined lengths.

As a result of the irregularity of the surface, intercellular gaps (G, Fig. 2) are common even in cell clusters in which the cells are obviously held together. Usually, however, in cell clusters it was possible to find patches of cell surfaces where the two plasma membranes ran parallel.
DISCUSSION

We wish to comment principally on two aspects of the cytology of these cells, both of which are in confirmation of the earlier impressions gained from light microscopy: the generalized character of the cells and the evidence of unusual surface activity associated with a lack of intercellular adhesion.

Normally differentiated adult cells are characterized not only by a typical pattern of essential cell organelles, nucleus, mitochondria, Golgi membranes, etc., but also by the presence of the products of the activity of the cells or by certain structural developments associated with their function. These may be recognized in some detail by the electron microscope as fibrils, secretion granules, arrays of membranes, etc. Certain cells, such as embryonic cells, or in the adult various germinal (or stem) cells, lack these differentia. The Walker ascites cell, which is devoid of differentiated inclusions, clearly belongs to this group. Characteristic of these undifferentiated cells, still capable of further division, is the population of small dense free particles (macromolecules, diameter 150-200 A) in their cytoplasm (23, 28). These particles have been shown, by Palade (21) and Palade and Siekert (22) in particular, to contain ribonucleic acid and protein and to be largely responsible for the basophilia of the cytoplasm. In cells which secrete protein these particles are largely attached to membranes which may be elaborately developed and form a well defined organelle, variously called the endoplasmic reticulum (24), the ergastoplasm (13), or clusters of α-cytomembranes (27). It seems well established (22) that this system of membranes and particles is associated with the synthesis and collection of the proteins secreted by these cells.

In the generalized cells, in contrast, the particles are free (or loosely held in small clusters), and there is only a sparse development of the system of particle-covered membrane (23, 3 and 28). Thus, it seems that the membrane system is required for the organized collection of the products of the cell when these are destined to leave the cell. In the embryonic cell proteins are probably required for “home use,” and in them the basophilic reticulum may appear as the cells differentiate (28). Other cells, such as muscle cells and keratinizing cells (3 and 17, 23), allow their proteins to accumulate in the cytoplasm where they form substantial fibrous masses not enclosed in membranes.

The anaplastic tumors, of which the Walker is typical, display this pattern of free particles and relative absence of membrane systems. The particles, which may be isolated as microsomes, are capable of protein synthesis (15), but clearly, since no substantial amounts of this collect, the proteins are those required to maintain the cell and to provide materials for future cell division.

The nucleus, often abnormally large and irregularly shaped, with its large nucleolus, is not in itself typical of tumor cells, since such appearances are also met with in embryonic mammalian material and in invertebrates—although the rarer, more bizarre, forms are found only in tumors—and probably reflects conditions of rapid growth and synthesis whatever the purpose.

Surface activity and intercellular contact.—That anaplastic tumor cells and perhaps tumor cells in general are poorly adhesive is an impression gained from the fact of tumor cell spread and the relative ease of dispersion of tumor tissue, and is apparently supported by direct measurement of intercellular adhesion (6). Observation of certain types of tumor cell in the light microscope shows that the surfaces of these cells may display a persistent activity shown by pseudopod formation, by “frills,” and by a movement of the entire cell which is not checked by contact with other cells. For a review of this phenomenon see Abercrombie (1) and Weiss (30).

Electron microscope images, which corroborate the existence of this surface activity by revealing in sections profiles of a highly convoluted surface, are to be noted in numerous published electron micrographs, but the authors, although they may mention the observation, have not emphasized it. However, our observations described above, and others made on other tumors in this Institute (5), reveal this irregularity of the surface as being a very common property of tumor cells. The irregular surface contours and the frequency of projecting villi are in themselves evidence that the cells are poorly adhesive. Intercellular gaps are the rule, and, in certain situations, one gains the impression that the villi themselves are actively preventing the surfaces from approaching.

