Virchows Arch [Pathol Anat] (1982) 398:33–43

*Virchows Archiv A*


# Divergency Between Incidence of Microscopic and Macroscopic Metastases*

R. Keller[1] and M.W. Hess[2]

[1] Immunobiology Research Group, Institute of Immunology and Virology, University of Zürich, Schönleinstrasse 22, CH-8032 Zürich, Switzerland

[2] Institute of Pathology, University of Bern, Freiburgstrasse 30, CH-3008 Bern, Switzerland

**Summary.** In a rat fibrosarcoma model (D-12), the incidence of macroscopic metastases was generally low but critically dependent on the site of the primary tumor implant; surgical removal of the primary tumor either induced or markedly enhanced the outgrowth of macroscopic metastases (Keller 1981). The present histological and biological findings indicate that dissemination of neoplastic cells and colonization of draining lymph nodes is a spontaneous, early occurring and continuing process. The incidence of micrometastases within lymph nodes by far exceeded the incidence of macroscopically evident metastases. Other evidence suggests that the growth characteristics of D-12 tumor cells derived from metastases are not measurably different from D-12 cells inducing primary tumor growth.

**Key words:** Metastasis – Lymph nodes – Tumor invasion – Tumor resistance

## Introduction

Most cancer treatment failures are not attributable to the locally growing primary tumor but rather to the inability to control its systemic spread and its establishment at secondary sites (Willis 1973; Sugarbaker and Ketcham 1977; Weiss 1977; Roos and Dingemans 1980). This experience is valid not only for highly malignant neoplasms of the lung, pancreas or stomach but apparently also for tumors with a lower degree of malignancy (Papaioannou 1981). In fact, what clinically appears to be a localized process is often already systemic at the time the primary tumor is diagnosed (Willis 1973; Weiss 1977; Mueller et al. 1978; Langlands et al. 1979; Sugarbaker 1979; Henderson and Cavellos 1980).

*Offprint requests to:* R. Keller at the above address

* This work was supported by the Swiss National Science Foundation (grants 3.173.77, 3.609.80 and 3.578.79) and the Canton of Zürich.

0340-1227/82/0398/0033/$02.20

Varied clinical and experimental evidence points toward detachment of tumor cells and their subsequent entry into the vascular and/or lymphatic tree as a continuing process (Griffith and Salsbury 1965; Griffith et al. 1973; Woodruff 1980). However, the likelihood of metastasis developing from cells gaining entry to the blood stream is rather small (Hewitt and Blake 1975; Van den Brenk et al. 1975; Woodruff 1980). It is noteworthy that surgical and other forms of intervention facilitate the development of tumors following intravenous injection of tumor cells at the site of the trauma and elsewhere (Fisher and Fisher 1959a; Robinson and Hoppe 1962; Alexander and Altemeier 1964; Vaitkevicius et al. 1965; Van den Brenk et al. 1974) as well as promote the outgrowth of metastases (Tyzzer 1912; Agosin et al. 1952; Crile 1956; Schatten 1958; Fisher and Fisher 1959b, 1965; Robinson and Hoppe 1962; Romsdahl 1964; Agostino and Cliffton 1964; Boeryd and Rudenstam 1967; Eccles and Alexander 1975; Lundy et al. 1979; Sugarbaker 1979; Woodruff 1980).

Earlier work in this laboratory with a dimethylbenz(a)anthracene-induced DA rat fibrosarcoma (D-12) has shown that subcutaneous, intramuscular or intraperitoneal inoculation of D-12 cells into the syngeneic host leads to progressive local tumor growth and to the demise of the host (Keller 1980a, 1980b, 1981). When interacted in vitro, D-12 cells are effectively killed by *C. parvum*-induced, activated macrophages but are resistant to NK-type killing (Keller 1980b). In the D-12 model, the extent to which spontaneous macroscopic metastases emerged was dependent on the site of the primary tumor cell inoculum (Keller 1981). Subcutaneous implantation of the primary inoculum into the back resulted only in progressive local tumor growth; macroscopic metastases emerged rarely. However, inoculation of these fibrosarcoma cells into the thigh frequently led to the outgrowth of macroscopic metastases in the draining lymph nodes; infiltration of lymph nodes by the progressively growing primary tumor was also observed. Metastases in other tissues were seldom found. The marked dependence of the incidence of macroscopic lymph node metastases on the site of the primary tumor and the rapidity with which these metastases emerged after surgical removal of the primary tumor was taken as indicating that invasion of the regional lymphatic system by tumor cells is an ongoing process, quite independent of surgical intervention (Keller 1981). It has moreover been argued that both the number and the rate at which tumor cells enter the regional lymph nodes is a critical factor in determining whether the secondary tumor cell spread will emerge (Keller 1981). The present work has further investigated this issue, utilizing histological and biological methods.

