Mechanism of the Invasiveness of Cancer

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DURING THE LAST SEVERAL YEARS AN attempt has been made in this laboratory to arrive at an explanation of the invasiveness characteristic of malignant tumors. In contrast to benign tumor cells, which remain restricted to their site of origin, malignant cells have the ability to infiltrate adjacent tissues and thus become locally disseminated. Furthermore, their capacity for invasiveness allows them to penetrate into the lumina of lymphatic and blood vessels, whereby they are transported to more distant parts. It was thought that physical and chemical differences must exist between benign and malignant neoplastic cells which permit the former to remain localized and the latter to permeate adjacent normal tissues.

Under the assumption that these differences between the types of cells were ascertainable by experimental methods, a series of investigations was made.[1]

The Decreased Adhesiveness of Cancer Cells

It was demonstrated by the writer (*Cancer Res.*, 1944, **4**, 625) that attached pairs of cancer cells could be separated from each other by micromanipulation, through the application of much less force than was required to separate normal or benign tumor cells. The mutual adhesiveness of the cells was determined by measuring the bend produced in a previously calibrated microneedle when subjected to the strain of detaching one cell from another. Thus, it was found that the mean force required to separate 50 pairs of normal squamous epithelial cells obtained from the lip was 1.42 mg. Similarly the value of adhesiveness for cells from skin papillomata (benign tumors) was 1.25 mg. On the other hand, the mean force necessary to separate 50 pairs of cells from squamous cell cancers of the lip was only one-third this value, or 0.47 mg.

This physical difference between cells of malignant and benign tumors composed of squamous epithelium affords the first requisite for an understanding of invasiveness. It is difficult to visualize any mechanism of invasiveness if tumors are composed of tightly adherent compact masses of cells. However, if the cells are but feebly attached to one another, facilitating complete separation, such separated cells are free to wander into adjacent parts by ameboid movement.

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Attempts were then made to find a chemical explanation of reduced adhesiveness. Normal squamous epithelial cells were subjected to various alterations in the chemical composition of the medium in which they were immersed while their adhesiveness was measured. In this way it was shown that absence of calcium from the medium caused reduction in adhesiveness of the cells (*Cancer Res.*, in press). For example, the mean force required to separate 100 pairs of cells in balanced salt solution was 1.34 mg., whereas the value for cells in calcium-free salt solution was only 0.96 mg., a significantly lower value. In this instance, as stated, calcium was absent from the medium surrounding the cells. In another experiment, methylcholanthrene, a substance shown by C. Carruthers and V. Suntzeff (*Science*, 1944, **99**, 245) to reduce the calcium content of epithelial cells directly, was applied to the cells and their adhesiveness measured. Under these conditions also, adhesiveness was reduced.

Since it has also been shown by A. Brunschwig, L. Dunham, and S. Nichols (*Cancer Res.*, 1946, **6**, 233) that cancerous tissue is abnormally low in calcium, and since the investigations just reviewed indicated that adhesiveness of normal cells was decreased when calcium was lowered experimentally, it was concluded that the decreased adhesiveness of cancer cells of the squamous epithelial variety is dependent upon their low calcium content.

The Ameboid Movement of Cancer Cells

A satisfactory chemical basis for the separation of cancer cells from each other having been found, the only additional requisite for invasiveness is the ability of the detached cells to move; that is, if the cells, no longer bound to each other, are capable of ameboid movement, their penetration of the adjacent tissues is understandable.

In a previous investigation in this series (*Cancer Res.*, 1942, **2**, 618) epithelial cells from human carcinomas were observed in tissue culture. It was found that individual cells frequently became detached from outgrowing sheets or clusters of epithelium and, further, that these detached cells were actively ameboid. Cells were seen to progress, in this way, some distance from the cluster from which they were derived and, by their proliferation, to build up new colonies. These observations confirmed earlier reports by W. H. Lewis and G. O. Gey (*Johns Hopk. Hosp. Bull.*, 1923, **34**, 369) of

ameboid movement in cells from both sarcomas and carcinomas.

Thus, it can be regarded as established that, once cancer cells have become detached, they are capable of ameboid motion.

