# Epidemiology, Multistage Models, and Short-term Mutagenicity Tests

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(See the list of minor proof changes appended to the end of the article.)

This section is intended as an introduction to, and apology for, multistage models. Such models occupy a curious position in the world of cancer research. It seems likely that multistage processes underlie the generation of a large majority of human cancers (the carcinomas), yet most research workers do not have any real interest in discussing the various alternative multistage models that attempt to describe these processes. These research workers can't all be wrong, so what is wrong with multistage models? The trouble is, I suppose, that the processes usually invoked are, in principle, extremely difficult to observe (phenotypically silent changes in a few scattered somatic cells), and that very similar predictions for the few things we can actually observe may follow from mathematical elaboration of various very different multistage models (each with its own implausible chalones, feedback mechanisms, exact numbers of "stages" to be progressed through, mutations, epigenetic switches, clonal growth rates, etc.).

However, laboratory investigations of various completely different aspects of the processes of cancer induction (viruses, mutagens, host control mechanisms, etc.) are already well established, and new lines of investigation, particularly of "cocarcinogenic" processes, may yet emerge. No single process is likely to be the whole truth, and we must hope that some grand synthesis of the known processes will eventually be put together which will describe all the essential features of human cancer induction. Although the eventual synthesis is not yet in sight, multistage models should at present be thought about to some extent, and their general features should be common knowledge, as the general framework of this eventual synthesis will (at least for that 90% of fatal human cancers which are carcinomas) almost certainly be some kind of multistage model. Moreover, despite all their present uncertainties, multistage models for carcinoma induction have already offered plausible answers to various questions concerning monoclonality, dose–response relationships under conditions of regular exposure, hypothetical "threshold" doses, the synergistic effects of different carcinogens, the role of luck, and, last but not least, the connection between cancer and aging. (This latter point has been

only partly resolved: multistage models give a very natural explanation for cancer being a hundred times rarer among young adults than among the elderly, but no plausible explanation has yet been offered for the fact that the risk of cancer in old age is not vastly different in species with very different life-spans.)

In the next three sections, I will recapitulate a few formulae that can emerge from certain multistage models. (Readers who don't like even simple algebra can pass over these sections.) Then I will discuss the epidemiology of lung cancer, because the general outlines of a synthesis of the rate-determining causes of human lung cancer are probably already visible, although many gaps remain. Finally, I will take one area of current experimental interest—the "carcinogens-are-mutagens" story—and will argue that if experimental scientists discussing this proposition do so in the perspective of epidemiology and multistage models, they will come to very different conclusions. Because I am trying to illustrate how one might, when considering new ideas, do so in the context of multistage models, I have introduced some ideas (particularly concerning the monoclonal origin of atherosclerotic plaques and the possible nature of certain carcinogenic processes) which eventually may be found to be false; but the context should make clear the distinction between established fact and tentative suggestion.

Multistage models derive considerable support from epidemiology, and specific aspects of multistage models for cancer induction can sometimes be tested by epidemiologic observation of populations subject to different dose rates of certain carcinogenic insults or, particularly, to *varying* dose rates of a carcinogenic insult. For example, the relationship between smoking and lung cancer among smokers who start later or earlier than usual is informative, and the pattern of lung cancer among ex-smokers is a very strong constraint indeed on postulated mechanisms for the induction of lung cancer.

The essential multistage hypothesis is that a few distinct changes (each heritable when cells carrying them divide) are necessary to alter a normal cell into a malignant cell, and that human cancer usually arises from the proliferation of a clone derived from a single cell that suffered all the necessary changes and then started to proliferate malignantly.

To reach any useful conclusions from these fundamental hypotheses, one has to add extra assumptions, and, according to the extra assumptions invoked, different variants of the multistage model follow. However, all have in common a stochastic approach rather than a deterministic one. Put another way, with multistage models, when all the predisposing factors have been allowed for, luck has an *essential* role (Peto 1977) in determining who gets cancer and who does not. The probability of one of my bronchial cells generating a fatal carcinoma can be predicted, but whether in actual fact one cell will do so cannot. (An analogous role for luck exists when predicting the genotype of a child using Mendel's laws and the genotypes of its parents.)