## Material and Methods

Inbred DA rats weighing 120–180 g were inoculated subcutaneously, either on the back or on the left thigh, with $10^4$ or $5 \times 10^4$ DMBA-induced, syngeneic fibrosarcoma cells, passaged in vivo in ascites form (D-12; Keller 1977 and 1981).

To ascertain the growth characteristics of the metastasizing tumor cells, cells derived from

macroscopically detectable lymph node metastases were suspended and approximately $5 \times 10^4$ tumor cells per animal were inoculated s.c. into the back of normal rats.

To determine whether tumor cells were introduced inadvertently directly into ruptured blood or lymph vessels during inoculation (Stackpole 1981), $5 \times 10^4$ D-12 cells were injected subcutaneously into the left thigh. After 1 h, the injected area was removed by amputation of the leg and the animals examined for macroscopic metastases after 4 weeks. Other animals were killed 1 h after the s.c. inoculation of $5 \times 10^4$ tumor cells into the left thigh; the inguinal, retroperitoneal and axillary lymph nodes were removed and the cell suspension prepared from the pooled lymph nodes of an individual animal were inoculated intraperitoneally into a control rat, and progressive ascites tumor growth assessed.

The eventual occurrence of metastatic tumor cell spread prior to surgery was investigated further in animals bearing primary tumor inocula in the thigh or on the back, utilizing histological and biological criteria.

*Histological Assessment of the Presence of Tumor Cells in Draining Lymph Nodes at Various Stages of Primary Tumor Growth.* Inguinal, retroperitoneal and axillary lymph nodes from animals with primary tumor cell inocula were obtained at various phases of primary and secondary tumor growth. Excised nodes, one to three per site and animal, were fixed in buffered, neutral (pH 7.4) 4% formaldehyde, embedded in methylmethacrylate, and sectioned on a Sorvall JB-4 microtome. Sections, 4 μm thick, were stained with Giemsa solution and examined for the presence of tumor cells by light microscopy.

*Biological Assessment of Viable Tumor Cells Within Draining Lymph Nodes.* Inguinal, retroperitoneal and axillary lymph nodes of macroscopically normal appearance were obtained at various intervals after subcutaneous inoculation of $10^4$ D-12 cells into the left thigh. Cell suspensions, prepared from the lymph nodes of individual animals were pooled, inoculated intraperitoneally into individual control rats and ascites tumor growth assessed. Repeated parallel experiments, in which increments of D-12 cells were inoculated i.p. into controls, had shown that $10^2$ to $5 \times 10^2$ D-12 cells sufficied to induce progressive growth of the ascites tumor and thus lead to the death of the host (Fig. 2A). Accordingly this was considered a reliable and sensitive test to detect functionally intact, live tumor cells.

## Results

In the present experimental model, varied findings suggested that macroscopic metastases, appearing rapidly after surgical intervention, were a consequence of the outgrowth of tumor cells present in lymphoid tissue prior to surgery, rather than to an induction of the secondary spread of cancer cells by surgery (Keller 1981). To assess this issue further, animals were inoculated subcutaneously with $10^4$ or $5 \times 10^4$ D-12 cells and the occurrence of metastases assessed after varying intervals, utilizing both histological and biological methods.

### *Histological Evidence for the Early and Continuing Lymphatic Spread of Tumor Cells*

In lymph nodes with metastases that were evident macroscopically, the parenchyma was consistently replaced by solid tumor tissue. However, even in lymph nodes of normal size and appearance, taken at different stages of progressive primary tumor growth, tumor cells could be identified in between 20 and more than 40% of the lymph nodes examined (Table 1). Tumor cells were present even in regional lymph nodes from animals with small primary tumor ($\sim$2 mm). Three grades of lymph node involvement could be distinguished (cf. Table 1):

grade 1: presence of individual tumor cells in marginal sinuses (Fig. 1a);
grade 2: presence of solid tumor confined to marginal sinuses and outer cortical area (Fig. 1b);
grade 3: lymph node parenchyma largely (Fig. 1c) or totally replaced by solid tumor tissue (Fig. 1d).

