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Understanding the Cancer Process: Some Aspects of the Spread of Cancer in Man and Experimental Animals

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ABSTRACT. The course of the illness in an untreated patient with cancer depends upon the cell of origin of the cancer and the organ in which the cancer arose. Although there are certain general principles concerning spread, malignant tumours comprise a large number of separate diseases rather than a single entity. Removal of the primary tumour (i.e. the tumour in the organ where it first appeared) can usually be achieved and it is the secondary deposits that prove resistant to removal by surgery or treatment by other means. The way these secondary deposits develop is therefore basic to the understanding of the cancer process as a whole and is needed for a rational approach to therapy. Many malignant tumours spread via the blood stream by way of the release of tumour cells into the circulation and the primary malignant tumour must reach a certain stage in development and size before tumour cells are released in significant quantities.

INTRODUCTION

One of the principal threads evident in human evolution from the time of recorded history has been an interest in the nature and of course, the methods of possible cure, of ailments that afflict man. There is a group of illnesses which are termed collectively cancers which consist of unusually rapid proliferation of cells to form abnormal newgrowths. In this group the mass of cells invades the adjacent tissues and often spreads by the dissemination of the abnormal cells.

Cancers were described in antiquity, being mentioned in the earliest literature of Iran and India, and in the Ebers papyrus (1500 B.C.) (Ewing, 1942). Hippocrates (460-375 B.C.) is recorded as having burnt out a cancer of the neck and was aware of cancers of skin, breast, uterus and certain internal organs. Galen of Pergamum (150-200 A.D.) regarded cancer as a concentration of black bile in his humoral system of the explanation of disease.

The elucidation of the circulation of the blood by Harvey in 1628 was the turning point in the rebuttal of the humoral system of disease. The discovery of the lymphatic system by Rudbeck (1652) suggested to LeDran (1685-1770) that breast cancer might spread by "cancer lymph". Morgagni (1682-1772) studied at autopsy many patients who had died of cancers and established major aspects of the pathological anatomy of many internal cancers.

Factual observations of the nature and progress of untreated cancers in the 18th century paved the way for modern concepts of the disorder. The advent of the microscope in the early 19th century allowed Schwann (1838) to enunciate his principle that tissues have a cellular structure. Schwann described the nucleus and nucleolus of the cell.

In 1838 Muller published a study of cancers and first described them as being composed of groups of cells with varying forms and proportions of stroma and cell masses (e.g. Figs. 1 and 2). Virchow founded cellular pathology on the basis that all cells are derived from cells. The doctrine of endogenous reproduction of cells laid the basis for the discovery by Thiersch that changes in normal cells gave rise to tumours. The last decades of the 19th century saw great activity utilising modern methods of processing and sectioning of cancer tissue so that detailed morphology was achieved at a light microscopic level (for historical review see Ewing, 1942).

Ewing (1942) advocated the proposition that cancer is a group of specific disorders, all of which have their own distinctive morphology, and natural history. Progress in therapy is dependent upon a clear understanding of the specific disease under treatment and its usual biologic course. The advent of the electron microscope and practical methods of preparation of tissues for examination in this specialized microscope in the early 1960's led to a great upsurge in the amount of information available at magnifications considerably beyond that realizable by the use of the light microscope.

Definitions

What is a cancer? The classes of cellular response to injury are limited. The cell may die, it may recover completely or it may undergo some permanent modification of structure and behaviour. The formation of cancers appears to be the result of such a permanent modification as a result of contact with a noxious agent. The word "cancer" is the most familiar term relating to newgrowths or tumours and refers to all types of malignant tumours. Normally the cells in the body go through a preordained progress with periods of maturation, full function and then senescence and death. There are both local and general control mechanisms which keep the relative parts of the body in register so that all are co-ordinated.

Ewing (1942) defines a tumour as an

autonomous new growth of tissue and Willis (1967) as an abnormal mass of tissue, the growth of which exceeds and is unco-ordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stumulus which evoked the change.

There are two major divisions of tumours - benign and malignant. The benign tumours grow locally and do not spread to distant sites. Malignant tumours (cancers) invade and destroy local tissues and spread by the lymphatic and blood circulations. A secondary deposit at a distance from the origin of the cancer is called a metastasis. It usually has the same structure as the primary tumour.