## Definitions

1. The *incidence rate* of cancer among people of age $t$, which may be written $I(t)$, is the probability that a person of age $t$ will develop a new cancer tomorrow.[1]

2. Among cells which have suffered certain heritable changes and are at risk of suffering a particular further heritable change, the *event rate, R,* of that further change is the probability that a cell which has been ready for such a change for some time, but hasn't yet suffered it, will do so tomorrow.[1]
3. If we number the heritable changes suffered by a particular cell en route from normality to malignancy 1, 2, . . . , $n$ (where $n$ might be, e.g., somewhere around 4 or 6), then we can call these changes *stages,* and we can call a cell that has suffered only the first two such changes a stage-II cell, and so on. A particular person might already contain several stage-I cells, some stage-II cells, and a few stage-III cells when the cell which eventually kills him suffers the first of its stages. Thus one cannot usefully say that a *person* has reached stage III, only that certain cells have done so. Confusion on this point is surprisingly common.
4. Let the event rate of the change into stage $i$ be called $R_i$. In some multistage models $R_i$ is constant, whereas in others it varies with age, or with the condition of the neighboring cells, or with the time since that cell suffered its previous change.
5. Any external agent that directly or indirectly increases any of the $n$ event rates, $R_1 \ldots R_n$, may be called a *carcinogen,*[2] as may any agent which makes it more probable that a fully transformed cell will proliferate successfully rather than be eliminated or held in check.
6. If, for a particular cancer type, $I(t)$ is approximately proportional to $t^k$, then, since a plot of log $I$ against log age for that cancer will yield an approximately straight line (with slope $k$), such a cancer may be called a *log/log cancer.*

## The Simplest Multistage Model

Suppose (without necessarily believing it) that the order in which the stages must occur is fixed; that all of $R_1 \ldots R_n$ are constant throughout life, and are small in comparison to the inverse of the life-span (Moolgavkar 1977); that the cells have straightforward kinetics; and that cells which have suffered some, but not all, of the relevant changes behave perfectly normally (as if

[1] Strictly speaking, definitions of "rates" need to be based on differential calculus, but the above definitions are accurate enough for our purposes. Multiplication of the rate per day by 365 yields the rate per year, of course. In many experimental or epidemiologic situations, there is a direct biological relationship leading to a fairly simple algebraic relationship between the carcinogenic stimulus and the age-specific cancer incidence rate, $I(t)$. However, the absolute risk of cancer per individual depends not only on $I(t)$ but also on the pattern of mortality from other causes, since, for example, early death from a traffic accident is a fairly effective way of avoiding cancer. The relationship between the carcinogenic stimulus and the total risk of cancer is therefore less simple and direct than the relationship with $I(t)$, and so it is the relationship of carcinogenic stimulus with $I(t)$, not with total risk, which should interest us.

[2] Some authors use the word "carcinogen" in a more limited sense and then refer to other agents, which I would also call carcinogens, as cocarcinogens, promoters, enhancers, etc. In this report, any agent, process, or habit that increases the risk of cancer among people of a particular age will be called a carcinogen whether or not it operates in one of the ways listed in definition 5.

they had suffered no changes). Given all this, it is easy to prove (Armitage and Doll 1961) that, at age $t$,

$$I(t) \propto R_1 R_2 \ldots R_n (t - w)^{n-1}, \quad (1)$$

where $w$ is the time a stage-$n$ (i.e., fully transformed) cell takes to proliferate into a pathological cancer that can be diagnosed. Since $w$ will often be a negligible proportion of the human life-span, this simple multistage model usually predicts that, approximately,

$$I(t) \propto R_1 R_2 \ldots R_n t^{n-1} \quad (2)$$

or, including the event rates in the constant of proportionality,

$$I(t) \propto t^{n-1}. \quad (3)$$

As is well known, a log/log relationship such as this (usually with an exponent of 4, 5, or 6) is observed for many human cancer sites, and this is often taken as evidence for simple multistage models with about six stages. That this inference may be ill-founded will be seen in the next section.

