FACTORS IN THE MECHANISM OF METASTASIS: A REVIEW

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THE phenomena displayed by malignant neoplasms of invading locally and disseminating widely have been observed almost since cancer has been recognized. The routes by which these tumors metastasize to distant sites have been frequently discussed in the medical literature. Also the more or less definite patterns of metastatic lesions in many malignant tumors have been observed and recorded. However, the mechanisms involved in the dissemination of tumors have only recently been studied by modern biochemical, microdissection, tissue culture and histologic technics. It is not possible to consider each type of malignant neoplasm separately, but to our knowledge the fundamental mechanisms to be discussed in this article are applicable to malignant tumors in general.

LOCAL INVASION

There is almost no malignant tumor in which local invasion is not observed to a greater or a lesser extent. In many this property is well developed and in others the local infiltration of tissues is desultory. Coman et el. and others[1-5] in considering this problem proposed that invasive growth depends upon three factors: (1) decreased adhesiveness of cancer cells; (2) ameboid movement; and (3) liberation of a spreading factor.

(1) Decreased Adhesiveness of Cancer Cells. The initial work on the cellular adhesiveness of cancer cells dealt with a comparison between squamous cells from normal lips and those from squamous cell carcinomas of the lip.[1,2] By means of a microdissection technic, the force required to separate cells from one another was gauged and cancer cells were shown to require less than half the force necessary to separate normal ones. During the process of separation normal cells showed distinct tension lines, became elongate, detached with a snap, and finally resumed their normal shape. In malignant squamous cells there was much less distortion and the separation was readily accomplished. Further experiments employing an agitating technic subjected samples of normal and neoplastic cells to varying degrees of violent shaking. The exfoliated single recognizable cells were then counted and in all cases of malignant tumors the cell counts were considerably higher. These experiments have included carcinomas of hollow viscera, cervix and breast. In all of these the results were constant, indicating that decreased adhesiveness is a property of cancer cells.

What is the reason for this decreased adhesiveness of cancer cells? Is it some

alteration in the chemical make-up of the cells? Coman, Brunschwig and others have shown that a decreased calcium content in cancers is a constant finding.[4, 6-8] By means of direct calcium determinations and flame spectophotometer examinations the calcium content of tumors and of normal adjacent tissue were compared. Potassium determinations were also made. In carcinomas of the colon the calcium content averaged 44 per cent less than the normal tissues and the potassium averaged 60 per cent higher. The decreased calcium content was interpreted by Coman to be an expression of invasiveness of cancer cells and a factor in their decreased adhesiveness. The elevated potassium content is more definitely understood. Rat experiments on regenerating liver tissue showed that potassium was elevated in tissues taken from actively growing zones in comparison with that from surrounding normal tissues. This elevated potassium was correlated with the numbers of mitoses present and was found to be directly proportional to the rate of mitosis.[8] Similar observations have been made on human tissues. Therefore, elevated potassium levels are an expression of cellular multiplication.

(2) **Ameboid Movement.** Active ameboid motility has been observed in cultures of both animal and human neoplastic tissue.[9] Many different types of tissues in tissue culture show cells which detach themselves from the central portion of the culture and exhibit ameboid locomotion. In studies on breast cancers this motility was appreciable and averaged 0.7 micra per minute.[9] Renal cell carcinomas, oncocytomas, and leiomyosarcomas all showed similar motility. However, as far as can be determined, ameboid activity of tumor cells in vivo has not been demonstrated although it may well occur. If it does, it would aid in the explanation of the behavior of malignant tumors both as to local invasion and as to the production of metastases.

(3) **Liberation of a Spreading Factor.** The presence of a spreading factor similar to hyaluronidase has been demonstrated in some tumors.[3,10] This substance hydrolyzes the hyaluronic acid of the intercellular cement substance of connective tissue and permits the penetration by malignant cells. In view of the fact that only some tumors produce hyaluronidase or a spreading factor, it cannot be regarded as a requisite for local invasiveness of cancer cells, but neither can it be disputed that the presence of such a factor would greatly facilitate the process of local invasion.

METASTASIS TO DISTANT SITES

The routes by which malignant neoplasms metastasize are well known and may be grouped under three headings: lymphatic, hematogenous and transcoelomic. It is axiomatic that tumor emboli gain access to lymphatics, to blood channels, or are cast free in any of the serous cavities by means of local invasion. However, certain purely physical factors aid emboli in gaining access to these structures. Studies by Young and Griffith[11] indicated that all tissue including neoplastic exemplify a differential pressure system in that they consist of vascular tubes invested by a semi-solid medium. Experiments showed that

emboli or other bodies cannot enter collapsible tubes or lymphatic or blood channels as long as the pressure within the channel is greater than the pressure surrounding it. Thus anything which increases the tissue or surrounding pressure favors embolization. The erosion and subsequent rupture of a vascular channel within a tumor may produce such circumstances. Active growth of a neoplasm confined to a more or less limited space will increase tissue pressure and provide the added physical factor. Manometric studies have confirmed this and have also shown that injection of saline or other fluid and digital compression increase tissue pressure.[12] From these facts it can be concluded that a malignant tumor in itself provides the locally invasive cancer cell or embolus and in many instances the required increased tissue pressure by its own growth. The unfortunate addition of further mechanical factors can only serve to augment an already undesirable set of circumstances.

