Transpulmonary Passage of Tumor Cell Emboli*

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The following report deals with an attempt to determine whether or not tumor cell emboli pass through the lung circulation. Metastases in many organs, such as adrenals and kidneys, are generally derived from emboli carried in arteries. How do these emboli get into the arteries from the primary neoplasm? Do they first lodge in the lungs, forming secondary tumors which later give off emboli into the arterial circulation? Or do they pass immediately through the lungs from the venous to the arterial side?

Judging from past observations on the behavior of tumors in man and experimental animals, it appears probable that transpulmonary passage does occur and that this depends to a great extent on the type of cancer cell. Thus, certain cancers in man, such as malignant melanoma, tend to produce widespread visceral metastases, sometimes even without visible pulmonary growths (8). In contrast, other human cancers—for example, osteogenic sarcoma—may metastasize via the blood stream to the lungs, but infrequently beyond the lungs (4). A similar difference in behavior is seen with transplantable animal cancers. The V_2 carcinoma of rabbits metastasizes spontaneously to lymph nodes and lungs, but rarely to other viscera; likewise, following an intravenous injection of tumor suspension, lung tumors are frequent, but tumors elsewhere occur in less than 5 per cent of the cases.[1] Yet, the Brown-Pearce carcinoma of rabbits often metastasizes to many organs besides the lungs, and, following intravenous injection, tumors in organs other than lungs occur in 90 per cent of the cases (3). Also, Walker rat carcinoma 256 (2) and mouse fibrosarcoma 241[2] produce tumors in the lungs after intravenous injection, but rarely in other viscera. These clinical and experimental observations suggest the possibility that tumor cell emboli of some cancers frequently pass through the lungs, whereas emboli of other cancers do so infrequently.

However, Willis (8) points out the lack of *direct* evidence for unarrested transpulmonary passage of tumor cell emboli and further states that, even in the absence of visible pulmonary neoplasms, systemic metastases may be derived from unseen minute growths. Walther (7) is of the same opinion, but suggests that small malignant cells may pass through the lungs without establishing local tumors first. Recently, Bierman *et al.* (1) offered evidence by cross-circulation experiments that lungs of individuals without leukemia arrest leukemia cells, although the lungs of leukemic patients may not be equally effective.

It would seem possible to illuminate this problem by simple, direct experiments designed to ascertain whether transpulmonary passage of tumor cell emboli is possible, and, in the event of a positive answer, to find out whether cells from widely metastasizing cancers pass through the lungs more frequently than do cells from cancers seldom metastasizing beyond the lungs. It is the purpose of this paper to report the details of such experiments.

MATERIALS AND METHODS

To determine whether or not emboli of tumor cells pass unarrested through the lungs, a suspension of the cells was injected into a vein while, simultaneously, the aortic blood of the animal was collected. The aortic blood was then injected intravenously into a second animal. Tumors developing in the second animal would indicate that the embolic cells had passed immediately through the lungs of the first animal.

The V_2 squamous-cell carcinoma and the Brown-Pearce carcinoma were used in mixed-breed rabbits and the Walker rat carcinoma 256 in albino rats. Suspensions were prepared by passing several pieces of tumor through a fine mesh, stainless-steel sieve into a solution containing equal parts of serum and salt solution. Cell counts of the suspensions showed that approximately the same number of cells was injected in all experiments. These counts averaged 14,000 single cells and 500 clumps per cubic millimeter. Each rabbit was anesthetized with 60 mg. of sodium Nembutal intravenously. Twenty mg. of heparin was injected intravenously to prevent clotting of the blood withdrawn from the aorta.[3] About 1.5 cc. of tumor sus-

* This investigation was supported by research grants from the Anna Fuller Fund, the Special Research Fund of the Committee on the Advancement of Research of the University of Pennsylvania, and the Division of Research Grants and Fellowships of the National Institutes of Health, U.S. Public Health Service.

[1] Personal observations of Dr. Charles Breedis.

[2] Authors' personal observations.

Received for publication June 16, 1952.

