Secondary Spread *of* Cancer

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R. W. BALDWIN

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The University of Nottingham
Cancer Research Campaign Laboratories
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Preface

The single most important characteristic of malignant cells is their capacity to metastasise, so producing tumours at distant sites. Indeed were it not for this property, many more patients could be cured by treatment of the primary growth. It is at first sight surprising therefore to find how little attention has been given until quite recently to the study of the processes of cancer cell dissemination. A closer examination of the problem indicates, however, how complex are these metastatic processes and their study is limited by the still rather rudimentary understanding of the control mechanisms involved in the growth of malignant cells. The primary objective of this book, therefore, is to present short authoritative accounts of several approaches for studying metastasis, and the treatment of secondary tumour growths. It will be immediately apparent to the reader how limited are the developments in many of these approaches under review and hopefully the challenge will be taken up by future research. One challenge for the experimentalist is to explain why with many animal tumours, such as those induced by extrinsic agents, e.g. chemical carcinogens, metastatic spread is not as common a feature as in clinical cancer. Does this mean that many of the experimental animal tumours lack an important property as analogues of human cancer, and, as reviewed in Chapter V, should other animal species be considered? Indeed, it would be a worthwhile study to compare the physio-pathological properties of tumours developing in one species with different metastatic potentials (Chapter I). This would surely include a closer look at the cell surface and particularly the accumulation (binding) of macro-molecules such as proteases, since many feel that the release of tumour cells from a primary tumour is controlled primarily by cell–cell interactions. Undoubtedly the tumour immunologist has much to offer in this type of investigation, although at present little effort has been made to turn loose the sophisticated immunological technology for characterizing metastasising and non-metastasising tumour cells (Chapter VI). Equally, these procedures will find use for studying what might be the more important question of how a disseminated tumour cell finds a "home" in which it is able to lie dormant, often for considerable periods of time, until an as yet undefined signal or set of signals induces this cell to proliferate progressively to become a metastatic growth (Chapter II).

Included in the book are a number of accounts of possible approaches to the treatment of metastatic disease. These include a consideration of the role of chemotherapy (Chapters IV and VII) where the point is made that too little attention has yet been given to agents which may influence the process of tumour cell dissemination (Chapter IV). It is perhaps necessary to work on the assumption that clinically when a primary tumour is treated, at least occult metastases are already present which also require treatment. The role of radiotherapy is considered in this respect (Chapter III) and the controversial view that radiation of tissues may under certain circumstances enhance metastatic disease requires challenge. The reader should also be cautioned about accepting too readily the view that immunotherapy holds out a ready and simple method for treating metastatic disease (Chapter VI). Nothing in tumour immunology is simple, and the principles of immunotherapy are still ill-defined. In fact there is to date little animal data to support the view that immunology is effective in treating metastatic deposits, apart from approaches aimed at depositing "macrophage-stimulating" agents such as bacillus Calmette Guérin (BCG) into lesions, and many of the clinical trials remain unproven.

The underlying message of the book is for a more basic and fundamental approach to the study of the processes involved in the release of tumour cells from a primary tumour, and for their subsequent deposition and development in secondary deposits. If as a result of our endeavours, investigators can be encouraged to study this multitude of questions, so providing a firmer basis for clinical studies, the book will have achieved its objective.

December 1977 R. W. B.

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Chapter I

General Pathology of the Metastatic Process

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"The peculiarity of cancer lies in its ability to create fresh starting points for a malignant mass when arrested somewhere along the system".

THOMAS HODGKIN (1848)

I. Introduction

The capacity for metastatic spread can reasonably be regarded as the single most important characteristic of malignant tumours. It is also the least understood. The overall march of events which characterize metastasis is easy enough to reconstruct, and competent analyses of

some features of the metastatic process were made by writers at the turn of the century (for review, *see* Wilder, 1956; Willis, 1973). But it will soon be apparent from the present chapter that purely descriptive accounts of certain critical phases of metastasis are still lacking and that investigations into underlying mechanisms have barely begun. Detection and treatment of metastatic disease in man continues to pose formidable problems; but slow improvements are being made and the older attitudes of therapeutic nihilism can, in several contexts, be legitimately questioned. It is therefore essential that clinical and experimental investigation into the general

