24. Kennedy KA. Hypoxic cells as specific drug targets for chemotherapy. *Anti-Cancer Drug Design* 1987, **2**, 181–194.
25. Vaupel P, Schlenger K, Knoop C and Hockel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurement. *Cancer Res* 1991, **51**, 3316–3322.
26. Workman P, Walton MI. Enzyme-directed bioreductive drug development. In: Adams GE, Breccia A, Fielden EM, Wardman P, eds. *Selective Activation of Drugs by Redox Processes*. New York, Plenum Press, 1990, 173.
27. Walton MI, Smith PJ, Workman P. The role of NAD(P)H: quinone reductase (EC 1.6.99.2, DT-diaphorase) in the reductive bioactivation of the novel indoloquinone antitumour agent EO9. *Cancer Commun* 1991, **3**, 199–206.
28. Jenkins TC, Naylor MA, O'Neill P, *et al.* Synthesis and evaluation of α-(((2-haloethyl)amino)methyl)-2-nitro-1H-imidazole-1-ethanol as prodrugs of α-((1-aziridinyl)methyl)-2-nitro-1H-imidazole-1-ethanol (RSU 1069) and its analogues which are radiosensitizers and bioreductively activated cytotoxins. *J Med Chem* 1990, **33**, 2603–2610.
29. Zeman EM, Brown JM, Lemmon MJ, Hirst VK, Lee WW. SR-4233: A new bioreductive agent with high selective toxicity for hypoxic mammalian cells. *Int J Radiat Oncol Biol Phys* 1986, **12**, 1239–1242.
30. Ratain MJ, Schilsky RL, Conley BA, Egorin MJ. Pharmacodynamics in cancer therapy. *J Clin Oncol* 1990, **8**, 1739–1753.
31. Egorin MJ, Van Echo DA, Olman EA, *et al.* Prospective validation of pharmacologically based dosing scheme for the cisplatin analog, carboplatin. *Cancer Res* 1985, **45**, 6502–6506.
32. Calvert AH, Newell DR, Gumbrell LA, *et al.* Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *J Clin Oncol* 1989, **7**, 1748–1756.
33. Mick R, Ratain MJ. Modelling interpatient pharmacodynamic variability of etoposide. *J Natl Cancer Inst* 1991, **83**, 1560–1564.
34. Stewart CF, Arbuck SG, Fleming RA, Evans WE. Relation of systemic exposure to unbound etoposide and hematologic toxicity. *Clin Pharm Ther* 1989, **50**, 387–393.
35. Thyss A, Milano G, Renee N, *et al.* Clinical pharmacokinetic study of 5-FU in continuous 5-day infusions for head and neck cancer. *Cancer Chemother Pharmacol* 1986, **16**, 64–66.
36. Collins JM, Zaharko DS, Dedrick RL, Bruce BA. Potential roles for preclinical pharmacology in Phase I clinical trials. *Cancer Treat Rep* 1986, **70**, 73–80.
37. Corbett TH, Valeriote FA and Baker LH. Is the P388 murine tumour no longer adequate as a drug discovery model? *Invest New Drugs* 1987, **5**, 3–20.
38. Double JA and Bibby MC. Therapeutic index: a vital component in the selection of anticancer agents for clinical trial. *J Natl Cancer Inst* 1989, **13**, 988–991.
39. Johnson RK. Screening methods in antineoplastic drug discovery. *J Natl Cancer Inst* 1990, **82**, 1082–1083.
40. Chabner BA. In defense of cell-line screening. *J Natl Cancer Inst* 1990, **82**, 1083–1085.
41. Grindey GB. Current status of drug development: Failure or limited success? *Cancer Cells* 1990, **2**, 163–171.
42. Sikic BI. Anticancer drug discovery. *J Natl Cancer Inst* 1991, **83**, 738–740.
43. Boyd MR. Status of the NCI preclinical antitumour drug discovery screen. *Principles and Practices of Oncology* 1989, **3**, 1–11.
44. Monks A, Scudiero D, Skehan P, *et al.* Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. *J Natl Cancer Inst* 1991, **83**, 757–766.

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Cell Kinetic Alterations During Epidermal Carcinogenesis

EACR—Mühlbock Memorial Lecture, 1991

Olav Hilmar Iversen

INTRODUCTION

Normality depends on a complex biological equilibrium

THE STRUCTURE of the higher living organisms is characterised on the one hand by complexity and strict organisation, and on the other by its dynamic character. Cells differentiate, mature, die and are constantly being replaced by new cells. While alive, all cells also exchange molecules and atoms, make use of them, excrete waste products and metabolites, and send out signal substances. Order in a state of perpetual change is maintained by a steady flow of information in control systems, which operate at the molecular, cellular, tissue and organ levels. All cells play a part in maintaining body equilibrium by communicating through biochemical and electrophysiological signals. The brain is the supreme director, and through the pituitary gland the hypothalamus is the conductor of the hormone orchestra. At the cellular level, everything depends on and is regulated by the information in the genes.

In the field of growth regulation there are basically two approaches, or two opposing paradigms. One is based on the theory that, in terms of cellular proliferation, living cells are naturally quiescent, like the Sleeping Beauty. They need a life-giving kiss from a growth factor in order to be activated to cell division. This way of thinking has been greatly encouraged by the discovery of a considerable number of stimulatory growth factors, and their receptors and genes; and by the fact that some growth factors are directly or indirectly associated with the oncogenes. Some adherents to the Sleeping Beauty theory also believe that cells produce factors to stimulate themselves, a hypothesis called autocrine growth stimulation [1].

The other theory, which I consider more probable, is that healthy cells possess a strong natural aptitude for division. This is clearly shown by the exponential growth phase in cell culture.

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A relatively rapid rate of cell division (a short cell cycle time and a high growth fraction) is thus the normal situation, which would be perpetually maintained if cells were not inhibited by external signals. Endogenous, inhibitory growth factors are physiological chemical signal substances in the body, which inhibit or restrain the cells' natural tendency to go into DNA synthesis and mitosis [2]. They operate in concert with growth stimulators and a multitude of modulating factors to maintain physiological growth balance.

This paper contains an overview of the most obvious kinetic alterations in cell proliferation provoked by carcinogenic chemicals, mainly in epidermal cells, which is my special field of knowledge.

Normal equilibrium is disturbed in malignant tumours

There is general agreement that cancer is associated with, and may be caused by, a partial and sometimes complete breakdown of the control mechanisms for cell proliferation, differentiation, maturation and recognition of tissue boundaries.

Studies of carcinogenesis have traditionally focused on disturbed growth regulation, in the sense of uncontrolled cell proliferation, because this has been thought to be the major malfunction in tumour growth. But it is important to realise that malignancy is in principle not always characterised by rapidly dividing cell populations. In fact, many tumours and leukaemias have a lower rate of cell birth than the mother tissue. Differentiation and maturation are also profoundly disturbed in malignant cells.

So the problem of carcinogenesis has to be approached in terms of general disturbance of cellular equilibrium, and cell kinetic methods are among the most suitable instruments for examining this issue.

CELL KINETIC ALTERATIONS DURING EPIDERMAL CARCINOGENESIS

Introduction

When approaching carcinogenesis through the instrument of cell kinetics, it may be fruitful to ask the following questions: Do carcinogenic agents alter the cell kinetics of tissues in a specific way? Is a rapid rate of cell proliferation in itself carcinogenic, or is this only a secondary phenomenon, often associated with, but not a causal factor in carcinogenesis? What influences a tissue's response to a carcinogen—the general rate of cell proliferation at the time of contact with the carcinogen, and/or the stage of the cell cycle in which the proliferating cells happen to be at the time of contact? Do carcinogens influence endogenous growth regulation systems (oncogene products, stimulatory growth factors, suppressor gene products and chalones), and if so, are the resulting changes causally involved in carcinogenesis?

As a general rule, it must be said that most of the experimental evidence concerning epidermal cell kinetics and the early effect of carcinogens is based on the effects of one single application of a chemical carcinogen or a single dose of radiation, both in rather large doses. High doses of carcinogenic agentia are almost always toxic and kill a large number of cells. Since cancer cannot arise from dead cells, and can arise only with difficulty from seriously injured ones [3], the real problem in carcinogenesis is to find out what happens to those cells that are critically altered by the carcinogen, without being killed or losing their vitality. The persistent changes in epidermal cell kinetics during long-term application of repeated doses of carcinogens are not so well documented, and here much more research needs to be done.