**Table 1.** Histological evidence for the presence of tumor cells in lymph nodes manifesting a macroscopically normal appearance. Animals inoculated subcutaneously in the back with $5 \times 10^4$ D-12 cells and developing progressive local tumor growth without macroscopic metastases were sacrificed preterminally (i.e. 6–12 weeks later), and the lymph nodes obtained. In animals inoculated with $10^4$ D-12 cells into the thigh, lymph nodes with normal appearance were obtained when the primary tumors had reached a size between 2 and 7 mm

| Site of tumor cell inoculation | Lymph nodes | Proportion of lymph nodes with tumor cells | Gradation of tumor cell involvement[a] | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 |
| Back | ALN[b] | 7/16 | 9 | 4 | 1 | 2 |
| | ILN | 2/18 | 16 | 2 | – | – |
| | RLN | 2/12 | 10 | 2 | – | – |
| Hind leg | ALN | 2/14 | 12 | 1 | – | 1 |
| | ILN | 9/17 | 8 | 2 | 1 | 6 |
| | RLN | 4/14 | 10 | 2 | 1 | 1 |

[a] See text
[b] ALN: axillary lymph nodes; ILN: inguinal lymph nodes; RLN: retroperitoneal lymph nodes

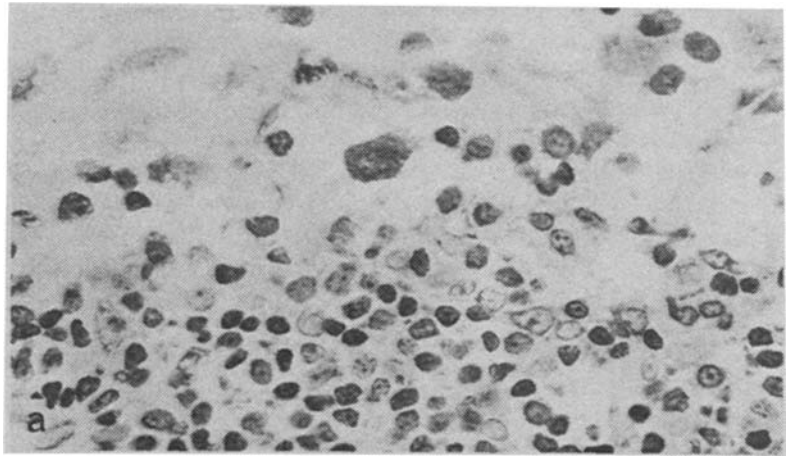


**Fig. 1a–d.** Grade of tumor cell involvement in normally appearing inguinal lymph nodes of DA rats. Tissue specimens were obtained three weeks after s.c. implantation of D-12 fibrosarcoma cells into the left thigh (methacrylate sections, 4 µm, Giemsa staining): **a** *grade 1:* Single tumor cell in marginal sinus (× 425). **b** *grade 2:* Marginal sinus distended and containing large numbers of pale-staining tumor cells; solid tumor growth in lymph node cortex (× 125). **c** *grade 3:* Large portion of lymph node parenchyma replaced by solid tumor tissue (× 125). **d** *grade 3:* Total replacement of lymph node parenchyma by solid tumor tissue (× 425)

