Observations on Lymph Node Metastases

A. C. WALLACE, R. L. JOSEPHSON* and N. K. HOLLENBERG**

Although lymphatic metastasis of human tumours has been given a great deal of study because of its frequency and importance in prognosis and management, experimental lymphatic spread has attracted less attention than blood-borne metastasis in laboratory animals. The difficulty in visualizing and utilizing lymphatic vessels, and perhaps the lack of suitable experimental models have been partially responsible. There have been few comprehensive reviews available on the experimental work; they include those of STRÄULI, (1970), ONUIGBO, (1972) and VAN DE VELDE and CARR, (1977).

There are important differences between lymphatic and hematogenous spread. The lymphatic fluid is normally very poor in fibrinogen and platelets, and so in comparison with blood-borne tumour cells, which are rapidly enmeshed in a platelet-fibrinogen-fibrin clump (JONES & WALLACE, 1969 and 1971, WARREN, 1973, CHEW & WALLACE, 1976), tumour cells arrive at the subcapsular sinuses of the afferent portion of the lymph nodes relatively naked. In this stage, they may be more vulnerable to their surroundings, although the sinuses may be a less hostile environment than pulmonary capillaries and arterioles; also the entire process may be less influenced by the clotting factors that have received such massive study in recent years with regard to experimental pulmonary metastases.

Another distinctive feature of lymphatic metastasis is the variable course possible once tumour cells have entered lymphatic channels. While the precise anatomical route of blood-borne metastases to lung or liver may vary, their immediate destination is seldom in doubt. Lymph-borne cells may settle directly in a single regional node; they may, especially in man, be distributed to a large number of associated nodes; they may enter a major trunk such as the thoracic duct, bypassing regional nodes, gaining entrance to the bloodstream (ENGESET, 1959) or they may possibly enter the bloodstream by lymphatic-venous communications that have long been documented (JOB, 1918) but whose significance in tumour spread still remains uncertain. There is also great variation in how rapidly different experimental tumours traverse lymph nodes by the sinusoids and reach efferent channels. The result of all these variable factors is not only great discrepancies in results of experimental studies, but also uncertainty in clinical situations as to whether local lymph node involvement represents the only problem or is part of a more widespread dissemination even at an early stage. Inevitably, doubt is sometimes expressed whether experimental studies have any validity in application to human lymphatic spread.

Human studies, nonetheless, have limitations: they can and have demonstrated the pattern usually present in the lymphatic spread of human cancer and have estimated the relation of this to prognosis in many instances, but controlled studies on the effect of various factors and the sequence of events in the process can only be pieced together from many cases. Experimental workers have utilized tumour models that mimic the human picture, and in addition to analyzing the process in a time framework have attempted to establish critical factors determining the extent of spread. There has been no agreement on how faithfully the experi-

* Present Address: Resident in Otolaryngology, University of Toronto, Canada
** Present Address: Shields Warren Radiation Lab., Harvard Medical School, Boston, Mass., U.S.A.

Metastatic Tumor Growth
edited by E. Grundmann
Gustav Fischer Verlag · Stuttgart · New York · 1980

mental model must follow the human one to provide useful information. It has been claimed that only spontaneous tumours in syngeneic strains are of any value (HEWITT & BLAKE, 1975, HEWITT, 1976); that only spontaneous metastases from tumours are suitable for study (VAN DE VELDE & CARR, 1977) and that even these may be actually induced metastases if cell suspensions are injected (IMAI et al., 1975, TAKAZAWA & SHIMIZU, 1976). In the long run, any extrapolation from animal to human may be fallaceous, and many workers are inclined to use any model they believe will give an answer or a clue to an answer, accepting that any conclusions must be extremely guarded.

In nearly all of the studies that are presented here the Walker 256 tumour was used in Sprague-Dawley rats. This is not a syngeneic system but in our hands regression of the tumour is almost unknown. Most of the observations that would be influenced by an allograft reaction were completed in 48 hours, by which time this reaction would probably not have developed. Early spread usually occurs from a primary site to regional nodes, while pulmonary metastases appear later *(Table 1)*. As reported previously (WALLACE & HOLLENBERG, 1965) the tumour requires fewer cells to transplant by lymphatic channels than intravenously *(Table 2)*. These findings are in contrast to those of ZEIDMAN, (1965) who concluded the opposite with a different tumour system. This point emphasizes individual tumour variations; the two carcinomas tested by us required fewer cells for intralymphatic transplantation, while a sarcoma and a leukemia failed to show any difference between the two routes. Another reason for the use of Walker 256 tumour in the later experiments, was the distinctive appearance of the cells in plastic sections and on transmission electron microscopy (TEM).

Some unpublished studies paralleled those of others in attempting to find factors that increased or decreased spontaneous lymphatic metastases. There is abundant evidence in the literature that passive movements decrease lymphatic flow (e.g. MCMASTER, 1937) and that immobilization will decrease flow (e.g. BARNES & TRUETA, 1941). Massage has been reported to increase blood-borne metastases (MARSH, 1927) and lymphatic spread (TJERNBERG &

Table 1: Showing incidence of tumor in nodes and lungs of Sprague-Dawley rats from tumor transplant in testes.

Spread of W256 from Primary Site in Testes in 4 Weeks		
Abdominal Nodes	Mediastinal Nodes	Lung
6/6 +	4/6 +	0/6 +

Table 2: Showing number of lung tumors following intravenous injection and lymph node tumors following testicular lymphatic injection (From Wallace & Hollenberg, 1965).

Transplantation of Walker 256 Tumour by Intravenous and Intralymphatic Routes			
	Number of animals with tumours		
Number of cells inoculated	Intravenous route	Intralymphatic route	
1.000	0/8	7/8	P = <0.001
10.000	3/8	9/10	P = >0.05
100.000	9/10	8/9	P = >0.05

ZAJICEK, 1965) and to h
to decrease popliteal no
nodes and lungs (STOCKE

Experiments were don
of Walker tumours from
were divided in the thigh
of Walker 256 tumour v
and 15 days popliteal no
results of these transpla
between the two sides a
influence lymph node spr

Another group of rats
each animal was manua
and 15 days *(Table 4)* ag
obvious that many varia
the Walker 256, that has
may not be influenced si
effect from these factors.

Most of the rest of o
lymphatic channels. For
used, in which testicula
technique have been put
made an attempt to corre
particulate material into

Table 3: Incidence of positiv
with and without denervatio

Effect of Denervation	po
+ Node Transplants at 8 day	
+ Node Transplants at 15 da	
Total	

Table 4: Incidence of positive
with and without massage of

Effect of Massage on Spontan	
+ Node Transplants at 8 days	
+ Node Transplants at 15 da	
Total	

ZAJICEK, 1965) and to have no effect (WALLACE, 1956). Passive movement has been reported to decrease popliteal node spread from tumour in the foot but to increase spread to other nodes and lungs (STOCKER, 1969).

Experiments were done to test the effect of massage and of denervation paralysis on spread of Walker tumours from a primary transplant in the leg. The sciatic nerves of a group of rats were divided in the thigh on one side, leaving the limb paralyzed below the knee. A suspension of Walker 256 tumour was made subcutaneously on the dorsum of both hind legs, and at 8 and 15 days popliteal nodes from both sides were transplanted to normal rats. *Table 3* shows results of these transplants from normal and denervated sides. There was no difference between the two sides and it appears that, at least with this model, immobilization does not influence lymph node spread.

Another group of rats had similar bilateral Walker 256 leg transplants, and one foot of each animal was manually massaged daily for two minutes. Popliteal node transplants at 8 and 15 days *(Table 4)* again showed no effect of the massage on degree of nodal spread. It is obvious that many variables could influence studies of this type; for example, a tumour like the Walker 256, that has a fairly constant and inexorable pattern of invasion and metastasis may not be influenced significantly while one of more marginal qualities might show more effect from these factors.

