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New Developments in Cancer Research

Advances in Tumour Pathogenesis

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An appraisal of progress in science, judged from proceedings at Congresses, becomes more and more difficult, with the ever-increasing number of papers submitted and the practise of holding many sessions concurrently. Thus, from the VIIth International Cancer Congress (London, July 6–12, 1958), any single observer could derive only an incomplete picture of contemporary advances in cancer research. On the other hand, the titles and brief summaries presented before the Congress do give a fairly clear impression of developments and shifts in emphasis in the field, by indicating what are the problems that are *currently* considered important. With this as a guide, and from a more detailed survey of the literature, an up-to-date picture of contemporary progress can be made.

The present article will be restricted to *tumour pathogenesis*, covering carcinogenesis in its various manifestations; genetic, hormonal, and other intrinsic factors influencing tumour development; and some of the more fundamental principles of neoplastic transformation, including the role of tumour viruses. Within the limited space available, I shall confine myself to trends of current research, quoting specific investigations only as illustrations or key references.

New developments in science, or changes in emphasis or interest, arise in one of two ways: (1) in response to an outside challenge, and (2) as an outcome of the scientific work itself. The former extends the scope of enquiry by presenting essentially new problems for study; the latter, by bringing to light new possibilities, at more fundamental levels, within the framework of existing knowledge. Two important examples of the first category, in the field of tumour pathogenesis, are the relation of tobacco smoking and air pollution to human lung cancer, and the problem of radiation hazards with respect to human leukaemia. A striking example of the second category is the demonstration of a causative virus in mouse leukaemia. Without denying the practical importance of the former two problems, it is the latter (mouse leukaemia virus) problem which is likely to have the most profound influence on our thinking in the period immediately ahead of us.

Though the theoretical concept of a viral origin of cancer is fairly old—its first practical demonstration dating back to 1908, when ELLERMANN and BANG[1] succeeded in transmitting fowl leukosis by a virus—it was not until 1951 that GROSS[2] demonstrated a viral agent involved in *mouse* leukaemia. It was, in fact, only after the confirmation of GROSS' results (by FRIEND[3], STEWART[4], GRAFFI[5], and others) that the significance of this finding became generally apparent.

The critical factor for the initial transmission of mouse leukaemia by cell-free (virus) extract was the use of new-born mice as recipients. In 1933, it was shown that in the case of the 'milk factor virus' for mammary cancer in mice, successful transmission was only possible soon after birth[6]; a viral agent had long been suspected in mouse leukaemia; yet an interval of 20 years elapsed before the lesson learnt from the study of mammary cancer was applied to the problem of leukaemia. The fact that other known tumour viruses (e.g. of the ROUS sarcoma in the fowl[7] and of the SHOPE papilloma in the rabbit[8]) could be transmitted to the adult, was possibly responsible for this delay. All the same, our failure to follow so obvious a lead is, when considered in retrospect, truly astonishing, and a cause for humble self-criticism.

This new work on mouse leukaemia virus has opened up afresh the controversy on the 'virus theory of cancer', and given it a new lease of life (see the review by FURTH and METCALF[9]). It has also given an impetus for the more energetic application to cancer research of a number of modern techniques, such as (i) electron microscopy studies of tumour viruses, (ii) the growth of tumour viruses in tissue culture, (iii) improved methods for the purification of viruses, and (iv) new approaches

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for the immunological analysis of weak antigens; as well as encouraging the re-investigation of other tumour types previously considered non-filtrable. These developments, already apparent in some of the reports at the VIIth International Cancer Congress, will undoubtedly loom large in the next Congress in 1962.

Among the interesting outcomes of this work are the changes observed in the properties of the mouse leukaemia virus after growth in tissue culture[4]. Such material induces a wide range of solid tumours of different kinds, notably in the parotid gland but also in other organs, and tends to be effective even in foreign species. Whether these changes are attributable to an increase in virulence of the virus, or to a marked increase in its concentration, or to the virus having undergone a mutation, or even to the presence of multiple viruses whose relative proportions have changed during passage in tissue culture, has not yet been determined. Further work in this field is, therefore, eagerly awaited.