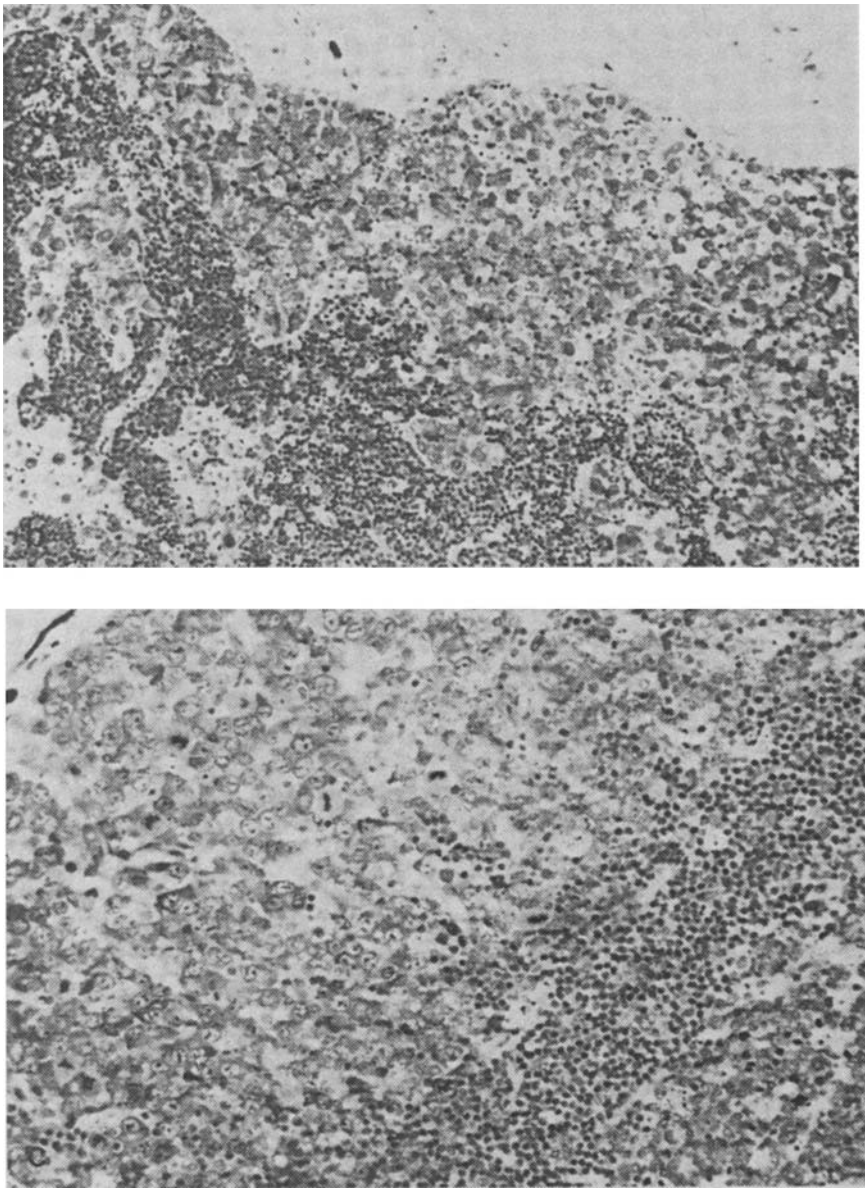

Fig. 1

In rats receiving primary tumor inocula in the back or in the hind thigh, approximately 50% of the regional (axillary or inguinal) lymph nodes contained tumor cells (Table 1). In rats with tumor inocula in the thigh, lymph nodes more remote from the primary tumor site were colonized by tumor cells more frequently.

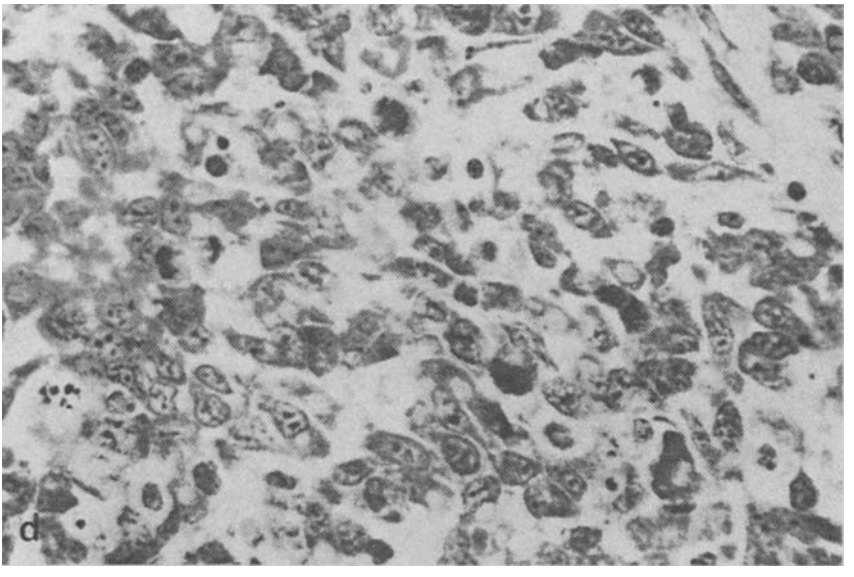

Fig. 1

*Biological Evidence That Spontaneous Secondary Spread of Tumor Cells Occurs Prior to Surgery*

*No Evidence That Tumor Cells Were Introduced Into Lymph Vessels During Their Inoculation.* Animals were briefly immobilized by ether inhalation, and $5 \times 10^4$ D-12 ascites tumor cells in 0.3 ml Hank's BSS was injected subcutaneously into the left thigh, 2–3 mm proximal to the knee. After 1 h, the left hind leg was removed by amputation. Animals were sacrificed 30 days later and examined for macroscopic metastases. No macroscopic metastases were detected in any of 40 animals examined (not shown).