Most of the rest of our studies, including current ones, have involved inoculation into lymphatic channels. For this purpose the method described by ENGESET, (1959) has been used, in which testicular lymphatics are entered with a fine needle. Details of our own technique have been published previously (WALLACE & HOLLENBERG, 1965). Initially we made an attempt to correlate observed anatomical channels with evidence for actual entry of particulate material into the venous system. A suspension of bacteria, Serratia Marcescens,

Table 3: Incidence of positive transplants from popliteal nodes from rats with Walker 256 tumor in legs, with and without denervation of leg.

Effect of Denervation on Spontaneous Metastasis of Walker 256 Tumour in the Leg		
	Denervated	Not Denervated
+ Node Transplants at 8 days	2/6	2/6
+ Node Transplants at 15 days	5/7	5/7
Total	7/13	7/13

Table 4: Incidence of positive transplants from popliteal nodes from rats with Walker 256 tumors in legs, with and without massage of the foot.

Effect of Massage on Spontaneous Metastasis of Walker 256 Tumour in the Leg		
	Massaged Side	Not Massaged
+ Node Transplants at 8 days	5/16	4/16
+ Node Transplants at 15 days	1/13	3/13
Total	6/29	7/29

Table 5: Recovery from right ventricle of bacteria following Serratia Marcescens injection into testicular lymphatics.

Serratia Marcescens in Bloodstream of Sprague-Dawley Rats Following Intralymphatic Injection		
Time	0 minutes	10 minutes
Right ventricular culture	7 ++++ 1 ++ 8 + 2 −	3 +++ 1 ++ 8 + 6 −

++++ More than 100 colonies per plate
+++ 50–100 colonies
++ 25– 50 colonies
+ 1– 25 colonies
− 0 colonies

which forms easily recognized red colonies on agar, was injected by testicular lymphatics and blood cultures were taken from the right ventricle immediately and at 10 minutes. This injection was then followed by mercury injection into the same channel, and animals were x-rayed. The results *(Table 5)* show that immediate heart cultures of only 2 of 18 animals were entirely negative, while 7 had massive bacteremia. The number of organisms fell markedly within 10 minutes presumably because of trapping in capillary beds. It would be logical to expect that those animals with the largest bacteria count in the right ventricle had direct communications between testicular lymphatics and thoracic duct, while the negative cultures came from animals with lymphatics terminating in abdominal nodes. In fact, x-rays showed that only 1 of the 7 +++ animals had a direct shunt to thoracic duct while in the other 6, the mercury went only to an abdominal node *(Fig. 1)*. Contrariwise, of 8 animals with only + cultures, 2 showed mercury entering the thoracic duct immediately *(Fig. 2)*. This is at variance with the impressive results of ENGESET (1959) who, using tumor cell injections, found that animals with direct communications to thoracic duct had tumour in lungs only; those with mercury reaching only abdominal nodes had metastases only in that area, while those in which channels to nodes and thoracic duct had pulmonary and nodal metastases. It is likely that smaller arteriovenous communications may be more accessible to bacteria than to large tumour cells, and in any case, it is also likely that the human pattern may be considerably different from the animal (see STRÄULI, 1970).

Current studies have centred on the fate of Walker 256 tumour cells reaching lymph nodes with a comprehensive and detailed examination of the histological features at various stages. It is remarkable how little electron microscopy of this process has been reported by others.

In all studies, the Walker 256 tumour was injected into testicular lymphatics of Sprague-Dawley rats. Cell suspensions were prepared by mincing and sedimenting solid tumour and 3×10^5 cells, (viable by Trypan blue exclusion) were injected. The inoculum was in 0.05 ml. of Hanks solution and was made over a period of at least 1 minute to avoid disruption of lymphatic channels or sinuses. The injected testicle was removed. A total of 120 rats were used, and sequential studies of regional nodes were made by light microscopy on 1 micron Epon and by TEM. Time intervals covered were 1 minute to 6 days, but only findings at intervals from 1 minute to 48 hours will be described here, since later times showed the development of massive confluent node involvement and interpretation became of limited value. Although other workers (JOB, 1915, ENGESET, 1959) reported that the ipsilateral renal

Fig. 1: X-ray of rat inject
as paraortic nodes and
ately after bacterial inject

node is the most com
injection that the ipsi
Both nodes were tak
ipsilateral posterior p
of each node and abo

The involvement o
number of tumour cel

Recognition of tum
trouble in identificat
Walker 256 and no
existed as to their ide

Fig. 1: X-ray of rat injected with mercury through the testicular lymphatics. The mercury goes only as far as paraortic nodes and yet this animal's right heart cultures were strongly positive for bacteria immediately after bacterial injection into the lymphatic channel.

node is the most common primary drainage of testicular lymphatics, we found by trypan blue injection that the ipsilateral posterior paraortic node was slightly predominant in our rats. Both nodes were taken from both sides from all rats, but the data below concern only the ipsilateral posterior paraortic nodes. Generally, 6 blocks were required for a complete study of each node and about 8 sections were examined from each block.

The involvement of various areas of the nodes was recorded with a rough estimate of the number of tumour cells present in each compartment (see *Fig. 3*).

Recognition of tumour cells was not difficult as a rule, but a few single cells caused some trouble in identification; TEM of the same area usually showed clear differences between Walker 256 and normal nodal cells. There remained one or two instances where doubt existed as to their identity and these cells were regarded as non-malignant.

Fig. 2: X-ray of another rat studied in same way as in Fig. 1. In this case, only a few bacterial colonies grew from heart culture but mercury is seen entering thoracic duct.

Table 6 summarizes the results of this study from 1 minute to 48 hours on the ipsilateral posterior paraortic nodes. The scores for cells present represents the *highest* for any of the blocks for each rat rather than the average, since it was found that nodal involvement was remarkably focal in early stages, and it was felt that the maximum involvement was the important point to identify. The focal involvement reflects the limited access the testicular channels have to a node, and also the temporary compartmentalization maintained in the subcapsular sinuses by trabeculae that slow down but do not totally prevent movement along the sinuses as shown by scanning electron microscopy (SEM) *(Fig. 4).*

The following points summarize the findings in Table VI and the observations made by light and electron microscopy:

Table 6: Observed incidenc
phatic of cells.

Walker 256 Cells in Lymph

Time	No. of Cells	Sub Sin
1, 3 & 5 minutes 18 rats	+++	12
	++	3
	+	3
	–	0
10, 15 & 30 minutes 18 rats	+++	10
	++	2
	+	4
	–	2
3, 6 & 12 hours 14 rats	+++	–
	++	2
	+	–
	–	1
24 hours 6 rats	+++	–
	++	–
	+	–
	–	–
48 hours 6 rats	+++	–
	++	
	+	
	–	

+++ >100 cells present
++ 25–100 cells pre

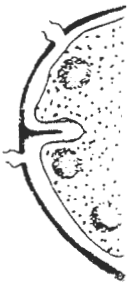


Fig. 3: Diagram showi
portion of the subcapsul

se, only a few bacterial colonies

to 48 hours on the ipsilateral
nts the *highest* for any of the
l that nodal involvement was
aximum involvement was the
e limited access the testicular
ntalization maintained in the
otally prevent movement along
ig. 4).
and the observations made by

Table 6: Observed incidence of Walker 256 cells in posterior paraortic lymph nodes following intralymphatic of cells.

