CIRCULATING CANCER CELLS

GRIFFITHS AND SALSBURY

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Foreword

OUR LIVING CHEMISTRY SERIES was conceived by Editor and Publisher to advance the newer knowledge of chemical medicine in the cause of clinical practice. The interdependence of chemistry and medicine is so great that physicians are turning to chemistry, and chemists to medicine in order to understand the underlying basis of life processes in health and disease. Once chemical truths, proofs and convictions become sound foundations for clinical phenomena, key hybrid investigators clarify the bewildering panorama of biochemical progress for application in everyday practice, stimulation of experimental research, and extension of postgraduate instruction. Each of our monographs thus unravels the chemical mechanisms and clinical management of many diseases that have remained relatively static in the minds of medical men for three thousand years. Our new Series is charged with the *nisus élan* of clinical wisdom, supreme in choice of international authors, optimal in standards of chemical scholarship, provocative in imagination for experimental research, comprehensive in discussions of scientific medicine, and authoritative in chemical perspective of human disorders.

Mr. Griffiths and Dr. Salsbury present the newer knowledge of the mode of spread of tumors via the blood stream and the method of examination of blood for malignant cells. Cancer cells, biologically different from host cells, reveal a marked tendency to escape from the original locus of the tumor through the circulation to another tissue where the lesion grows into a secondary tumor mass. Ma-

lignant tumors thus spread by direct infiltration of a neighboring tissue, by extension along lymphatic channels, by transport in the blood stream and by implantation. Most distant metastases are therefore the result of bloodborne dissemination of tumor cells from both small and large cancers. Early in the development of most cancers and, perhaps, before they can be recognized clinically, cancer cells may thus be widely disseminated throughout the body. The relationship between the development of metastases and the infiltration of blood vessels by a primary malignant tumor was formulated by Cruveilhier (1829), confirmed by Ashworth (1869), and established by von Recklinghausen (1885). Metastases follow the transport of viable malignant cells in the blood, hence Langenback (1889) asked "What determines the organ that shall suffer in a case of disseminated cancer? When a plant goes to seed its seeds are carried in all directions but they can only live and grow if they fall on congenial soil." This concept of the relation between the circulating tumor cell and the organ in which it becomes arrested persists to this day.

Blood films of a cancerous patient may reveal cancer cells in transit from the primary tumor to the metastatic site. Quantitative studies indicate that circulating cancer cells rarely exceed 10/ml blood, hence few are detectable amongst the millions of red and white cells. Identification thus requires concentration of cancer cells in blood to remove the red cells and possibly the white cells from the preparation before applying Papanicolaou's staining techniques. Routine blood examination is unwarranted except in selected cases because of the low incidence of cancer cells, sporadic appearance in the blood, and prolonged scanning of blood concentrates. Blood examination for malignant cells is of inestimable value in increasing our

knowledge of the mode of spread of tumors and in revealing the dangers of liberating bloodborne cells by certain procedures—biopsy, curettage and tumor manipulation. Malignant tumors vary in their ability to form bloodborne metastases but the cells found in the blood usually arise from local veins draining the tumor. The presence of cancer cells in peripheral blood indicates widespread dissemination and transorgan passage. Most of the circulating cancer cells are destroyed in the blood stream without lodging in any organ. Escape of the malignant cells into the circulation does not necessarily indicate establishment of metastases. Thiersch (1865) first indicated that few of the circulating tumor cells were viable enough to form metastases, an hypothesis that stood the test of time for a century.

The fate of most embolic cancer cells which are lodged in the arterioles and capillaries is death but some manage to survive and establish metastatic growths. The course of a circulating malignant cell to a metastic site involves adherence of malignant cells to the vascular endothelium aided by thrombus formation around the cells and irreversible penetration of the endothelium by cancer cells. The question is no longer what makes the cancer shed its cells into the circulation but rather why do not all cancer cells that enter the blood stream survive to produce metastases. The basic approach is not mere removal of the tumor before it has spread to the blood stream or even to remove it so widely that every cancer cell is excised. The mechanical objectives of surgery or localized radiation therapy are no longer attainable because of the entry of cancer cells into the blood stream partly due to their increase in motility and lack of cohesion. The real problem is to remove the primary tumor and *at the same time* prevent the circulating cancer cells from implanting themselves and growing.

Differentiation between benign and malignant tumors may present great diagnostic difficulty. Neoplastic cells found in the blood stream are necessarily malignant because a benign tumor does not metastasize. It is possible for benign cells to escape into the blood from various tissues, especially after trauma but such cells are unable to multiply or produce metastases. The release of cells from the local and contact mechanisms that normally restrain their growth appears to be the common denominator of carcinogenesis. It is the irony of Nature that the phenomenon responsible for so many deaths is indissolubly connected with life. The fate of man is not like a game of chess dependent on skill, but like lottery dependent on chance.

" — non sarebbe
qualche nuova conquesta?
Io lo devo saper, per porla in lista."

I. NEWTON KUGELMASS, M.D., PH.D., SC.D., *Editor*

Preface

INCREASING INTEREST IS BEING shown in the techniques recently developed for the detection of cancer cells in the blood. Much information has accumulated over the last few years, and we felt that there was some need for a book which could view the results impartially, and assess their significance. It must be appreciated that techniques for the isolation of circulating malignant cells are still, relatively, in their infancy. The full import of some reports may not yet be apparent, and it is not surprising that other results are contradictory. We hope to be able to draw certain broad conclusions from the evidence at present available, to draw attention to sources of possible error, and to suggest ways in which these techniques may further our knowledge of the concept of malignancy.