In other experiments, 45 DA rats were inoculated with $5 \times 10^4$ D-12 cells in 0.3 ml Hank's BSS s.c. in the left thigh, killed 50 min later, and the inguinal, retroperitoneal and axillary lymph nodes excised. Pooled cell suspensions from the lymph nodes of individual animals were inoculated into the peritoneal cavity of individual control rats. These animals were autopsied 12 weeks later. In no case was local tumor growth discernible. Other controls were injected intraperitoneally with graded numbers of the same tumor cells and the growth of the ascites tumor assessed. Intraperitoneal inoculation of D-12 ascites tumor cells induced progressive local tumor growth, in a dose-dependent manner (Fig. 2A). In 12 of 15 experiments, the inoculation of 50–100 D-12 cells sufficed to induce tumor growth and to lead to the demise of the host within 3–4 weeks. When ascites tumor cells from the same pool were first mixed in vitro with a cell suspension from inguinal, retroperitoneal and axillary lymph nodes from an individual control rat and this mixture then inoculated i.p. into another individual control, tumor growth was measurably enhanced in 14 out of 15 experiments. Results of a typical experiment are depicted in Fig. 2B which show

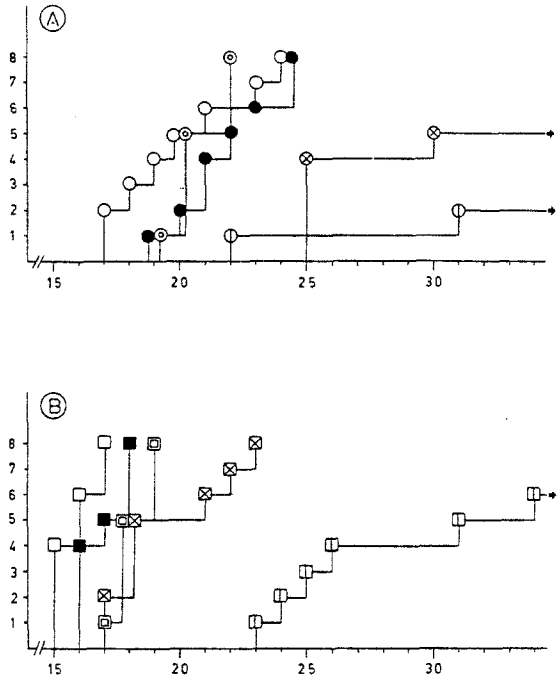


**Fig. 2A, B.** Induction of progressive ascites tumor growth by progressive increments of D-12 tumor cells. Tumor cells were inoculated i.p. into 3 m old DA rats on day 0. Symbols indicate the time of death of the animals. →Remaining animals survived for a further two months without manifestation of tumor growth. **A** Tumor cells only (*circular symbols*). **B** Tumor cells to be inoculated into individual rat were first mixed in vitro with a cell suspension derived from inguinal, retroperitoneal and axillary lymph nodes of an individual animal, and this mixture injected i.p. (*square symbols*). *Ordinate*: number of animals killed by the progressively growing tumor. *Abscissa*: time (d) after challenge. o $10^3$ D-12 cells; • $5 \times 10^2$ D-12 cells; ⊙ $10^2$ D-12 cells; ⊗ $5 \times 10$ D-12 cells; ⓪ 10 D-12 cells

that not only immune (Fidler 1973; Prehn 1976) but also normal lymph node cells may promote tumor growth.

*Biological Evidence for the Presence of Live Tumor Cells in Regional Lymph Nodes Prior to Surgery.* The present experiments have taken advantage of the capacity of D-12 cells consistently to induce progressive ascites tumor growth upon intraperitoneal inoculation of a relatively small challenge dose ($10^2 - 5 \times 10^2$ tumor cells per animal; Fig. 2). leading to the death of the host within a few weeks. Within a dose range of $10^2$–$10^4$ D-12 cells, the number of cells injected is proportionate to the time interval between challenge and the occurrence of death.

When cell suspensions from regional lymph nodes obtained from individual tumor-bearing rats were inoculated i.p. into individual controls, local tumor growth was induced in a considerable percentage of the animals. The results of a typical experiment, given in Table 2, show that viable, biologicallly active tumor cells are indeed present in the draining lymph nodes of animals bearing primary tumor inocula of different size, but not manifesting macroscopically evident metastases. Roughly, the number of live tumor cells present in the draining lymph nodes was between $10^2$ and $5 \times 10^3$ per animal. It is particularly noteworthy that a level of biologically detectable tumor cells is occasionally already reached within the draining lymph nodes at a time when the primary tumor is so small as to be just visible (diameter 2–5 mm). Comparison of results of parallel experiments show that the incidence of micrometastases was much higher (>80% in animals with tumors larger than 10 mm; Table 2) than that of animals with macroscopically evident metastases (~5%; legend to Table 2).

