wall of the lymph capillary also starts to undergo alteration, i.e. differentiation, behind the first valve in the adult organism. This, however, only confirms that the phase of embryogenesis from the 9th to 14th day of incubation, in which we studied the initial development of lymphatic drainage of the wall of the heart up to formation of the first valves, is defined entirely naturally.

References

1 *Leak, L.V., J.F. Burke:* Fine structure of the lymphatic capillary and the adjoining connective tissue area. Amer.J.Anat. 118 (1966) 785-809

2 *Leak, L.V., J.F. Burke:* Electron microscopic study of lymphatic capillaries in the removal of connective tissue fluids and particulate substances. Lymphology 1 (1968) 39-52

3 *Lauweryns, J.M., L. Boussouw:* The ultrastructure of pulmonary lymphatic capillaries of newborn rabbits and of human infants. Lymphology 2 (1969) 108-129

4 *Wegmann, R.:* Histochimie et histoenzymologie des voies lymphatiques. Pp. 146-158 in New trends in basic lymphology, J.M. Collette et al. ed., 235 pp., Birkhauser, Basel and Stuttgart (1967)

5 *Casley-Smith, J.R.:* The fine structure, properties and permeabilities of the lymphatic endothelium. Pp. 19-30 in New trends in basic lymphology, J.M. Collette et al. eds., 235 pp., Birkhauser, Basel and Stuttgart (1967)

6 *Csillik, B.:* Discussion to Wegmann R., see 4.

7 *Pfleger, L., F. Kaindl, E. Mannheimer, B. Thurnher:* Morphology of lymphatic vessels. Pp. in New trends in basic lymphology, J. M. Collette et al. eds., 235 pp., Birkhauser, Basel and Stuttgart (1967)

8 *Descartes, R.:* Discours de la méthode. Leyden 1637. According to a czech edition, Praha (1947)

9 *Rychter, Z., R. Jelinek, E. Klika, L. Antalíková,* Development of the lymph bed in the wall of the chick embryo heart. Physiol.bohemoslov. 20 (1971) 533-539

10 *Clark, E.R.:* Observations on living lymphatics in the tail of the frog larva. Anat. Rec. 3 (1909) 183-198

11 *Kuprianov, V.V.:* Some features of the initial lymphatic vessels in their interrelation with blood capillaries. Acta anat. 73 (1969) 69-80

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Trapping of Calibrated Microspheres in Experimental Rat Lymph Node Metastases

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Summary

The position in rat lymph nodes of microspheres relative to embolized tumor cells and micrometastases was studied histologically to evaluate some aspects of intralymphatic cancer treatment.

Walker carcinosarcoma cells were injected into afferent lymphatic channels of rat lymph nodes. Calibrated, nonradioactive microspheres were also injected, either together with the tumor cells or after 3-day or 7-day intervals. Twenty-four lymph nodes were available for study. In 18 of the 24 positive lymph nodes, microspheres were intermingled with tumor cells or micrometastases. However, in every instance, most of the microspheres were found in the uninvolved portions of the lymph nodes, both near the tumor-cell infiltrates and micrometastases and at a distance from them. Even when microspheres were in contact with tumor cells, the number of microspheres relative to the tumor cells was very small. Lymph node metastases probably cause diversion of the lymph flow and thus prevent optimal contact of tumor cells and microspheres.

Radioactive microspheres may localize in human inguinal and iliac lymph nodes and may be used for intralymphatic radiation therapy (1). The effectiveness of such treatment depends on the type and size of the metastatic lesions and the behavior of the microspheres, that is, their radioactive properties, the duration of their lodgement in lymph nodes, and the sites where they lodge in relation to the lesions.

The microspheres that have been used in a few instances in humans were 1 to 4 μ in diameter (1, 2). A recent experimental study in rats showed that larger spheres (5 to 10 μ and 15 μ) might be better suited for intralymphatic therapy because of their slower passage through the lymph node (3). The study presented herein describes the site of lodging of such microspheres in relation to early experimental tumor metastases.