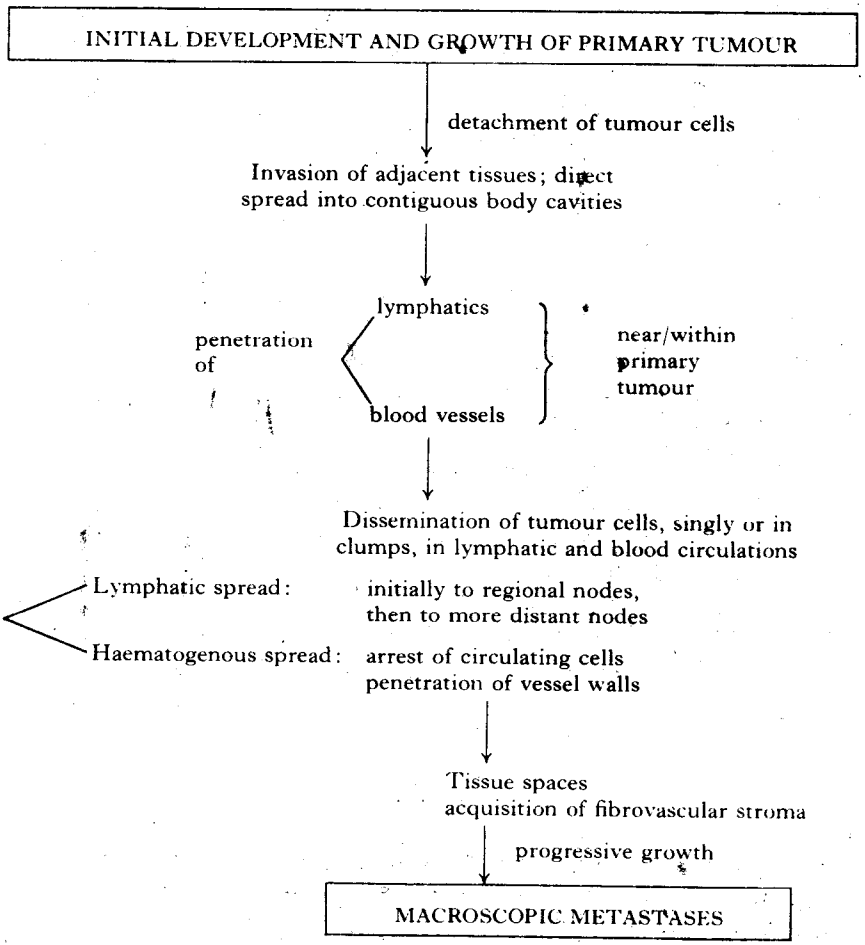

Fig. 1. The natural history of metastatic growth

pathology—or perhaps what might be called the natural history—of the metastatic process is aggressively pursued.

It is convenient to regard metastasis as a series of events each of which can be analysed separately (Fig. 1). It is self-evident that each phase involves a measure of interaction between tumour cells and host elements of various kinds, and it is important to note that the host elements which respond to metastasising tumour involve far more than the immune system, preoccupation with which has tended to obscure the role of stromal reactions, coagulation mechanisms and probably inflammatory responses.

II. The Primary Tumour

Appraisal of the likely metastatic potential of a primary tumour is still little short of rudimentary. In addition to purely clinical aspects, some clues will be afforded to the pathologist by the anatomical site of the tumour and cell of origin, its size, extent of local spread and—in particular— its histological appearances. With most tumours, it is reasonable to link increasing risk of metastasis with decreasing morphological differentiation as judged by conventional light microscopy. Such an association is empirical and based largely on accumulated experience. Specific morphological features are sometimes singled out and used for grading schemes which, in certain tumours, may give more precise prognostic information. Histological signs of local lymphatic or blood vessel invasion by tumour usually provide a clear indication that the metastatic process is already in train. It is, however, well recognized that there are several tumours where microscopy is no guide to metastatic behaviour: the most notable examples occur among cancers of the thyroid, adrenal and ovary. Here, seemingly well-differentiated lesions may metastasise but—paradoxically—histological evidence of local vascular involvement does not necessarily indicate that metastasis has occurred. Again, some malignant neoplasms, irrespective of their degree of morphological differentiation, invade locally but rarely metastasise to distant sites: examples include basal cell tumours, gliomas and thymomas. Exceptionally, embryonal tumours may show morphological signs of maturation with a corresponding improvement in prognosis and decreased risk of spread. The best documented examples are neuroblastomas, some teratomas and retinoblastomas and various other childhood malignancies (Smithers, 1969). The mechanisms for this excessively rare event are unknown.

The presence of host cell infiltrates of lymphocytes, plasma cells and macrophages in and around certain primary tumours—medullary carcinoma of the breast, malignant melanoma, choriocarcinoma, seminoma—is commonly regarded as a favourable prognostic feature; but the relevance of such infiltrates vis-à-vis local invasion and metastasis is unclear. This topic is discussed further in *Chapter II.*

Non-morphological criteria for assessing malignant potential are largely speculative at the present time.

(1) Loss of ABH isoantigens was described by Davidsohn and his co-workers (*see* Davidsohn, 1972) in human primary tumours arising in several different sites: oral cavity, stomach, large intestine, cervix, lung, pancreas, bladder and ovary. Some interesting initial findings were reported, but a recent paper from Davidsohn's group (Lill *et al.*, 1976) casts considerable doubts on the results previously obtained in intraepithelial carcinomas of the cervix. Loss of ABH isoantigens must, therefore, now be viewed with reserve.