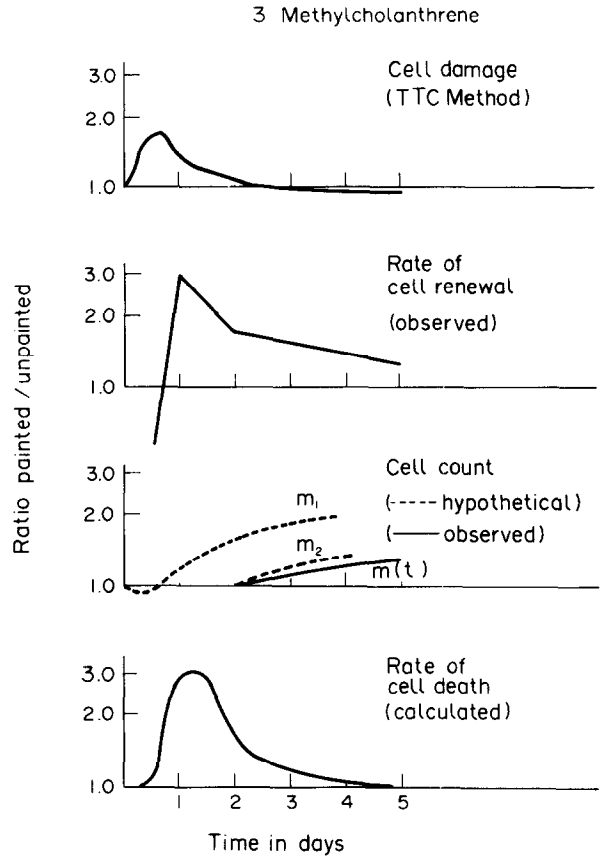

Fig. 1. Cell population kinetics in epidermis during first 5 days after application of 50 µl 1% 3-methylcholanthrene (MCA). *y*-axis = relative deviation from normal. The top panel shows intensity of cell injury measured by tetrazolium reduction method. Initial block in cell renewal was followed by increased cell proliferation from first day (second panel). In third panel from top, unbroken line [$m(t)$] = observed cell count. If rate of cell renewal is like that in second panel and assuming no extra cell loss, cell count would have increased according to m_1; m_2 depicts theoretical cell mass if rate of cell death had increased, but had normalised after 2 days. Calculated rate of cell death is based on intensity of cell injury, observed rate of cell renewal and observed development of hyperplasia. Reproduced from [7] by kind permission of Norwegian Universities Press.

Alterations in cell kinetics provoked by chemical carcinogens

Let us now examine how a single carcinogenic dose of a chemical carcinogen alters the cell kinetics of the epidermis. A single application of a strong hydrocarbon carcinogen in a sufficiently high dose leads to rapid alterations in the cell kinetics. Signs of cell toxicity will appear initially even at relatively moderate doses (Fig. 1). The cell cycle variables indicate a toxic shock phase lasting only a few hours at small doses and for many hours at higher doses [4–7]. During this period DNA synthesis and mitotic activity are more or less completely arrested. These reactions reach a peak 16–24 h after the initial contact with the carcinogen [6,7]. Signs of acute cell injury with oedema and membrane changes can be noted in all cell layers and in the dermis. The cell injury caused by the toxicity of a single carcinogen application also leads to a short period of delayed maturation [8–10] and a subsequent increased cell mortality; which can be measured from the 2nd to the 5th day. During this period an abnormally high number of cells are lost from the epidermis by scaling and maybe by apoptosis [7, 11] (Fig. 1).

When the basal cells recover from the primary shock, a

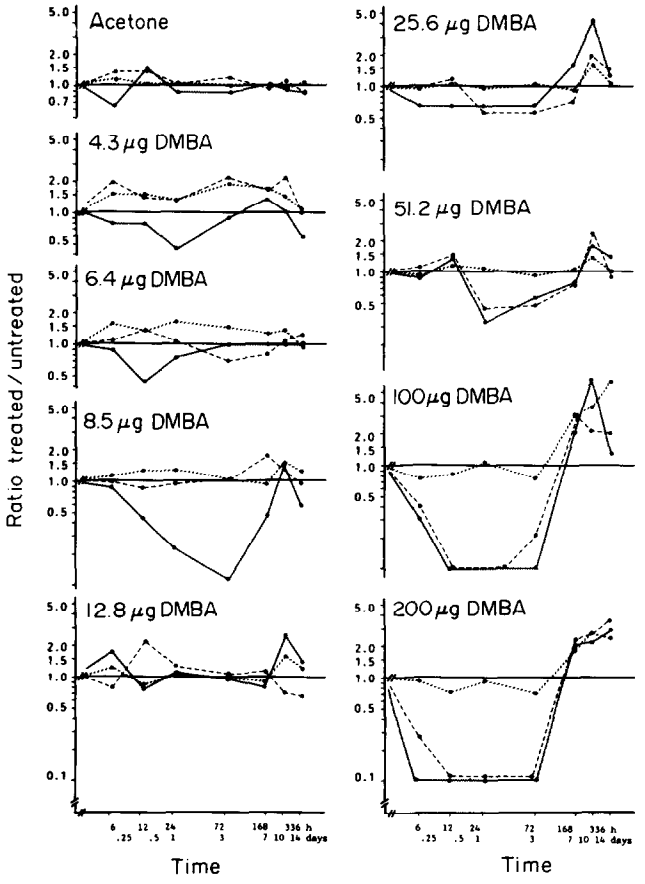

Fig. 2. Number of suprabasal cells [indicating hypoplasia or hyperplasia (dotted line)], alterations in DNA synthesis (broken line), and mitotic rate (unbroken line), after application of acetone and various doses of DMBA over time. There was deeper and longer lasting depression of proliferative variables at high doses, especially 51.2, 100 and 200 μg DMBA. At highest doses there was considerable hyperplasia with increasing cell numbers, starting on day 3 and peaking on days 7–10. There was initial slight hyperplasia after small doses (probably due to delayed maturation with reduced cell loss). Reproduced from [6] by kind permission of *APMIS*.

proliferative wave starts, reaching a peak 3–10 days after the application, depending on the dose (Figs 2–4). During this period DNA synthesis and mitotic rate are increased three to five times, and the mitotic index becomes high. This is partly due to the high rate of cell proliferation and partly to a prolongation of the mitotic duration [11]. As a consequence of the increased rate of proliferation, a transient hyperplasia develops, which in the hairless mouse epidermis lasts for about 3–5 weeks (Fig. 2).

All these reactions are dose-dependent [6] (Figs 3 and 4). My own belief is that they are mainly provoked by the general toxicity of the carcinogen, which destroys cells and therefore the growth inhibitory factors in the epidermis (see below), and leads to the wave of regenerative proliferation. However, whether this reaction is specific is another question. We also know by a series of articles from Rohrbach *et al.* [12–15] that the amount of growth inhibitory substances in the epidermis varies inversely with the state of proliferation in the epidermis (Figs 5–7). This has been shown to be so whether the epidermal cells were removed by tape stripping, or injured by methylcholanthrene or croton oil. This supports the idea that cell toxicity and cell death lead to lack of physiological growth inhibitors, and the reaction is rapid proliferation to compensate for the injury. Several studies, especially those of Clausen [16–18] have shown that such reactions occur both after carcinogens [16], after the non-carcinogenic skin irritant cantharidin [17] and even after adhesive tape stripping [18] (Fig. 8). So the secondary wave of proliferation is, mainly at least, a non-specific regenerative reaction to the cell-damaging effect of the carcinogen.