Approaches to the cancer problem

Cancer is placed second of the causes of death in New South Wales. The approaches to this disorder can be listed under three main headings which are - prevention, early diagnosis and effective treatment which may include a combination of surgery, radiotherapy and chemotherapy. Ideas regarding the prevention of cancers stem mainly from descriptions of "industrial" cancers and epidemiological surveys. One of the earliest industrial diseases was the scrotal cancers of chimney-sweeps described by Pott in 1775 (Willis, 1967). This skin cancer was due to the application of soot to the skin over a period of many years. Modern methods of travel and communication have led to the discovery of the wide divergence in the incidence of cancers of various organs in different countries (Higginson, 1960). The difference is frequently striking and has led to specific comparative studies of different races under differing cultural influences. For example Japanese who immigrate to the U.S. show a cancer incidence between that of the Japanese in Japan and the rest of the U.S. population. The great number of studies of this type has led Higginson to state that perhaps 70% of all cancers are environmentally induced (Higginson, 1980). It is naturally up to the individual to arrange his own habits to diminish the risk of this disease. Reduction or abolition of cigarette smoking, reduction of excess exposure to sunlight and abolition or marked reduction of exposure to industrial carcinogens and radiation are high on the list of desirable measures to be taken.

Early diagnosis

A slowly enlarging lump or bleeding from any orifice which is not related to trauma are abnormal findings requiring medical attention. Dr. George Papanicolaou, a Greek born physician who spent most of his working life in the U.S., is credited with founding Cytopathology. With Dr. Traut he was able to show that individual cancer cells possess structural features which are characteristic for malignancy (Papanicolaou and Traut, 1943). The study of cells exfoliated from a specific surface has recently given further information regarding the method of progression of certain cancers particularly carcinoma of the cervix and bronchus.

Problems of treatment

The methods used in the treatment of neoplastic disease depend upon the type of cancer involved. Therapy may consist of surgery, radiotherapy or chemotherapy or any combination of these. Probably the major problem of therapy is the development of secondary deposits (metastases) at a distance from the primary tumour.

Investigation of the process of tumour metastasis

My own work on tumour metastases arose out of an interest in the initial lodgment, growth and vascularization of tumour cell clumps released from the main mass of the tumour (also called the primary tumour). The biology of the metastatic cycle consists of release of tumour cells from the primary tumour, the reaction of the tumour cells with the blood components and their circulation in the blood, followed by impaction and growth of the surviving cell clumps in tissues which are susceptible to involvement by metastatic deposits of tumour. This is a complex sequence of events and is difficult to study because of the alteration of the natural mechanisms imposed by the very nature of the means of study.

The release or shedding characteristics of a tumour are difficult to quantify because of the multiplicity of naturally occuring blood vessels supplying the primary tumour in most instances. The blood supply to experimental tumours, may, however be manipulated by encasing an organ, such as the kidney, which has a clearly identifiable vascular pedicle in a capsule of an inert substance such as wax and allowing the parenchyma of the organ to be replaced by tumour tissue. The tumour tissue is then supplied by the renal artery and vein and vascular studies may proceed using these vessels. This was the technique used by Gullino and Grantham (1962) to study the reaction of the blood vessels in the tumour mass to various stimuli.

It appeared to me, that, if progress were to be made the metastatic cycle (diagram 1) would have to be broken down into manageable segments for study and that only then could the conditions of the experiments be sufficiently defined to allow of firm conclusions. My earlier work on endothelium (Warren, 1963) prompted me to start with the phase of attachment of the tumour cell to the vessel wall. Baserga and Saffiotti in 1955 had noted that tumour cells in the lungs actually lodged in the small vessels by adhering to their endothelium. Later studies by Wood (1964) using rabbits showed that when single carcinoma cells (of the ascitic V2 line) were injected into vessels these cells clumped together and fibrin was formed around them. With a German exchange student working with me at the time (F-H Guldner) I decided to study this phenomenon by taking a suspension of a human tumour line (HeLa cells) which were readily available because of their use in the viral laboratory in Oxford and to add this suspension to human vein walls. During the operation of varicose vein stripping many segments of vein are removed from the leg as part of the treatment and and these segments include normal lengths of vein which are present between the varicose regions and the normal segment of vein

at the upper end of the specimen. Half of these normal rectangles of vein were left undamaged and half were damaged by light mechanical trauma. To these segments HeLa cells in suspension were added by pipetting the suspension onto the endothelial surface of the vein segments. These preparations were incubated in tissue culture medium at 37 degrees Centigrade. They were then fixed and studied by electron microscopy.