Time	No. of Cells	Walker 256 Cells in Lymph Node Compartments: Subcapsular Sinus	Intermediate Sinus	Cortical Pulp	Medullary Sinus	Efferent Lymphatics	Extranodal Involvement
1, 3 & 5	+++	12	2	0	0	0	
minutes	++	3	5	1	0	0	0
18 rats	+	3	3	3	0	0	
	−	0	7	14	18	18	
10, 15 &	+++	10	7	0	0	0	
30 minutes	++	2	3	2	0	0	0
18 rats	+	4	5	8	0	0	
	−	2	3	8	18	18	
3, 6 & 12	+++	4	2	1	0	0	
hours	++	2	1	2	0	0	0
14 rats	+	8	8	7	0	0	
	−	0	3	4	14	14	
24 hours	+++	2	4	3	0	0	0
	++	2	0	1	0	0	2
6 rats	+	2	2	2	0	0	0
	−	0	0	0	6	6	4
48 hours	+++	4	4	4	0	1	0
	++	1	1	1	3	1	1
6 rats	+	0	1	1	0	1	0
	−	1	0	0	3	3	5

+++ >100 cells present
++ 25–100 cells present
\+ 1–25 cells present
− No cells identified

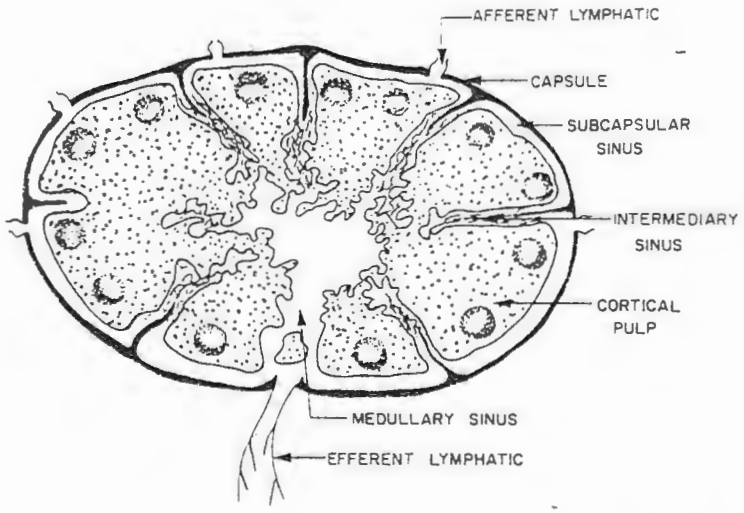


Fig. 3: Diagram showing the areas of lymph nodes assessed for tumor cells in Table VI. Only a small portion of the subcapsular sinus was usually entered by tumor cells from the testicular channels.

(1) There was immediate entrance into some compartments of the subcapsular sinuses by the tumour cells, along with some leukocytes and a small amount of debris.

(2) Tumor cells were able to move into some intermediary sinuses at early intervals. The nodes varied in degree of compactness and some channels to these sinuses permitted easy entry while in other cases cells were deformed and appeared to be entering by diapedesis *(Figs. 5 and 6)*.

(3) Occasional tumor cells invaded the cortical pulp from the sinuses at an early stage. These were usually scattered single cells and they moved in by diapedesis *(Figs. 7 and 8)*.

(4) Spread through the capsule of the node into surrounding fat occurred as early as 24 hours.

(5) Rapid proliferation of tumour cells occurred in the intermediary sinuses by 24 hours. This became more pronounced than that seen in the subcapsular sinuses and was accompanied by extensive invasion of cortical pulp *(Fig. 9)*.

(6) Medullary sinusoids were entered relatively late. Only by 48 hours was there definite entry of tumour cells into this compartment. By light microscopy the reticuloendothelial cells appeared to form an effective block until massive growth appeared to displace these cells. Once the medullary sinusoids were reached, the efferent lymphatics were readily entered *(Fig. 10 and 11)*.

It is difficult to exclude the possibility that rare single cells may have migrated through the compartments earlier, although the hundreds of sections examined might be expected to detect these if the occurrence was frequent. It appeared, in any case, that the sinusoidal lining cells and associated macrophages in the intermediary sinuses formed a remarkably effective barrier, broken down mainly by a growing mass of tumour cells. Even then, some tumour cells entering the medullary sinusoids still had reticulo-endothelial (R.E.) cells adherent to them. If the architecture of this area of the node is examined by SEM (as previously shown by FUJITA et al., 1972) these cells are seen to form a series of discontinuous plates or baffles *(Fig. 12)*. It is generally believed that these cells and their arrangement allow for a slow movement of leukocytes through the intermediary sinuses described as "percolation". This concept of a simple action of baffles, which would be even more effective against large tumour cells, probably explains some of the delay in reaching the medullary sinuses. Electron microscopy, however, revealed that the tumour cells were adhered to, and entangled and partially encircled by the cells in the sinuses in a way that may be much more impeding *(Fig. 13 and 14)*. The question of the importance of cell surface activity by the R.E. cells and its effect on tumour cells is thus raised. Attention is further drawn to this by the fact that at 24 hours, nodes of 4 of 6 rats showed striking rosette formation between the R.E. cells and erythrocytes that were present in the nodes, apparently associated with small hemorrhages *(Fig. 15)*. By TEM *(Fig. 16)* it is seen that the cells do not form the typical rosette seen with T lymphocytes and sheep erythrocytes (MANDACHE et al., 1978) but display very long processes more reminiscent of a phagocytic function. The tumour cells do not appear to be directly involved in the process.

Rosette formation by R.E. cells is known to occur (PARKES & STUART, 1978) and does tend to resemble our present findings more than that seen with T cells. Also, rosette formation with autochthonous RBC has been described (CHARRIERE & BACH, 1975, KOLB, 1978, PARKES & STUART, 1978) but the rosettes are usually unstable. We have been unable to find any description of these occurring in tissue sections although they apparently are known to occur in vivo (DUFFUS & ALLEN, 1971); nor can we find reports of the presence of this phenomenon in lymph nodes containing tumour. Since the phenomenon occurs rapidly in an animal with no primary tumour, it is unlikely to represent an immunological mechanism of the usual type. Sinus hyperplasia has been reported in tumourous nodes and has given rise to much interest in human tumours, especially breast cancer. In experimental models, it has been reported

Fig. 4: SEM photograph o solution through lymphati partially divided by trabec ment. × 300.

Fig. 5: Light microscopy lymphatic injection. They diapedesis. × 480.

subcapsular sinuses by
bris.
s at early intervals. The
sinuses permitted easy
ring by diapedesis (*Figs.*

nuses at an early stage.
esis (*Figs. 7 and 8*).
occurred as early as 24

ary sinuses by 24 hours.
sinuses and was accom-

hours was there definite
ne reticuloendothelial cells
ed to displace these cells.
s were readily entered (*Fig.*

have migrated through the
ned might be expected to
e, that the sinusoidal lining
med a remarkably effective
s. Even then, some tumour
ial (R.E.) cells adherent to
EM (as previously shown by
continuous plates or baffles
angement allow for a slow
ribed as "percolation". This
more effective against large
e medullary sinuses. Electron
nered to, and entangled and
nay be much more impeding
activity by the R.E. cells and
n to this by the fact that at 24
n between the R.E. cells and
ated with small hemorrhages
the typical rosette seen with T
out display very long processes
do not appear to be directly

& STUART, 1978) and does tend
ls. Also, rosette formation with
, 1975, KOLB, 1978, PARKES &
been unable to find any descrip-
ntly are known to occur in vivo
resence of this phenomenon in
rs rapidly in an animal with no
l mechanism of the usual type.
nas given rise to much interest in
models, it has been reported