## More General Multistage Models

Multistage models that are more plausible than the over-simplified multistage model I have just outlined will also lead, approximately, to the log/log relationship characterized by

$$I(t) \propto t^k, \quad (4)$$

but in some of these models $k$ will not be equal to $(n - 1)$. For example, suppose that stage-$(n - 1)$ cells have a slight selective advantage over their normal neighbors (which is quite likely). Such cells will proliferate as limited clones of $(n - 1)$-stage cells, some or all of which will be at risk of the last step into full malignancy. Suppose that the number of cells in such a growing clone increases roughly as the *square* of the time since that clone originated (Fisher 1958), i.e., that for a particular clone which originated $x$ years ago,

$$\text{present number of cells in clone} \cong C \cdot x^2. \quad (5)$$

Something like this could be roughly true, especially since in epithelia one sometimes observes "carcinoma in situ," a monoclonal precancerous lesion. Equation 5 predicts that as the clone increases, its doubling time slows down —but then, transplanted tumors don't grow exponentially (Laird 1964), so why should in situ premalignant clones?

This particular multistage model can be shown to imply that

$$I(t) \propto R_1 R_2 \ldots R_n C (t - w)^{n+1} \quad (6)$$

or, approximately, that $I(t) \propto t^{n+1}$, where the constant of proportionality depends not only on the event rates for the $n$ stages, as previously, but also on $C$. In this or any other model in which partially altered cells have a selective advantage, any agent (perhaps even a simple irritant, although Berenblum [1974] and others have emphasized that the classical promoting agents do not act simply as irritants or as mitogens) that helps them proliferate[3] will increase $I(t)$ and therefore is a carcinogen. In this, as in all the other models described

so far, there is no age dependence of the rate constants for the different stages or processes. This may seem a surprising assumption, especially in view of the extent to which cancer is more common in the elderly. However, experimental systems have been studied in which the event rates really do seem to be independent of age (Peto et al. 1975), and one of the conceptual attractions of multistage models is the way in which they explain away the age dependence of carcinomas without the need for having to understand the biology of aging first. (This is somewhat facile, and the relevance of the natural life-span will be discussed more seriously in a later section.)

The model just described is an example of a multistage model which yields $I(t) \propto t^{n+1}$. Other models can also be constructed: Fisher (1958) devised a rather implausible one in which $I(t) \propto t^{3(n-1)}$, and Pike (1966) proved that for a wide range of utterly dissimilar biological models, an approximate log/log pattern might emerge. One conceptually trivial source of different log/log models is the algebraic fact that if $I(t)$ is proportional to $(t - w)^{n-1}$ and $w$ is moderately large, then $I(t)$ is also, to a very good approximation, proportional to $t^k$ for some $k$ which exceeds $(n - 1)$ (Peto and Lee 1973).

Thus a log/log relationship with exponent $k$ between age and the incidence rate of a certain type of cancer does not imply, or even suggest, that a simple multistage model is biologically true, neither does it imply nor suggest that there are $(k + 1)$ stages[4] en route from normality to malignancy. Unfortunately, a sort of two-way idealization is often applied to age-specific cancer incidence rates. The well-known existence of the simple multistage model predicting $I(t) \propto t^{n-1}$ leads to idealization of the data by an undue concentration on log/log cancers at the expense of the numerous instructive exceptions. This belief that nearly all cancers are log/log cancers then lends undue credence to the simple multistage model with about six stages at the expense of equally plausible models which yield similar predictions.

## Epithelial and Other Cells

The kinetics of mitosis in human tissues are complex and differ markedly from tissue to tissue. Some populations of cells, such as heart muscle cells and neurones, rarely or never divide in adult life, and so perhaps the mechanisms

[3] Cairns (1975) has suggested that there are territorial constraints on stem cells (e.g., their restriction to the crypts in the small intestine) and that interference with these constraints may enhance the selective advantage of partially altered stem cells.