(2) Expression of new antigens is a still more problematic determinant of metastatic behaviour. Synthesis and release of previously-suppressed fetal antigens, typified by carcino-embryonic antigen (CEA), may perhaps reflect some tumours' innate metastatic potential as well as serving as a means of monitoring the overall tumour burden. The role of true tumour-specific transplantation antigens in metastatic growth is equally speculative (*see* Chapter VI), though an association between a scanty glycocalyx, low immunogenicity and a high capacity to metastasise has been described in mammary tumours in rats (Kim, 1970; Kim *et al.*, 1975). Corroboration in other experimental tumour systems is needed before human implications can reasonably be considered.

(3) The elaboration and release of certain tumour-associated products may be an important determinant of metastatic behaviour. Examples include factors which stimulate local vasoproliferation or which dissolve bone, both of which are discussed later in this chapter.

It is reasonable to postulate, though difficult to prove, that the neoplastic cells which comprise a tumour are functionally heterogeneous and that certain elements in such a mixed population of malignant cells will have a particular proclivity to metastasise. This problem has been investigated by Fidler (1973a, 1975, 1976) in studies which have produced persuasive evidence that subpopulations of cells with enhanced metastatic potential can be

demonstrated within tumours. Using the B16 melanoma which had been adjusted to grow in tissue culture as well as *in vivo*, Fidler (1973a) set up the following system. B16 cells were grown for a short period *in vitro* and then injected intravenously into syngeneic (C57B1) mice where they gave rise to pulmonary deposits. Cells from these deposits were grown up again *in vitro* and injected into normal mice, and the cycle was repeated several times. The incidence of lung tumours increased with each tumour line derived from successive pulmonary metastases. These results suggest the operation of intrinsic properties of the individual tumour cell lines rather than any extraneous factors: and attempts have been made to characterize high- and low-metastasising B16 cell lines in more detail. Fidler (1975) found that the high-metastasis lines showed more invasiveness in the subcutaneous tissues, more trapping in the lungs, more pulmonary metastases and a greater tendency to form clumps with platelets. Bosmann *et al.* (1973) have reported comparative chemical and biophysical investigations with the two lines and, in sparse cultures, these authors found differences in electrophoretic mobility and—significantly—in surface glycoprotein, glycosyltransferases, glycosidases, transferases and proteases. Recently, Winkelhake and Nicolson (1976) have compared the *in vitro* adhesive properties of Fidler's high- and low-metastasising B16 cell lines. They find that the high metastasising lines have a relatively greater capacity to attach to homotypic or heterotypic monolayers—in this case, monolayers of the same B16 cells or 3T3 cells. Subsequent studies with other target organs have proved particularly interesting (Nicolson *et al.*, 1976): the high metastasising B16 lines adhered rapidly and strongly to lung cells but interacted weakly with liver, spleen, heart and other cells. The low metastasising B16 line adhered slowly to all heterotypic cell substrates, showing no target specificity.

A. *Stroma*

The supporting fibrovascular stroma plays an essential role in establishing and maintaining a growing primary tumour and in facilitating its local and distant spread. It has, indeed, been suggested that the rate of proliferation of vascular endothelium is an important factor in limiting the rate of tumour growth (Tannock, 1970)—a proposal that applies equally to primary and metastatic lesions (cf. Section VI). The kinetic turnover of unstimulated capillary endothelium is normally measured in months; endothelium in a transplantable rat mammary adenocarcinoma has a turnover time of

about 50 hr, comparable to that found in proliferating endothelium in the vicinity of a 3-day old fracture (Tannock, 1970; Tannock and Hayashi, 1972).

The stimuli which induce endothelial proliferation are likely to be diverse, but one important element is released by the tumour itself—