**Table 2.** Tests of cell suspensions from combined regional inguinal and retroperitoneal lymph nodes for capability to induce ascites tumors. 118 DA rats, 3–4 m of age, were inoculated subcutaneously with $5 \times 10^4$ D-12 cells into the left thigh. When the primary tumor had reached the size indicated in the Table, 68 rats were autopsied and combined cell suspensions from left inguinal and from retroperitoneal lymph nodes obtained from individual tumor-bearing animals (which did not manifest macroscopic metastases) were inoculated into individual 3 m old controls, the occurrence of death registered, and progressive ascites tumor growth verified. Animals with verified progressive tumor growth died within 4 weeks after challenge. Other animals were autopsied 10 weeks after challenge and were tumor-free. The remaining 50 animals were autopsied when their primary tumor had reached a size of approximately 20 mm, and examined for metastatic foci. Among these 50 animals, only 2 had developed macroscopic metastases which were located in the draining inguinal lymph nodes

| | Size of the primary subcutaneous tumor on the left thigh at the time of autopsy | | | |
|---|---|---|---|---|
| | 2–5 mm | 10 mm | 15 mm | 20 mm |
| Number of animals | 15 | 24 | 15 | 14 |
| Incidence of progressively growing ascites tumors | 1 (7%) | 22 (92%) | 12 (80%) | 12 (86%) |

*Growth Characteristics of Tumor Cells Derived From Metastases.* There is considerable evidence that neoplasms do not necessarily consist of homogenous populations of tumor cells (Fidler 1978; Fidler and Cifone 1979; Fogel et al. 1979; Tao et al. 1979; Weiss 1979). To explore the proposition that, in the present model, tumor cells capable of inducing metastasis involve a selected subpopulation with unique properties, the growth characteristics of tumor cells obtained from lymph node metastases were analyzed. These investigations have shown that single inoculation or repeated passage of tumor cells from metastases into the subcutaneous tissue of normal DA rats consistently induced tumor growth. However, such tumors invariably remained localized; moreover, their growth characteristics were not measurably different from the primary tumor, and in no instance did macroscopic metastases occur (not shown).

## Discussion

Previous findings in the present model, based on a large body of experiments, had shown that the outgrowth of macroscopic metastases is critically dependent on the site of the primary tumor implant (Keller 1981). Surgical removal of the primary tumor either induced or markedly enhanced the outgrowth of macroscopic metastases. As amputation was the means used for removal of the primary tumor on the leg, we could exclude the possibility that remnants of the primary tumor accounted for the spread of tumor cells having occurred before surgical removal of the primary tumor (Keller 1981). Utilizing both histological and biological methods, the present work has pursued this issue further.

As the histological evaluation of lymph nodes with normal appearance was based on examination of randomly selected sections, the results are

at best semiquantitative and consequently probably represent an underestimate of actual tumor cell involvement. The histological findings nevertheless indicate that dissemination of tumor cells occurs frequently from small primary tumors but is an almost obligate event with larger primary tumors (Table 2). The proportion of animals with live tumor cells within draining lymph nodes of macroscopically normal appearance was distinctly higher than the proportion of animals manifesting macroscopic metastases. Additional biological experiments attest that colonization of regional lymph nodes is a spontaneous rather than an artificial process (Stackpole 1981). Accordingly, the present findings indicate that surgical removal of the primary tumor was almost always performed at a time when live tumor cells had already colonized the draining lymph nodes.

Clearly, most experimental tumor models reflect only some of the diverse aspects of a primary interaction between host and tumor. The D-12 ascites is likely to represent a highly selected cell population; thus a selection process, apparently an important step (Fidler 1978; Fidler and Cifone 1979; Fogel et al. 1979), is probably not involved in the present model. This may be the cause for the finding that the growth characteristics of tumor cells derived from metastatic foci were not measurably different from those of the primary tumor (not shown). Furthermore, tumors transplanted as cell suspensions can hardly be regarded as adequate models of primary tumors. Taking into consideration these restrictions, it is noteworthy, however, that conclusions comparable to those in the present work have been reached in a variety of experimental and clinical situations (Crile 1956; Lewis and Cole 1958; Schatten 1958; Buinauskas 1965; Gershon et al. 1968; Kurokawa 1970; Cochran et al. 1972; Gershon 1974; Carr and McGinty 1976; Saba 1976; Mosley et al. 1978; Sugarbaker 1979). These conclusion are that the spread of tumor cells is an early and ongoing process, that lymph node micrometastases must not necessarily develop into macroscopic metastases, and that surgical removal of the primary tumor either or markedly enhanced the outgrowth of macroscopic metastases. It is thus conceivable that the present findings truly reflect processes critically involved in the dissemination of tumor cells and their outgrowth at secondary sites. It remains to be determined whether the enhanced outgrowth of the micrometastases elicited upon removal of the primary tumor is due to a loss of some inhibitory effect excerted by the primary tumor (Simpson-Herren 1976; Sugarbaker et al. 1977; Gorelik et al. 1978), to its compromising effect on a form of host defense (Tyzzer 1912; Agosin 1952; Fisher and Fisher 1959a; Robinson and Hoppe 1962; Agostino and Cliffton 1964; Romsdahl 1964; Van den Brenk et al. 1976), or to some other mechanisms.