[3] In some experiments the heparin was not injected intravenously but was contained in the syringe used for the collection of aortic blood. The presence or absence of heparinemia made no significant difference in the experimental results.

pension was then injected into the ear vein, and, during the injection procedure, the abdominal aorta was cut. Blood escaped into the peritoneal cavity and was collected in a syringe. About 30–50 cc. of this blood was then injected into the ear vein of a second rabbit. These latter rabbits were sacrificed 3–5 weeks after injection, and the organs were examined for tumors; histologic sections were used to confirm diagnoses made grossly. With the Walker rat carcinoma 256, 1 cc. of tumor suspension was used, and approximately 5 cc. of blood was collected after the aorta was cut.

RESULTS

Immediate transpulmonary passage of tumor cell emboli occurred with all three cancers used. The results are shown in Table 1. The injection of aortic blood led to tumor formation in ten out of twenty experiments with Brown-Pearce carcinoma. Usually, the tumors were numerous and widely distributed (Table 2), and the eye was the most frequent site. Other frequently involved sites were liver, lungs, kidneys, and adrenals. Evidently, cells of this tumor passed through the pulmonary circulation in large numbers.

V_2 carcinoma cells also passed immediately through the lungs, but in only two of fifteen experiments. Thus, the two rabbit cancers exhibited a different incidence of transpulmonary passage, and by statistical analysis with the Chi square method this difference was found significant. The P value was 0.03, and, therefore, the difference in incidence would occur by chance in only two or three out of 100 such experiments.

The Walker rat carcinoma behaved like the V_2 carcinoma in passing through the lungs in one out of eleven experiments. Thus, embolic cells from all three cancers passed immediately through the lungs, and the Brown-Pearce carcinoma revealed a significantly higher incidence of transpulmonary passage than did the V_2 carcinoma.

TABLE 1

IMMEDIATE PASSAGE OF EMBOLIC TUMOR CELLS THROUGH THE PULMONARY CIRCULATION

Type of cancer	No. animals injected with aortic blood	No. animals developing tumors
Brown-Pearce rabbit carcinoma	20	10
V_2 rabbit carcinoma	15	2
Walker rat carcinoma 256	11	1

TABLE 2

TUMORS PRODUCED IN NORMAL RABBITS FOLLOWING INTRAVENOUS INJECTION OF AORTIC BLOOD CONTAINING TUMOR CELL EMBOLI WHICH HAVE TRAVERSED THE PULMONARY CIRCULATION

Brown-Pearce carcinoma	V_2 carcinoma
Eye +,* lung +, adrenals +	Lung +
Eye +, lung ++, adrenals +	Eye +, ear +, lung +, brain +
Eye +, lung +, adrenals +, kidney +	
Eye +, lung +++, liver ++++, kidney +	
Eye +, adrenal +	
Eye +, adrenal +, liver ++, retro-orbital fat +	
Eye +, adrenal +, lung ++++, liver ++++, kidney ++	
Liver +	
Kidney +	
Liver +	
Negative, ten rabbits	Negative, thirteen rabbits

* One plus indicates one to twenty tumor nodules; four pluses indicates at least 100 tumor nodules.

DISCUSSION

The above results become even more significant in view of the fact that the experimental method employed offers so many hazards to cell survival. The preparing of a suspension by passing the tumor through a sieve causes a high percentage of cell deaths (9). Only a few of the viable cells injected ever develop into tumors, as most of them are destroyed in the body of the host (6, 9). The collection of blood in the abdominal cavity, the subsequent withdrawal into a syringe, and the reinjection into a second rabbit are further hazards to cell survival. The use of only 30–50 cc. of aortic blood would decrease the chances of picking up tumor cells which passed through the pulmonary circulation. In light of these obstacles, the positive results obtained are surprisingly high, particularly in the experiments with Brown-Pearce carcinoma. Therefore, it would seem necessary to revise our concepts concerning the transpulmonary passage of tumor cell emboli. The conclusion that such passage occurs frequently rather than rarely would seem inescapable.

