news and views

Cell culture studies provide new information on tumour promoters

from I. Bernard Weinstein and Michael Wigler

MANY factors influence tumour incidence. Among these are the chemical carcinogens which, as a class, may act as mutagens. Tumour promoters are factors of an entirely different nature. They can be defined as agents which increase tumour incidence when administered after a suboptimal dose of carcinogen, but which are not in themselves carcinogenic. Recently, reports on the effects in cell culture of the most potent known promoting agents, the phorbol diesters, have raised expectations that an understanding of the action of these compounds *in vivo* is near.

The existence of tumour promoters was most clearly demonstrated more than 30 years ago by Berenblum and others who found that an oil extracted from the seed of *Croton tiglium L.* dramatically enhanced the incidence of skin tumours in mice pretreated with carcinogens. Croton oil was not by itself tumorigenic. The exposure of skin to carcinogens, was called 'initiation' and the subsequent step, the repeated application of croton oil or other agent, which elicited the growth of tumours from initiated cells, was called 'promotion'. Early work in this field established that the initiated state is extremely stable, extending perhaps for the lifetime of the animal. In fact, mice can be initiated with carcinogens *in utero* and, at some time after birth, tumours elicited by topical application of promoters (Goerttler & Lochrke *Virchows Arch.* **A372**, 29; 1976). Promotion generally leads to the development of benign papillomas; prolonged exposure results in the appearance of carcinomas. In 1968, the active principles of croton oil were isolated and their structures elucidated (Hecker *Cancer Res.* **28**, 2338; 1968; Van Duuren *Prog. exp. Tumour Res.* **11**, 31; 1969). They are fatty acid diesters of a tetracyclic plant diterpene alcohol, phorbol. Macrocyclic plant diterpene esters of related structure, some of which are active as tumour promoters, are widespread in the plant kingdom. Other compounds and crude extracts, notably phenols, anthralin and extracts of tobacco tar, have tumour-promoting activity when tested on mouse skin, but no agents studied so far approach the phorbol esters in potency.

Recent observations on the action of the phorbol esters *in vitro* can be subsumed under two principles. The first is that nanomolar concentrations of tumour-promoting phorbol esters (but not their inactive analogues) induce changes in cultured cells which resemble those seen on transformation with either chemical carcinogens or tumour viruses, and further enhance the expression of these transformation-specific phenotypic features in already transformed cells. In chicken embryo fibroblasts (CEF), phorbol esters alter cellular morphology (Driedger & Blumbers *Cancer Res.* **37**, 3257; 1977), increase plasminogen activator synthesis (Wigler & Weinstein *Nature* **259**, 232; 1976), alter the composition of glycopeptides obtained from cell membranes (Weinstein *et al.*, in *Origins of Human Cancer, Cold Spring Harbor Symp. quant. Biol.*, in the press), increase deoxyglucose uptake (Driedger & Blumberg *op. cit.*), and cause loss of LETS, the large external transformation sensitive protein (Blumberg *et al. Nature* **264**, 446; 1976). Similar changes are also seen when CEF are transformed by Rous sarcoma virus (RSV). The transformation mimetic effects of the phorbol esters are not all secondary to growth stimulation since they occur in CEF under conditions where growth stimulation does not occur. Phorbol esters enhance the expression of transformation-associated properties in transformed cells. The clearest example of this is the combined effect of the Rous sarcoma genome and phorbol esters on plasminogen activator production in CEF. Each factor alone increases the synthesis of the enzyme. Together they have a multiplicative effect (Weinstein *et al. op. cit.*). Synergistic action is also observed in alterations of the membrane glycopeptides of CEF (Weinstein *et al. op. cit.*). O'Brien and Diamond have reported similar findings with respect to phorbol ester-induced ornithine decarboxylase levels in chemically transformed and normal hamster cells (*Symposium on Mechanism of Tumour Promotion and Cocarcinogenesis* Gatlinburg, 1977, in the press). If phorbol esters can enhance the expression of transformed properties *in vitro*, perhaps they can do so *in vivo* and thus provide a preferential growth advantage to latent tumour cells.