Materials and Methods

Microspheres. Sterile, nonradioactive, complete spheric, homogeneous ceramic microspheres (3M Co., St. Paul) were used. The sizes of the microspheres were designated as 5 to 10 μ and 15 $\pm$ 5 μ. The actual sizes of these microspheres varied between 2 and 10 μ and between 12 and 24 μ, respectively (3).

Tumor Cells. Walker-256 carcinosarcoma cells were used. The preparation of tumor-cell suspensions from monolayer cultures has been described (4). The suspensions of tumor cells contained about 100,000 cells/0.1 ml. For this study, no radioactive label was added to the culture medium.

Animals. A total of 55 male, albino Sprague-Dawley rats, weighing 250 to 350 gm, were used. They were housed under standard conditions at a temperature of 72 F, with free access to a standard laboratory pellet diet (*Rockland*) and water. For injections of tumor-cell suspensions and microspheres, the rats were anesthetized with a solution of pentobarbital (Pentobrocanal) administered intraperitoneally in a dose of 4 mg/100 gm of body weight. They were killed with ether. A complete autopsy was done on each rat, and all regional lymph nodes were removed for histologic study.

Injection Procedure. Anatomic variations of the lymphatic drainage from the left testis and techniques of intralymphatic tumor-cell or microsphere injection have been previously described (3, 4). Either the tumor cells and microspheres were mixed and injected together or the tumor-cell suspension was injected first, followed after 3 or 7 days by the injection of microspheres. In every instance, an attempt was made to inject the microspheres into the same lymphatic vessel that had previously been used for the tumor-cell injection. The rats were killed 1 day after the injection of the microspheres.

Histologic Techniques. Fixation, embedding, and staining procedures have been described previously (3, 4).

Results

The lymph nodes of 15 rats, that is 27% of the animals studied, showed both microspheres and tumor-cell infiltrates or micrometastases (the term "micrometastasis" has been defined in a previous publication) (4). Thirteen rats (24%) showed lymph nodes with microspheres but without tumor cells, and four rats (7%) showed lymph nodes with tumor cells but without microspheres. In the remaining lymph nodes, neither tumor cells nor microspheres were found. In nine rats, two lymph nodes were positive for both microspheres and micrometastases or tumor-cell infiltrates. Therefore, a total of 24 lymph nodes were available for study (Figure).

Of the 15 rats whose lymph nodes were studied in detail, 3 had received simultaneous injection of tumor cells and microspheres. In nine rats, microspheres had been injected 3 days after tumor-cell injection, and in three rats, 7 days after tumor-cell injection.