The HeLa cells were seen to adhere to the regions of damaged vessel wall where the endothelial cells had been abraded off by the previous trauma. The tumour cells stuck to the exposed layer of the vein wall - the basement membrane of the endothelial cell by means of multiple microvilli and the cells eventually flattened themselves against the basement membrane (Warren and Guldner, 1969).

My next studies (1970) were on the reaction of the blood to circulating tumour cells. Because of the intricacies of both the haemostatic/thrombotic processes and the physical design of the circulation, the size and nature of the particles in which the tumour cells travel in the circulation bears directly on the adhesion and impaction events which occur between the tumour containing mass and the damaged vessel wall. Thus single cells flowing over intact smooth endothelium would pass through capillary beds. On the other hand a mass of platelets attached to tumour cells and forming a mass consisting of a number of tumour cells with activated platelets would tend to impact in the capillary bed and also to stick to damaged vessel wall.

In the early work on the ultrastructure of thrombi the Chandler tube apparatus was used. This was a plastic tube into which a ml. of whole blood was placed. The tube was formed into a circle by means of a small connecting piece of tubing placed between the ends, and the circle itself with contained blood rotated on a turntable. A solid mass was formed at the leading edge of the column of blood and this possessed the structure found in a thrombus. This was a platelet, leucocyte and fibrin rich head and a red cell and fibrin tail (Poole, 1959). This system was used to study the interaction of tumour cells and blood. Tumour cell suspensions of Walker 256 carcinoma, which is a tumour cell line derived from a tumour of the mammary region of a rat and a mouse mammary adenocarcinoma were used. Platelet rich plasma suspensions were prepared from rats and mice. 0.5 ml of platelet rich plasma were added to 0.5 ml of a suspension of tumour cells and the mixture rotated in the Chandler tube apparatus. By electron microscopical examination this method was shown to produce particles consisting of a loosely knit body of tumour cells, tumour cell debris, platelets and activated platelets in the case of Walker 256 tumour cells and rat platelet rich plasma. The mouse mammary adenocarcinoma preparations produced a denser body of tumour cells, fibrin and platelets (Warren, 1970).

It is thus evident that at least with some tumours, the tumour cells in the body do not circulate in the blood stream as single cells but as masses of tumour cells, debris, fibrin and platelets.

The next stage was to examine the attachment of "circulating" tumour cells to damaged vessel walls. This was done by injecting tumour cells in suspension into the inferior vena cavae of rats and mice after mechanically damaging by pressure the vessel wall upstream. Suspensions of Walker 256 carcinoma and a thymic lymphoma of mice were injected into rats and mice respectively. Two types of adherent tumour emboli were found. They were distinguished by the presence or absence of endothelial damage of the underlying vessel wall. Where damage had resulted in removal of the endothelial cells, fibrin formed the adhesive between the tumour cells and the vessel wall. In areas where endothelium was intact, tumour cells were attached to platelets and a small amount of fibrin which enclosed the tumour-platelet mass (Warren and Vales, 1972).

Migration of the tumour cells following the more secure adhesional site provided by damaged vessel wall, occurs by insinuation of the tumour cells into the deeper layers of the vessel wall (Warren, 1976). Scanning electron microscopy of human tumours has revealed microvilli on the surface of epidermoid carcinoma cells and to a much lesser extent on human adenocarcinoma cells (Warren, 1978). Veins draining renal adenocarcinomas in man contain activated platelets, tumour cells and debris (Warren, 1978).

