EXPERIMENTAL STUDY OF THE COURSE AND REGULATION OF TUMOUR GROWTH

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by

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RECENT EXPERIMENTAL STUDY of the course and regulation of tumour growth is less extensive and systematic than the evident importance of the subject deserves. The difficulty of finding suitable experimental material is one of several explanations. Some general principles seem to be emerging, however, from disconnected inquiries, and I shall review four of these, namely the study by various workers of fibroadenoma of the breast in rats, the investigation, especially by Rous and his colleagues, of induced tumours of the skin in rabbits and mice, and personal observations on induced tumours of the bladder and spontaneous tumours of the breast in mice.

Mammary fibroadenoma in rats

Fibroadenoma is the commonest spontaneous tumour of the breast in rats. Although these tumours are admittedly benign, some are transplantable in series in normal rats and, despite difficult transplantation and extremely slow growth, repay investigation on account of unusual properties, especially a capacity to respond to stimulation by sex hormones and a tendency to develop into sarcoma.

The structure of primary tumours is liable to diverge widely from that of atypical fibroadenoma. The epithelial component may increase at the expense of the connective tissue, leading to the histological appearance of adenoma or secreting adenoma. This happens during pregnancy and lactation or after the administration of oestrogens, and under these circumstances the histological changes in the tumour roughly correspond with those in the surrounding breast tissue. The tumour responds to the physiological stimulus in much the same way as the normal tissue from which it derived. On the other hand, the connective tissue component may overgrow the epithelium to produce a fibroma or, rarely, a sarcoma. This is apt to occur in long-standing tumours in old rats, possibly because growth of the epithelium depends on oestrogenic stimulation which becomes inadequate in old rats.

When atypical fibroadenoma is transplanted, the daughter tumours for many successive generations may be entirely similar, but structural variations are common. Like the primary tumours, and under similar hormonal conditions, the transplanted tumours may consist predominantly of adenoma or secreting adenoma. In other, undefined,

circumstances the epithelium may differentiate into squamous, sebaceous or sweat gland type; the connective tissue may show chondroid, osteoid or adipose differentiation or chronic mastitis may be simulated. All these variations from the typical are temporary and reversible; if the variant tumours be transplanted they revert to fibroadenoma. Two other variations, to fibroma and sarcoma respectively, are not reversible; transplantation does not restore the typical structure of fibroadenoma.

Sarcoma, rare in primary tumours, is common in transplanted ones; it may develop early or late in the course of serial transplantation, directly from fibroadenoma or indirectly from fibroadenoma to fibroma and thence after a longer or shorter interval to sarcoma; the change may be completed within one transplanted generation or spread over many generations. A comparable irreversible change in the epithelial component, leading through adenoma to carcinoma, occurs but rarely.

The plasticity and variability of structure and behaviour of the fibroadenoma is thus accompanied by a tendency to irreversible change towards a more stable and more aggressive tumour and especially towards sarcoma. Fibroadenoma, it seems, is a tumour in a stage of uncompleted development; given time and opportunity, it changes into something different. In compensation for the experimental inconvenience of their sluggish growth, these tumours reveal, as though in "slow-motion", processes which are less apparent in more vigorous growths. They show conspicuously two phenomena with which this lecture is particularly concerned; the first is reactivity to extraneous stimuli for which I shall use the term *responsiveness* and the second is the development, which I shall call *progression*, through successive stages as a result of irreversible qualitative changes in the tumour itself. Progression implies not a mere augmentation of size or of properties already present but the irreversible development of new properties. The irreversible change from fibroadenoma to sarcoma is an example of progression; the reversible change from fibroadenoma to secreting adenoma is not. The observations on fibroadenoma show that different paths of progression are available, leading from similar starting points to diverse end-points, for example carcinoma and sarcoma. Progression may be halted for a long time at any point along the chosen path and it does not necessarily reach an end-point within the life-time of the host. If incomplete when the animal dies, progression continues to the end-point in transplanted tumours.