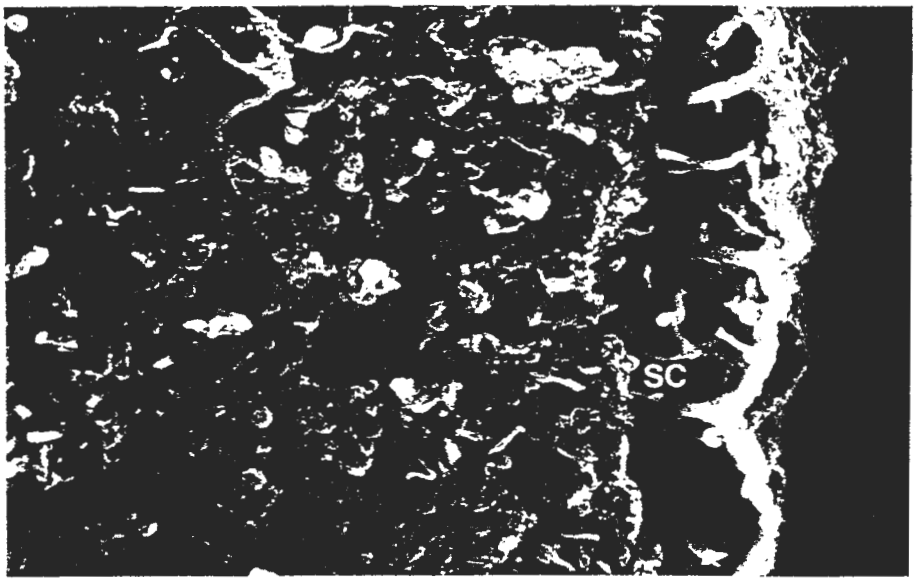


Fig. 4: SEM photograph of capsular area of rat posterior peritoneal lymph node, flushed with Hanks' solution through lymphatic vessels before fixation. It can be seen that the subcapsular sinuses (SC) are partially divided by trabeculae that inhibit but do not eliminate movement in the subcapsular compartment. × 300.

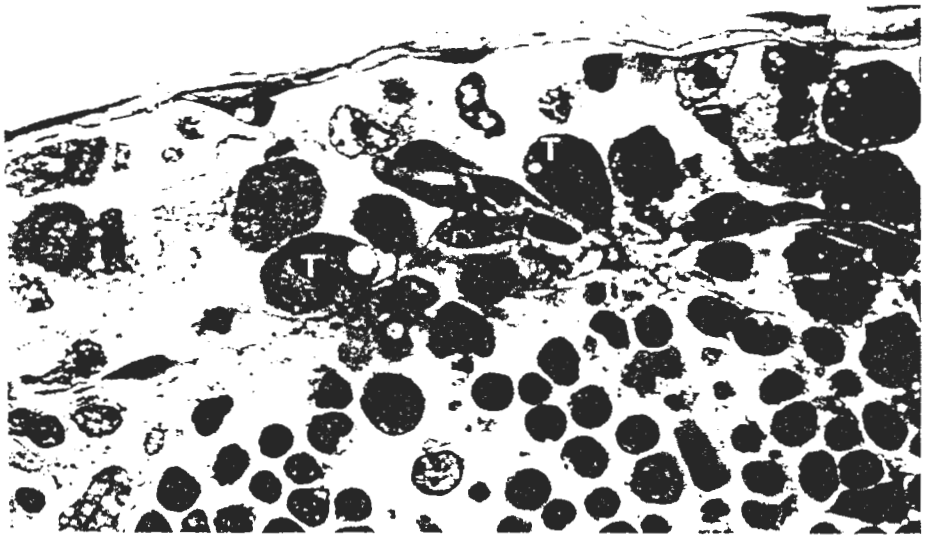


Fig. 5: Light microscopy of Walker 256 tumor cells (T) leaving subcapsular sinus at 1 hour after lymphatic injection. They are extending pseudopods and appear to be entering intermediate sinuses by diapedesis. × 480.

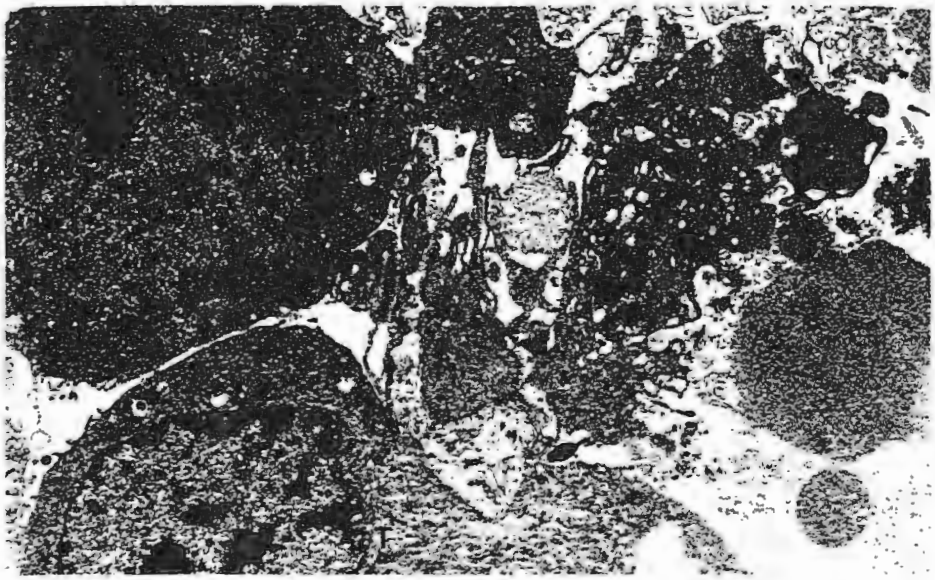


Fig. 6: TEM field of 2 tumor cells (T) passing from subcapsular sinus by diapedesis. × 4875.

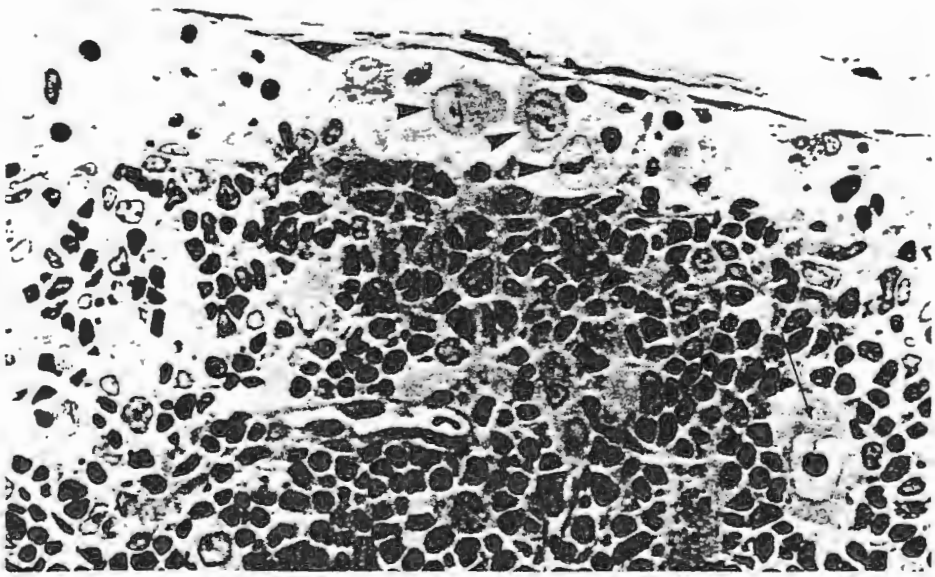


Fig. 7: Light microscopy of Walker cells (arrowheads) in subcapsular sinus at 6 hours. A single tumor cell (arrow) is clearly within cortical pulp at this time. × 300.

Fig. 8: TEM photograph o
× 12750.

