Tissue Interactions in Carcinogenesis

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edited by

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Preface

The main purpose of this book is to provoke discussion and to advocate that study of the behaviour of the tumour as a whole be reintroduced into cancer research. It is a well-known maxim that when progress in a particular field is slow one should re-examine the concepts which are generally taken for granted. It is my belief that such is the present position in cancer research, for while it is now many years since Yamagiwa and Itchikawa discovered how to produce tumours at will in animals, we still do not know how carcinogenic agents act. Therefore this book attempts to begin a challenge to some of the well-established dictums of this field such as the view that cancer is simply the result of uncontrolled cellular multiplication caused by breakdown of "growth control" mechanisms. This kind of statement does not satisfactorily explain the overall properties of tumours such as their histological disorganisation, invasiveness and propensity to metastasize. It is therefore time to re-examine the whole problem and to try to develop new explanations for the phenomena we observe.

The interpretations and hypotheses presented in this book may prove to be wrong or too simple but if it stimulates discussions and a critical reassessment of generally held basic assumptions, its aims will have been achieved.

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Introduction: Rationale of a New Approach to Carcinogenesis

Despite the passage of nearly 200 years since it was first convincingly argued that carcinogenesis can be induced by environmental agents (Pott, 1779) and of over half a century since the discovery of methods for the experimental production of neoplasms (Yamagiwa and Itchikawa, 1915) the underlying disturbances responsible for the development of cancer remain unknown.

In this time there has, nevertheless, been considerable progress resulting in the identification of several chemicals, viruses and types of radiation as so-called "causes" of cancer. Strictly speaking, however, these are causative *agents* and the processes involved in eliciting the carcinogenic response from the affected tissues are still unknown.

This is not intended to detract from the immense prophylactic value of this knowledge or of the work which has led to its acquisition but, it is already apparent from the profusion of agents found to be capable of inducing tumours, that the complete exclusion of such factors from the human environment is not going to prove a practical proposition. It is evident, therefore, that satisfactory long-term solutions to the cancer problem will depend on understanding how the disease arises, and thence of finding methods of blocking its development or of curing it once it has become established. Meanwhile, of course, the continued assessment of the carcinogenic potential of environmental agents is extremely valuable.

For a long time now, research devoted to unravelling the mechanism of carcinogenesis has concentrated mainly on the study of cellular multiplication in tumours. Although it has yielded much valuable information this approach has not so far provided a basis for understanding the overall properties of cancerous tissues or the way in which these new traits arise. Moreover, it is unlikely that such an exhaustive analysis of a single aspect of tumour biology ever can.

Alternatively, it is perhaps possible to gain new insights into the mechanism of carcinogenesis by reconsidering the characteristic features of the tumour as a whole. After all, a tumour is not simply a collection of "cancer cells" but also an organoid structure, composed of a number of different tissues, the arrangement of which becomes progressively disturbed during its maturation.

It may be thought facile to suggest that, in order to know more about

tumours, we should actually look very carefully at exactly what intact tumours do, but consideration of this sort is needed in the planning of further experiments to elucidate the nature of cancer. One of the major problems in adopting this approach is the great variation in structure and behaviour which different neoplasms display. Nevertheless, it is possible to select certain properties which are sufficiently common to merit description as typical of tumours in general. These are listed below. Although none of these properties is, on its own, absolutely characteristic of neoplasia every tumour possesses one or more of them.

Common Characteristics of Neoplastic Tissues

1. Increased rate of growth and of cellular proliferation
2. Development of cellular pleomorphism
3. Disturbance of cellular arrangement; gradual disruption of specific histological structure
4. Invasion of adjacent tissues
5. Metastasis

Considering each of these, briefly, in turn:

Increased rate of growth and of cellular proliferation

Active neoplasms are almost invariably recognised by a localised and progressive increase in size in the tissue involved. Microscopical examination reveals that the swelling is much more cellular and frequently contains more mitotic figures than the normal tissue in the rest of the organ. It is accepted, therefore, that marked cellular proliferation is an integral feature of carcinogenesis. The increase in number of cells in the affected area has been attributed by many to a disturbance of factors controlling the rate of cellular multiplication. It has been explained above that much research has already been conducted on the kinetics of cellular proliferation in neoplastic tissues and their normal counterparts, and in seeking new approaches to the problem it is perhaps appropriate to turn more attention to other properties. This should not be taken as a recommendation that studies of cellular proliferation in tumours should stop; merely that investigation of the cancer problem should be expanded to include greater consideration of other aspects.