It is interesting to note (Table 2) that in five of the ten experiments with Brown-Pearce carcinoma, there were no detectable lung tumors in the second animal, although tumors appeared in the eye, kidney, adrenal, and elsewhere. It seems likely that the cancer cells traversed the pulmonary systems of two different rabbits without arrest. Also, the incidence of transpulmonary passage by both rabbit cancers corresponds to the previously noted incidence of metastases in organs beyond the lungs produced either spontaneously or by intravenous injection. This suggests that many spontaneous metastases appearing in organs other than the lungs may be produced by emboli which have immediately passed the pulmonary circulation, rather than by emboli from secondary pulmonary growths. The possibility remains that in the above experiments tumor cell emboli passed through arterio-venous anastomoses (5) or a patent foramen ovale. Such passage probably oc-

curs infrequently if at all, for the intravenous injection of V_2 carcinoma cells rarely leads to the appearance of tumors beyond the lungs.[4]

This difference in the incidence of transpulmonary passage exhibited by Brown-Pearce carcinoma and V_2 carcinoma requires further examination. The phenomenon cannot be explained by differences in the number of tumor emboli originally used for the intravenous injection, since approximately the same number of tumor cells was used in all experiments with both cancers. Conceivably, the cells of the Brown-Pearce carcinoma may be smaller than those of V_2 carcinoma, and, hence, the cells of the former may traverse pulmonary capillaries more easily. To test this explanation, cells of both types of cancer were measured with the aid of an ocular micrometer. The width of the smallest rectangle fitting around each cell was measured, as this width would be the critical dimension for transcapillary passage. As seen in Table 3, the cells of Brown-Pearce carcinoma were certainly not smaller than V_2 carcinoma cells. Thus, difference in cell size cannot explain the difference in incidence of transpulmonary passage. However, other explanations are still possible. While the same number of cells was used for each experiment, the viability of the two types of cancer cells may not be alike under the same conditions. Also, the possibility that the Brown-Pearce carcinoma cells may release a pulmonary vasodilator has not been excluded. Finally, Brown-Pearce cells may be more pliable, and, hence, may traverse the pulmonary capillaries by elongating. More work is in progress to explain the difference in incidence of transpulmonary passage exhibited by two rabbit cancers under the same experimental conditions.

TABLE 3

SIZE OF BROWN-PEARCE AND V_2 CARCINOMA CELLS

No. cells	Type of cell	Mean size* (μ)	Range (μ)
100	V_2 carcinoma	10.7	6.5–17.6
100	Brown-Pearce carcinoma	12.6	6.9–19.4

* By size is meant the width of the smallest rectangle fitting around a cell, as this width would be the critical one for passage through a capillary.

SUMMARY

Experiments were designed to determine whether tumor cell emboli can pass immediately through the pulmonary circulation. V_2 carcinoma and Brown-Pearce carcinoma were used in rabbits, and Walker carcinoma 256 in rats. A suspension of tumor cells was injected into an ear vein while the animal was bled simultaneously through the abdominal aorta. The aortic blood was then injected intravenously into a second animal of the same species. Tumors developing in the second animal indicated immediate passage of tumor cells through the lungs of the first animal. All animals were sacrificed 3–5 weeks after the injection of aortic blood, and a search for tumors was made. Tumors were found with all three types of cancers, indicating that the embolic tumor cells had passed through the lungs without delay. The positive results obtained with Brown-Pearce carcinoma were significantly higher than those found with the V_2 carcinoma. This difference in incidence of transpulmonary passage corresponds to the greater tendency of Brown-Pearce carcinoma to metastasize to many organs, in contrast to the behavior of V_2 carcinoma which rarely metastasizes beyond the lungs.

It is concluded that tumor cell emboli are able to pass immediately through the pulmonary circulation of both rabbits and rats and that differences in incidence of such activity apparently depend on some as yet unexplained property of the cancer cell. These results suggest that transpulmonary passage of tumor cell emboli probably occurs far more frequently in man than heretofore suspected.

[4] Personal observations of Dr. Charles Breedis.

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