[4] What is a "stage"?—or rather, what is not to be classed as a "stage"? If, after one change, a cell is likely to suffer another change in the near future, they would not both count as stages. Stages have to be *slow* and *improbable,* since Equation 3 holds exactly only if the number of cells in a particular stage is not depleted appreciably by progression of some of them on to the next stage. A rough general rule is that if a change is not likely to have happened within 10 years of a cell being ready for it, then it would count as a stage, but if it is likely to take less than a year it would not. (For example, the process of DNA damage and faulty repair would only comprise one stage, not two.) The reason for this distinction is that slow changes affect the kinetics of the whole process (increasing the exponent of $t$ in $I(t)$, etc.) but quick ones do not. If the probability of faulty, as opposed to accurate, DNA repair is $10^{-4}$, for example, this would affect the rate constant for the single stage consisting of mutation plus faulty repair but would not change the exponent of $t$.

that restrain them from mitosis are fundamentally different from the mechanisms that restrain cells such as those in the hemopoietic system, where division must occur rapidly when needed and must stop when sufficient. Because many particular reticuloendothelial cells are not as physically localized as cells in other tissues are, their decisions as to whether to differentiate and divide must often be controlled by diffused stimuli, such as erythropoietin, and their kinetics may ultimately be completely explained by fairly straightforward probability rules. These rules, however, may prove to be very different from the rules that govern the divisions (if any) of the melanocytes in the skin or the divisions of the epithelial cells lining the milk ducts of the breast.

Since cancer induction involves (among other biological abnormalities) impairment of the ordinary control of mitosis, the mechanism of cancer induction is likely to be fundamentally different in different types of cells whose normal mechanisms of mitotic control differ fundamentally. Therefore, when formulating multistage models, we cannot ask about *the mechanism* of cancer induction; instead, we must ask separately about the mechanism in cells with a particular kind of normal growth control, and then ask separately about the mechanism in cells with a different kind of normal control, and so on. For these purposes, it is useful to divide the human body into epithelial cells and other cells.[5] From this, the cell types of the human body can be divided into three groups:

1. *Sex-specific epithelial cells:* The epithelial cells of the breast, cervix, vagina, endometrium, ovary, prostate, testis, vulva, penis, scrotum, etc.

[5] The term "epithelial," although widely used, has no universally agreed upon definition; some define it embryologically, some topologically, and some morphologically. For most cell populations, there will be general agreement, no matter which definition is adopted, as to whether the cells are epithelial or not, but there will be differences in the classification of a few (mostly minor) cell types. In general, epithelial cells have characteristic methods of controlling their growth. Typically, there is a basement membrane with nonepithelial cells underneath it and a layer of epithelial cells on top of it. If the layer of epithelial cells is several cells deep, then the cells in contact with the membrane are called the basal layer of epithelial cells. The epithelial cells above the basal layer are moribund; they may or may not divide again, but they will ultimately be pushed up, out, and away to death by more cells moving up following mitoses in the basal layer. The ability of epithelial cells to divide is strongly influenced by contact with the nonepithelial cells below the basement membrane.

To the extent to which the concept of a "stem cell" can be usefully carried over from reticuloendothelial kinetics to epithelial kinetics, all the epithelial stem cells probably lie in the basal layer, although some of the cells in the basal layer are not stem cells. (If there were stem cells elsewhere in the body capable of migrating to and colonizing the basal layer, this would be very relevant to cancer induction mechanisms, but no evidence exists either for such migratory epithelial stem cells or for migratory "control cells.") Epithelia sometimes do have a few nonepithelial cells, such as melanocytes or connective-tissue cells, intermixed with their epithelial cells, but, apart from this, there is a general simplicity about the distribution of epithelial and nonepithelial cells in the body.