Induced tumours of the skin of rabbits and mice

The original investigation of the carcinogenic action of coal tar was carried out with rabbits but most later workers used mice. Rous and his colleagues, however, have re-investigated the tumours induced on rabbits' skin by tar or carcinogenic hydrocarbons. Tar evokes warts which have the histological structure of benign papillomas but differ

from neoplasms, as usually defined, by persisting only so long as applications of the carcinogenic substance are continued. Rous describes the warts as " conditional " tumours, their growth being dependent and conditional upon extraneous stimulation. Some of the conditional tumours are locally invasive and histologically resemble malignant growths.

When the carcinogenic stimulus is withdrawn, conditional tumours disappear. They reappear, in their earlier form, and at the same site, in response to renewed applications of the carcinogen but they reappear also in response to non-specific stimuli such as wound healing or irritants like turpentine that never by themselves elicit tumours from normal skin. Tar or other carcinogens, therefore, induce a permanent change in rabbits' skin, and permanently altered cells, which Rous calls cells in the subthreshold neoplastic state or latent tumour cells, remain at the site of conditional tumours that have regressed, and respond to non-specific stimuli by growing in the form of benign, or sometimes apparently malignant, tumours. Rous distinguishes two factors in the induction of tumours, namely (1) *initiating factors* which induce the initial change from normal cells to latent tumour cells in the subthreshold neoplastic state, and (2) *promoting factors* which stimulate these altered cells to proliferate.

Tar warts in rabbits like the epithelial component of fibroadenoma in rats are responsive to extraneous stimuli; the effective stimulus for the one is external and artificial and for the other natural and physiological. The tar warts further resemble the fibroadenomas in representing a stage of uncompleted development; they are liable to progression towards a definitive stage of unresponsive carcinoma. In rabbits, progression to carcinoma is long delayed. It results, according to Rous, from a secondary step-like change in a papilloma and the change is not a mere exaggeration of the preceding papilloma but a wholly new event—the genesis of a neoplasm distinct from its predecessor.

Similar general principles apply, as Mottram and Berenblum have shown, to induced skin tumours of mice although with some differences in detail. In mice, progression to carcinoma occurs earlier and more frequently. Berenblum, like Rous, infers a sequence of step-like changes, corresponding with my definition of progression, from normal epithelium to the subthreshold neoplastic state, thence to papilloma and from papilloma to carcinoma.

Tumours induced in the bladders of mice by acetylaminofluorene

My own attention was drawn to the phenomenon of progression by observations on tumours of the bladder induced in mice by oral administration of the carcinogenic substance acetylaminofluorene. (Foulds, 1950.)

The induced tumours of the bladder are varied in structure ranging from localized intra-vesical papilloma to malignant sarcoma-like growths

invading the bladder wall and disseminating in the peritoneal cavity. One of the localized papillomas (AF4) was transplanted and in the first generation it resembled squamous carcinoma in structure. In the second transplanted generation similar squamous epithelial tissue was mixed with sarcomatoid tissue, the term sarcomatoid being used to evade the question, irrelevant to the present discussion, whether the sarcoma-like tissue was true sarcoma or atypical epithelioma. In the third and all subsequent generations the tumours were wholly sarcomatoid and no further progression occurred. Another tumour (AF7), which had invaded the bladder wall and spread in the peritoneal cavity, was sarcomatoid before transplantation and continued unchanged through several transplanted generations.

In its definitive, stable phase the tumour AF4 looked almost identical with tumour AF7, but AF7 attained this state in the primary tumour whereas AF4 reached it through intermediate steps in the course of serial transplantation. Other tumours in the same experiment corresponded roughly with the earlier generations of transplanted AF4 tumours and there is apparently a graded series of developmental stages in these tumours. A tumour does not always reach the end-point of its development in the original host as did AF7. Progression may be halted at any stage and haemorrhage or urinary obstruction may kill the animal before progression is far advanced. If progression is uncompleted when the animal dies, as in tumour AF4, it advances to the end-point in transplanted tumours growing in normal mice. This demonstrates the important fact that progression is not dependent on continued action of the carcinogen that initiated the primary tumour.