Carcinogens act in two ways: as cell destroyers and as agents that lead to a higher rate of cell proliferation (see later). I believe that some chemicals, like strong hydrocarbon carcinogens, have the ability to transform large numbers of cells into malignant cells, combined with a relatively moderate cell toxicity. These are the substances that are traditionally known as strong or complete carcinogens. Other chemicals, like the phorbol esters, have a high cell toxicity, with many measurable pleiotypic effects even in small doses, but they also have a less pronounced, but definite ability to transform cells to malignant cells. This may be the characteristic of the substances which are often called promoters. If an animal has a large number of transformed cells due to previous contact with a carcinogen, felt wheel abrasion followed by rapid regenerative proliferation is enough for promotion, and the ensuing hyperplasia will be precarcinogenic. Finally, a pure physical trauma like felt wheel abrasion alone, with no additional carcinogenic influence, leads to a regenerative, more or less normal hyperplasia without any risk of true carcinogenesis [19]. The main increase in cell proliferation after contact with a carcinogenic influence is probably secondary to cellular regeneration after injury, as mentioned above. However, since it is dose-dependent, this reaction may be a mixture of massive regeneration due to the cell injury, and a low degree of primary mitogenesis. It is difficult to exclude completely a true, primary mitogenic effect caused by very low doses of a carcinogen. There are, however, only weak indications of this [6]. (For a general discussion on the two types of mitogenesis, see later.)

The initial blocking of DNA synthesis seems to follow contact with all types of carcinogenic agents, and there was earlier much discussion about whether this block is causally related to the carcinogenesis process. Markard *et al.* [20–22], for instance, showed that in synchronised fibroblasts in cell cultures, two carcinogens were able to transform the cells in G_1 and S, but not in G_2 or M. The induction of malignant transformation in S-phase cells was greater than in G_1. This observation was thought to be significant in relation to the initial block in DNA synthesis seen in epidermis, but non-specific blocks may also be seen after exposure to non-carcinogenic agents [18]. The other disturbances in the cell kinetics show an interesting difference, namely that carcinogens induce a much longer-lasting increase in the mitotic duration than non-specific irritants do [11]. Since a single application of a high enough dose of a complete chemical carcinogen may lead to many skin tumours in a sensitive mouse strain, it cannot be excluded that some of the changes in the cell kinetics induced by a single application of a carcinogenic substance may be causally associated with carcinogenesis. At present, however, we do not know whether or not these changes are entirely specific. Maybe a genuine genotoxic carcinogen without other toxic effects on cells (if such a substance exists) could be a pure mitogen and mutagen. Farber [23] stresses that initiation does not occur unless it is followed by a round of cell proliferation. Much research is being done to try to find out whether oncogenes become activated, and whether growth suppressor genes are deleted, and if so, at what stage in the process, and so on [24].

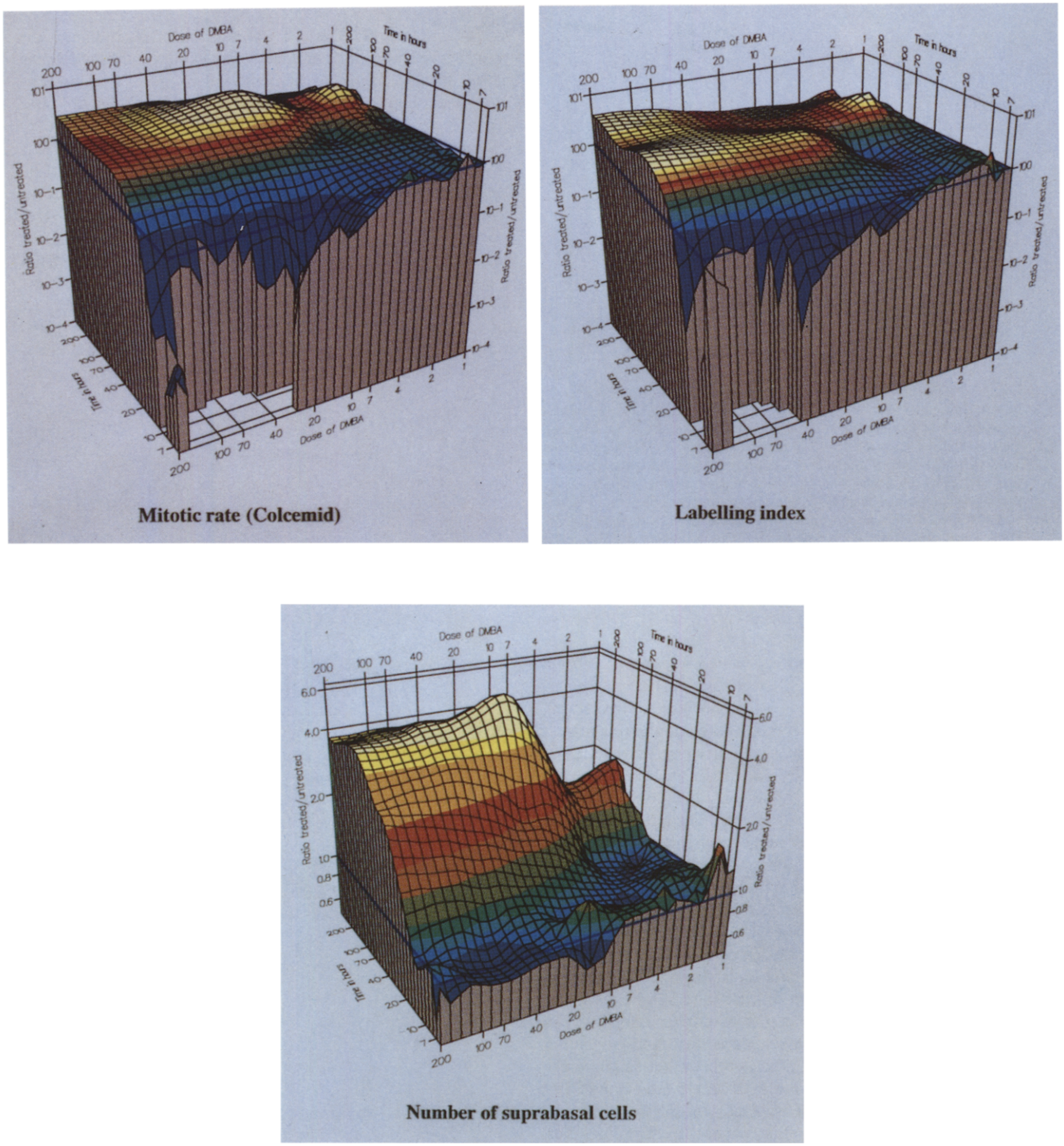

Fig. 3. 3-D illustration of mitotic rate, labelling index and number of suprabasal cells after single application of various doses of DMBA over time. Highest doses of DMBA led to immediate complete block in mitotic rate and in DNA synthesis, but after smallest doses there was no block. After some time, block was released, and after about 70 h both mitotic rate and labelling index were higher than normal. Higher doses reduced number of suprabasal cells in the epidermis, but after about 70 h, considerable hyperplasia developed.

Is rapid cell proliferation in itself carcinogenic?

It is self-evident that a tumour cannot grow without cell division. Most, but not all, precancerous conditions are associated with periods of sustained hyperplasia and an increased rate of cellular proliferation. The idea that carcinogenesis is usually associated with an increased rate of proliferation and sustained hyperplasia due to regenerative reactions is an old one, but it has been taken out and shined up again quite recently.

There are probably at least two types of triggering increased cell proliferation as alluded to above. One is a secondary reaction to cell injury which also leads to reduction of growth-inhibitory factors, and this is what is seen after all sorts of removal of cells

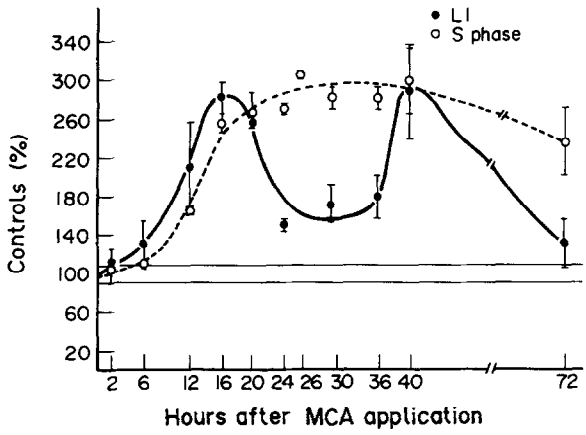

Fig. 4. Proportion of cells with S-phase DNA content measured by flow cytometry, and in [^{3}H]TdR labelling index (LI) of isolated epidermal basal cells after methylcholanthrene application. Discrepancy in values from 20 to 40 h indicates block in DNA synthesis. Value for cells with S-phase DNA content was about twice as high as that of LI, which again was 40% higher than normal, which means that while an increased number of cells synthesise DNA as measured by LI, there are also many replicating cells with very slow or blocked DNA synthesis. This is typical of effect of relatively high doses of hydrocarbon carcinogens on the skin. Reproduced from [16] by kind permission of Virchows Archiv, Springer.

or serious injury to cells. It may, however, also be a true primary reaction due to the known effect of classical mitogens such as certain oncogene products, platelet-derived growth factors, lectines, minute doses of TPA on lymphocytes in cell culture, etc. Some authors mention that cell proliferation in this respect may be both a quantitative and a qualitative phenomenon [25].