In the future, one may well expect further advances in the detection of circulating cancer cells, but the basic difficulty will almost certainly remain — the problem of positively identifying a single cell in the blood as malignant. At the present, there seems no possible means by which this desirable end will be achieved.

Our thanks are due to Mr. C. Naunton Morgan and Dr. Warren H. Cole who stimulated our interest in this work and have been helpful at all times; to the Department of Medical Illustration at the Royal Marsden Hospital and to Mr. P. Cull, of St. Bartholomew's Hospital, for the preparation of photographs; and to Miss D. Cross and Mr. P. Crocker, of the Department of Medical Photography, St. Bartholomew's Hospital for assistance with the photo-

graphs. Dr. P. Smart and Dr. A. McKinna developed the perfusion apparatus described in Chapter Three, and the Editor of the *British Journal of Cancer* has given us permission to republish Figures 2 to 7 in Chapter Four. Part of the Authors' work referred to in this book was submitted by one of us (A.J.S.) as a Thesis for the Degree of M.D., Cambridge.

J. D. G.
A. J. S.

Introduction

The Concept of the Circulating Cancer Cell

THE BLOODBORNE SPREAD OF malignant disease has been appreciated since the idea of secondary dissemination was first conceived. Apart from extensive permeation of lymphatic channels, spread in the bloodstream was the only method by which distant metastases could be produced. This implied that the primary tumour must at some stage in its growth invade blood vessels, or that tumour cells must pass by some other means into the vascular networks surrounding the tumour.

Tumour invasion of blood vessels was first associated with the development of remote metastases by Cruveilhier[1] in 1829. He suggested that intravascular growth liberated a specific 'cancerous juice' which travelled in the circulation to produce metastases. The concept of free malignant cells or emboli was propounded by Von Recklinghausen[2] in 1891. He also postulated that carcinomata metastasised via the bloodstream.

Differentiation between benign and malignant tumours can occasionally be of great difficulty. However, by definition no benign tumour can metastasise; therefore neoplastic cells found in the bloodstream must be deemed to be malignant. It is possible for benign cells from various tissues to escape into the blood, usually following trauma, but these cells are unable to multiply and produce metastases.

Malignant tumours vary in their ability to form bloodborne metastases — some are prone to vascular dissemination at an early stage in their development, whereas others,

such as basal cell carcinoma, very rarely metastasise via the bloodstream.

The leukaemias possess most of the attributes of malignant disease, but are not dealt with in this book, as leukaemic or 'malignant' cells are almost invariably found in unconcentrated blood, and require no special techniques for their demonstration. Apart from this exception, the authors propose to refer to all types of malignant disease.

The use of the word 'circulating' with regard to cancer cells must be qualified to specify the site from which blood was taken. It has been shown by many investigators that malignant cells are more frequently found in blood from local veins draining a tumour than in peripheral blood. The presence of cancer cells in peripheral blood is indicative of widespread dissemination and probable trans-organ passage.

The circulation of malignant cells and the establishment of distant metastases presupposes that some, at least, of these malignant cells are viable, and are capable of implantation with the formation of a metastasis resembling the primary tumour. However, it is probable that the majority of circulating cancer cells are destroyed in the bloodstream, without their lodgement in any organ.

Cells in the blood resembling those found in malignant skin tumours were described at autopsy by Ashworth[3] in 1869, but on further examination of his original description, it is difficult to be certain that these were indeed malignant cells. Positive identification of circulating cancer cells was not demonstrated until recently, when Engell[4] in 1955 and Roberts and his colleagues[5,6] in 1958 found tumour cells in blood samples from patients suffering from malignant disease.

The escape of malignant cells into the circulation does not necessarily signify the establishment of metastases.

Thiersch[12] suggested that only few of the circulating tumour cells were viable and capable of forming metastases – an assumption which has withstood the test of time, and has been amply confirmed by later investigators, as will be described in the following chapters.

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Note: There are many references to the Symposium on vascular dissemination of cancer held at Geneva in June 1963. Summaries of papers presented at this meeting have now been published by Schwabe and Co. Verlag. Basel/Stuttgart in book form as *Krebsmetastierung auf dem Blutwege*.

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Entry of Malignant Cells Into The Blood

IT IS POSSIBLE FOR THE MALIGnant cell to enter the circulation in one of four possible ways. Cells may become detached from tumour invading a vein in which the circulation is still present. Intravasation of free malignant cells through a blood vessel wall can occur, particularly through the capillary plexus of the organ in which the tumour is growing. Tumour cells may also pass from the lymphatic system draining the tumour area and from lymph nodes containing metastases into the blood and in the case of intra-abdominal tumours, by passage of free malignant cells from the peritoneal cavity to the blood. Naturally, a combination of more than one means of bloodstream entry could occur in the same patient. The entry of cancer cells into the blood will be considered in more detail below.

Venous Invasion by Malignant Tumours

This phenomenon frequently occurs, and probably accounts for the majority of bloodborne metastases. The clusters of cancer cells occasionally found in peripheral blood, and circulating large malignant emboli are almost certainly a consequence of venous invasion.

Over a century ago, surgeons and pathologists noted the invasion of veins by malignant tumours. Billroth[1] in 1855 first described tumour cells in veins draining a primary neoplasm, finding a testicular teratoma growing along the