The second principle is that the phorbol esters reversibly inhibit terminal differentiation. This effect was noted independently in three laboratories using two systems: chicken embryo myoblasts undergoing myogenesis (Cohen *et al. Nature* **266**, 538; 1977), and Friend erythroleukaemia cells (FEC) undergoing either spontaneous or induced erythroid differentiation (Rovera *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 2894; 1977; Yamasaki *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3451; 1977). These initial observations have been extended to the differentiation of 3T3 cells to lipocytes (Diamond *et al. Nature* **269**, 247; 1977), chondrogenesis of chicken embryo chondroblasts (Pacifici & Holtzer *Am. J. Anat.* **150**, 207; 1977), and differentiation of neuroblastoma cells in culture (Ishii *et al. Science*, in the press). These observations and their generalisation, if applicable to mouse skin, provide a seductively simple interpretation of initiation and promotion. The stem cells for epidermis are continually dividing, yet the tissue as a whole is in a state of balanced growth, and a stable stem cell pool size is maintained. This is possibly achieved by a regular asymmetric division of the stem cell: one daughter cell becoming a stem cell and one daughter cell terminally committed to differentiate, irreversibly losing its growth potential. If a potential tumour cell were restrained to the stem cell mode of division, it could not increase its proportion in the stem cell pool. If, however, the stem cell division mode were interrupted by the action of a promoting agent, a potential tumour

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cell could increase its proportion in the cell population and give rise to a tumour. Two cautions must be noted in the exercise of this hypothesis. First, phorbol esters greatly potentiate transformation by chemical carcinogens in cell culture systems (Lasne *et al. Nature* **247**, 490; 1974; Mondal *et al. Cancer Res.* **36**, 2254; 1976) where the stem cell concept may not be applicable. Second, phorbol esters have not been shown to inhibit terminal differentiation of mouse skin.

The two generalisations of recent data on the action of phorbol esters in cell culture, that they mimic and enhance the tumour phenotype and that they inhibit terminal differentiation, are preliminary but provocative. The factors which determine tumour incidence, latency and rates of tumour progression are largely unknown. Studies on the cellular and molecular basis for the action of the phorbol esters and similar compounds may clarify these aspects of carcinogenesis and suggest novel approaches to the control of neoplasia. □

Limits to similarity among coexisting competitors

from Henry S. Horn and Robert M. May

About 50 years ago, the theoretical work of Lotka and Volterra and the laboratory experiments of Gause, Park and others led to the formal enunciation of the 'competitive exclusion' principle': species that make their livings in identical ways cannot coexist. Although this principle may appear fundamental, it is not really very helpful. As stressed by Hutchinson, in his classic 'Homage to Santa Rosalia, or why are there so many kinds of animals?' (*Amer. Nat.* **93**, 145; 1959), the meaningful question is rather how similar can species be, yet persist together? What are the limits to similarity?

Most competitive situations involve many different ecological factors, which are too difficult to disentangle with present methods. But insight can be gained from those special situations where competitors sort themselves out mainly along a single resource axis, such as food size or foraging place. Hutchinson catalogued many examples, drawn from both vertebrates and invertebrates, of sequences of competing species in which the average individuals in successive species have weight ratios around 2. This implies ratios of about the cube root of 2, or 1.3, between typical linear dimensions (for example, beak length) of successive species. Many other examples have subsequently been tabulated, particularly for birds, lizards and frogs, by MacArthur, Diamond, Cody, Schoener, Pianka, Toft and others. In an extensive study of guilds of birds on various West Indian islands, Faaborg (*Amer. Nat.* **111**, 903; 1977 and in the press) has shown that the weight ratio of roughly 2 holds for passerines, but that non-passerine sequences typically have smaller weight ratios.