Clinical examples are legion. In classical lists of causes of cancer, chronic irritation and chronic inflammation have always been listed as causes of cancer. Already Rudolf Virchows himself adhered to an irritation theory involving hyperplasia and high mitotic activity as a cause of human cancer [26], and he maintained that tumours start with an irritation stage. Oral cancer, for example, is said sometimes to be due to irritation from a sharp, decaying tooth, and tropical ulcer in Africa has a very high frequency of squamous cell carcinoma (Fig. 9) [27]. In 1980 Deschner [28] described preneoplastic events occurring in the colon mucosa in patients at risk of developing cancer. He found proliferative abnormalities with enhancement of the labelling indices in the crypts and believed that the combination of an already existing defect in cell proliferation among high-risk colon cancer patients along with an elevated rate of cell renewal increases the probability that a neoplasm will arise. Cohen and Ellwein [29] believe that increase in cell proliferation can account for the carcinogenicity of non-genotoxic compounds, which possess the property of increasing cell proliferation in the target organ. Recently, the same authors [25] discussed cell proliferation, genetic errors and carcinogenesis. They point to the fact that cancer arises mainly due to genetic alterations, and that more than one of these is required. At the same time DNA replication is not 100% precise, and thus cell replication can in itself contribute to carcinogenesis by an indirect mechanism providing somatic mutations more easily to occur. In 1990 Preston-Martin *et al.* [30] hypothesised that increased cell division *per se* stimulated by external or internal factors is associated with the development of many human tumours. They maintained that a higher rate of cell division increased the risk of mutations, translocations and amplifications of oncogens taking place and being fixed. They published three tables of cancer sites in humans, where they considered a physiological high rate of proliferation to be a contributing cause.

When we come to experimental evidence and theory, we can go back to Deelman [31] who as early as 1927 stated that regeneratory processes after cell injury by tar treatment had a significant effect on the occurrence of malignant tumours. He later reported in the Royal Society the conclusion that injury has a direct influence on tumorigenesis in an extensive area of epithelial cells that are already susceptible to tumour formation. Mackenzie and Rous [32] concluded that when cells are injured, the stimulus of wound-healing will suffice to make some of them multiply and form tumours, provided that they have also been treated with tar. Mottram [33] said in 1944 that his experiments

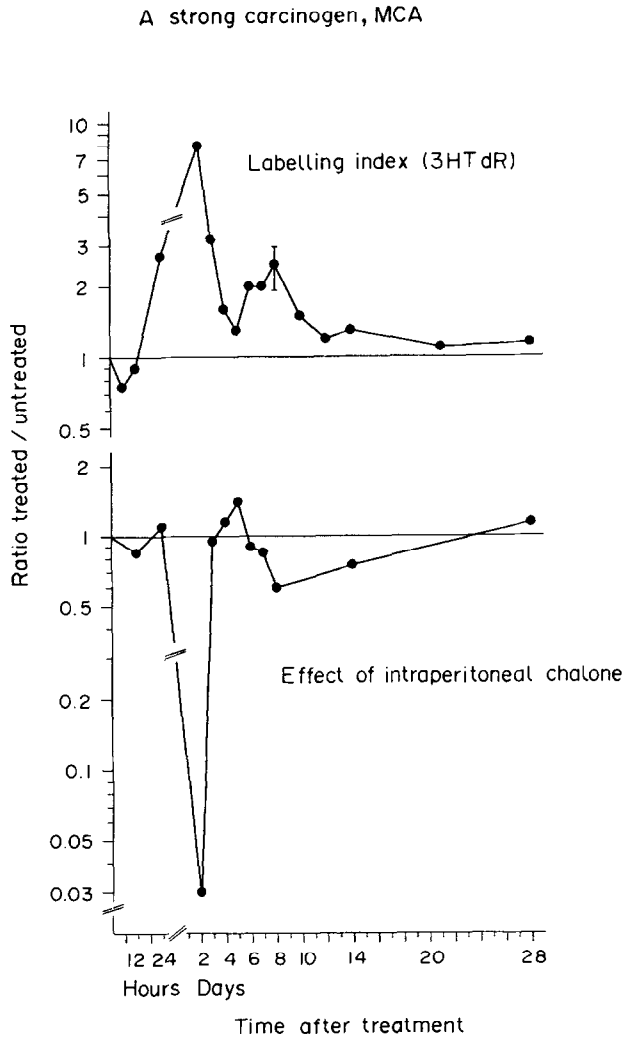

Fig. 5. Labelling index and chalone effect on mouse epidermis after single application of 0.02 ml 1% acetone solution of methylcholanthrene, a strong carcinogen. Lower panel shows mitosis-inhibitory effect on normal mice after intraperitoneal injection of epidermal chalone produced from mice treated on whole back by methylcholanthrene at above strength. Effect of chalone was low when labelling index was high, which may confirm the idea that increased proliferation is due to injury to cells leading to reduced chalone content. Adapted from [13] by kind permission of the author and Gustav Fischer.

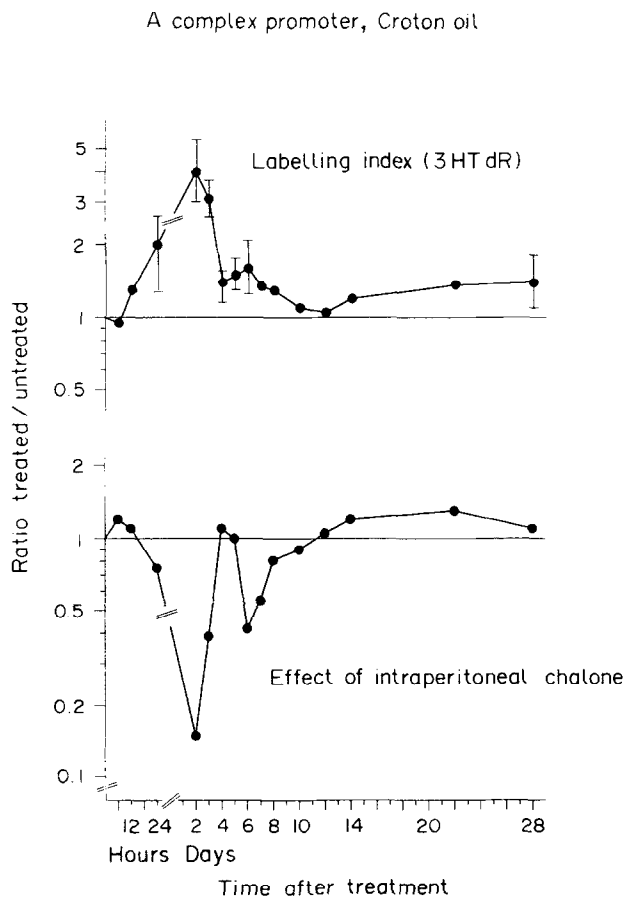

Fig. 6. Labelling index and chalone effect after single application of 0.02 ml 0.25% acetone solution of croton oil, as a complex promoter. As in Fig. 5, labelling index peaked when chalone content in similarly treated epidermis was low. Adapted from [14] by kind permission of the author and Gustav Fischer.

showed how important hyperplasia and all other conditions of active cell proliferation are in the natural occurrence of tumours. In 1934 Rous and Beard [34] published a paper on the progression of virus-induced carcinomas in the rabbit, and concluded that the better the papillomas grew, the more likely it was that cancer would occur. They came to the conclusion that all the carcinogenic substances were agents of cell destruction, and that they induced constant reparative processes leading to hyperplasia and increased rates of cell proliferation. In 1980 Melzer [35] discussed in detail what he called the paradox of carcinogens being both cell destroyers and proliferation inducers.

