that AEV replicates and induces the expression of p75 in macrophages, and that MC29 replicates and is expressed in erythroblasts (Graf *et al.*, in press).

These results are compatible with the following model of DLV-induced leukaemogenesis and target-cell specificity (Graf & Beug, 1978; Graf *et al.*, in press; Graf *et al.*, 1979). (1) DLVs induce a leukaemic transformation by blocking the differentiation of their haemopoietic target cells. (2) The differentiation block is the consequence of a competitive inhibition of the viral transforming protein synthesized in large amounts by a hypothetical homologous but non-identical cellular differentiation-specific protein. (3) That a competitive inhibition of transforming protein is only possible in those cells expressing the putative cellular counterpart protein (*e.g.* AEV p75 in erythroblasts and MC29 p110 in immature macrophages) explains the target-cell specificity of DLVs.

We are currently trying to test our hypothesis by searching for the postulated cellular counterpart proteins of the transforming proteins of DLVs, and to study their tissue distribution.

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EARLY CARCINOGENESIS, DIFFERENTIATION AND PROMOTION

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THE NORMAL mammalian urinary bladder is lined by highly differentiated epithelium known as the urothelium, which has been described and illustrated in detail (Hicks, 1975; Hicks *et al.*, 1974; Severs & Hicks, 1977). The markers for normal differentiation include development of highly specialized, polyploid surface cells, with a uniquely structured surface membrane. This particular form of transitional-cell differentiation is in a state of balance which is very easily disturbed. Furthermore, the urothelium is a multipotential tissue (Hicks, 1975) and has the option of other forms of differentiation. Thus, if subjected to regular irritation

as from a bladder calculus, the normal differentiation is disturbed and switches from transitional-cell differentiation to epidermalization with the synthesis of gross keratin plaques. Similarly, epidermalization is also produced if the animal is made vitamin A-deficient, when again the genome for normal transitional differentiation is apparently switched off, and that for differentiation to squamous metaplasia is switched on or amplified (Hicks, 1968, 1969, 1975, 1976). The frequency with which epidermalization or squamous metaplasia of the urothelium is seen suggests that the genome responsible for keratinization, though normally repressed,

must be readily accessible and easily de-repressed. An alternative type of differentiation which is also seen in the urothelium is mucous metaplasia, and this frequently develops in mild pathological conditions (*e.g.* chronic cystitis). Again, this form of differentiation is seen sufficiently frequently to suggest that the area of the genome concerned with mucous production, though normally repressed, is in a position or state where it is readily inducible. These 3 forms of differentiation in the normal adult mammalian urothelium all differ morphologically from the more primitive differentiation of the urothelium seen in the foetus (Firth & Hicks, 1970). These gross manifestations of differentiation observed in the normal or nearly normal urothelium must represent minor variations at the level of transcription in the control of the genome in the normal urothelial cell.

Biogenesis of urothelial cancer, though it occasionally occurs as a result of exposure to a high dose of a complete carcinogen, is more usually the outcome of a multi-stage process of initiation, promotion and propagation (Hicks *et al.*, 1978; Hicks & Chowanec, 1978). In this sequence of events the initiator, which in man is probably a small amount of the activated form of a urine-borne chemical carcinogen, reacts with and produces some permanent alteration to the DNA so that the DNA carries abnormal information. It has been shown by Yuspa *et al.* (1969) that initiators do not preferentially react with replicating rather than non-replicating DNA and therefore, since most of the genes are in a repressed state, the modified areas of DNA are more likely to be in an unexpressed rather than in a transcribing area of the genome. No difference in phenotypic expression will therefore necessarily be seen in the urothelial cells after an initiating event. However, the initiating event is relatively permanent, and will leave some cells harbouring misinformation even though they are phenotypically identical to their unaffected neighbours, as judged by their functional proteins and membrane structure. After the initiating event, although the cell may carry altered information, so long as the altered area remains unexpressed no change in phenotype will occur even when the cell divides in the normal course of events. Before a change in phenotype can be seen, a promoting event is required which will allow the altered area of genome to become inducible, *i.e.* the affected

area must be morphologically altered into a transcribing rather than a non-transcribing form.

