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## Lymphatic Dissemination of Cancer Cells in Mice Transplanted Intratibially with Ehrlich Carcinoma\*

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### Summary

Ehrlich carcinoma cells implanted in the marrow cavity of the tibia in Swiss mice induce the formation of visible metastases in axillary lymph-nodes. The pattern of the lymphatic vessels in the tumor implantation area was investigated. Cancer cells were found in lymph samples taken from the subcutaneous inguino-axillary lymphatic trunk. The blockade of this lymphatic vessel by a polyvinyl resin resulted in a different distribution of lymphnodal metastases, while the hematic dissemination of cancer cells was unaffected. The results are discussed in relation to the pathogenesis of the axillary metastases in the considered experimental model.

Previous studies (1) indicated that Ehrlich carcinoma implanted in the marrow cavity of the tibia in Swiss mice induced the formation of visible metastases in distant lymph-nodes such as axillary, paraortic, thoracic, paravertebral, and cervical nodes. These findings suggested a study to identify the lymphatic circulation in the posterior leg of the mouse after the intratibial transplantation of Ehrlich carcinoma and to analyse the distribution of lymphnodal metastases after inducing lymphodynamic changes.

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*Anatomical Notes*

The normal pattern of the lymphatic vessels in the mouse represented in Fig. 1 was extensively studied by *Ottaviani* (3), by injecting in the foot paw of the animals Direct Skyblue 40%. The anterior network of the posterior leg drains lymph from the dorsal side of the foot and from the anterior part of the leg and reaches the inguinal region. On the other hand, the posterior networks drain lymph from the plantar side of the foot and from the posterior part of the leg. The lateral subcutaneous trunk which connects directly the inguinal lymph-node to the axillary lymph-node is illustrated in Fig. 2. Fig. 3 reports the lymphodynamic changes after the blockade of the lateral subcutaneous lymphatic trunk by injecting a polyvinyl resin (Neoprene Latex) into the inguinal node. The lymphatic flow in posterior vessels is markedly increased because of the stasis produced by the blockade in the anterior area of the leg.

Fig. 4 shows the changes induced by the tumoral mass on the lymphatic circulation when Ehrlich carcinoma has been implanted in the marrow cavity of the tibia, but the blockade has not been performed. Stasis is produced only in the distal area of the anterior part of the leg. Consequently, the lymphatic flow in the posterior vessels is increased. The inguinal node and inguino-axillary trunk drain lymph from the tumor area. The lymphodynamic situation after the blockade of the inguinal node and inguino-axillary trunk in animals bearing Ehrlich carcinoma in the tibia is represented in Fig. 5. The lymph from tumor area is drained by a collateral way represented by a neoformation produced by the stasis in the prelymphnodal area in a period of about 10 days after the tumor transplantation.

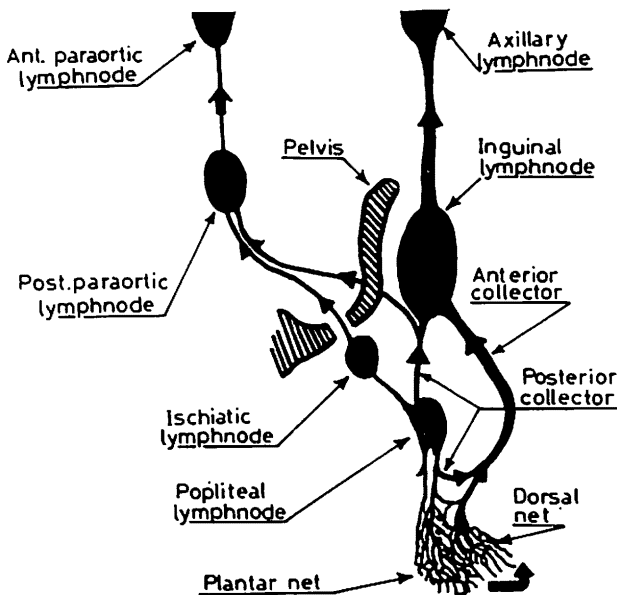


Fig. 1 Diagram to illustrate the lymphatic circulation of the posterior leg of the mouse.