In August 1990 Ames and Gold [36, 37] claimed that we have too many rodent carcinogens because in animal experiments we use the maximum tolerated dose. This is so high that tissue toxicity induces cell divisions, and these themselves may provoke DNA mutations from which tumours eventually develop. Ames said that the endogenous rate of DNA damage is enormous and that mitogenesis in itself is mutagenic in numerous ways. He also thinks that suppression of intercellular communication causes mitogenesis, and that mitogenesis from exogenous and endogenous factors can cause cancer. In this connection he also mentioned chronic infections, hormones and toxicity leading to regenerative increase in the rate of cell division. This theory provoked a lively discussion [38–40], in which Weinstein [41] maintained that mitogenesis is only one factor in carcinogenesis.

In 1990 the Chemical Industry Institute of Toxicology produced a model [42] proposing that exposure to a carcinogen results in a certain target tissue dose, usually smaller than the exposure dose (Fig. 10). This again may affect the cells with cytotoxicity and receptor-mediated mitogenesis and DNA reactivity. Mitogenesis leads to cell division, and if there are no mutations, normal cells are born. If there are mutations, cytotoxicity can also induce a higher rate of cell division which in itself may increase the risk of mutation. Intermediate cells with only one mutation may arise, which may die without giving rise to a tumour, but with further mutations malignant cells can occur and lead to a tumour. In this model cell division plays an important, but not critically decisive role, and at present it might be an interesting way of attacking the problem. Similar views have been expressed by Ashby and Morrod [43] and Trosko *et al.* [44], who also think that both spontaneous or induced mutation may occur during cell proliferation.

Thus, my own conclusion is that the carcinogenic process is linked with an increased rate of cell proliferation, usually combined with sustained hyperplasia. I think much of this

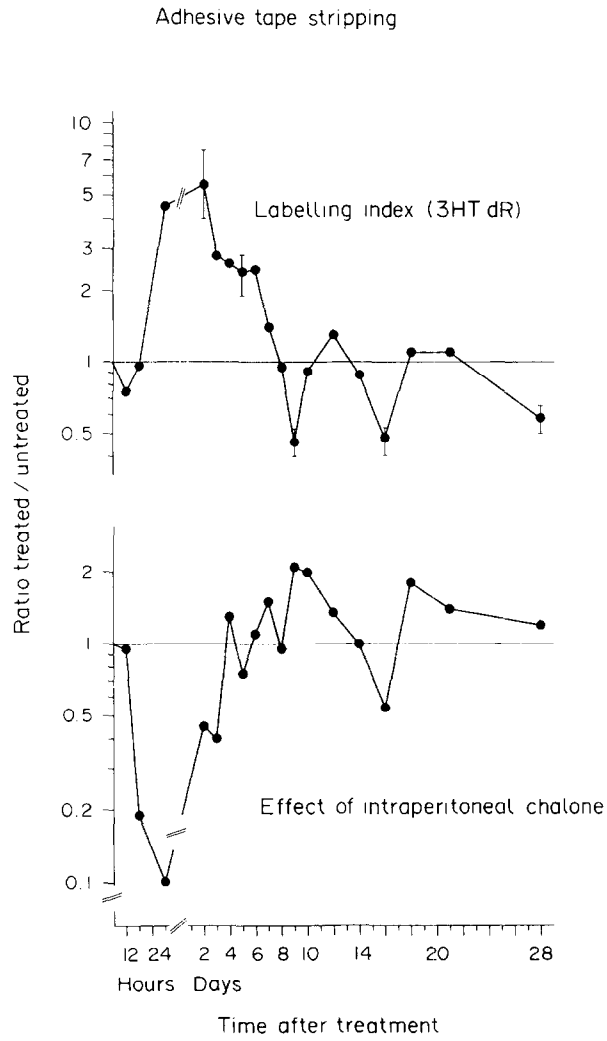

Fig. 7. Labelling index and chalone strength after six strippings of epidermis with adhesive tape. Peak in labelling index generally coincided with low chalone content in skin extract, showing that this type of effect is non-specific, follows cell loss and cell injury, and is not necessarily related to carcinogenesis. Adapted from [15] by kind permission of the author and Gustav Fischer.

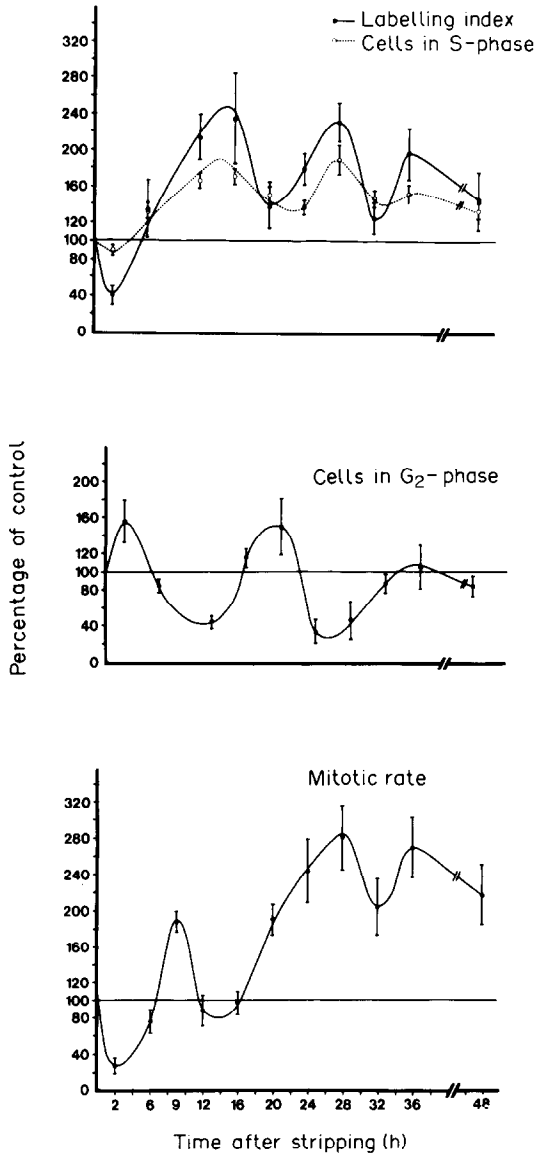

Fig. 8. Cell kinetic variables after adhesive tape stripping of mouse epidermis. First reaction is block in labelling index and cells in S phase (upper panel), and block in mitotic rate (bottom panel). Then follow three waves of increased regeneration with fluctuation in labelling indices, number of cells in S phase, cells in G_2 phase and mitotic rate. These results support the idea that similar reactions seen after carcinogens are due to cytotoxic, harmful effect of carcinogens on the cell, and are not specifically carcinogenic. Adapted from [18] by kind permission of the authors and *Cell and Tissue Kinetics.*

may be a non-specific regenerative reaction, although in some situations it may be linked to genuine carcinogenesis, as explained above. Many believe, however, that a critical genetic change in DNA is more easily fixed and propagated in a rapidly-dividing cell population [23], and in this way non-specific secondary mitogenesis contributes to an increased risk of cancer. This may be an inherent error-proneness of DNA replication or the presence of weak carcinogenic influences, e.g. background radiation. Personally, I believe that a rapid rate of regenerative, normal cell proliferation caused by cell loss provoked by non-carcinogenic cell destroyers is not strongly carcinogenic in itself. On the other hand, the promoter TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) produces sustained hyperplasia [45] with rapid cell proliferation. The skin takes at least 20 weeks to return to a quiet, normal morphological and kinetic situation after a course of TPA treatment [46]. I thus believe that there are conditions of non-carcinogenic, sustained hyperplasia and a high rate of cell proliferation, e.g. in the epidermis during increased physical wear and tear. The carcinogenic risk of such hyperplasia is negligible, because the transforming error proneness of DNA replication is low. But there are also conditions of precarcinogenic hyperplasia, like that provoked by TPA, and this hyperplasia has an increased risk of developing into real malignant tumours because TPA also acts as a complete carcinogen [47]. Urethan has interesting effects on the mouse skin. It acts as an initiator, a complete carcinogen, and as a