From the now extensive literature on the fine structure of cell contact we may summarize the structural characteristics as follows: (a) cells are bounded by a dense plasma membrane < 100 A in width1 which, when the cells are in adhesive contact, is seen over a large part of the surface.

1 Concerning the fine structure of this membrane, see Robertson (25) and Mercer (18). It need not concern us here.
of contact to be parallel to the membrane of the second cell but separated from it by a lighter layer of the order of 150–200 Å thick. The rather constant thickness of this intercellular space suggests that it is occupied by a layer of material of low intrinsic scattering power for electrons. After the specimen is stained with phosphotungstic acid or lead hydroxide, this material appears more dense than the adjacent cell cytoplasm. This intercellular layer, which contains protein (9), could constitute an intercellular cement.

b) In addition to this layer of adhesive material there may appear in most cellular tissues areas of localized enhancement of contact which may range from areas of a simple increased density of the two opposed membranes to elaborate organelles in which many dense layers of material both in intercellular space and in the cytoplasmic space behind the areas of contact participate (11). These latter and more elaborate organelles are referred to as desmosomes.

Over the greater part of the surfaces of the Walker tumor cells these two structural signs of adhesive contact are missing. We have never noted a desmosome. However, in the solid islands limited areas of contact of the simple type are to be seen (C, Fig. 3). These are not extensive, and they may be temporary; but they appear sufficient to maintain the cell colony. A very similar situation has been noted by one of us (E. H. M.) in a number of embryonic tissues, but embryonic cells entering into the formation of cellular tissues rapidly develop desmosomes suggestive of the emergence of a more permanent attachment.

This surface activity and poor adhesion should not be regarded as definitive of the tumor cell, since there are tumors containing well differentiated regions in which the cell contacts resemble those of a normal epithelium (5), and, conversely, in mesenchymal tissues there exist vast populations of nonadhesive, persistently active cells, e.g., lymphocytes, macrophages, and fibroblasts, which form no permanent contacts. Rather, it would seem more correct to say that "free living" cells, produced either as end-products of a special line of differentiation or as a result of a loss of the property of adhesion in becoming cancerous, exhibit a persistent surface activity, simply because the cell membranes are free. This activity is suppressed temporarily or permanently when they are able to associate themselves with another cell surface.

It is perhaps desirable to emphasize that we have noted no particles in the Walker cells which could be described as virus. Such particles have been frequently described and are common in the Ehrlich ascites cell (26), which, in other respects, much resembles the Walker cell. Some authors have supposed that the surface activity is associated with the transfer of virus particles from one cell to another, as is indeed convincingly illustrated in the recent paper by Moore et al. (19). Our cells exhibit much the same surface activity and in particular the formation of long thin villi, but we have not found evidence of particle transfer and must conclude that the surface activity exists in the absence of virus particles.

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Fig. 2.—Section through a Walker tumor mesenteric nodule showing the cell membrane $M$, gaps ($G$) between the cells, numerous projecting microvilli ($V$), and occasional irregular pseudopods ($W$). Part of a nucleus ($N$), mitochondria ($m$), vesicles ($R$), covered with dense particles, dense particulate material ($P$), and fat droplets ($F$), may be seen, and also a vacuole ($S$) enclosing a number of smaller vacuoles.
FIG. 3.—Section through a Walker tumor mesenteric nodule showing close cell contacts (C). Part of a nucleus (N), particle-covered vesicles (R), a fat droplet (F), mitochondria (m), dense particulate material (P), projecting microvilli (V), and the cell membrane (M) may be seen.
Fig. 4.—Section through a Walker ascites tumor island showing the cell membrane (M), abnormal forms of mitochondria (m), a vacuole (S) containing numerous smaller vacuoles, particle-covered vesicles (R), and an inclusion (L) showing the pattern of concentric circles characteristic of phospholipides.
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