## References

Agosin M, Christen R, Badinez O, Gasic G, Neghme A, Pizarro O, Jarpa A (1952) Cortisone-induced metastases of adenocarcinoma in mice. Proc Soc Exp Biol Med 80:128–131

Agostino D, Cliffton EE (1964) Anesthetic effect on pulmonary metastases in rats. Surgery, St. Louis 88:735–739

Alexander JW, Altemeier WA (1964) Susceptibility of injured tissue to hematogenous metastases: An experimental study. Ann Surg 159:933–944

Boeryd B, Rudenstam C (1967) Effect of heparin, plasminogen inhibitor (EACA) and trauma on tumor metastasis. Acta Pathol Microbiol Scand 69:28–34

Buinauskas P, Brown ER, Cole WH (1965) Inhibiting and enhancing effect of various chemical agents on rat's resistance to inoculated Walker 256 tumor cells. J Surg Res 5:538–546

Carr I, McGinty F (1976) Neoplastic invasion and metastasis within the lymphoreticular system. In: Friedman H, Escobar MR, Reichard SM (eds) The reticuloendothelial system in health and disease. Advances in experimental medicine and Biology, vol. 73 B. Plenum Press, New York. pp 319–329

Cochran AJ, Spilg WGS, Mackie RM, Thomas CE (1972) Post-operative depression of tumor directed cell mediated immunity in patients with malignant disease. Br Med J IV:67–70

Crile G (1956) The danger of surgical dissemination of papillary carcinoma of the thyroid. Surg Gynecol Obstetr 102:161–165

Eccles SA, Alexander P (1975) Immunologically mediated restraint of latent tumour metastases. Nature (Lond) 257:52–53

Fidler IJ (1973) In vitro studies of cellular mediated immunostimulation of tumor growth. J Natl Cancer Inst 50:1307–1312

Fidler IJ (1978) Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res 38:2651–2660

Fidler IJ, Cifone MA (1979) Properties of metastatic and non-metastatic cloned subpopulations of an ultraviolet-light-induced murine fibrosarcoma of recent origin. Am J Pathol 97:633–648

Fisher B, Fisher ER (1959a) Experimental studies of factors influencing metastases. III. Effect of surgical trauma with special reference to liver injury. Ann Surg 150:731–744

Fisher B, Fisher ER (1959b) Experimental evidence in support of the dormant tumor cell. Science 130:918–919

Fisher ER, Fisher B (1965) Experimental study of factors influencing the development of hepatic metastases from circulating tumor cells. Acta Cytol 9:146–159

Fogel M, Gorelik E, Segal S, Feldman M (1979) Difference in cell surface antigens of tumor metastases and those of the local tumor. J Natl Cancer Inst 62:585–588

Gershon RK (1974) Regulation of concomitant immunity. Activation of suppressor cells by tumor excision. Israel J Med Sci 10:1012–1023

Gershon RK, Carter RL, Kondo K (1968) Immunologic defense against metastases: impairment by excision of an allotransplanted lymphoma. Science 159:646–647

Gorelik E, Segal S, Feldman M (1978) Growth of a local tumor exerts a specific inhibitory effect on progression of lung metastases. Int J Cancer 21:617–625

Griffith JD, Salsbury AJ (1965) Circulating cancer cells. CC Thomas, Springfield, Ill.

Griffith JD, McKinna JA, Rowbotham HD, Tsolakidis P, Salsbury AJ (1973) Carcinoma of the colon and rectum: circulating malignant cells and five-year survival. Cancer 31:226–236

Henderson IC, Cavellos GP (1980) Cancer of the breast. The past decade. New Engl J Med 302:17–30

Hewitt HB, Blake ER (1975) Quantitative studies of translymphnodal passage of tumor cells naturally disseminated from a non-immunogenic murine squamous carcinoma. Br J Cancer 31:25–35

Keller R (1977) Abrogation of antitumor effects of *Corynebacterium parvum* and BCG by antimacrophage agents. J Natl Cancer Inst 59:1751–1753

Keller R (1980a) Distinctive characteristics of host tumor resistance in a rat fibrosarcoma system. In: van Furth R (ed) Mononuclear phagocytes. Functional aspects. M Nijhoff, The Hague. pp 1725–1740

Keller R (1980b) Regulatory capacities of mononuclear phagocytes with particular reference to natural immunity against tumors. In: Herberman RB (ed) Natural cell-mediated immunity against tumors. Academic Press, New York pp 1219–1269