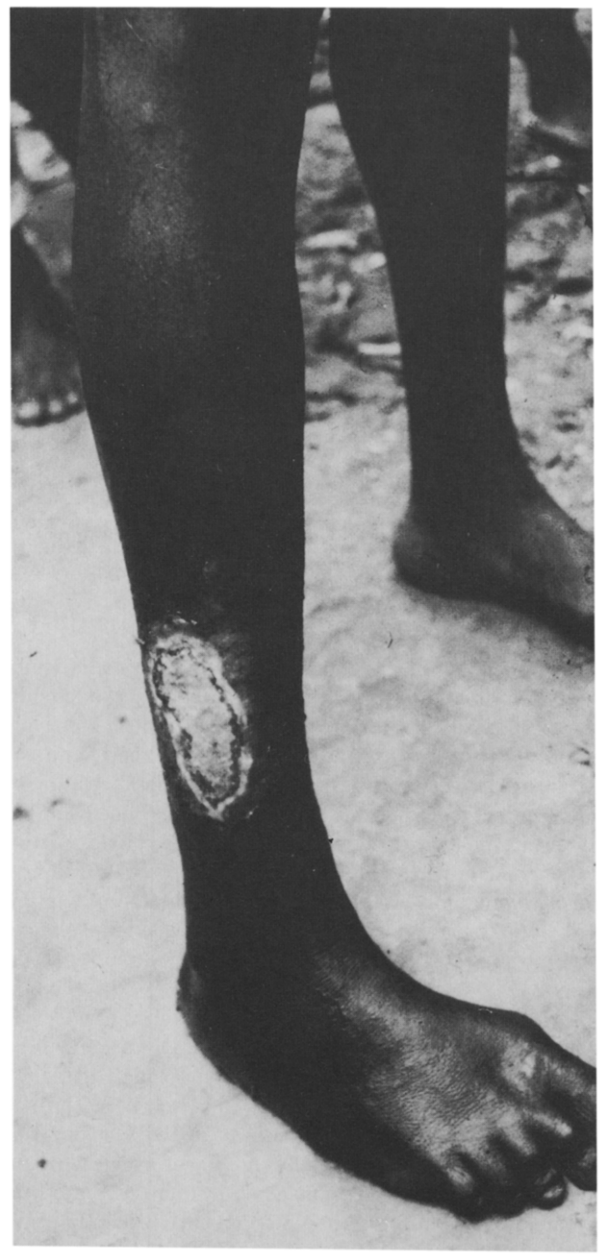

Fig. 9. Typical tropical ulcer in an African boy. Tropical ulcer is liable to become malignant, and about 10% of chronic ulcers will eventually develop into tumours. If tropical ulcers were always cured in early stages of development (easily possible with antibiotics), malignant tumours of skin in Africa would probably become rare. Reproduced from [27] by kind permission of Springer.

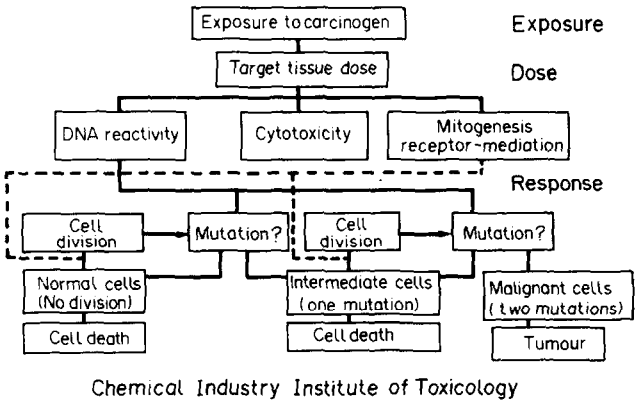

Fig. 10. Exposure–dose–response paradigm for chemical carcinogenesis, developed by Chemical Industry Institute of Toxicology. Reproduced from [41] by kind permission of CIIT.

promoter [48, 49]. I could not, however, observe any development of hyperplasia. The situation is really complex, and until more is known it would be wise to avoid any sweeping conclusions.

Cell cycle specificity of carcinogenic action?

Is there a certain stage in the cell cycle during which cells are most sensitive to carcinogens? This has been discussed in the literature for many years [50–53]. Mottram [54] suggested that the sensitivity of mouse skin to a carcinogen depends on the number of cells in mitosis when the carcinogen is applied. However, the results were difficult to analyse because most of these studies were performed with carcinogens that require an unknown amount of time to be metabolised to the ultimate carcinogen.

Recent studies using the carcinogen methylnitrosourea (MNU), which has a biological half-life of only 30 min, have shown that when epidermal cells are blocked in the cell cycle at the G_1–S transit, the tumour incidence is greatly increased compared to situations without cell blocking or with a block in mitosis only. This reaction is also dose-dependent. This is so whether the blocking is done by the epidermal chalone, or by hydroxyurea [55–57], and it can also be seen when the cells are blocked by the synthetic growth-inhibitory pentapeptide (epidermal chalone) [58] (Figs 11 and 12). We concluded that the sensitivity of epidermal cells to the alkylating substance MNU is higher when cell proliferation is blocked in such a way that a large cohort of resting or slowly progressing cells is in late G_1 or early S. This may be due to an increased binding of MNU to form MNU–DNA adducts in late G_1 and early S. A subsequent compensatory increased rate of DNA replication caused by the toxicity of MNU in cells recruited from the blocked cells at the G_1–S border, may then contribute to fix DNA injury or prevent repair. This is shown only for MNU, but it may also explain the well-known fact that rapidly proliferating tissues (be it naturally as in the renewing cell populations, or secondary as in regeneration) are most prone to cancer development. The G_1–S transit phase lasts several hours even during rapid proliferation. Hence, in such situations there may be a large number of cells in this phase either owing to a traffic jam (block), or because, although fast, the traffic is heavy, i.e. there is a rapid cell cycle progression in a cell population with a short mean generation time.

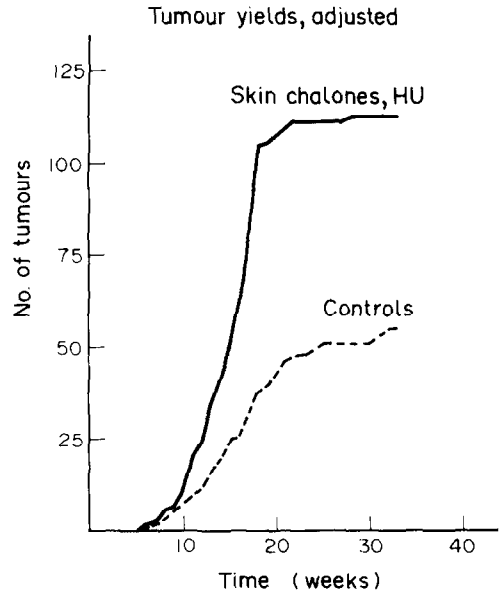

Fig. 11. Average tumour yields from hairless mouse skin after single application of 2 mg MNU after various pretreatments. Thick line = pretreated with intraperitoneal injections of skin extract or hydroxyurea (HU). Dotted line = controls, no pretreatment, or intraperitoneal injection of saline, colcemid or liver or heart muscle extract. Difference was highly significant, and shows that when epidermal cells are delayed or blocked in late G_1–S phase, they are more sensitive to carcinogenic effect of MNU. Reproduced from [55] by kind permission of *Carcinogenesis* and IRL.

Do carcinogens disturb normal growth control in the epidermis?

Finally we come to the problem of disturbed growth control in carcinogenesis [59–61]. The most obvious examples can be found in hormonal systems. It is known that ovarian tumours can be produced in castrated rats or mice in a piece of ovary transplanted to the spleen [62]. This phenomenon has been explained on the basis that hormones produced by the ovary normally pass into the bloodstream and act as an inhibitor for the production of gonadotrophic hormones by the pituitary

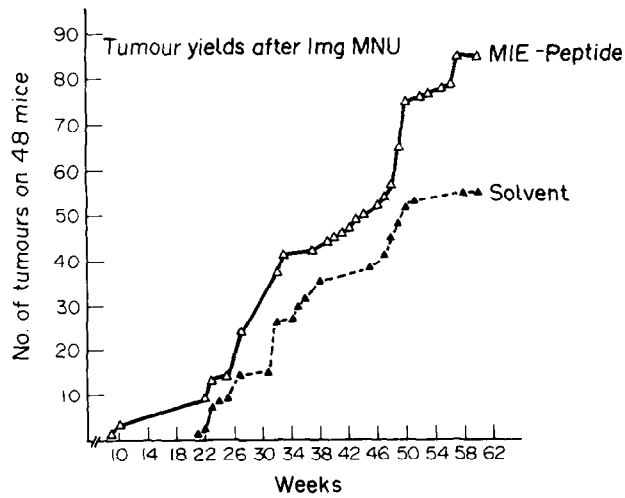

Fig. 12. Similar results to those shown in Fig. 11, showing that purified, synthesised mitotis-inhibitory epidermal peptide (MIE peptide) increases tumour yield. Reproduced from [58] by kind permission of *Carcinogenesis* and IRL.

gland. When an ovary is transplanted to the spleen, its hormones pass via the splenic vein to the portal vein and to the liver, where it is inactivated. Thus, no or very little inhibiting information from the ovary reaches the pituitary, which continues to produce excess gonadotrophic hormone, stimulating the ovarian transplant first to hyperplasia and later to neoplasia. Modern research has revealed that the picture is somewhat more complicated, but the main chain of events is as described. In cybernetic terms, the transplantation of ovarian tissue to the spleen in castrated animals corresponds with what has been called "opening up the loop".