Because studies of the vasculature of tumours (Warren, 1979) had led me to consider that the wide bored, thin walled vessels (giant capillaries) found at the edge of tumours possessed "porous" properties with regard to movement of tumour cells, I examined these vessels in experimental tumours by electron microscopy. Identification of these vessels was readily possible if a mm cube fragment of a transplantable tumour is grown on the cheek pouch membrane of a hamster and this membrane and tumour fragment is encased in a transparent chamber which allows observation of the vessels by light microscopy in the living state. The morphology of movement of melanoma cells through the endothelium of these thin walled vessels is by a series of stages. First the tumour cell becomes positioned beneath the basement membrane of the vessel. Erosion of the basement membrane of the vessel next occurs and the tumour cell insinuates itself through the break in the basement membrane to lie beneath the endothelium. The overlying endothelium becomes attenuated or separates and the tumour cell emerges into the vascular lumen often via a dumb-belllike stage (Warren, Shubik and Feldman, 1978). This method of intravasation is not unlike the movement of lymphocytes from the vascular compartment into lymph nodes.

Metastasis via the blood stream is an important route of dissemination of malignancy. The demonstration of tumour cells in the blood stream in the 1960s indicated that tumour embolism is only a stage in a long process before tumour metastasis is established and it was not possible after exhaustive study to correlate prognosis of the patient with the presence or absence of circulating tumour cells (for review see Warren,1981). Why certain tumour emboli proceed to metastasis formation and others do not is unknown. It appears from animal work that the larger the tumour mass involved the more likely it is that

metastasis will result.

If showers of emboli are derived from tumours then recurrent blockage of capillary beds would result in areas of localized damage with fibrin on their surfaces. Such areas following re-routing of the blood stream or re-opening of the capillary bed would provide preferential sites for the lodgment and migration of tumour cells (the micro-injury hypothesis of the formation of micrometastases, Warren, 1981b).

The diagram of the metastatic cycle indicates in outline form the complexities of the process and how difficult it is even in the model situation in animals to study the specific dynamic processes at the moment of adhesion and or impaction of the tumour cell embolus which will progress to tumour metastases. The animal work can give vitally important clues and indications of the processes involved but eventually final studies must be devised for the human situation, with of course due regard for the overwhelming importance of the treatment and welfare of the patient.

Basic studies on the structure and function of the microcirculation of various organs, particularly those in which metastases readily occur (such as the liver, a scanning electron micrograph of which is shown in Fig. 3) are also likely to be of importance in the unravelling of the detail of the metastatic process.

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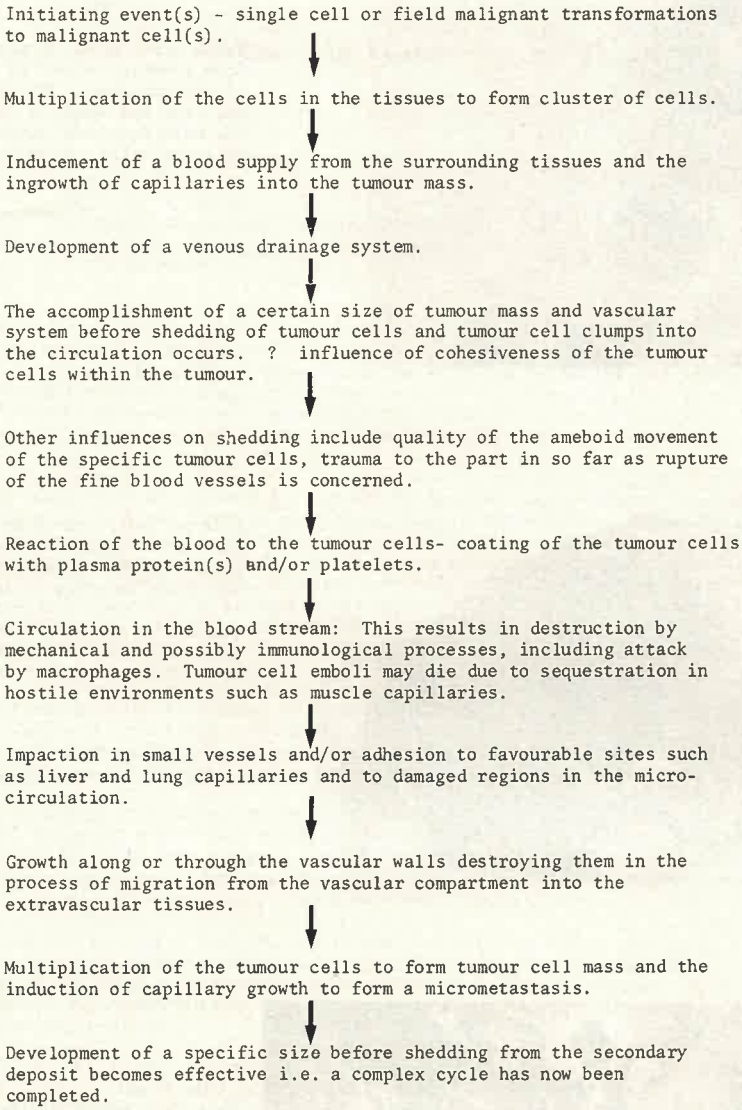