The Role of Spreading Factors

The detached ameboid malignant cell is physically adapted to invasion of surrounding tissues. However, invasion would be facilitated further if the normal tissues were made more permeable to the cancer cells. This suggested the hypothesis that malignant tumors contain spreading factors, such as hyaluronidase, which, by softening the intercellular cement substance of adjacent normal tissues, render these tissues more susceptible to penetration by the cancer cells (*Amer. J. med. Sci.*, 1946, **211**, 257).

Experiments designed to test this hypothesis were of two kinds. First, an analysis of human tumors was made to determine whether they contained significantly greater amounts of spreading factors than did normal tissues (*Cancer Res.*, in press). Extracts were made from a variety of normal and tumor tissues by grinding, freezing, and thawing the tissue and extracting it with sodium acetate. The acetate extract was used for the assay of spreading factor.

Two methods of assay were employed. By one method, the effect of the tissue extracts upon the spread of hemoglobin in the rabbit skin was determined. The other method depended upon measuring the reduction in viscosity of hyaluronic acid, the substrate upon which the enzyme, hyaluronidase, acts. By these methods it was found that several of the malignant tumors examined did contain spreading factors. In most instances the spreading factor content was not great, and in some it was lacking. When considering the presence of spreading factors in malignant tissues, it must be emphasized that the source of the spreading factors within the tumors has not been determined. It is possible that hyaluronidase was present in certain tumors because of infection by bacteria which were the source of the enzyme. If this is the only source of hyaluronidase in malignant tissue, then its presence is coincidental, even though it conceivably operates to facilitate invasion by the tumor cells by rendering the adjacent tissues more susceptible to penetration. It has yet to be demonstrated that the cancer cell itself contains hyaluronidase. Regardless of the ultimate source of the spreading substance, our analysis of human tumors revealed, in several instances, significant amounts obtainable from the tumors, so that support is lent to the hypothesis that spreading factors may facilitate the invasiveness of cancer cells.

In the second set of investigations, hyaluronidase was injected into transplantable sarcomas in mice, and into virus-induced papillomata in rabbits (*Cancer Res.*, in press). The object of these *in vivo* experiments was to determine whether an excess of hyaluronidase would increase the invasiveness of tumors. The mouse sarcomas were invasive and metastasized to the lungs. It was thought that if hyaluronidase increased the invasiveness of these sarcomas, there would be earlier fixation of the tumors to the adjacent normal tissues and an increased frequency of pulmonary metastases. Neither indication of augmented invasiveness was demonstrable. The rabbit papillomata are primarily benign tumors which may become malignant in some instances when allowed to grow for a long time. It was thought that local injection of hyaluronidase might increase the incidence of malignancy, as judged by invasiveness and distant metastases. Again, confirmative evidence was not obtained. The negative results in these experiments force the conclusion that spreading factors of the hyaluronidase type, though they may be found in malignant tumors, are not essential to invasiveness. The mouse tumors, for instance, were strongly invasive to start with, and their invasiveness apparently could not be enhanced by an excess of hyaluronidase experimentally introduced.

In these *in vivo* experiments, in which spreading factor was injected into the animals daily over long periods, the formation of antienzymes must be considered. Such antienzymes, which inhibit the action of the enzyme on its substrate, have been reported by E. Haas (*J. biol. Chem.*, 1946, **163**, 63, 89, 101), and it will be necessary to await further developments in this field before better-controlled experiments can be designed.

The concept of invasive growth that has resulted from the experiments summarized above depends upon a triad of factors:

(1) Decreased adhesiveness of cancer cells, dependent upon local calcium deficiency. Decreased adhesivensss facilitates the separation of cells from each other so that they become detached units.

(2) Ameboid movement, by which the malignant cells are enabled to wander into the surrounding parts to establish new colonies.

(3) Liberation of spreading factor (hyaluronidase), which acts upon adjacent normal tissues. Hydrolysis of the hyaluronic acid of the intercellular cement substance of connective tissue opens the tissue spaces for penetration by the malignant cells. It is quite possible that this third factor is not requisite for invasive growth, but that when it does operate, it augments the facility with which invasion occurs.

Of the three factors the first two are of greater importance for invasive growth. It is of interest in this regard that the most invasive of all normal cells, macrophages, polymorphonuclear leucocytes, and lymphocytes, are all detached cells, rarely showing any evidence of mutual adhesiveness and all having great ameboid activity. The cancer cell possesses these same attributes, coupled with its capacity for unlimited proliferation.