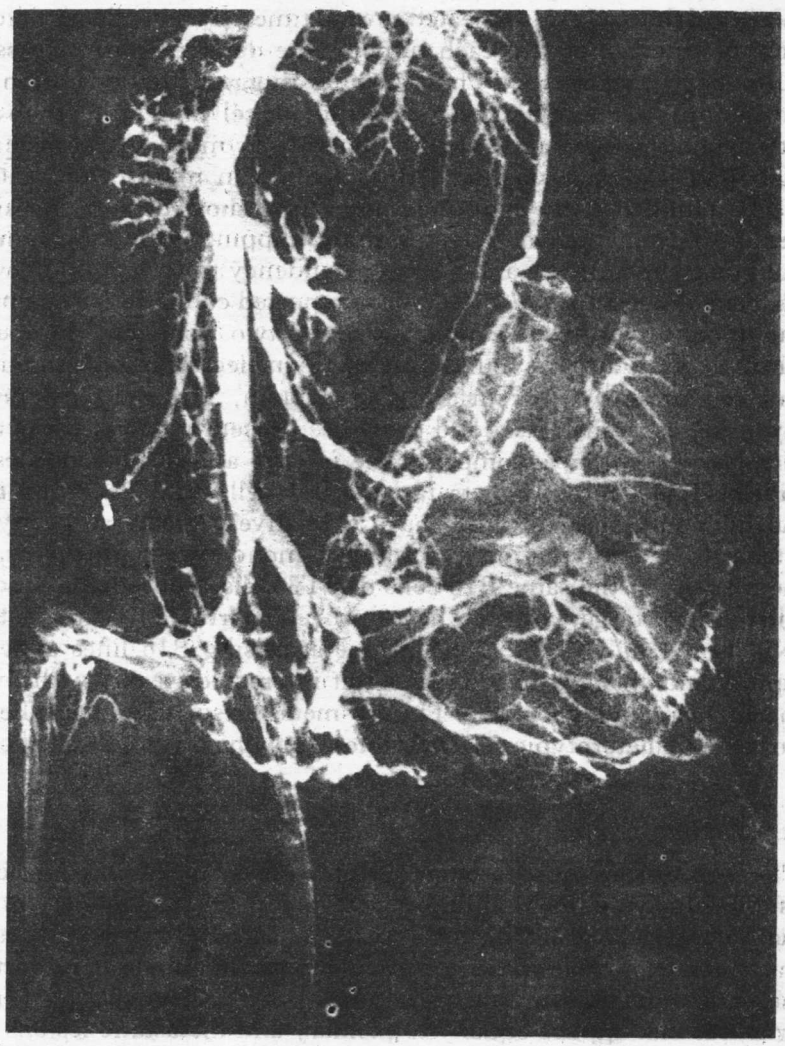

FIG. 2. Venogram of a Lewis carcinoma (3LL) in a C57B1 mouse, 21 days after implantation. Highly vascular zones containing tortuous blood vessels alternate with regions with little or no demonstrable blood supply. (Illustration kindly supplied by Dr. Anne Atherton and Professor K. Hellmann)

the *tumour angiogenesis factor* (TAF) of Folkman (*see* Folkman, 1974, 1975). A reciprocal interaction is thus established between tumour cells and capillary endothelium, aptly described by Folkman as "a highly integrated ecosystem".

Many tumours are richly vascular (Fig. 2) but the detailed anatomy of their blood vessels is still imperfectly understood. Thiersch, in 1865, commented on the abundant growth of capillaries in the stroma of carcinomas, and the current view (*see* Willis, 1973) suggests that most tumour vessels consist only of "irregular endothelium-lined channels with scanty perivascular connective tissue". Investigations with experimental tumours confirm this view (Underwood and Carr, 1972; Papadimitriou and Woods, 1975) and interesting serial studies on vascular morphology and blood flow in developing and regressing Walker tumours in rats have been reported by Oikawa *et al.* (1975).

Blood vessels in experimental tumours have been shown to be highly permeable (Underwood and Carr, 1972; Papadimitriou and Woods, 1975) and susceptible to a wide range of vasoactive compounds such as adrenaline and noradrenaline, acetylcholine, 5-hydroxytryptamine, bradykinin and kallikrein (Cater *et al.*, 1966; Cater and Taylor, 1966). It becomes, however, increasingly difficult to separate host and tumour vasculature and some of the alleged characteristics of tumour blood vessels may reflect non-specific local conditions within the tumour such as necrosis, haemorrhage and infection. Nevertheless, the details of blood flow within and near tumours is important since the vasculature represents a major channel of dissemination and, in consequence, an obvious target for measures to reduce metastatic spread. Folkman (1975) has discussed the development of possible "anti-angiogenesis" factors, and there is recent evidence that such factors may exist in normal cartilage (Brem and Folkman, 1975; Langer *et al.*, 1976)—a tissue which is strikingly resistant to invasion by extraneous tumour. It has been shown experimentally that the dioxopiperazin compound, ICRF 159, may prevent blood-borne metastases, possibly by acting on the vascular endothelium (Le Serve and Hellmann, 1972; Salsbury *et al.*, 1974; Atherton, 1975) though the underlying mechanisms are uncertain (cf. Peters, 1975). In particular, ICRF 159 has been shown to be effective in only certain tumour systems (cf. Pimm and Baldwin, 1975): it clearly does not act non-specifically on all tumour-associated capillary endothelium.

It is uncertain whether tumours have lymphatics within their substance (Futrell and Pories, 1975) though dilated lymph vessels are commonly seen in the adjacent stroma. The lymphatic and blood