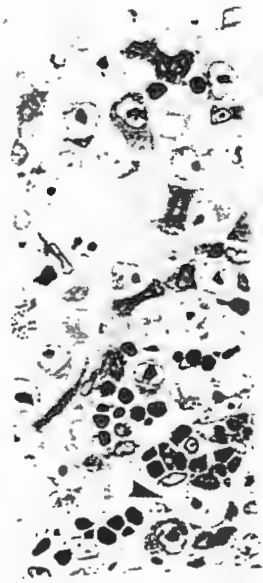
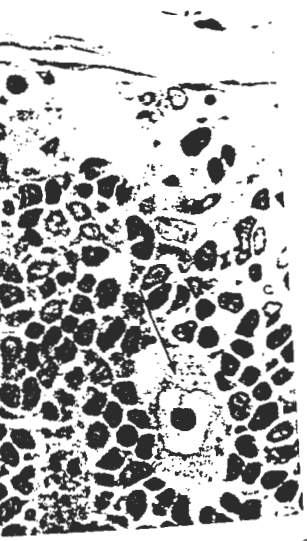


Fig. 9: Light microscopy
zones, the boundary of co
into cortical pulp (arrowhe

apedesis. × 4875.



r sinus at 6 hours. A single tumor cell

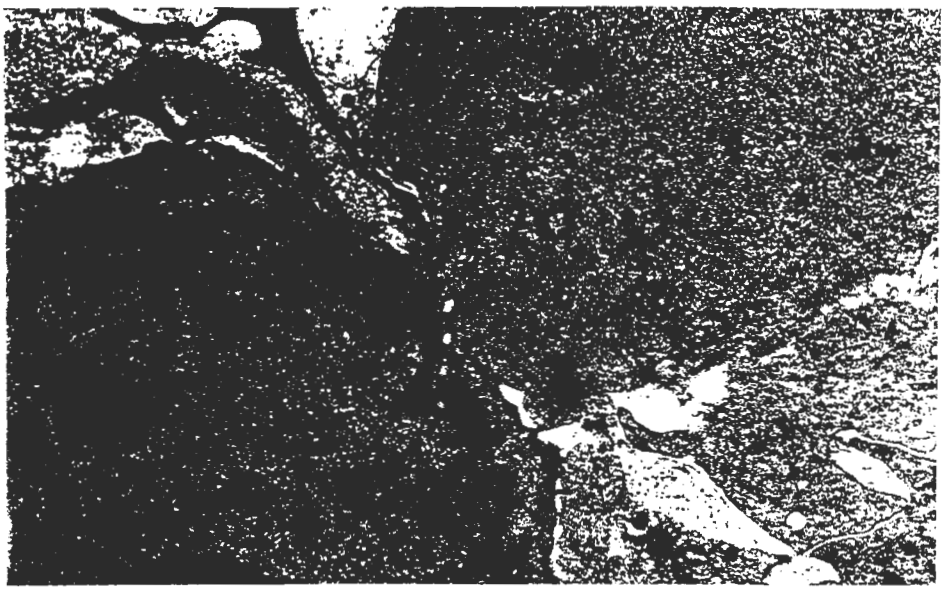


Fig. 8: TEM photograph of tumor cell (T) passing from subcapsular sinus into cortical pulp by diapedesis. × 12750.

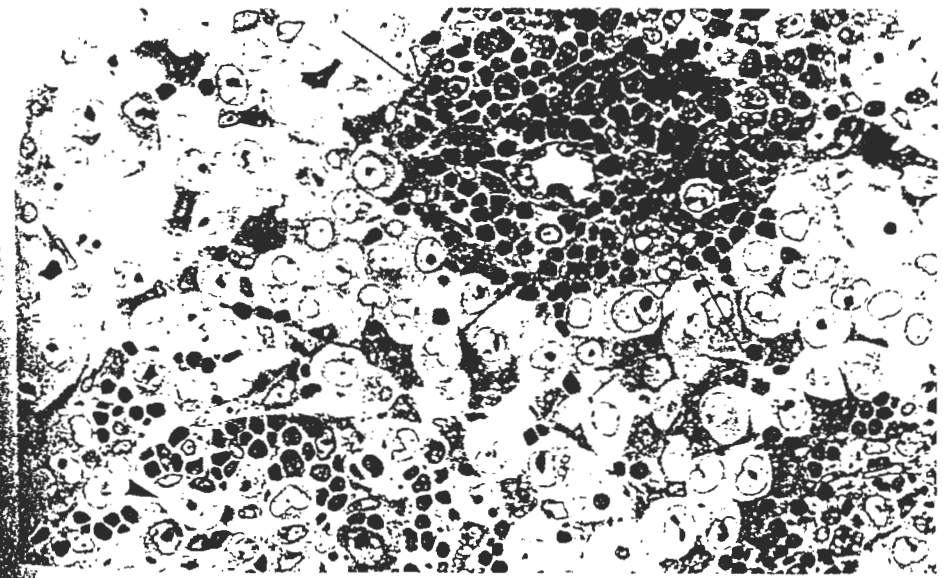


Fig. 9: Light microscopy showing massive involvement of intermediate sinuses by 24 hours. In some zones, the boundary of cortical pulp is still intact (arrows) but in other areas there is entry of tumor cells into cortical pulp (arrowheads). × 300.

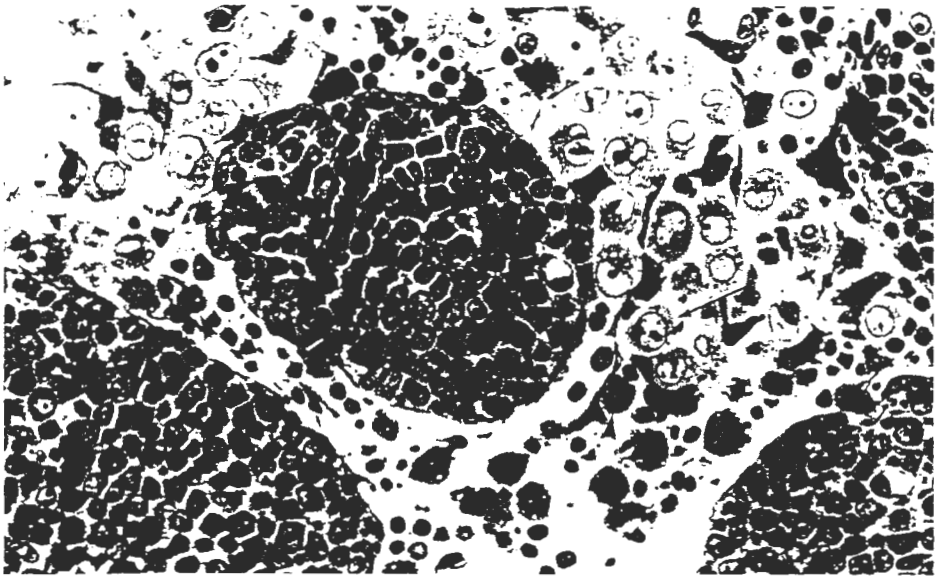


Fig. 10: Light microscopy of junction of intermediate and medullary sinuses at 48 hours. Tumor cells (arrows) have nearly reached the latter, but are still impeded by R. E. cells (arrowheads). × 300.