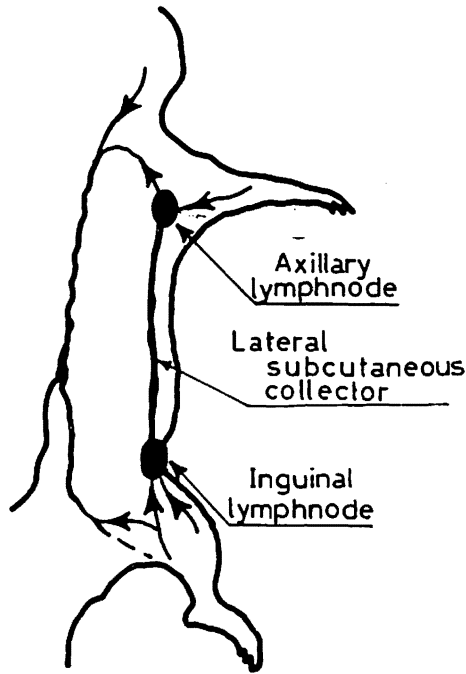


Fig. 2 The lateral subcutaneous lymphatic trunk.

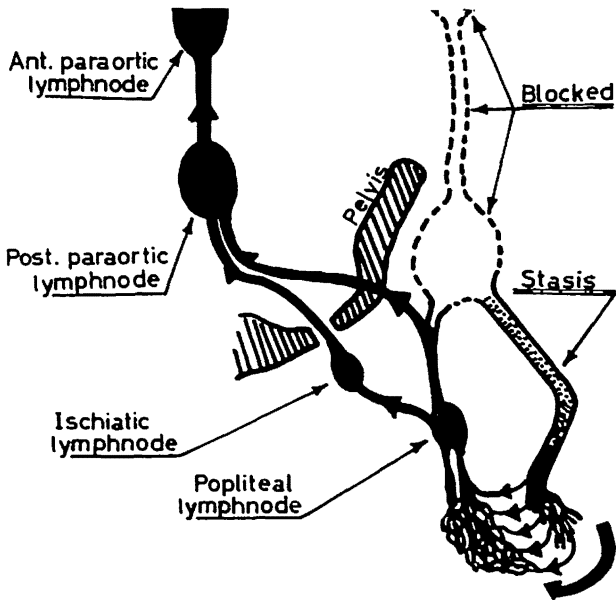


Fig. 3 Modification in lymphatic circulation induced by blocking the subcutaneous inguino-axillary trunk.

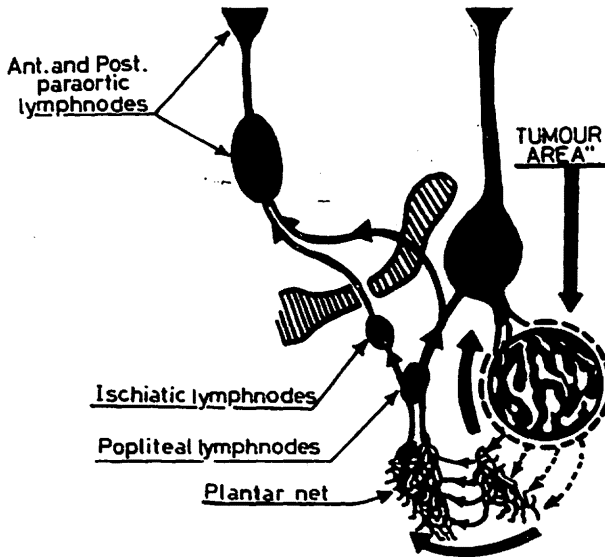


Fig. 4 Changes induced by the tumoral mass on the lymphatic circulation.

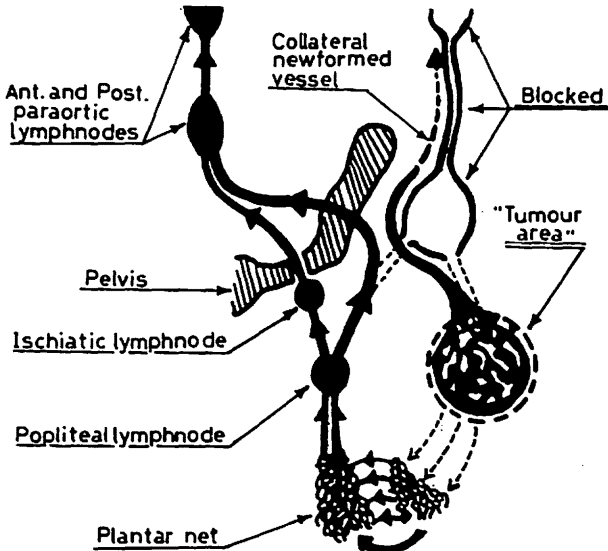


Fig. 5 Changes in lymphatic circulation induced by blocking the lateral subcutaneous trunk and implanting the tumor intratibially.