The "topological" definition of what is epithelial and what is not is that all body surfaces which connect, however tortuously, with the outside of the body are epithelia, composed mainly of epithelial cells, and that nearly all human cells not on these surfaces are nonepithelial. (Two exceptions, generally classified as epithelial, are the ovaries, which do not quite connect with the fallopian tubes, and the thyroid ducts, which close off from the outside just before birth.) By this definition, for example, epithelia line the urethra, bladder, ureter, and renal tubules, but not the renal capillaries.

2. *Other epithelial cells:* The epithelial cells of parts common to both male and female, including bladder and kidney.
3. *Nonepithelial cells:* Neural cells, germ-line cells, melanocytes, reticulo-endothelial cells, blood vessels, bones, muscles and other connective and soft tissues, together with any cells that have never differentiated into epithelium, such as those cells which give rise to the various teratomas and blastomas.

## Data for Human Cancer

Many organs are intimate mixtures of epithelial and nonepithelial cells, and, unfortunately, national mortality statistics, with the notable exception of those from Denmark (Clemmesen 1964), often do not tell us which tumors of these organs arise from the epithelial cells. (Carcinoma means,[6] by definition, tumor arising from an epithelial cell, and, for example, ICD 152 includes small intestine sarcomas as well as carcinomas.) However, it appears that in Britain today, and in all other developed countries, the cells of origin of fatal malignant tumors are distributed approximately as follows: (1) 20% of fatal malignant tumors are carcinomas arising from the sex-specific epithelial cells; (2) 70% are carcinomas arising from the other epithelial cells; (3) 10%, including all the leukemias and sarcomas, arise from nonepithelial cells and are therefore not carcinomas.

Human incidence data suggest that if an inbred population of humans were to be exposed throughout life to constant levels of the types of environmental insults we now suffer, the separate incidence rates of *all* carcinomas in group 2 (other epithelial cells) (with the possible exceptions of the liver, which exhibits a mode in countries where liver cancer is very common, and naso-pharynx, which is slightly more common among children than it ought to be) would *each* increase as the fourth, fifth, or sixth power of age up to at least age 75, in conformity with the predictions from several multistage models.[7]

[6] The slight uncertainty in the definition of epithelial means there is also a slight uncertainty about the definition of carcinoma.

[7] In practice, divergences from a simple power-law relationship governing all the epithelial tumors in group 2 are caused by:

a. Genetic heterogeneity—for example, patients with untreated polyposis coli die of colon cancer in early adult life, causing a substantial deviation from the log/log relationship for carcinoma of the colon between the ages of 15 and 35. Heterogeneity of susceptibility will only cause appreciable deviations, however, if *most* of the highly susceptible individuals die.
b. Environmental heterogeneity—in extreme old age, the proportion of smokers is reduced by death (also, the very old tend to cut down on the number of cigarettes they smoke). This produces a slight flattening of the age-incidence curve for carcinoma of the lung in old age.
c. Age-related changes in insult—cigarette smokers usually begin smoking between the ages of 15 and 20; so whereas the incidence rate of carcinoma of the lung among non-smokers rises as $(\text{age})^4$, among smokers it rises approximately as $(\text{age} - 20)^4$, which is much steeper. (This also affects the rates for other carcinomas which are strongly related to smoking, e.g., lip and larynx.)
d. Cohort effects—in Britain, older people have been more exposed to pipe smoking, spirit drinking, and uncured syphilis, and perhaps to other causes of oral cancer which we do not yet know; thus their risks for these cancers are even higher, in comparison

(*continued on following page*)

Reasonable extensions of one or another of the possible multistage models could therefore well explain the age-incidence relationships for all carcinomas of group-2 (other epithelial) tissues. However, the size, structure, or habitual use of each of the sex-specific organs changes sharply at different times in one's life—at puberty, when sexual intercourse starts, at pregnancy, and at menopause—and some of these changes cause or correlate with sharp changes in the event rates of the stages of carcinogenesis in these organs. Even if the mechanisms of carcinogenesis in the sex-specific epithelial tissues (group 1) and the other epithelial tissues (group 2) are similar, as may well be the case, multistage models would not lead us to expect, nor do we generally find, log/log relationships between incidence and age for carcinomas of the group-1 tissues. For example, carcinoma of the uterine cervix, which is related to some aspect of sexual intercourse, depends on age just as carcinoma of the lung would if people smoked only from early adult life to middle age and then gave up smoking (see the section below on effects of giving up smoking).