Spontaneous mammary tumours in mice

The mammary tumours developing spontaneously in certain strains of hybrid mice which I bred for another purpose gave unusual opportunities for the study of responsiveness and progression. A high percentage of the female mice developed mammary tumours at a relatively early age and most of them had multiple tumours. Less than half of the tumours grew steadily ; the others grew conspicuously during pregnancy and regressed, partially or completely, after parturition. Tumours were measured frequently and their growth recorded on graphs which showed also the approximate times of parturition. Most of the females were continuously with males and their litters were removed soon after birth so they had pregnancies in rapid succession. Figures 1-4 show examples of the several hundred charts made.

The tumours are divisible into two main groups. The first comprises tumours that grow steadily and progressively from their first appearance and whose course is represented graphically by an approximately straight line. (Figs. 1, 2 and 4.) They are designated *unresponsive tumours* to distinguish them from the second group of *responsive tumours* that respond to reproductive activity of the host by growing during pregnancy and

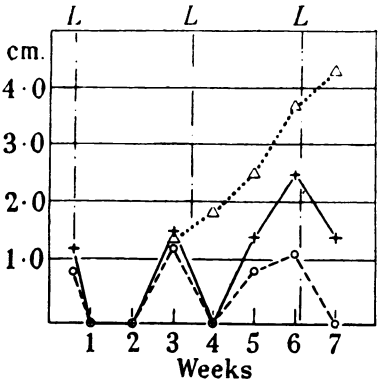

Fig. 1. Showing the growth curves of 3 tumours in the same mouse. The size of the tumours is recorded as the sum of two diameters measured in centimetres and the vertical broken lines headed " L " indicate the dates of parturition. One tumour, represented by a broken line, is responsive Type I, one (continuous line) is responsive Type II, and the third (dotted line) is unresponsive.

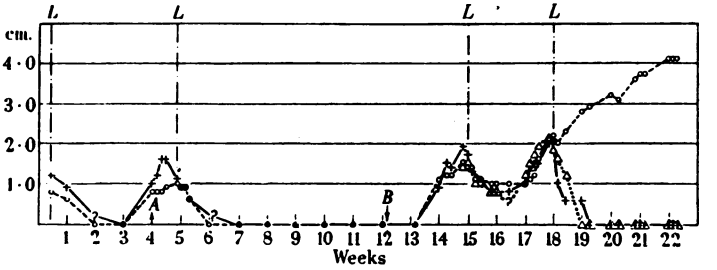

Fig. 2. Showing 2 responsive tumours which regressed after parturition and remained in abeyance during an intermission of breeding. The mouse was separated from males between the points marked A and B. One of the tumours (broken line) subsequently became unresponsive. The other early tumour (continuous line) and a later one (dotted line) remained responsive.

regressing after parturition. The growth of responsive tumours is shown graphically by waves with peaks at or near the times of parturition. (Figs. 1-4.) During intermissions of breeding responsive tumours usually remain in abeyance but they recur promptly when the mouse becomes pregnant again. (Fig. 2.) The behaviour presumably depends on hormones but the mechanism is obscure. Apparently it is not due to a straightforward action of oestrogens or progesterone. The possible intervention of pituitary or placental hormones needs much more study. The justifiable inferences are that the responsive tumours are conditional ones like the tar warts of rabbits and that systemic changes, dependent on pregnancy, act as promoting factors which stimulate latent tumour cells to proliferate.

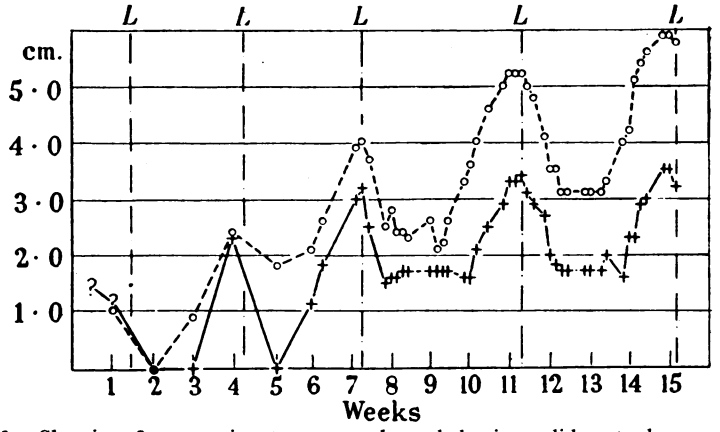

Fig. 3. Showing 2 responsive tumours whose behaviour did not change during observation for 15 weeks.