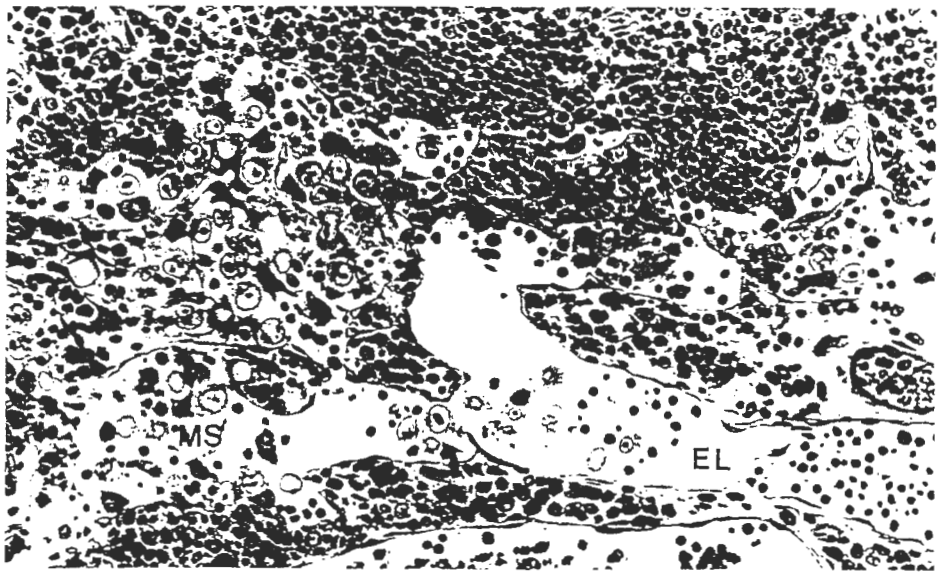


Fig. 11: Light microscopy at 48 hours showing tumor cells reaching medullary sinuses (MS) and efferent lymphatic (EL). Some tumor cells have adherent R. E. cells (arrows). × 120.

Fig. 12: SEM photograph
and sinusoidal lining cells
450.

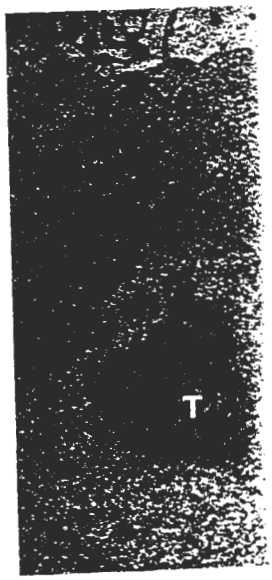


Fig. 13: Walker tumor cel
the latter appear to adher

uses at 48 hours. Tumor cells
rrowheads). × 300.

edullary sinuses (MS) and efferent
120.

Fig. 12: SEM photograph of intermediate sinus of posterior peritoneal node of a rat. The macrophages and sinusoidal lining cells (S) form a series of baffles, as previously described by Fujita et al. (1972). × 450.

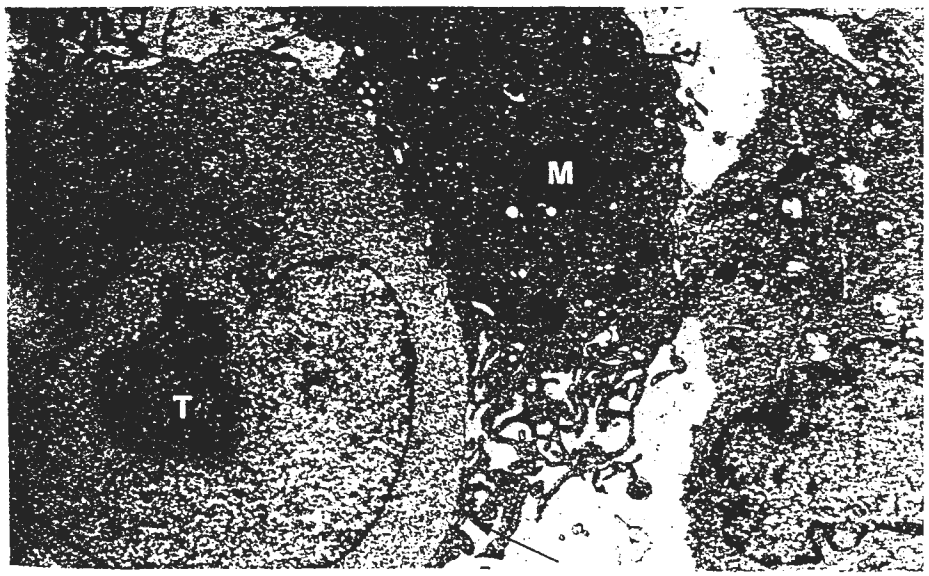


Fig. 13: Walker tumor cell (T) with adjacent macrophage-type of cell (M). Many long cell processes from the latter appear to adhere to the tumor cell at many points (arrows). × 4875.

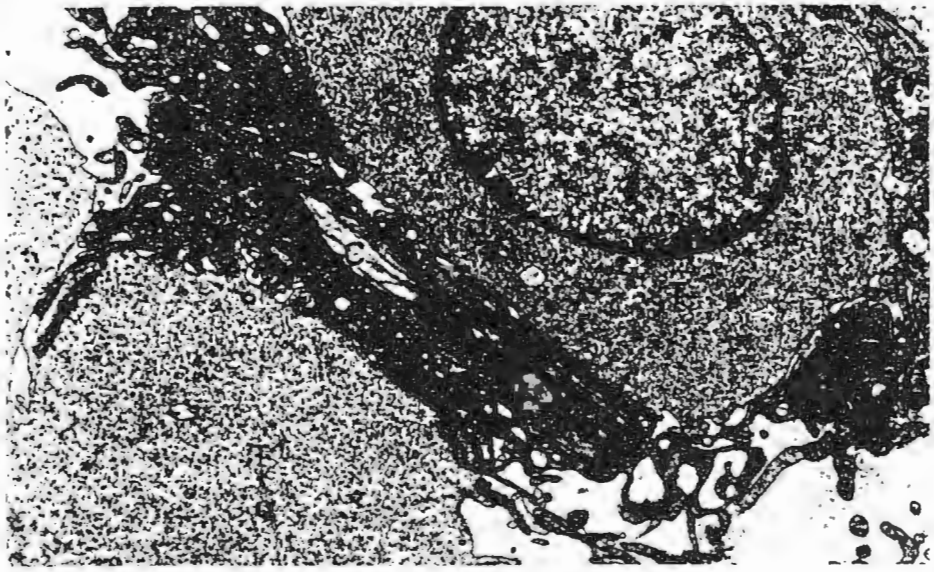


Fig. 14: Two Walker tumor cells (T) almost enveloped by the processes of one or more R.E. cell. The presence of a collagen trabecula (C) indicates that the cell in this area is a sinusoidal lining cell. × 7875.

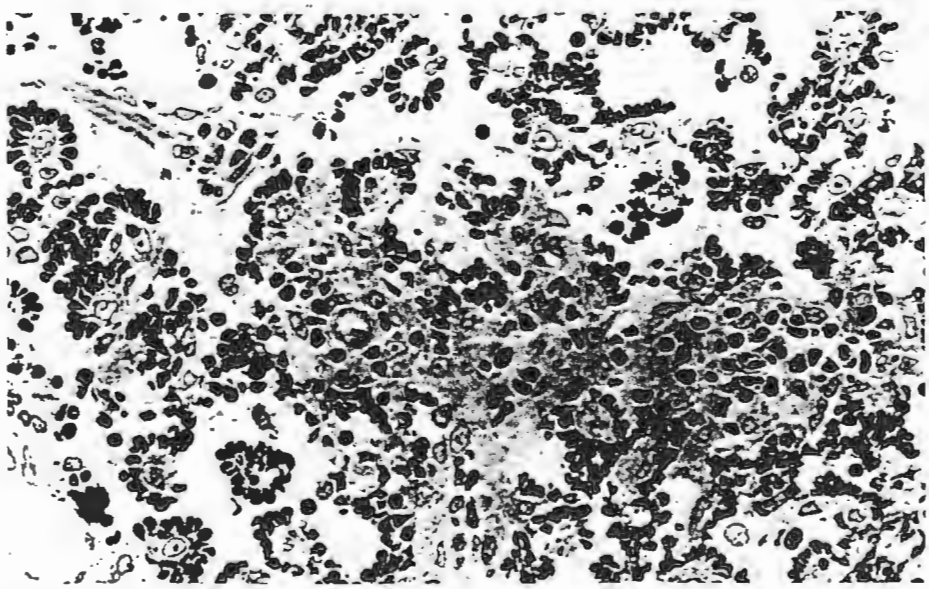


Fig. 15: Light microscopy of node near junction of intermediary and medullary sinuses at 24 hours. Red blood cells are forming rosettes with the sinusoidal lining cells and macrophages. Tumor was present in the node adjacent to this field but the tumor cells did not directly participate in the process. × 120.