Pleomorphism

The presence of morphological differences amongst cells of similar origin is a common feature of neoplastic tissues. Thus, in a carcinoma

the individual epithelial cells may show marked variation in properties such as nuclear size, distribution of chromatin and cytoplasmic basophilia. The degree of variation between the neoplastic cells is different in different tumours and even in different parts of the same tumour. Pleomorphism is a property that is easily recognised with the light microscope but less readily with the electron microscope. Indeed, electron microscopical examination of the epithelial cells in carcinomas shows no consistently abnormal feature which distinguishes them from normal cells of equivalent type. Detailed study of this property has therefore failed to provide insight into the mechanisms by which carcinomas arise and does not seem as promising as others for further work.

Disturbance of tissue architecture

The characteristic histological pattern of an organ becomes disturbed in the region giving rise to a neoplasm. The degree of disruption of this pattern varies considerably in different tumours. Although disturbance of tissue organisation cannot be said to be absolutely specific for carcinogenesis, for it is sometimes seen in other conditions such as inflammatory processes and wound healing, this property constitutes one of the most constant features of neoplasia.

Recent work has shown that interactions between different tissues of an organ are necessary for the development and maintenance of its normal histological structure (see Chapters 1, 2 and 3). It therefore seems likely that study of the structural and functional interrelationships between different tissues in a region undergoing carcinogenesis will offer opportunities for obtaining new information on the nature of cancer.

Invasion

Penetration of adjacent tissues is another pattern of behaviour commonly seen in tumours. Non-neoplastic tissues do sometimes manifest invasive activity as in the growth cycle of the hair follicle, the growth of the mammary ducts in pregnancy and the implantation of the ovum. Such examples are few, however, and are specifically related to controlled alterations in tissue architecture. In this type of regular and orderly penetration the invading cells remain in close contact and cease to invade after a short time. In carcinomas, however, invasion is random, progressive and unrestrained. It contributes greatly to the disturbance of local tissue architecture characteristic of this condition. Such invasion clearly indicates that pre-existing relationships between the components of the neoplasm and the immediately adjacent tissues

are disturbed and the nature of this change deserves further investigation.

Metastasis

It is well known that malignant tumours have the propensity to disseminate neoplastic cells or tissue fragments to distant parts of the body where they continue unrestrained growth and produce secondary tumours. However, it is completely unknown why some types of tumours commonly metastasize and others remain only locally invasive or simply expand within a compressed "capsule" of normal tissue (i.e. remain "benign"). Other important aspects which are as yet unexplained include the fact that with each type of malignant tumour there are some sites to which it will commonly metastasize and others to which it will not. The interrelationships between the tumour cells and the tissues of each of these types of sites clearly deserves investigation because it might provide information useful in the control of metastatic spread. Also, as the emergence of *each* metastatic deposit represents the formation of a new tumour, albeit initiated by a unique agent (i.e. a cell or cells from an existing tumour), careful studies of their location development and behaviour may well reveal fundamental principles involved in the formation of tumours in general.

The structure of secondary tumours usually bears some resemblance to that of the primary from which they arise (see Willis 1952, for review). The secondaries of many tumours (including, for instance, those of papillary adenocarcinomas of the thyroid and the ovary) even possess distinctive patterns of cellular arrangement, or organoid structure, typical of the primary tumour. This raises the issue of whether all the cell types of a primary tumour participate in metastasis or whether one type usually spreads on its own. If the latter is true one must consider whether the tissues of the host organ are coerced to participate in the formation of the secondary tumour and provide the missing elements of the organoid pattern.

Metastasis constitutes the major clinical problem of cancer because surgical removal of the primary tumour and other methods of treatment usually fail to cure if widespread dissemination has occurred. The indications for detailed study of this property are therefore great, especially as it has so far been comparatively neglected.

Motivated by considerations similar to these, the contributors to this book have studied the structural and functional properties of tumour tissues and related problems. Their work, involving the use of morpho-

logical, biochemical and transplantation techniques, has produced substantial evidence which is consistent with the interpretation that interactions between different tissues are disturbed in the region of a developing neoplasm. Whether this disturbance is causally associated with the development of cancer or is merely the consequence of the disease is at present unknown, but the concept is worth exploration because, whichever is the case, such investigation may provide information which can be used in the control of the carcinogenic process.