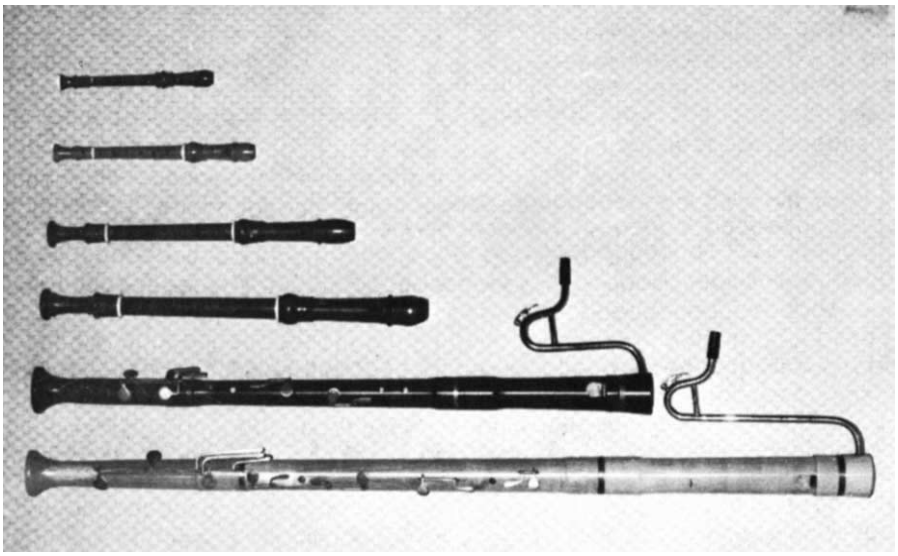

Fig. 1 The conventional ensemble of recorders, whose lengths roughly obey the '1 : 3 ratio rule'.

Recent studies of the workings of the rule among congeneric sequences of invertebrates tend to be more equivocal. Uetz (*J. Anim. Ecol.* **46**, 531; 1977: see also Enders, *Environ. Entomol.* **5**, 1; 1976) has shown that part of the structure of his guild of 10 species of wandering spiders in an oak–maple woodland in the eastern United States conforms to the 1.3 ratio; some of the species with very similar sizes further subdivide their habitat by flourishing at different times in the season. Similar stories are told of carabid beetles and guilds of spittlebugs (by Southwood and Halkka, respectively, *Royal Entomological Society Symp. on Insect Faunal Diversity*, Imperial College, September 22–23, 1977). Again the 1.3 length ratio plays a part for those species that are very similar in other respects.

The magic 1.3 ratio is no newcomer to the biological literature. Dyar (*Psyche* **5**, 420; 1890) long ago noted that successive larval instars of many insects have weight ratios of 2, and linear ratios of 1.3. This provoked much discussion, which seems to have been forgotten, and even speculations that the underlying mechanism was a doubling of the number of cells between instars (later shown to be not generally the case; for a review, see Bodenheimer, *Q. Rev. Biol.* **8**, 92; 1933).

This empirical relationship is still not understood. When the size of food is considered, various lines of argument lead to the expectation that the average difference in food size between two species should not be appreciably less than the characteristic range of food sizes utilised by either species. This, however, does not explain why the typical intraspecific range of food items spans a weight ratio of around 2.

Before too much time is spent theorising about the Dyar–Hutchinson rule, it should be noted that it appears in many other contexts. In the conventional ensemble of recorders, the lengths of sopranino, descant, treble, tenor, bass and great bass are in the ratios 1.2, 1.5, 1.3, 1.6, 1.4. For the consort of crumhorns, the heights of descant, treble, tenor and bass go as 1.2, 1.5, 1.4. The usual consort of viols have typical lengths of treble (61 cm), tenor (86 cm) and bass (110 cm) in the ratios 1.4, 1.3; the alto (70 cm) and viola d'amore (85 cm) are competitively excluded and are relegated to solo roles.

The four string instruments of the modern orchestra—violin, viola, cello, double bass—are grossly discrepant with the rule, having length ratios 1.2,

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