Finally, what of group 3? There were no a priori reasons not to expect simple log/log relationships here, but in many cases they do not exist. Instead, we find almost everything imaginable—childhood tumors (e.g., medulloblastomas, Wilm's tumor, acute lymphoblastic leukemia), tumors with very shallow age slopes (e.g., Hodgkin's disease, tumors of connective and soft tissues), simple fifth-power log/log tumors (e.g., chronic lymphatic leukemia, myelomatosis), and even tumors with clear modes (e.g., teratoma, osteosarcoma, glioma).

No single set of mechanisms is likely to govern all these disparate age distributions, and an important mechanistic inference is that *90% of malignant human cancers are carcinomas (i.e., epithelial tumors), and*

(*Footnote 7 continued*)
with the risks suffered by the young, than age alone would lead us to expect. This, as in c above, causes a steepening of the cross-sectional age-specific incidence rates. (Lesser cohort effects also affect the British rates for carcinoma of the stomach, pancreas, colon, and, among males, kidney.)

e. Undercertification among the old—failure of physicians to investigate old people who are seriously ill as thoroughly as they investigate young people with similar illnesses leads to relative underrepresentation in cancer registry data and on death certificates of certain cancers among the aged.

f. Growth time—that is, the time a tumor takes to grow from a fully transformed, but still microscopic, lesion into a pathological cancer. Using $w$ to denote this terminal growth time, if $w$ were similar for all carcinomas of a particular type, then the incidence rate of those tumors would be proportional not to age$^k$ but to (age $- w$)$^k$, which has a steeper slope. Of course, $w$ would not really be similar for all carcinomas of a particular type, but even with variable $w$, a steepening of the slope (and some divergence from an exact log/log relationship) is still to be expected if the growth times are appreciable in comparison with the total life-span.

g. Cellular event rates not all small—Moolgavkar (1977) has emphasized that, in a *multi*stage model, the log/log relationship only follows if all the event rates per cell are small in comparison with the inverse of the life-span. (Moolgavkar is correct in this matter, and the specific event rates cited in our reply to his claim should have been smaller.) If this condition does not hold, there will in general be some downward curvature of the predicted log/log relationship, especially at older ages, this curvature being even greater if the stages are less strictly ordered than Armitage and Doll (1961) supposed. (Call the product of the age times the average of the $n$ event rates per cell $e$. Ignoring higher order terms, log $I$ will fall below its predicted straight-line relationship with log age by some multiple of $e$. This multiple is 0.5 $(n + 1)$ if no restrictions on order exist, and Moolgavkar (pers. comm.) has shown that it is unity if the stages all have to occur in a certain fixed sequence.)

*although common mechanisms may well underlie the development of most carcinomas, one should not try to infer those mechanisms from observations on other tumors (such as leukemias or sarcomas).* Research on viruses that cause leukemias, lymphomas, or sarcomas may therefore be irrelevant to the large majority of malignant human cancers.

The age distributions of the group-2 carcinomas seem likely (if the various biases already mentioned are allowed for) to be so similar that *mechanisms found to be relevant to particular epithelial cancers (except, perhaps, carcinoma of the liver or nasopharynx) are likely to be relevant to most or all other epithelial cancers, although not necessarily to sarcomas, etc.*

However, in view of the heterogeneity of the age distributions of the group-3 cancers, *there are probably many completely different sets of mechanisms involved in the generation of the various different nonepithelial tumors.*