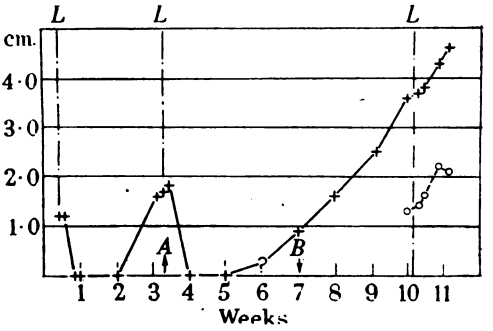

Fig. 4. Illustrating progression during an intermission of breeding. A responsive tumour regressed but recurred during the period between the points A and B, when the mouse was separated from males, and was then unresponsive as shown by its behaviour after the subsequent pregnancy.

There is no regularity in the proportion or order of appearance of responsive and unresponsive tumours in individual mice. Responsiveness is not proportional to the size or duration of a tumour; it persists sometimes through repeated pregnancies covering a large fraction of the lifespan of a mouse and in relatively large tumours. (Fig. 3.)

Some responsive tumours, designated Type I, grow to similar peaks in successive pregnancies and usually regress completely between; they achieve no net increase in size over long periods of time. (Fig. 1.) Other responsive tumours, designated Type II, reach progressively higher peaks in successive pregnancies, and the intervening regressions are often incomplete. (Figs. 1 and 3.) It is possible to resolve the growth curve of a Type II tumour into an approximately straight line with superimposed waves corresponding with the pregnancies. The slope of the straight line indicates the rate of net increase in size, or the *intrinsic*

growth rate, and the amplitude of the waves measures the degree of responsiveness to pregnancy. Comparison of numerous curves shows that responsiveness and intrinsic growth rate are independent variables; any degree of the one may be linked with any degree of the other.

Many responsive tumours undergo progression to the unresponsive state. The change occurs unpredictably after short or long periods of responsive behaviour; usually it seems abrupt and is permanent. The phenomenon is illustrated in Fig. 2. Two tumours of nearly the same size appeared at the same time towards the end of a pregnancy. After parturition, they regressed together, remained in abeyance during an intermission of breeding and recurred together in the ensuing pregnancy when a third tumour developed. The three tumours regressed partially and recurred during the next pregnancy until the end of which their growth curves were so alike that it is difficult to distinguish them when charted on a single diagram. At the end of this pregnancy, two tumours regressed promptly and completely and one continued to grow with a growth curve characteristic of an unresponsive tumour. The altered tumour was one of the two that appeared earliest and that for 4½ months had run parallel courses. The abrupt change in the behaviour of one out of three similar tumours in the same mouse must be ascribed to a change in the tumour itself and not to a change in the environment. The tumour behaves differently because it has become different.

It has been repeatedly confirmed that, as shown in Fig. 2, when multiple tumours are present only one of them undergoes progression at a time. It seems to be a general rule that tumours in the same animal undergo progression independently of each other. This rule of the independent progression of tumours implies that progression depends more on the intrinsic properties of the tumour cells than on the environment to which they are exposed.

Progression is manifested most impressively by the frequent abrupt change from responsive to unresponsive growth. It is shown also by a change from Responsive Type I to Responsive Type II and by conspicuous acceleration of the growth rate without apparent alteration of the responsiveness. Different characters in the same tumour thus undergo progression independently of each other.

Progression often becomes evident at the end of a pregnancy when the expected regression of a hitherto responsive tumour does not occur. (Fig. 2.) Pregnancy seems to exert a " trigger-action," making apparent a change that occurred some time before, but it is not an indispensable " cause " of progression. Some previously responsive tumours recur as unresponsive ones during intermissions of breeding (Fig. 4.). Progression occurs therefore in latent tumour cells whilst growth is suppressed. Progression is not dependent on growth.