#### *Cancer cell dissemination and metastases*

These anatomical findings can explain the lymphatic dissemination of cancer cells and the metastases at the axillary nodes from Ehrlich carcinoma implanted intratibially. In fact, lymph samples collected from the lateral subcutaneous lymphatic trunk, show

the presence of cancer cells in tumor bearing animals. More evidence for a lymphatic dissemination of cancer cells came from the evaluation of lymphnodal metastases distribution when the subcutaneous lateral trunk was blocked by "Neoprene Latex". Table 1 shows that the weight of the tumor implanted intratibially, the total weight of metastases and the percent of mice with metastases, is unaffected by the blockade of the inguinal lymph-node. However, the average weight of axillary lymph-nodes is only 0.2 g for mice with blockade of the inguinal-axillary trunk as opposed to 1.2 g for control mice. Furthermore, the average weight of paraortic lymph-nodes is increased from 0.5 g for control mice to 1.2 g for mice with the block of the inguinal axillary lymphatic vessel.

The metastatic involvement for inguinal lymph-node in the blocked mice can be interpreted considering that the cancer cells can grow in the lymph-node, since there is no possible way to enter the post-lymphnodal pathway.

Metastatic involment of cervical, scapular, thoracic and paravertebral nodes has disappeared in the blocked mice, because of the decreased metastatic involvement of the axillary nodes.

Table 1 Ehrlich carcinoma transplanted intratibially in mice after blockade of inguino-axillary collectors by "neoprene latex".

	Controls (n=29)	Blocked (n=27)
Av. primary tumor weight g $\pm$ S.E.	12.14 $\pm$ 1.8	11.65 $\pm$ 1.2
% of mice with metastases	79	70
Total weight of metastases g $\pm$ S.E.	46.3 $\pm$ 0.8	41.8 $\pm$ 1.2
Distribution of lymphnodal metastases		
A		
number	10	15
weight (g $\pm$ S.E.)	1.8 $\pm$ 0.9	0.2 $\pm$ 0.04*
PO		
number	22	28
weight (g $\pm$ S.E.)	0.5 $\pm$ 0.1	1.2 $\pm$ 0.3*
I		
number	0	11
weight (g $\pm$ S.E.)		0.3 $\pm$ 0.04
PV		
number	2	0
weight (g $\pm$ S.E.)	2.1	
TH		
number	1	0
weight (g $\pm$ S.E.)	0.4	
C		
number	4	0
weight (g $\pm$ S.E.)	2.6 $\pm$ 0.9	
SC		
number	1	0
weight (g $\pm$ S.E.)	1.1	

A = axillary, PO = paraortic, I = inguinal, TH = thoracic, PV = paravertebral, C = cervical, SC = scapular.

Animals have been killed 50 days after tumor implantation.

\* t Student test  $P < 0.05$ .

$\chi^2$  test for the analysis of metastases distribution in the two groups is significant at  $P < 0.05$ .

Since data previously reported (1) show that the tumor growing intratibially disseminates into the blood stream it seemed interesting to establish if the lymphatic blockade could affect also the hematic dissemination of neoplastic cells. The presence of cancer cells in blood and lungs determined by a bioassay technique, described elsewhere (2) was not substantially affected by the blockade of the inguinal lymph-node (see Table 2).

Table 2 Number of takes in recipient normal animals following subcutaneous transplantation of blood clot and lung from mice inoculated intratibially with Ehrlich carcinoma (100,000 cells/mouse).

Group	Time after inoculation (days)	No. of takes	
		Blood from femoral vein	No. of transplantations Lung
normal	29	5/10	4/10
blocked	29	5/10	4/10
normal	36	7/10	4/10
blocked	36	5/10	5/10

Considering the reported experimental data, it seems then possible to conclude that the metastatic involvement of lymph-nodes is mostly related to a lymphatic dissemination of neoplastic cells, when Ehrlich carcinoma is implanted intratibially.

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