In many other such systems, tumorigenesis has been provoked simply by disturbance of the hormonal equilibrium in: (a) the pituitary–thyroid system, (b) the pituitary–gonad system, and (c) the pituitary–mammary gland system [63, 64]. Thus, there seems to be good evidence that disturbed growth control *per se* can be carcinogenic in hormone-regulated systems, maybe due to endogenous mutations occurring during the hormone-induced mitogenesis.

During recent years another system of growth control, the chalones, has begun to interest carcinogenesis researchers [65]. As shown in Figs 5 and 6, relatively high doses of carcinogens destroy the amount of chalone in the epidermis, but this is so also after adhesive tape stripping with removal of maturing cells (Fig. 7). Hence, this is a non-specific reaction. All seem to agree that malignant tumours produce the chalone of the tissue of origin, and that the mitotic rate of tumours can be influenced by their own specific chalones. However, tumours may be less sensitive than normal tissue to chalone inhibition of cell division [66]. As regards the possible role of chalones in carcinogenesis, there is no agreement. There is one theory which stated that a disturbance in the chalone mechanism is probably a basic event [66]. Another view, which I support, is more sceptical, and states that there is no proof that a disturbance in the chalone mechanism is basic or primary for carcinogenesis [65]. It may well be that the disturbances in the chalone mechanism are secondary events. This problem will have to be solved experimentally.

We thus have good evidence that growth control systems become disturbed by carcinogenic influences, but we have no exact information about the nature of the disturbances. We must hope that more evidence about oncogenes, suppressor genes, and chalones and their putative genes, will provide firmer grounds for conclusions.

GENERAL CONCLUSIONS

Carcinogens profoundly affect the variables of cell proliferation kinetics, but it is difficult or impossible to distinguish between non-specific and specific alterations. Many of the alterations are obviously due to the toxicity of the carcinogens, and there is no firm proof that specific alterations occur.

The existing conditions of cell proliferation kinetics in each tissue influence carcinogenesis. Rapidly renewing tissues are generally more prone to cancer development, but there may be cell cycle specific phases, and one may suspect that the late G_1 and early S are such sensitive areas. Many cells may be in this area when they are blocked in early S, or when the cells are proliferating.

Certain disturbances of hormonal growth control systems seem to be carcinogenic in themselves, and there is good evidence that carcinogenic substances also disturb the chalone systems, primarily or secondarily.

Increased rate of cell proliferation is definitely associated with carcinogenesis, but, in my opinion, usually in connection with other, probably genotoxic, carcinogenic influences. Mitosis in itself is certainly necessary in order for a tumour to grow, but a normal process of DNA replication and mitosis is not in itself a direct and primary carcinogenic factor.

What does this mean for prevention of cancer? Most people believe that we should avoid all contact with all carcinogens, but no one would disagree that a moderate dose of sunshine, for example, improves health and quality of life. The positive health effects are obvious, the risk of carcinogenesis very small. Whether similar hormetic doses also exist for ionising radiation and some chemical carcinogens, is not proven, but cannot be excluded. On the other hand, it does seem reasonable to avoid as far as possible all carcinogens that even in moderate doses induce cell injury with compensatory regenerative reactions (increased rate of cell proliferation and sustained hyperplasia) in addition to their transforming ability.

When testing for carcinogens, doses as small as possible should be used—to avoid non-specific tumorigenesis due to cell toxicity and regeneration.

1. Todaro GJ. Autocrine growth in carcinogenesis. In Iversen OH: ed. *Theories of Carcinogenesis* p. 61. Washington, Hemisphere, 1988, 61.
2. Iversen OH. What is new in endogenous growth stimulators and inhibitors (chalones)? *Path Res Pract* 1985, **180**, 77.
3. Iversen OH. "Dead cells never produce cancer". The problems of dose in relation to toxicity and genuine carcinogenicity of carcinogenetic substances tested. *Proc Skin Carcinogenicity Symp* Rouen, France, 1984. Centre Henri Becquerel, 104.
4. Iversen OH. Cell proliferation kinetics and carcinogenesis: a review. *Fifth Int Symp Biological Characterization of Human Tumours* Bologna, 1973. 21. Amsterdam, International Congress Series No. 321, 1973, 21.
5. Iversen OH. Kinetic characterization of malignant tumours. *Sixth Int Symp Biological Characterization of Human Tumours*, Copenhagen 1975. Amsterdam, Excerpta Medica International Congress Series No. 375, 1975, 173.
6. Iversen OH. The early effects of a single application of acetone and various doses of 7,12-dimethylbenz(α)anthracene on CD-1 and hairless mouse epidermis. *APMIS Suppl.* 1988, **96**, 7.
7. Iversen OH and Evensen A. Experimental skin carcinogenesis in mice. Thesis, Oslo, Norwegian Universities Press, 1962.
8. Knutsen HK. Måling av overflatetap av hornceller normalt og etter applikasjon av carcinogene og/eller hudirriterende stoffer. Thesis, Oslo, University of Oslo, 1989.
9. Thrane EV. Histologisk kvantitering av celletap fra hornlaget i museepidermis, normalt og etter kreftfrembringende og ikke-kreftfrembringende hudirritamenter. Thesis, Oslo, University of Oslo, 1990.
10. Skjæggestad Ø. Experimental epidermal hyperplasia in mice. Relation to carcinogenesis. Thesis, Oslo, Norwegian Universities Press, 1964.
11. Elgjo K. Epidermal cell population kinetics in chemically induced hyperplasia. Thesis, Oslo, Norwegian Universities Press, 1966.
12. Rohrbach R, Elgjo K, Iversen OH, Sandritter W. Effects of methylcholanthrene on epidermal growth regulators. I. Variations in the M-factor. *Beitr Path Bd* 1972, **147**, 21.
13. Rohrbach R, Elgjo K, Iversen OH, Sandritter W. Effects of methylcholanthrene on epidermal growth regulators. II. Variations in the S-factor. *Beitr Path Bd* 1976, **158**, 145.
14. Rohrbach R, Iversen OH, Elgjo K, Riede UN, Sandritter W. Effects of croton oil on epidermal growth regulators (chalones). *Beitr Path Bd* 1976, **159**, 143.
15. Rohrbach R, Iversen OH, Riede UN, Sandritter W. Effects of cellophane tape stripping of mouse skin on epidermal growth regulators (chalones). *Beitr Path Bd* 1977, **160**, 175.
16. Clausen OPF. Regenerative proliferation of mouse epidermal cells following application of a carcinogenic skin irritant (MCA). Microflow fluorometric DNA measurements and 3H-TdR incorporation studies of isolated basal cells. *Virchows Arch* 1978, **27**, 205.