Thus far we have considered local invasion and the mechanical factors in metastasis. What are the influences governing the number of metastases?

Many if not most tumor emboli fail to survive the embolism. Zeidman, McCutcheon, Coman,[13] and others have studied this problem extensively using transplantable mouse tumor. Determined concentrations of cells of mouse sarcoma 241 were prepared, injected intravenously into C57 mice, and 18 days later the resultant metastases counted. Relatively large numbers of tumor cells were required indicating that many of the tumor cells failed to survive the embolism. But in humans, emboli are given off probably at a sporadic but more or less constant, slow rate quite dissimilar to an injection. Therefore, a corollary experiment was performed in which large and small implants of the same tumor were placed in the flanks of mice and the animals killed at intervals. The results showed that the duration of the primary was a determinant in the number of metastases and that greater numbers of metastases resulted from large than from small implants. However, there was no definite correlation between the final size of the primary and the number of metastases.

It is a common observation that thyroid, spleen, and skeletal muscle are seldom the seat of metastases. Coman[14] pointed out, though these structures have often been considered as offering "poor soil," experimentally this is not true since intra-arterial injections of a suspension of V_2 rabbit carcinoma into rabbits produced neoplasms in skeletal muscle as well as in any other location in the body. Injections into the left side of the heart produced metastases in myocardium, skin, skeletal muscle, lungs, kidneys, liver and other viscera. In this instance metastases were frequent in the muscles of the trunk and became less frequent in the distal portions of the extremities. This tumor can grow in all tissues of the body, yet of itself the neoplasm rarely metastasized beyond the regional nodes and the lungs. Injections of the tumor in the femoral vein produced no tumors beyond the lungs indicating that some of the cells failed to survive the embolism and the remainder were arrested in the lungs.

The question then arises: How effective are the lungs in filtering out tumor cells and do cancer cells pass through the pulmonary circuit without being arrested? Experiments using three different types of experimental cancers were

employed to solve this problem.[15] Cell suspensions of the V_2 squamous carcinoma, the Brown-Pearce carcinoma, and the Walker rat carcinoma 256 were injected into the veins of one group of rabbits and almost immediately the aortic blood collected. The aortic blood was then injected into a second group of rabbits. Autopsies done on the second group three to five weeks later showed some interesting results. With the Brown-Pearce carcinoma 10 of 20 rabbits developed metastases to distant sites and in five of the ten no pulmonary lesions were found. The V_2 carcinoma and the Walker 256 carcinoma produced lesions in 2 of 15, and 1 of 11 respectively. In view of these results it is inescapable that some tumor cells can pass through the pulmonary circuit and enter the systemic circulation. These experiments are doubly remarkable since only 50 cc. of aortic blood was employed.

Since certain types of experimental tumors passed through the pulmonary circuit more readily than others, the same authors examined these tumors for differences in cell size and found no significant difference. Passage of tumor cells through an arteriovenous shunt or through a patent foramen ovale could be excluded since so few cells of the V_2 carcinoma and the Walker carcinoma were able to pass through the lungs. It can be concluded that some types of tumor cells are better able to effect transpulmonary passage than others. The reason for this difference is still obscure although it is perhaps due to variations in pliability and viability of tumor cells.

In order to explain the reasons for the rarity of secondary tumors in some organs, Coman et al.[16] conducted the following experiments. Tumor cells stained with iron hematoxylin were injected into the left side of the heart of rabbits, and the animals killed in one to three minutes following injection. Multiple sections were then taken and examined for the presence of stained cells. The cells were counted and it was noted whether they appeared in capillaries or in arterioles. Emboli were found everywhere, but in the spleen and thyroid they were arrested in arterioles with only a few in capillaries. Emboli were rare in muscle. When viable tumor cells were used and the animals autopsied in one to three weeks, a close correlation between the number of emboli lodging in capillaries and the number of metastases was noted. When emboli in arterioles were included the correlation was lost. In the spleen and thyroid almost all of the emboli were in arterioles and secondary neoplasms not produced. In addition tumors most often developed when emboli of four or less cells were present. It is therefore established that tumors most often develop when emboli of four or less cells are arrested in capillaries and they are rare where emboli are arrested principally by arterioles.

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