One of the oldest branches of modern cancer research is that concerned with the role of genetics in the origin of animal tumours. This field has, perhaps, not played so prominent a part at this Congress as at previous ones; yet significant advances have been made in recent years, both in amplification of earlier work and in the development of new fields (see the recent Symposium on 'Genetic Concepts for the Origin of Cancer'[10]). Some phases of special interest in the more recent studies are concerned with (i) genetic aspects of transplantation immunity, (ii) chromosomal aberrations in response to the action of carcinogenic, mutagenic, and radiomimetic drugs, and (iii) the role of genetics in drug resistance (to tumour chemotherapy).

The problem of chemical carcinogenesis has changed radically since the early work on the locally-acting polycyclic aromatic hydrocarbons, dramatically presented at the IInd International Cancer Congress in 1936[11], and the discovery, about the same time, of *remotely-acting* carcinogens. There are now more than 500 known carcinogens, representing over 20% of all compounds tested for such action (see Supplement to the 'Survey'[12]). New carcinogens do not, therefore, arouse much interest any longer, unless they happen to belong to entirely new classes of compounds (e.g. the epoxides[13]), or throw new light on occupational or environmental cancer in man (e.g. in relation to pharmaceuticals, food additives, etc.[14]), or unless they illustrate some new principle in carcinogenesis (e.g. the discovery that urethane acts as a pure initiator for skin carcinogenesis[15]). It should be noted, however, that a systematic study of polycyclic aromatic hydrocarbons is being continued, as an extension of the 'electron distribution' hypothesis of carcinogenesis[16].

The main interest in chemical carcinogenesis is, however, nowadays directed to the metabolic fate of carcinogens in the body, and to their mechanism of action.

With the introduction of isotopically-labelled compounds for *in vivo* metabolism studies, important advances became possible for investigating the fate of carcinogens in the body. While the earlier work, with unlabelled compounds, gave interesting information about the phenolic derivatives metabolically produced from polycyclic aromatic hydrocarbons[17], the more recent studies by HEIDELBERGER and his group, using C^{14}-labelled hydrocarbons, demonstrated that the body can, in addition, break open the ring structure of such compounds, with the formation of dicarboxylic acid and other derivatives[18]. An extension of recent metabolic studies has led to the demonstration of protein-binding between carcinogens, or some of their (unidentified) metabolites, and the tissues[19]. In comparing the carcinogenicity of various hydrocarbons with their protein-binding capacity, some correlation was observed, though with at least one striking exception[20]. Considerable progress has also been made on the metabolism of azo dye carcinogens[19], 2-acetylaminofluorene[21], and other carcinogens, with evidence of protein-binding.

The study of the metabolism of urethane (ethyl carbamate)—a carcinogen of relatively simple chemical structure, capable of inducing adenomas of the lung, but only the initiating phase of carcinogenesis in the skin—has prompted some interesting speculations, based on indirect evidence only, about the possible implication of pyrimidine metabolism in the process of carcinogenic action[22].

From a critical appraisal of the significance of the metabolism of carcinogens in relation to their mechanisms of action (presented at the Congress[23]), it became

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clear that the available evidence on the subject was as yet too scanty, and of too indirect a character, to justify some of the theories currently held.

Eventually, the 'mechanism' of carcinogenesis will undoubtedly have to be interpreted in terms of chemical interactions between carcinogens (or their metabolites) and the tissues acted upon, and even take into account physico-chemical considerations—e.g. the spatial configurations of the molecules, the forces of interaction, etc. (see ALEXANDER[24], HADDOW[25], etc.). But in the meantime, the more modest approach, of investigating the mechanism of carcinogenesis in *biological* terms, might provide a better chance of reaching some understanding of how the transformation from the normal cell to the established tumour is brought about.