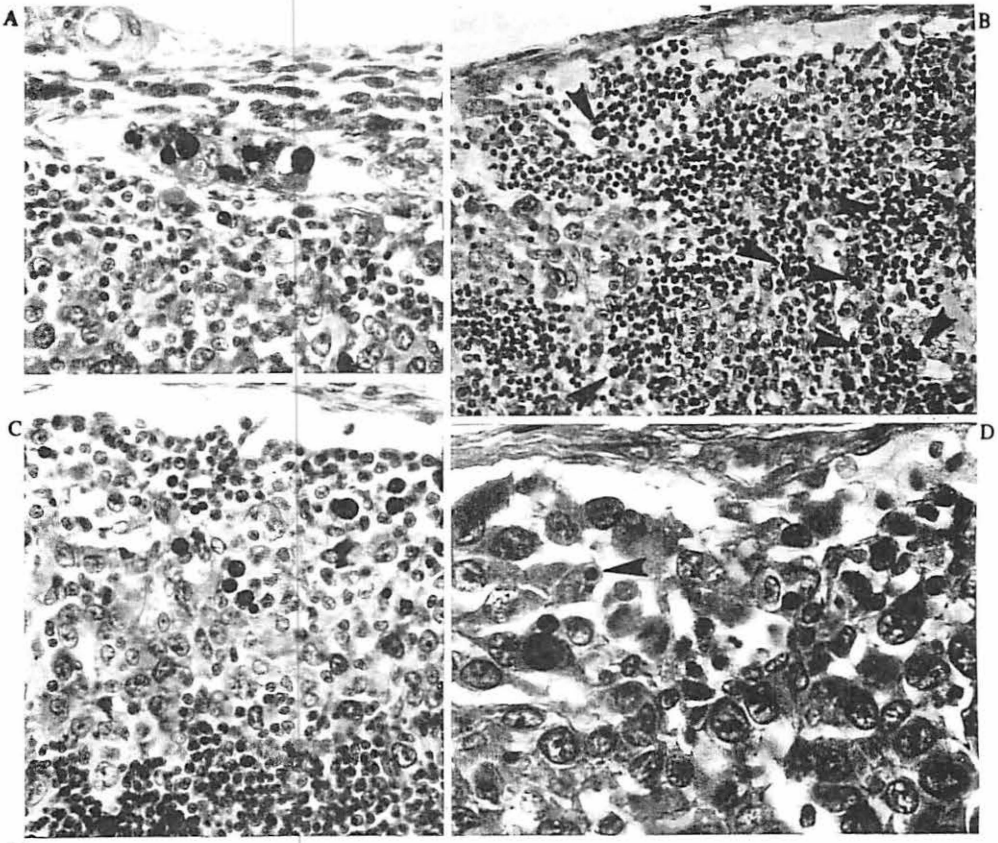

Fig. 1 A-D. Rat lymph nodes 4 days after intralymphatic injection of Walker-256 carcinosarcoma cells and 1 day after injection of 2- to 10-μ microspheres. A) Tumor cells and microspheres in afferent lymphatic channel of renal lymph node. (Hematoxylin and eosin; reduced from x 400.) B) Tumor cells and microspheres (arrows) infiltrating cortical pulp. (Elastic van Gieson; reduced from x 250). C) Microspheres intermingled with tumor cells of micrometastasis growing in cortical pulp just beneath subcapsular sinus. (Hematoxylin and eosin; reduced from x 400). D) Tumor cells and microspheres in cortical pulp. Small sphere (arrow) phagocytized by tumor cell. (Hematoxylin and eosin; reduced from x 600.)

The rats had been killed 1, 4, or 8 days after tumor-cell injection, respectively. Microspheres in the 15-μ size range were present in the lymph nodes of the three rats that had received simultaneous injection of microspheres and tumor cells. Microspheres of 2 to 10 μ had been injected into the lymph nodes of the other 12 rats.

The size of the microspheres (5 to 10 μ versus 15 μ) and the interval between tumor-cell injection and injection of microspheres did not seem to significantly affect the distribution of the spheres, relative to the tumor cells.

In 18 of the 24 positive lymph nodes, some microspheres were in contact with tumor cells (in 6 lymph nodes, Fig. B) or micrometastases (in 12 lymph nodes, Fig. C). In all 18 lymph nodes, however, most of the microspheres were found in uninvolved portions of the lymph nodes, both near the tumor cells and at a distance from them. In

six lymph nodes, microspheres and tumor cells had completely failed to come in contact. In three of these, some microspheres were found close to the tumor-cell infiltrates.

The micrometastases tended to grow just beneath the membrane, between the subcapsular (marginal) sinuses and the cortical pulp (Fig., C). Microspheres that were close to these metastatic lesions lodged in the adjacent subcapsular and intermediary sinuses or in the adjacent cortical pulp.

In every instance, the extent of the malignant growth outweighed by far the number of microspheres, so that often only few microspheres were found in large sheets and clusters of tumor cells (Figure). Furthermore, the microspheres that were within the micrometastases usually lodged in the periphery of the tumor nodule. Some 2- to 5-μ microspheres were phagocytized by tumor cells (Fig., D).