As a contribution towards this end, most of the currently available information on this subject has been assembled and coordinated in this book, in the hope that it may serve as a useful source of reference and stimulate further work on tissue interactions in carcinogenesis.

Briefly, the composition of the book is as follows:

The first two chapters are intended to acquaint readers with the general properties (Chapter 1) and mechanisms (Chapter 2) of tissue interactions in embryogenesis.

Chapter 3 reviews examples of interactions between tissues in adult organs.

The remainder of the book presents and discusses information pertinent to the relationships between different tissues in precancerous states and in established tumours and is subdivided as follows:

Chapters 4–8 Morphological studies (histological and ultrastructural) on human neoplastic conditions and on experimentally induced ones in animals.

Chapters 9–14 Biochemical and transplantation studies on the mechanisms of carcinogenesis and of invasion.

Finally, it is important to explain that the scope of this book is limited to a consideration of *local* tissue interactions in the area of a developing neoplasm and excludes systemic effects, humoral or immunological. This is because there is very little information on the relationships between systemic and local factors and it is not profitable to speculate until more knowledge is available.

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Contents

1. Tissue Interaction During Embryonic Development: General Properties

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I. INTRODUCTION

In the development of any multicellular organism a great variety of specialized cell types is formed. The origin and regulation of this diversification within the progeny of a single cell, is one of the fundamental problems of developmental biology.

Frequently, an inhomogeneity is already seen to exist in the egg cell itself and during cleavage these regional differences of its cytoplasm are passed on to different groups of cells. It is assumed that the fertilized egg contains cytoplasmic factors responsible for the regulation of gene function during subsequent embryonic development. These factors, or "morphogenetic determinants", are unequally distributed in the egg cytoplasm and thus become separated by the cell divisions of early embryogenesis (see Davidson, 1968, pp. 103 ff.). A well known example is the presence in some eggs of a distinctive "germinal cytoplasm" giving rise to the germ cell line (for reviews, see Davidson, 1968; Blackler,

1970). In the so-called "mosaic" type of development, diversification of cell types appears to be entirely due to differences in the inherited egg cytoplasm, and the differentiation of an individual cell therefore proceeds independently of its environment.

Such purely intracellular control of cytodifferentiation is, however, rather exceptional with higher organisms and, even where it does occur, it is operating only during a relatively short period in early embryogenesis. In most cases, the intrinsic control system of a cell is subject to influences coming from its environment. By far the most important source of such extrinsic influences are other, adjacent cells. In particular, the development of vertebrate embryos is highly dependent on interaction between like and unlike cells. It is obvious that the integrated behaviour of individual cells within a tissue requires some sort of intercellular communication. However, embryogenesis is also characterized by a precise spatial arrangement and temporal coordination of diverse tissues participating in the formation of complex structures, such as organs. This perfection is achieved by the development of one tissue being under the control of its partner, this regulation frequently operating in both directions.

The decisive influence of one tissue on the developmnet of another has long been known as *embryonic induction*. It was first demonstrated in the formation of the lens under the influence of the optic vesicle (Spemann, 1901). This was followed by the discovery (Spemann and Mangold, 1924) of the induction of the medullary (neural) plate in gastrula ectoderm by the underlying chordamesoderm, a process that became known as "primary embryonic induction" (Saxén and Toivonen, 1962). Due to the high complexity of the response, this system—still representing the most dramatic and spectacular instance of embryonic induction —has so far contributed little to our understanding of inductive processes. It is therefore not surprising that other interacting systems came into focus; only they did so much later, when the development and refinement of organ culture techniques allowed new approaches to be taken. These investigations, concentrating on "late" or "secondary" inductive processes operating in the formation of vertebrate organs, were initiated by the studies of Grobstein in the early fifties.

Organogenesis in fact provides particularly illustrative examples of regulative tissue interaction. With very few exceptions, the organs of vertebrates consist of at least two tissue components, in many cases deriving from different germ layers. These components, having been initially separate, very early in embryogenesis, and having followed quite different developmental pathways, become secondarily associated and subsequently integrated into new morphological and functional

units. It was shown that the two components are interdependent in their development, and these two-way organogenetic tissue interactions are equally referred to as being inductive, as expressed by Grobstein (1956b): "Inductive or morphogenetic tissue interactions take place whenever in development two or more tissues of different history and properties become intimately associated and alteration of the developmental course of the interactants results." It is evident that according to this wide conception, "inductions" may regulate quite different developmental processes in various systems.