Some progress has already been made in this direction with regard to skin carcinogenesis (see BERENBLUM[26]), with little extension, as yet, to locally-acting carcinogenesis in *other* organs. In the field of remotely-acting carcinogenesis, for which the methods of approach are very different, considerable advances have been made, especially in connection with the mechanism of total body irradiation (see KAPLAN[27]) and hormonal carcinogenesis (see BIELSCHOWSKY and HORNING[28]).

From earlier studies on the biological mechanism of skin carcinogenesis[26], a two-stage action is envisaged, comprising (1) *initiation*, which constitutes a sudden, irreversible transformation of a normal cell into a 'dormant' or 'latent' tumour cell, and (2) subsequent *promotion*, a slower process (mainly responsible for the length of the latent period of carcinogenesis) which encourages the dormant tumour cells to develop into progressively growing tumours, presumably by a process of delayed maturation. Though both initiating action and promoting action normally take place locally—i.e. at the site of application—it has recently been shown that *initiating* agents can also act indirectly on the skin—i.e. by being administered intraperitoneally, or by other routes.

The fact that locally-acting carcinogenesis involves a component (initiation) which can operate systemically, brings the locally-acting and the remotely-acting patterns closer together, and thus raises the possibility of a common underlying mechanism. It must be admitted that the evidence for a two-stage mechanism operating in remotely-acting carcinogenesis (e.g. for the thyroid, liver, and possibly the mammary gland) is rather tenuous. But this may be due to technical difficulties in demonstrating the process for tissues other than the skin. In fact, until recently, the field has hardly been explored.

With regard to radiation carcinogenesis, apart from some systematic tests for the carcinogenic properties of radioactive fission products of atomic reactors[29], the most interesting recent advances have come from the study of total body radiation in relation to leukaemogenesis[27], on the one hand, and to tumour induction of endocrine organs[28], on the other.

Malignant lymphoma and leukaemia, which occur spontaneously in certain strains of mice and can be artificially induced by the systemic action of carcinogenic hydrocarbons or oestrogens, can also be induced by total body irradiation—even in strains in which the spontaneous or chemically-induced disease is rare. The incidence of radiation-induced lymphomas can be lowered by thymectomy; the effect reversed by re-implantation of normal (homologous) thymus tissue *after* the irradiation; yet when the thymus alone is irradiated, lymphomas do not develop; and even total body irradiation becomes ineffective when one limb is shielded, or when homologous bone-marrow is injected after such treatment. The possibility that a virus is involved in radiation-induced lymphoma (and leukaemia) is now being considered by various investigators—i.e. in line with virus involvement in spontaneous mouse leukaemia; and the fact that unirradiated thymus tissue, implanted in irradiated *thymectomised* mice, raises the lymphoma incidence, is theoretically in keeping with this possibility.

The involvement of yet another factor—hormonal imbalance—in radiation-induced lymphomas, stressed by KAPLAN *et al.*, is even more apparent in tumour induction of endocrine organs by total body irradiation. Here, one is confronted with an intricate, normal, physiological system of inter-relationship between the different endocrine organs in the body, and especially with 'feed-back' mechanisms with respect to the pituitary gland. It is, therefore, not surprising that such feed-back mechanisms enter into play in the case of *carcinogenesis* of endocrine organs. The classic example of this is seen in the development of tumours in normal ovarian tissue grafted into the spleen (BISKIND and BISKIND[30]). Recent work by FURTH[31] on tumour induction by total body irradiation, with special reference to the pituitary gland, has opened up an important new field of study, both with respect to the identification of the distinctive types of pituitary tumours induced (as judged by their hormonal effects on the respective endocrine target organs), and to the role of feed-back mechanisms in the process of carcinogenesis.

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Another aspect of this work is concerned with the problem of tumour dependence: The earlier studies of FOULDS[32], FURTH, and others[32], on the 'dependence' of certain tumours, for their growth and existence, on their hormonal surroundings, and the possibility of such tumours eventually losing this dependence and becoming hormonally 'autonomous' (the two conditions also being referred to as 'responsiveness' and 'progression', respectively), have done much to clarify the vague clinical concept of 'tumour autonomy', and to explain some of the curious behaviour patterns of tumours in man. The subject is, however, at the fringe of tumour pathogenesis, and bordering on the field of the properties of *established* tumours, which is beyond the scope of this review.