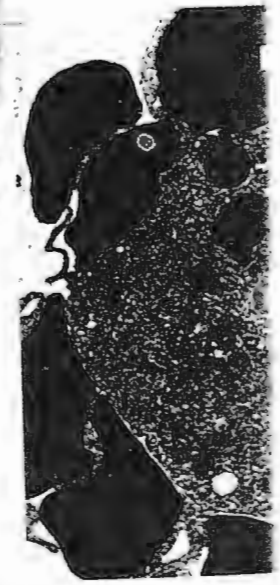
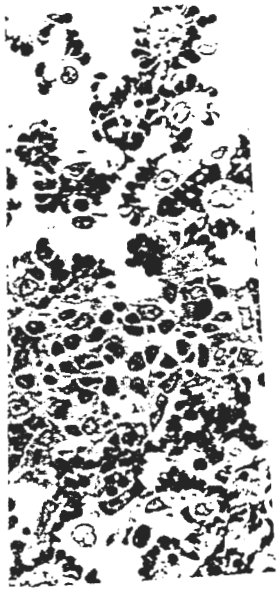


Fig. 16: TEM of same blo
(M) and a sinusoidal linin
with T cell rosettes. × 431

(KUROKAWA et al., 19
study.

The observations on
with those of ZEIDMA
pelvic nodes, of LUDW
labelled cells, of CARR
foot tumour transplan
took a matter of many
with those of FISHER
tumour cells in fluid fr
label from nodes, of K
identify large number
BLAKE, (1975) who re
tion of nodal metastas
were completely elim
suggest at least that th
may depend on the pr
may warrant further e

t one or more R.E. cell. The
ısoidal lining cell. × 7875.



edullary sinuses at 24 hours. Red
crophages. Tumor was present in
pate in the process. × 120.

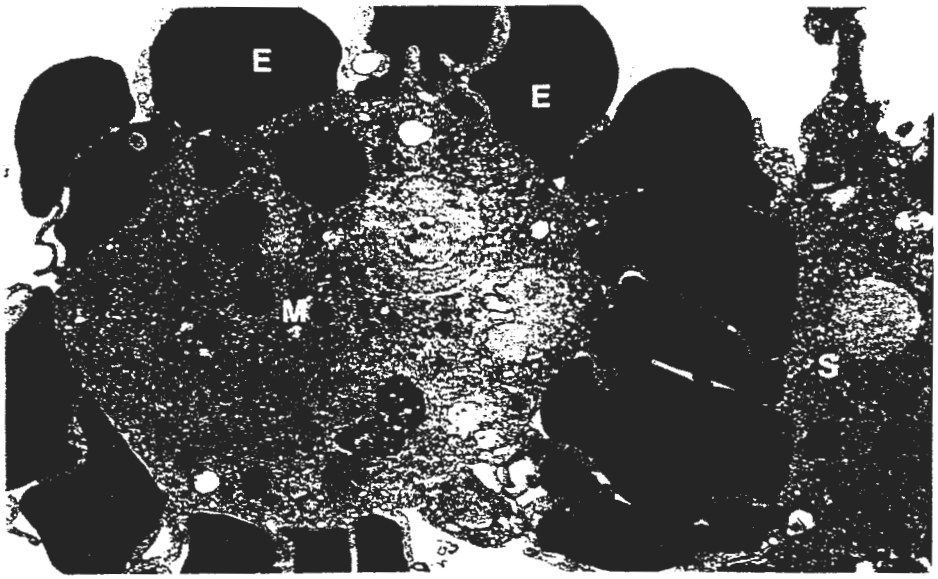


Fig. 16: TEM of same block seen in Fig. 15. Erythrocytes (E) are forming a rosette with a macrophage (M) and a sinusoidal lining cell (S). The long processes partially surrounding the red cells are not seen with T cell rosettes. × 4310.

(KUROKAWA et al., 1970, CARR et al., 1976) but was not seen to any extent in the present study.

The observations on tumour cell passage to medullary sinuses are more or less in keeping with those of ZEIDMAN & BUSS (1954) who measured the time of spread from popliteal to pelvic nodes, of LUDWIG & TITUS, (1967) who used intralymphatic injections of radiolabelled cells, of CARR and MCGINTY, (1974) who studied spontaneous metastases from a foot tumour transplant and of KUROKAWA, (1970); all of whom reported that tumour cells took a matter of many hours or days to reach efferent channels. The findings are at variance with those of FISHER and FISHER, (1966 and 1967) who reported visual identification of tumour cells in fluid from efferent lymphatics in a matter of minutes and rapid loss of radiolabel from nodes, of KOHNO et al., (1979) who used both labelling and transplant methods to identify large numbers of tumour cells in thoracic duct within 3 hours, and of HEWITT & BLAKE, (1975) who reported continuous transnodal passage of tumour cells without formation of nodal metastases. It is not clear in the first two of these reports whether nodal bypasses were completely eliminated as a means of reaching efferent channels. The present studies suggest at least that there is a partial barrier to lymph node migration by tumour cells that may depend on the properties of the sinusoidal lining cells and macrophages. Their properties may warrant further examination.

Summary

1. Any animal experiment of any type on lymph node metastases may be of limited relevance to human cancer; the use of transplanted tumors, of induced metastases, and of allogeneic systems may also reduce their validity. None-the-less, useful information may be obtained if it is interpreted cautiously.
2. The pattern and extent of lymphatic metastases varies not only with the anatomy of different species but of individual animals and with different tumors. Important factors influencing the process may be masked by highly malignant tumors and large tumor doses.
3. Sequential studies of induced lymphatic spread of Walker 256 tumor in Sprague Dawley rats are described. The progress of tumor cells through the nodes from afferent channels was followed using light and electron microscopy. There appeared to be a substantial barrier to entrance into medullary sinuses, involving the reticulo-endothelial cells. It is suggested that the shape, arrangement and surface properties of these cells may be important in impeding transnodal spread. Their effectiveness probably varies with the individual host and tumor.

Acknowledgements

This work was supported by grants-in-aid from the National Cancer Institute of Canada and the Petherick Fund, University of Western Ontario. The skillfull technical assistance of Mrs. Elaine Hunter is gratefully acknowledged.

References

Barnes, J.M. and Trueta, J.: «Absorption of bacteria, toxins and venoms from the tissues». Lancet *1:* 623–626 (1941).

Carr, I. and McGinty, F.: «Lymphatic small metastases and its inhibition: An experimental model». J. Path. *113:* 85–95 (1974).

Carr, I., Price, P. and Westby, S.: «The effects of tumor extract on macrophage proliferation in lymph nodes». J. Path. *120:* 251–254 (1976).

Charriere, J. and Bach, J.F.: «Binding of autologous erythrocytes to immature T-cells». Proc. Nat. Acad. Sc. U.S.A. *72:* 3201–3205 (1975).