In the following pages *some* examples of inductive tissue interaction will be presented and discussed. Primary induction, which has been treated extensively by Saxén and Toivonen (1962) and more recently by Saxén and Kohonen (1969) is not reviewed here. Instead, emphasis is placed on organogenetic tissue interactions occurring during the development of epithelio-mesenchymal organs. Lens and cartilage induction are included mainly to illustrate some features not as clearly observed in the former group. Even among epithelio-mesenchymal interactions some aspects had to be neglected, the most important of them—at least in the context of this volume—being the ability of dissociated cells of both tissues to reaggregate, sort out, and eventually to rearrange into organotypic structures (Moscona, 1960; Moscona and Garber, 1968; Weiss and Taylor, 1960; Grover, 1963).

II. PRINCIPAL METHODS*

Essentially, the requirement for inductive interaction between two tissues is tested by comparing their development in isolation after separation† with that obtained after reassociation. The specificity of inductive influences is assayed by observing developmental variations of a tissue in combination with various "heterogenous" partners, i.e. with tissues derived from other regions or organ rudiments. The onset and duration of inductive activity in one tissue, and of the ability to respond ("competence") in the other, are determined by combining partners of different developmental stages ("heterochronic" combinations).

The earliest studies on embryonic induction were conducted almost exclusively in amphibians, as their embryos are easily accessible, and have proved able to withstand surgical manipulations. The morphogenetic role of a presumed inductor region was tested by observing the effect of its extirpation on the development of adjacent tissues, or by

* This brief outline is only intended to aid the reader in evaluating the experimental findings with regard to possible ambiguities resulting from the methods employed.

† Or before they first become associated in development.

the creation of artificial tissue associations within the embryo through transplantation of either inducing or responding tissue to different sites. The now classical examples of primary induction and of lens induction have been worked out mainly with these methods (reviewed in Spemann, 1938).

The analysis of many organogenetic tissue interactions is, however, not as practicable in whole embryos. These later interacting systems have become accessible to study by through the introduction of organ culture methods. Simultaneously, and due to the possibilities and limitations of this new technique, preference has shifted from lower to higher vertebrates, with the chick and mouse embryo becoming the favourite objects of research. Consequently, our present knowledge of organogenetic tissue interactions is based almost entirely on work done in birds and mammals.

Several methods have been developed for the *in vitro* culture of vertebrate organ rudiments, differing in the nature of the substrate and in the composition of the nutrient medium. Organ rudiments are either explanted on the surface of coagulated plasma, as in the original method of Fell and Robison (1929) or on nutrient medium solidified with agar (Wolff and Haffen, 1952), but they can also be placed directly on the surface of the culture vessel. Most explants develop best at the air-liquid interface, on a raft (e.g. of lens paper—Chen, 1954) or on a disc of membrane filter suspended over the liquid medium (Grobstein, 1956a). These various methods yield essentially comparable results, although the physical substrate has a great influence on cell attachment and migration. For example, explants on agar remain

Fig. 1. The experimental procedure for the study of tissue interaction in organ culture (method of Grobstein, 1953a, 1956a), schematically demonstrated on the submandibular salivary gland. The rudiment is excised from a 13-day mouse embryo, part of its capsular mesenchyme is removed mechanically (for use in recombination cultures), and the remaining gland is incubated in a 3% trypsin-pancreatin solution. After about 3 min, the epithelium and the mesenchyme can be separated by repeated flushing through a fine bore pipette. The tissues can be recombined either in direct contact or placed on either side of a thin (25 μm) membrane filter ("transfilter"-recombination). Explants are cultured on a filter disc suspended over liquid medium. The photographs at the bottom show the typical development of the intact rudiment, and of experimental recombinations after 3 days *in vitro* (living cultures, approx. × 25). Note that the isolated epithelium is unable to develop unless mesenchyme is added, and that the mesenchyme supports the development of the epithelium even when separated by the filter (for histological sections of transfilter-cultures, see Figs. 1 and 2 in the following contribution by Saxén). — In experimental tissue recombinations, organ-specific mesenchyme may be replaced by mesenchyme from various sources. In the salivary gland, for instance, such an exchange would result in complete absence of epithelial development.