## Atherosclerotic Plaques; Other Classifications

There is, however, one large exception that may eventually have to be made to the general thesis that "what matters is carcinomas." In developed countries, vascular disease involving occlusion of the cerebral or coronary arteries causes more deaths than all malignant cancers put together. In many patients, the underlying cause of fatal arterial occlusion appears to be the formation of atherosclerotic plaques in the wall of the artery, in which the smooth muscle cells in the arterial wall proliferate sufficiently to occlude the vessel themselves or, more commonly, to initiate the formation of an occlusive thrombus. Benditt and Benditt (1973) (see also Benditt 1977) and other workers have discovered by G6PDH typing that these plaques are monoclonal, i.e., that they appear to result from the limited proliferation of one smooth muscle cell that has partially escaped from its normal mitotic controls. If so, atherosclerotic plaques should perhaps be thought of as multiple independent benign leiomyomas, especially since they can be produced in chicken arteries by a few of the classical carcinogens such as dimethylbenz(a)anthracene (DMBA) (Albert et al. 1977), and questions about their initiation and promotion should be asked just as for other cancers. (One obvious question is why, if they really are benign tumors, do they so rarely progress to malignancy in unessential arteries?) If atherosclerotic plaques are to be included in the domain of the National Cancer Institute, then, in view of the enormous numbers of people killed by such plaques, the multistage-modeller's classification of fatal neoplasms will eventually have to be revised to (1) carcinomas of sex-specific organs; (2) other carcinomas; (3) tumors, excluding plaques, of nonepithelial cells; and (4) atherosclerotic plaques.

An alternative approach might be to forget about exactly what is or is not epithelial and to classify tumors by their age distributions under conditions of constant exposure. This might lead to a classification such as:

a. Cancers of sex-specific organs. This is almost the same as "carcinomas of sex-specific organs," except that it includes teratomas, various odd ovarian tumors, and a few sarcomas.
b. Adult log/log cancers with an exponent, under conditions of constant exposure, of about 5. This is almost identical with "other carcinomas,"

except that it also includes chronic lymphatic leukemia and, possibly, melanoma[8] (see Elwood and Lee 1975).

c. Adult log/log cancers with exponents below 4.
d. Other adult cancers.
e. Childhood cancers.

However, the aim should be to get biologically uniform categories, and the earlier classification into epithelial and other tumors, for all its uncertainty, is probably more relevant than the five-part classification above. The stem-cell hierarchies in different types of epithelia, however, may differ quite markedly from each other—in many epithelia, for example, there may be no analog of the localization of stem cells to the crypts of the small intestine—and these differences among epithelia might be relevant to oncogenesis but are as yet very incompletely understood. If they were understood, perhaps we could usefully subdivide carcinomas by the stem-cell kinetics in the epithelia from which they arose. In the meantime, all carcinomas are pooled in the hope that what is relevant for one of them is relevant for most of them. I am somewhat encouraged in this hope by the similarity of their age distributions.

## General Considerations about Mutagenicity and Mitosis

Although epithelial cells are less numerous than nonepithelial cells, mitoses are far more frequent in epithelial cells than in most other types of cells. In addition, since most mitoses that occur in the human body occur in epithelial cells, it is not surprising that uncontrolled mitosis (cancer), when it occurs, usually arises from an epithelial cell. However, the simple number of mitoses that occur in an organ cannot be the sole determinant of the susceptibility of that organ to cancer induction. The small intestine, in which cancer is exceedingly rare, is a large organ and turns over its cells more rapidly than any other epithelium. However, there is a precise program for this rapid turnover, involving stem cells in crypts with low or moderate turnover rates shedding daughter cells which proliferate rapidly and are then lost before they have time to give rise to carcinomas. Perhaps, then, what is dangerous for human organs (and particularly human stem cells) is suffering more rapid mitosis than they would normally have suffered in our animal ancestors some $10^7$ or $10^8$ years ago. If epithelia such as those in the small intestine are subject to rapid turnover in all animals, perhaps we have therefore evolved stem-cell hierarchies and territorial imperatives, based on crypts and villi, that allow this without much risk of cancer, whereas perhaps no such mechanisms have been evolved to protect the bronchi because apes and coelocanths did not smoke cigarettes. The relevance of mutagenicity and mitosis to cancer risk might then be fairly similar for all epithelial stem cells, but different epithelia may differ in the accessibility of their stem cells to insult. This general line of argument suggests that any cancer which is common somewhere today is probably being caused inter alia by some divergence of our current habits from those of our hunter and gatherer ancestors of $10^5$ years ago or their precursors of $10^7$ years ago. This suggestion is supported by the fact that whereas some of the rare human tumors show little geographic variation, all tumors that are