Chew, E.C. and Wallace, A.C.: «Demonstration of fibrin in early stages of experimental metastases». Cancer Res. *36:* 1904–1909 (1976).

Duffus, W.P.H. and Allan, D.: «The kinetics and morphology of the rosette-forming cell response in the popliteal lymph nodes of rats». Immunology *20:* 345–361 (1971).

Engeset, A.: «An experimental study of the lymph node barrier – injection of Walker Carcinoma 256 in the lymph vessels». Acta Un. Intern. Contre le Cancer *15:* 879–883 (1959).

Fisher, B. and Fisher, E.R.: «Transmigration of lymph nodes by tumor cells». Science *152:* 1397–1398 (1966).

Fisher, B. and Fisher, E.R.: «Barrier Function of lymph nodes to tumor cells and erythrocytes. I. Normal nodes». Cancer *20:* 1907–1913 (1967).

Fujita, T., Miyoski, M. and Murakami, T.: «Scanning electron microscope observation of the dog mesenteric lymph node». Z. Zellforsch. *133:* 147–162 (1972).

Hewitt, H.B.: «Projecting from animal experiments to clinical cancer. *In* L. Weiss (Ed.) Fundamental Aspect of metastases». Amsterdam, North Holland Publ. Co., 343–357, 1976.

Hewitt, H.B. and Blake: «Quantitative Studies of translymphnodal passage of tumor cells naturally disseminated from a non-immunogenic murine squamous carcinoma». Brit. J. Cancer *31:* 25–35 (1975).

Imai, R., Ashizawa, T. an
Yoshida sarcoma inocula
Job, T.T.: «The adult anat
9: 447–458 (1915).
Job, T.T.: «Lymphatic-ver
Anat. *24:* 467–491 (191
Jones, D.S. and Wallace, A.
Assn. Cancer Res. *10:* 44
Jones, D.S., Wallace, A.C.
256 tumor: light, immu
46: 493–504 (1971).
Kohno, K., Yamaguchi, T
through the lymph node
Kolb, H.: «Characteristics
erythrocytes». J. Cellular
Kurokawa, Y.: «Experime
tumor cells, with special
Gann *61:* 461–471 (197
Ludwig, J. and Titus, J.L.:
(1967).
Mandache, E., Moraru, I.
patterns of indicator cel
91–97 (1978).
Marsh, M.C.: «Tumor ma
McMaster, P.D.: «Change
tions». J. Exper. Med. 6
Onuigbo, W.I.B.: «Histor
(1972).
Parkes, A.C. and Stuart, A
ous erythrocytes in hum
Stokes, T.A.M.: «The effe
lymphatic system». Brit.
Sträuli, P.: «The barrier f
Eds. Bern., Hans Huber
Takazawa, H. and Shimiz
403–406 (1976).
Tjernberg, B. and Zajicek,
spread». Acta Cytologic
Van de Velde, C. and Carr
Wallace, A.C.: «The occ
724–732 (1956).
Wallace, A.C. and Hollen
tic routes». Brit. J. Canc
Warren, B.A.: «Environm
(1973).
Zeidman, I.: «Comparison
Proc. Amer. Assn. Canc
Zeidman, I. and Buss, J.
Effectiveness of the lym
403–405 (1954).

Author's address:
Dr. A.C. Wallace, Dept. P

Imai, R., Ashizawa, T. and Nakamura, N.: «An experimental postoperative metastasis system using Yoshida sarcoma inoculation subcutaneously into footpad». Gann *66:* 69–73 (1975).
Job, T.T.: «The adult anatomy of the lymphatic system in the common rat». (E. Norvegicus) Anat. Rec. *9:* 447–458 (1915).
Job, T.T.: «Lymphatic-venous communications in the common rat and their significance». Amer. J. Anat. *24:* 467–491 (1918).
Jones, D.S. and Wallace, A.C.: «A study of fibrin in transplanted and metastatic tumors». Prov. Soc. Am. Assn. Cancer Res. *10:* 44 (1969).
Jones, D.S., Wallace, A.C. and Fraser, E.E.: «Sequence of events in metastases of experimental Walker 256 tumor: light, immunofluorescent, and electron microscopic observations». J. Nat. Cancer Mat. *46:* 493–504 (1971).
Kohno, K., Yamaguchi, T. and Takahashi, T.: «An experimental study of the spread of tumor cells through the lymph node». Tohuku, J. Exper. Med. *127:* 183–188 (1979).
Kolb, H.: «Characteristics of spontaneous rosette formation by mouse erythrocytes with autologous erythrocytes». J. Cellular Immunol. *37:* 127–133 (1978).
Kurokawa, Y.: «Experiments on lymph node metastasis by intralymphatic inoculation of rat ascites tumor cells, with special reference to lodgement, passage and growth of tumor cells in lymph nodes». Gann *61:* 461–471 (1970).
Ludwig, J. and Titus, J.L.: «Experimental tumor cell emboli in lymph nodes». Arch. Path. *84:* 304–311 (1967).
Mandache, E., Moraru, I., Sjoquist, J. and Ghetie, V.: «II. Ultrastructural differences in attachment patterns of indicator cells to target cells in the rosette-forming process». J. Immunolog. Methods *22:* 91–97 (1978).
Marsh, M.C.: «Tumor massage and metastases in mice». J. Cancer Res. *11:* 101–107 (1927).
McMaster, P.D.: «Changes in human cutaneous lymphatic flow under normal and pathological conditions». J. Exper. Med. *65:* 347–352 (1937).
Onuigbo, W.I.B.: «Historical data on the dynamics of lymphatic metastases». Cancer Res. *26:* 505–514 (1972).
Parkes, A.C. and Stuart, A.E.: «Ultrastructural studies of leukocytes which form rosettes with homologous erythrocytes in human autoimmune hemolytic anemia». Scand. J. Hematol. *20:* 129–140 (1978).
Stokes, T.A.M.: «The effect of cortisone therapy and limb exercise on the dissemination of cancer via the lymphatic system». Brit. J. Cancer *23:* 132–135 (1969).
Sträuli, P.: «The barrier function of lymph nodes». *In* Surgical Oncology. F. Saegesses and J. Pettavel, Eds. Bern., Hans Huber 1970. P. 161–176.
Takazawa, H. and Shimizu, S.: «An experimental model for lymphatic metastases in rats». Gann *67:* 403–406 (1976).
Tjernberg, B. and Zajicek, J.: «Cannulation of lymphatics leaving cancerous nodes in studies on tumor spread». Acta Cytologica *9:* 197–202 (1965).
Van de Velde, C. and Carr, I.: «Lymphatic invasion and metastases. Experientia *33:* 837–843 (1977).
Wallace, A.C.: «The occurrence of metastases in a group of related rat tumors». Brit. J. Cancer *10:* 724–732 (1956).
Wallace, A.C. and Hollenberg, N.K.: «The transplantability of tumors by intravenous and intralymphatic routes». Brit. J. Cancer *19:* 338–342 (1965).
Warren, B.A.: «Environment of the blood-borne tumor embolus». J. Med. Exper. and Clin. *4:* 150–177 (1973).
Zeidman, I.: «Comparison of metastatic growth produced by tumor cell emboli in veins and lymphatics». Proc. Amer. Assn. Cancer Res. *6:* 71 (1965).
Zeidman, I. and Buss, J.M.: «Experimental studies on the spread of cancer in the lymphatic system I. Effectiveness of the lymph node as a barrier to the passage of embolic tumor cells». Cancer Res. *14:* 403–405 (1954).

Author's address:
Dr. A.C. Wallace, Dept. Pathology, University of Western Ontario, London/Ont. N6A 5C1, Canada