Keller R (1981) Induction of macroscopic metastases via surgery. The site of the primary tumor inoculum is critical. Invas. Metas 1:136–148

Kurokawa Y (1970) Experiments on lymph node metastases 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

Langlands AO, Pocock SJ, Kerr GR, Gore SM (1979) Long-term survival of patients with breast cancer: A study of the curability of the disease. Br Med J 2:1247–1251
Lewis MR, Cole WH (1958) Experimental increase of lung metastases after operative trauma (amputation of limb with tumor). Arch Surgery 77:621–626
Lundy J, Lovett EJ, Wolinsky SM, Conrau P (1979) Immune impairment and metastatic tumor growth. The need for an immunorestorative drug as an adjunct to surgery. Cancer 43:945–951
Mosley JG, Sadler TE, Castro JE (1978) Effects of amputation and *Corynebacterium parvum* on tumour metastases in mice. Br J Cancer 37:571–575
Mueller CB, Ames F, Anderson GD (1978) Breast cancer in 3558 women: age as a significant determinant in the rate of dying and causes of death. Surgery 83:123–132
Papaioannou A (1981) Preoperative chemotherapy for operable solid tumours. Eur J Cancer 17:263–269
Prehn LM (1976) Immunostimulation of highly immunogenic target cell tumor cells by lymphoid cells *in vitro*. J Natl Cancer Inst 56:833–838
Robinson KP, Hoppe E (1962) The development of blood-borne metastases. Effect of local trauma and ischaemia. Arch Surg 85:720–724
Romsdahl MM (1964) Influence of surgical procedures on development of spontaneous lung metastases. J Surg Res 4:363–370
Roos E, Dingemans KP (1980) Mechanisms of metastasis. Biochem Biophys Acta 560:135–166
Saba TM, Antikatzides TG (1976) Decreased resistance to intravenous tumour-cell challenge during reticuloendothelial depression following surgery. Br J Cancer 34:381–389
Schatten WE (1958) An experimental study of postoperative tumor metastases. I. Growth of pulmonary metastases following total removal of primary leg tumor. Cancer 11:455–459
Simpson-Herren L, Sanford AH, Holmquist JP (1976) Effects of surgery on the cell kinetics of residual tumor. Cancer Treat Rev 60:1749–1760
Stackpole CW (1981) Distinct lung-colonizing and lung metastasizing cell populations in B16 mouse melanoma. Nature (Lond) 289:798–800
Sugarbaker EV (1979) Some characteristics of metastasis in man. Am J Pathol 97:623–632
Sugarbaker EV, Ketcham A (1977) Mechanisms and prevention of cancer dissemination: an overview. Semin Oncol 4:19–32
Sugarbaker EV, Thornthwaite J, Ketcham AS (1977) Inhibitory effect of a primary tumor on metastasis. In: Day SG, Laird Myers WP, Stansly P, Garattini S, Lewis MG (eds) Cancer invasion and metastasis: Biologic mechanisms and therapy. Progr. in Cancer Research and Therapy, vol. 5. Raven Press, New York. pp 227–240
Tao RW, Matter A, Vogel K, Burger MM (1979) Liver-colonizing melanoma cells selected for B-16 melanoma. Int J Cancer 23:854–857
Tyzzer EE (1912) Factors in the production and growth of tumor metastases. J Med Res 18:309–333
Vaitkevicius VK, Sugimoto M, Reed ML, Brennan MJ (1965) Effect of acute tissue injury on transplantability of autologous human cancer. Cancer 18:665–670
Van den Brenk HAS, Stone M, Kelly H, Orton C, Sharpington C (1974) Promotion of growth of tumour cells in acutely inflamed tissues. Br J Cancer 30:246–260
Van den Brenk HAS, Burch WM, Kelly H, Orton C (1975) Venous diversion trapping and growth of blood-borne cancer cells en route to the lungs. Br J Cancer 31:46–61
Van den Brenk HAS, Stone M, Kelly H, Orton C, Sharpington C (1976) Lowering of innate resistance of the lungs to the growth of blood-borne cancer cells in states of topical and systemic stress. Br J Cancer 33:60–78
Weiss L (1977) A pathologic overview of metastasis. Semin Oncol 4:5–17
Weiss L (1979) Dynamic aspects of cancer cell populations in metastasis. Am J Pathol 97:601–608
Willis RA (1973) The spread of tumours in the human body, 3rd edn. Butterworths, London
Woodruff MFA (1980) The interaction of cancer and host. Its therapeutic significance. Grune and Stratton, New York

Accepted September 2, 1982