With regard to general theories of tumour causation, less attention seems to be paid at present to the somatic cell mutation theory (but cf. BURNET[33]) and more to the virus theory; while two new concepts have appeared on the horizon—one attributing the neoplastic properties of a tumour to some protein deletion in the cell, and the other attempting to explain neoplasia on immunological principles.

The 'protein delection' hypothesis comes essentially from the known fact that a tumour cell is less specialised than a normal cell. Functionally, it is less able to produce its specialised products (e.g. keratin, cartilage, bone, etc., or specific hormones), while its enzymic pattern is usually simpler than that of the corresponding normal cell (see GREENSTEIN[34]). Structurally, a tumour is also less differentiated than a normal tissue. All this has now been more succinctly formulated as denoting that neoplasia represents *a loss rather than a gain in composition*—presumably enzymic in nature; and the observation of protein-binding between carcinogens and tissues has been adduced in favour of this concept. It should be realised, however, that the evidence is remote and indirect, and hardly sufficient to establish the validity of the hypothesis. Moreover, in at least one respect—namely, in connection with virus-induced tumours—neoplasia represents a *gain* in composition.

The other hypothesis, based on immunological principles (see GREEN[35]), is somewhat more complex, and dependent for its possible validity on (a) the theoretical possibility of isologous tissues acting as (weak) antigens in the host, (b) some indications of specific tumour antigens (evidence rather weak), and (c) the demonstration that carcinogen-protein complexes (synthetically produced) can act as antigens in the original host. The implications of the hypothesis are too complicated to be reviewed here.

Conclusions

In discussing 'trends' in scientific development, one is naturally tempted to confine oneself to issues that somehow fit into a pattern. One is also committed, in such a case, to a highly personal judgement of relative merits, usually of work still in progress, whose outcome cannot be foretold. This does not mean that what has been left out is necessarily considered to be unimportant.

An attempt has been made both to analyse in retrospect, by noting the changes in emphasis and direction of established fields of research in tumour pathogenesis, and to peer a little into the future, by drawing tentative conclusions from the accumulated data that seem well-established. A balance sheet of this kind is never complete and final; for what seems clear and simple, in the light of existing knowledge, will inevitably assume complexity as more information becomes available. Yet, to refuse to appraise and speculate is tantamount to affirming that the purpose of scientific enquiry is merely the accumulation of data.

What, in fact, are the recognisable trends in tumour pathogenesis?

(1)—The search for new carcinogens is no longer the objective for carcinogenic study, since we already have an over-abundance of tumour-inducing agents. The main emphasis is now on (a) the metabolic fate of carcinogens in the body, and (b) their mode of action.

(2)—There is a noticeable change in interest from the more simple 'locally-acting' to the more complex 'remotely-acting' patterns of carcinogenesis, with particular emphasis on the effects of (a) ionising radiation, and (b) hormonal disturbances.

(3)—In continuing the exploration of the two-stage mechanism of locally-acting carcinogenesis of the skin, one component (initiation) has been shown to operate systemically. The extension of the two-stage hypothesis to remotely-acting carcinogenesis is, however, still in its infancy.

(4)—Through the study of remotely-acting carcinogenesis, the psychological barrier between spontaneous and induced tumours is gradually breaking down.

(5)—Though steady progress is being made in the study of the metabolism of carcinogens in the body, its precise role in the mechanism of carcinogenesis is still elusive.

(6)—With the discovery of a viral agent responsible for mouse leukaemia, the 50-year-old controversy concerning the 'viral origin of cancer' is opened up afresh. With the new methods of study, there seem to be good prospects of important discoveries being made in this field in the not-too-distant future.

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[33] F. M. BURNET, Acta Unio Internat. Cancrum 15, 31 (1959).

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[35] H. N. GREEN, Brit. med. Bull. *14*, 101 (1958).