DIAGRAM 1. The metastatic cycle of the blood-borne spread of carcinoma.

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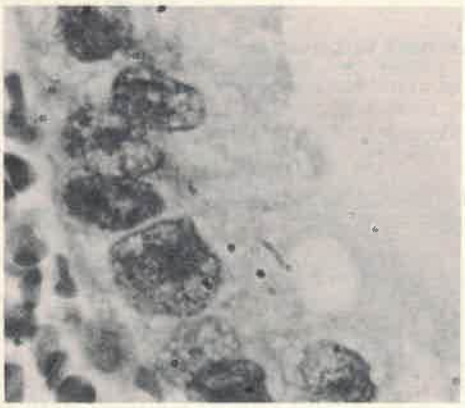

FIG. 1.

These are a group of adenocarcinoma cells from a needle biopsy of an adenocarcinoma of the pancreas. The great variability of the size and shape of the nuclei as well as the molding of the nuclear shapes by the adjacent cells can be seen.

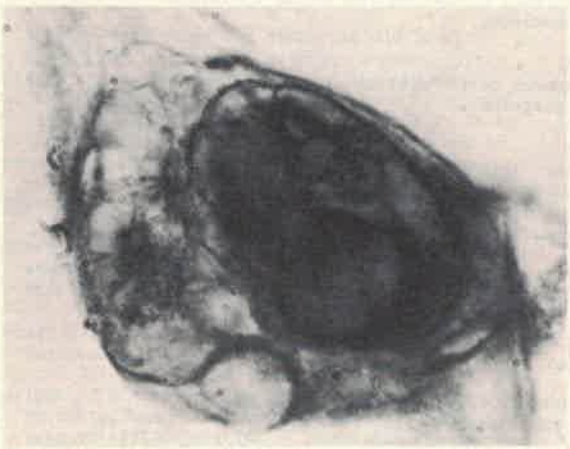

FIG. 2.

A large single adenocarcinoma cell from a pancreatic adenocarcinoma fills this photograph. There is indentation of the nucleus and mucus vacuoles in the cytoplasm. This is from a smear preparation prepared and stained by the Papanicolaou technique. In this technique the whole of the cell is seen not a thin section of the cell.

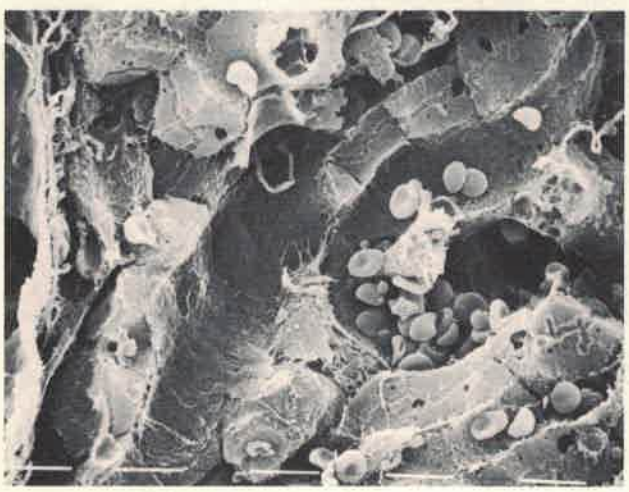

FIG. 3.

This is a scanning electron micrograph of the cross sectional surface of the liver. The microcirculation of the liver is shown. In two of the sinusoids groups of red cells are present.

The sinusoids of the liver-are a frequent site for the development of tumour metastases. The endothelium is incomplete in the sinusoids and allows direct contact of the tumour cell embolus with some of the collagen components of the sinusoidal wall.