The action of promoters in carcinogenesis has been extensively studied in the skin, and we have extended some observations to the urinary bladder. In general, promoters increase the opportunity for abnormal phenotypic expression by accelerating the rate of mitosis; this also helps to propagate the tumour. In addition, by randomly altering gene expression, they increase the opportunity of expressing any altered information previously introduced into the genome by exposure to an initiator. Furthermore, by inhibiting terminal differentiation of the tissue, they increase the time required before a cell will pass into the "differentiate and die" pathway, and retain the cells for longer in the cell cycle where they can grow and divide. In the biogenesis of bladder cancer, vitamin A deficiency clearly acts as a promoting stimulus. Vitamin A deficiency causes an increase in DNA synthesis and increased mitotic activity in the basal cells of the urothelium which causes hyperplasia. Coincidentally with this rather nonspecific stimulation of cell division and tumour promotion, vitamin A deficiency also specifically affects the genes controlling the differentiation of the cell, so that bladder tumours in vitamin A-deficient animals are also heavily keratinized. The same is true where cancer growth is associated with mechanical irritation, as in the presence of a urinary calculus or in bilharzial bladder in which the wall is filled with calcified ova, for irritation predisposes to squamous metaplasia. In both these instances, keratinization is concomitant with but not an essential part of the biogenesis of the neoplasms. The keratinization demonstrates that vitamin A deficiency or irritation produce a disturbance in the normal control of transcription. If such factors also encourage random transcription of the genome, they will automatically increase the chance of transcribing potentially carcinogenic changes in the genome introduced by previous exposure to an initiator. We have demonstrated that saccharin also acts as a promoter of the growth of bladder cancer in animals previously subjected to an initiating dose of the carcinogen methyl nitrosourea (MNU) (Hicks *et al.*, 1975; Hicks & Chowanec, 1977). These observations have now been confirmed by Friedell and his co-workers, using an initiating dose of another bladder

carcinogen, the nitrofurantoin FANFT (Cohen *et al.*, 1978). It is not known how saccharin acts as a promoter, but undoubtedly tumours promoted by saccharin show a wide variety of differentiation patterns both within the tumour and in adjacent, non-neoplastic areas of the urothelium. This suggests that saccharin in some way disturbs the normal control of differentiation and, like vitamin A deficiency, encourages random gene transcription, thus increasing the chance of expression of any neoplastic modification of the growth-controlling genes introduced by pre-treatment with an initiator. Saccharin also causes urothelial hyperplasia in some but not all animals, and increased numbers of cells enter mitosis. Thus saccharin has two properties characteristic of skin promoters: it appears to increase the chance of random transcription of the genome and at the same time it encourages cell division, thus promoting and propagating tumour growth.

The final result of neoplastic transformation in any tissue is an altered phenotype, such that the cancer cell expresses certain properties which differ from those of normal cells from which they were derived. It appears to be a disease in which some concomitant phenotypic change in differentiation is obligatory. However, the changes which occur in a tissue during carcinogenesis may also illustrate many changes in differentiation which are not necessarily a part of the neo-

plastic growth syndrome. Promoters encourage random gene expression and the production of multiple phenotypes, among which may be the phenotype associated with cancer growth if the tissue has previously been exposed to an initiating carcinogen.

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PROPERTIES OF SARCOMA GROWTH FACTOR AND OTHER PEPTIDE HORMONES PRODUCED BY MOUSE AND HUMAN TUMOUR CELLS

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MOUSE 3T3 cells have been transformed in culture by a variety of agents, including DNA-containing viruses (SV40, polyoma), RNA-containing viruses (*e.g.* MSV, avian sarcoma viruses) as well as by various chemical carcinogens and radiation. Of these, the clones transformed by MSV specifically demonstrate a loss of cell-surface epidermal growth factor (EGF) receptors. The basis for this apparent loss of receptors is shown to be the production by the MSV-transformed cells of a low-mol.-wt peptide (9-11K) related to EGF. This sarcoma growth factor (SGF) stimulates

normal fibroblasts to assume a "transformed" morphology in monolayer cultures and to form progressively growing colonies in soft agar. EGF, under similar conditions, stimulates cell division in monolayer cultures but not anchorage-independent growth in semi-solid medium.

SGF has been purified from serum-free medium of MSV-transformed 3T3 cells. A critical step in the purification involves the use of formalin-fixed human carcinoma cells, designated A431, that have exceptionally high levels of EGF receptors (2.2×10^6 receptors/