17. Clausen OPF. Regenerative proliferation of mouse epidermal cells following application of a skin irritant (cantharidin). *Cell Tissue Kinet* **12**, 135 (1979).
18. Clausen OPF, Lindmo T. Regenerative proliferation of mouse epidermal cells following adhesive tape stripping. *Cell Tissue Kinet* 1976, **9**, 573.
19. Iversen OH, Astrup EG. The paradigm of two-stage carcinogenesis: A critical attitude. *Cancer Invest*, 1984, **2**, 51.
20. Marquardt H, Bendich A, Philips FS, Hoffmann D. Binding of [G-^{3}H]-7,12-dimethylbenz(α)anthracene to DNA of normal and of rapidly dividing hepatic cells of rats. *Chem-Biol Interactions* 1971, **3**, 1.
21. Marquardt H, Philips FS, Bendich A. DNA binding and inhibition of DNA synthesis after 7,12-dimethylbenz(α)anthracene administered during the early prereplicative phase in regenerating rat liver. *Cancer Res* 1972, **32**, 1810.
22. Marquardt H. Cell cycle dependence of chemically induced malignant transformation *in vitro*. *Cancer Res* 1974, **34**, 1612.
23. Farber E. Possible etiologic mechanisms in chemical carcinogenesis. *Environ Health Perspect* 1987, **75**, 65.
24. Burck KB, Liu ET, Larrick JW. *Oncogenes*. New York, Springer, 1988.
25. Cohen SM, Ellwein LB. Genetic errors, cell proliferation, and carcinogenesis. *Cancer Res* 1991, **51**, 6493.
26. Virchow R. *Vorlesungen über Pathologie*, 5. lecture. Berlin, August Hirschwald, 1863, Band 2, 72.
27. Iversen U, Iversen OH. Tumours of the skin. In: Templeton AC, ed. *Tumours in a Tropical Country*. Ch. 10, p. 180. *Recent Results Cancer Res* 1973, **41**, Berlin, Springer.
28. Deschner EE. Cell proliferation and colonic neoplasia. *Scand J Gastroenterol* 1988, **23** (Suppl. 151), 94.
29. Cohen SM, Ellwein LB. Cell proliferation in carcinogenesis. *Science* 1990, **249**, 1007.
30. Preston-Martin S, Pike MC, Ross RK, Jones PA, Hendersen BE. Increased cell division as a cause of human cancer. *Cancer Res* 1990, **50**, 7415.
31. Deelman HT. The part played by injury and repair in the development of cancer; with remarks on the growth of experimental cancers. *Br Med J* 1927, **1**, 872.
32. MacKenzie I, Rous P. The experimental disclosure of latent neoplastic changes in tarred skin. *J Exp Med* 1941, **73**, 391.
33. Mottram JC. A developing factor in experimental blastogenesis. *J Path Bact* 1944, **54**, 181.
34. Rous P, Beard JW. The progression to carcinoma of virus-induced rabbit papillomas (Shope). *J Exp Med* 1935, **62**, 523.
35. Melzer MS. Paradox of carcinogens as cell destroyers and cell stimulators. A biochemical hypothesis. *Eur J Cancer Clin Oncol* 1980, **16**, 15.
36. Ames BN, Gold LS. Chemical carcinogenesis: too many rodent carcinogens. *Proc Natl Acad Sci USA* 1990, **87**, 7772.
37. Ames BN, Gold LS. Carcinogenesis mechanisms: the debate continues. *Science* 1991, **252**, 902.
38. Cohen SM, Ellwein LB. Carcinogenesis mechanisms: the debate continues. *Science* 1991, **252**, 903.
39. Perera FP, Rall DP, Weinstein IB. Carcinogenesis mechanisms: the debate continues. *Science* 1991, **252**, 903.
40. Butterworth BE, Goldsworthy TL, Popp JA, McClellan RO. The rodent cancer test: An assay under seige. *CIIT Activities* 1991, **11**, 1.
41. Weinstein IB. Mitogenesis is only one factor in carcinogenesis. *Science* 1991, **251**, 387.
42. Chemical Industry Institute of Toxicology, Annual Report p. 6 (1990).
43. Ashby J, Morrod RS. Detection of human carcinogens. *Nature* 1991, **252**, 185.
44. Trosko JE, Change CC, Madhukar BV. Cell-to-cell communication: relationship of stem cells to the carcinogenic process. In: Stevensen DE, Popp JA, Ward JM, McClain RM, Slaga TJ, Pitot HC, eds. *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons*. New York, Alan R. Liss, 1990, 259.
45. Iversen OH. Hairless mouse skin in two-stage carcinogenesis. *Virchows Arch (Cell Pathol)* 1982, **38**, 263.
46. Iversen OH, Paulsen JE, Schjoelberg A. The time needed for normalization of hairless mouse epidermis after treatment with twice weekly skin applications of 10 nmol 12-*O*-tetradecanoylphorbol-13-acetate in acetone, or acetone alone, for 18 weeks. A morphologic and cell kinetic study. *Carcinogenesis* 1981, **2**, 1353.
47. Astrup EG, Iversen OH, Elgjo K. The tumorigenic and carcinogenic effect of TPA (12-*O*-tetradecanoylphorbol-13-acetate) when applied to the skin of BALB/cA mice. *Virchows Arch (Cell Pathol)* 1980, **33**, 303.
48. Iversen OH. Urethan (ethyl carbamate) alone is carcinogenic for mouse skin. *Carcinogenesis* 1984, **5**, 911.
49. Iversen OH. Urethan (ethyl carbamate) is an effective promoter of 7,12-dimethylbenz(α)anthracene-induced carcinogenesis in mouse skin two-stage experiments. *Carcinogenesis* 1991, **12**, 901.
50. Frei JV, Ritchie AC. Diurnal variation in the susceptibility of mouse epidermis to carcinogen and its relationship to DNA synthesis. *J Natl Cancer Inst* 1964, **32**, 1213.
51. Hennings H, Smith HC, Colburn NH, Boutwell RK. Inhibition by actinomycin D of DNA and RNA synthesis and of skin carcinogenesis initiated by 7,12-dimethylbenz(α)anthracene or β-propiolactone. *Cancer Res* 1968, **28**, 543.
52. Iversen U, Iversen OH, Hennings H, Bjerknes R. Diurnal variation in the susceptibility of mouse skin to the tumorigenic action of methylcholanthrene. *J Natl Cancer Inst* 1970, **45**, 269.
53. Pound AW. Carcinogenesis and cell proliferation. *NZ Med J* 1968, **67**, 88.
54. Mottram JC. A diurnal variation in the production of tumours. *J Path Bact* 1945, **57**, 265.
55. Iversen OH. Enhancement of methylnitrosourea skin carcinogenesis by inhibiting cell proliferation with hydroxyurea or skin extracts. *Carcinogenesis* 1982, **3**, 881.
56. Iversen OH. Hydroxyurea enhances methylnitrosourea skin tumorigenesis when given shortly before, but not after, the carcinogen. *Carcinogenesis* 1982, **3**, 891.
57. Kirkhus B, Iversen OH, Kristensen A. Carcinogenic doses of methylnitrosourea induce dose response related delay in transit through S and G_2 phases in mouse epidermis: a cell kinetic study. *Carcinogenesis* 1987, **8**, 369.
58. Iversen OH, Elgjo K, Reichelt KL. Enhancement of methylnitrosourea-induced skin tumorigenesis and carcinogenesis in hairless mice by pretreatment with a mitosis-inhibiting epidermal pentapeptide. *Carcinogenesis* 1989, **10**, 241.
59. Furth J. A meeting of ways in cancer research: thoughts on the evolution and nature of neoplasms. *Science* 1959, **19**, 241.
60. Prescott DM. Biology of cancer and the cancer cell: normal and abnormal regulation of cell reproduction. *Cancer* 1972, **22**, 262.
61. Smithers DW. In: Teir H, Rytömaa T, eds *Control of Cellular Growth in Adult Organisms*. London, Academic Press, 1967, 357.
62. Biskind GR, Biskind MS. Atrophy of ovaries transplanted to the spleen in unilaterally castrated rats; proliferative changes following subsequent removal of intact ovary. *Science* 1948, **108**, 137.
63. Furth J. Pituitary cybernetics and neoplasia. In: *The Harvey Lectures, Series 63*. New York, Academic Press, 1969, 47.
64. Kirschbaum A. In: Nowinski WW, ed. *Fundamental Aspects of Normal and Malignant Growth*. Amsterdam, Elsevier, 1960, 823.
65. Iversen OH. The hunt for endogenous growth-inhibitory and/or tumor suppression factors: their role in physiological and pathological growth regulation. In: *Advances in Cancer Research* 57 Klein G, Vande Woude G, ed. 1991, Chap. 12, 412.
66. Bullough WS, Laurence EB. Control of mitosis in rabbit V×2 epidermal tumours by means of the epidermal chalone. *Eur J Cancer Clin Oncol* 1968, **4**, 587.