From a detailed study of several hundred mammary tumours of mice (Foulds, 1949) and a survey of other investigations, the following general principles, or rules, of progression are suggested.

I. Progression occurs independently in different tumours in the same animal.

II. Progression occurs independently in different characters in the same tumour.

III. Progression is independent of growth.

There are two notable corollaries :—

(1) At its first clinical appearance a tumour may be at any stage of progression.

(2) Progression is independent of the size or clinical duration of the tumour.

IV. Progression may be abrupt or gradual.

V. Progression follows one of several available paths leading from the same starting point to different end-points.

VI. Progression may be halted at any stage and does not always reach its end-point within the life-time of the host.

These principles may help to lighten some of the mysteries in the pathology and therapeutics of human cancer. For example, prognosis based on a distinction between " early " and " late " tumours is sometimes unaccountably misleading ; a presumed " early " tumour withstands radical treatment and kills the patient whereas an apparently " late " tumour is unexpectedly cured. A possible explanation is that the prognosis depends to an important extent on the stage of progression which, according to the third of the above rules, is independent of growth. A tumour may be small in size and young in clinical duration but far advanced in the progression of aggressive characters. Conversely, a large tumour of long duration may remain in an early stage of progression and respond well to treatment.

The principle of the independent progression of characters holds important implications. It is surely a mistake to conceive " malignancy " as a single, indivisible character of the more dangerous tumours. Malignancy is a type of behaviour which results from a number of properties variable in proportion and degree. Rapid growth, invasiveness, dissemination, loss of histological differentiation and so on, are expected in malignant tumours, and in text-book examples they are developed proportionately, but it is not always so. As a consequence of the independent progression of characters, the several properties may develop disproportionately. Disproportionate, or " out-of-step " progression accounts, it may be, for the " locally malignant " tumours like rodent ulcers which invade but do not disseminate and the metastasising " benign " tumours which disseminate without notable local invasion.

Cancer of the prostate satisfies the accepted criteria for the diagnosis of a malignant tumour but it has, additionally, an unexpected responsiveness to hormones which, according to some definitions, would exclude it altogether from the family of neoplasms. Here again the independent

progression of characters is credibly responsible for the anomaly. The responsiveness is out of step with the other characters appropriate to a malignant tumour ; progression of one character is retarded. If this be true, further progression to the unresponsive state is predictable. It is not possible to increase the opportunities for progression by transplantation of the tumour, as in animals, but there is strong clinical evidence that it does occur for, sooner or later, most or all cancers of the prostate become refractory to hormonal control. The progression to unresponsiveness takes place, as might be anticipated from the third of the rules of progression, in tumours whose growth is inhibited by treatment.

The eventual failure of other methods of treatment whose immediate effects are satisfactory is possibly attributable, similarly, to progression of the tumour towards its end-point. Responsiveness to hormones is particularly amenable to experimental study but is reasonably expected only in tumours derived from tissues that normally respond to hormones. Other forms of responsiveness, using the term widely for reactivity to extraneous stimuli, may be equally or more important. All treatments based on responsiveness, however, are liable to eventual failure, as the result of progression of the tumour to the unresponsive state.

A tendency to progression towards an ultimate stage of unresponsive, unmanageable growth is possibly inherent in the nature of neoplasia. Progression is not dependent on continuance of the carcinogenic stimulus that initiated the tumour. The independent progression of multiple tumours in the same animal suggests that intrinsic properties of the tumour cells are more decisive than extraneous stimuli. Progression occurs frequently in transplanted tumours probably because transplantation prolongs indefinitely the time and opportunities available for it and also, by selective action, favours the predominance of the changed cells which, at first, may constitute only a small fraction of the whole tumour. Animal experiments, therefore, supply no evidence for a specific " cause " of progression but they do not exclude the possibility of acceleration or retardation by experimental or therapeutic procedures. The outstanding problems of responsiveness and progression are numerous and difficult but their investigation by observation and experiment may be rewarded by a deeper understanding of how neoplasms behave and how they might be controlled.

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