Comment

Microspheres may mingle with tumor cells and small metastatic lesions, at least in the early phase of metastatic tumor growth in lymph nodes. In this situation, radioactive microspheres may be expected to successfully radiate tumor cells that are nearby. However, most of the microspheres seem to lodge either outside the tumor-cell infiltrates or at a distance from them. This seems primarily due to lymphodynamic changes after intranodal tumor growth. As the metastatic lesions in the lymph node become larger, more lymph flows into the uninvolved portion of the node, thus diverting the embolized microspheres from their target and preventing optimal contact with the tumor cells. This also may happen when the microspheres fail to travel the same afferent lymphatic channels that were used by the tumor cells. Such a situation was probably reproduced experimentally when microspheres had to be injected into afferent lymphatic channels distant from the site of the tumor-cell injection. On occasion, this was necessary because of postoperative epididymal edema. Whatever the mechanism, histologic study of positive lymph nodes of the rat confirmed the observation in humans that microspheres tend to lodge in uninvolved portions of lymph nodes (5). Failure to detect microspheres or tumor cells in regional lymph nodes is most likely due either to the presence of lymphatico-venous anastomoses (6) and other anatomic variations (7) or to faulty technique.

Production of measurable radiation-induced histologic changes probably will require microspheres labeled with maximal amounts of densely ionizing nuclides. The experimental findings of this study do not imply that there is no place for treatment with radioactive microspheres of tumor cells or micrometastases in human lymph nodes. However, this technique probably does not permit sufficient exposure to ionizing radiation necessary for killing all tumor cells in such a lymph node.

References

1 *Ariel, I.M., M.I. Resnick, D. Galey:* The intralymphatic administration of radioactive isotopes and cancer chemotherapeutic drugs. Surgery, St. Louis 55 (1964) 355-363

2 *Ariel, I.M., W. Flynn, G.T. Pack:* Le possibilità attuali delle microsfere di ceramica radianti marcate con yttrio 90. Minerva Med. Paris 58 (1967) 4496-4498

3 *Ludwig, J.:* Trapping of calibrated microspheres in rat lymph nodes. Lymphology 4 (1971) 18-24

4 *Ludwig, J., J.L. Titus:* Experimental tumor cell emboli in lymph nodes. Arch.Path. 84 (1967) 304-311

5 *Jantet, G.:* Intralymphatic therapy. In: Lymphography in Cancer. Recent Results in Cancer Research, No. 23. (Ed. by Fuchs, W.A., J.W. Davidson, H.W. Fischer). Springer, New York 1969
6 *Job, T.T.:* Lymphatico-venous communications in the common rat and their significance. I. Lymphatico-venous communications in the common rat. Am.J.Anat. 24 (1918) 467-491
7 *Engeset, A.:* The route of peripheral lymph to the blood stream: An x-ray study of the barrier theory. J.Anat. 93 (1959) 96-99

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Activation of Fibrinolysis by a Lymphogenic Activator in the Rat

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Summary

The plasminogen concentration in rat lymph was found to be on average 40% of the plasma concentration. The antiplasmin level was only 27% of the plasma level. On heated fibrin plates a distinct fibrinolysis in the lymph, collected over a period of 7 days, was found. The simultaneous lack of enzymatic activity in the plasma suggested the presence of a lymphogenic activator. Further evidence for the importance of the lymphogenic activator in blood fibrinolysis was found: plasminogen determinations performed in rat plasma during a period of 7 days of continuous lymph flow revealed significantly higher values compared to a control group. The euglobulin lysis time in the plasma of cannulated animals was significantly prolonged. The plasminogen consumption in the control animals was interpreted as being due to a continuous stress-induced activation of blood fibrinolysis by the lymphogenic activator.

The presence of coagulation factors in the lymph of experimental animals and humans is well established (1) but rather little is known about the fibrinolytic system of the lymph. Although a high content of plasminogen activator in human lymph nodes has been reported (2) no data are available concerning its significance in lymph and plasma fibrinolysis. The present communication deals with determinations of some components of the fibrinolytic system in rat lymph and rat plasma during the course of a long-term cannulation of the thoracic duct.

Material and Methods

The investigations were carried out in female SIV-50 rats, weighing 200-250 g. After thiobarbital anaesthesia the abdominal part of the thoracic duct was cannulated with a polyethylene cannula according to a technique described by *Bollman* (3). For 7 days the animals were kept in semirestraining cages. Lymph samples were collected in glass tubes containing 50 units/ml heparin to prevent clotting. The sampling of each lymph specimen lasted from 60 to 120 minutes. Blood samples were obtained by aortic puncture.

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