The study of tumour pathogenesis has a clear objective: to enable us to understand *how a tumour arises*. For this, knowledge of the causative factors themselves (i.e. aetiology) is not enough. The crucial issue is concerned with *their mode of action* or, in terms of tissue response, with what happens in the cell during the process of neoplastic transformation. But for this, we must still learn much more about the way the normal cell functions.

Résumé

Le but de cet aperçu est d'examiner dans quelles directions se sont réalisés les progrès actuels de la pathogénie des tumeurs, notamment de la carcinogénèse dans ses différentes manifestations. Il traite aussi des facteurs génétiques, hormonaux et d'autres facteurs intrinsèques, influençant le développement des tumeurs, et de quelques-uns des principes fondamentaux de transformation néoplastique y inclus le rôle des virus.

Recent Advances of Tissue Culture in Cancer Research

By MARGARET R. MURRAY*

Tissue culture, as a body of methods directed towards maintaining the cells and tissues of multicellular organisms outside the parent body, is about as old as this century. However, in the period since World War II, there has occurred a great and rather sudden enrichment of the technical resources of tissue culture, an almost explosive expansion of its applications. With this has gone hand in hand a substantial increase in precision. On this account, and because of the unique kinds of information made available by these methods, it has seemed desirable to summarize recent contributions of tissue culture to cancer research.

The fundamental importance of this research method lies in the fact that it throws open the cells and organs of the higher organisms—including the human—to direct observation and experiment while in the living state. Normal and neoplastic tissues can thus be isolated from many complicating factors that are present in the whole body; they can be examined and photographed at magnifications up to 2000×; their behavior at the cellular level can be recorded by time-lapse cinematography; chemical determinations can be made, and experimental agents of all sorts can be applied to target tissues directly. At once it becomes potentially possible to assess the relative importance of nature and nurture as regards the basic units of multicellular life: to designate the properties of cells that are dependent or independent of the specific environment: to examine the same cell as an isolated individual or as a member of an aggregate, of greater or lesser complexity. These topics are the proper concern of any student of basic problems of neoplasia, of the causes, progress, and arrest of the tissue autonomy which takes place at the expense of the whole organism.

The early tissue cultures consisted of small explants 1 mm³ or less in dimensions, which were embedded in lymph or plasma clots on glass or mica coverslips. These cultures, usually taken from embryos, astonished observors by revealing that the cells of normally fixed tissues could and did move about in their new and roomier environment, as well as grow in size or number. They were stimulated to still greater growth and migration if fed with tissue extracts in addition to the sera. Tissue cultures therefore, had many points in common with a healing wound; and it was in this relationship that they first attracted the interest of the surgeon ALEXIS CARREL, by whom they were dramatized for the medical and scientific public. In addition to the above proclivities, however, tissues from embryos displayed in other hands, such as HARRISON's and the LEWISES', a capacity to continue their development *in vitro* with increased cyto-differentiation for a limited time. And gradually it became clear that growth or multiplication (possibly migration), and cell or tissue differentiation, were in a general way antagonistic processes; hence methods of culture that greatly encouraged the former type of cellular activity were unsuitable for the latter. Culture methods, then, fall roughly into two grand divisions: those that favor unlimited cellular proliferation, versus those that restrain growth and migration, and encourage the assumption by the cell of special functions and the elaboration of specialized structures or products—for example the highly organized striated micellar bundles of the sarcoblast in skeletal muscle[1], the secretions of glandular cells[2] or the myelin sheath of the axis cylinder[3].

The idea of growth has universal appeal—the more so if it is exemplified in animal cells artificially propagated outside the body. Hence it is not surprising that CARREL's chicken heart fibroblasts continuously proliferating for some 30 years, eclipsed in the scientific mind the differentiating tissues which were as continuously being described during that period[4]. It was no less to be expected that the most spectacular recent advances in culture techniques should be directed towards inducing cells to divide to the limit of their capacity, or—since uncontrollable growth is the endpoint of neoplasia—that workers in the field of cancer research should by and large be preoccupied with cell multiplication.

The extraordinary variety, precision, and effectiveness of these newer methods of cell propagation deserve some circumstantial comment. Though many devices

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