[8] Melanoma is probably in class c but might be in b or d.

presently common in one country are rare in some other country (Doll 1977).

The trouble with evolutionary arguments is that you can usually demonstrate whatever you want by a plausible evolutionary argument.[9] However, a final understanding of carcinoma induction must tie in the low event rates which humans suffer with evolutionary considerations (even though evolutionary considerations may not be the main clues which lead to this understanding), for in the evolutionary diversification of mammals enormous changes in cellular susceptibility to oncogenesis have developed. These may be nicely illustrated by a comparison of mice and men: A man has 1000 times as many cells as a mouse (although the ratio of our epithelial *stem*-cell numbers is not known), and we usually live at least 30 times as long as mice. Exposure of two *similar* organisms to risk of carcinoma, one for 30 times as long as the other, would give perhaps $30^4$ or $30^6$ (i.e., a million or a billion) times the risk of carcinoma induction per epithelial cell. However, it seems that, in the wild, the probabilities of carcinoma induction in mice and in men are not vastly different. Are our stem cells really, then, a billion or a trillion times more "cancerproof" than murine stem cells? This is biologically pretty implausible; if human DNA is no more resistant to mutagenesis in vitro[10]

[9] For example, it has been argued that since all exposed surfaces are epithelial, there must have been stronger evolutionary pressures to make epithelia resistant to cancer induction, and devices evolved in response to this need might account for the marked differences between the age distributions of carcinomas and sarcomas. This would be only a reason for seeking a certain special mechanism for cancer induction in epithelia, however, not a mechanism itself; and anyway, if it were true, one would expect to find in organs such as the kidney where epithelial and nonepithelial cells are in intimate contact with each other and have a common environment that cancer would be rarer in the epithelial cells, whereas in fact it is more common.

[10] Transformation of any epithelial cells in a petri dish is so difficult that no one can do it reliably, so I don't know if human epithelial cells are much more resistant to *chemical* (nonviral) transformation in vitro than the epithelial cells of shorter-lived mammals. If somebody knows the answer to this, please will they write and tell me? If not, will someone please do the necessary simple experiments and tell me the answer? Epithelial cells from donor species with a range of different life-spans (shrew, mouse, rat, cat, dog, sheep, baboon, human, etc.) can be studied in four different circumstances: in vivo on the intact donor, in vivo in a full-thickness skin transplant onto a nude mouse, in vivo in an epithelium-only transplant onto a nude mouse, and in vitro, growing in a petri dish on top of a feeder layer of 3T3 cells. In each case (except for human cells on intact donors!) two questions could be studied experimentally. First, how easily can extrinsic mutagens cause unrepaired damage to the DNA in the nonkeratinizing keratinocytes? (If radiolabeled polyaromatic hydrocarbons are used as mutagens, the label still on the DNA in particular basal layer cells at various times after application of a single dose can be counted as an index of unrepaired damage [F. J. Burns, pers. comm.].) Second, how easily can a given amount of unrepaired DNA damage lead on to a full transformation into a carcinoma? There are probably species differences in both these respects. The species differences in penetration of the mutagen through the impermeable overlying keratinized cells, the species differences in enzymatic activation and inactivation, and the species differences in DNA repair (indicated by Hart and Setlow 1974) would be of interest, but I doubt whether they alone could account for the whole of the billionfold differences between mice and men. I *hope* that such experiments would indicate that for a given degree of unrepaired basal-cell DNA damage, there are vast differences in the probability of a carcinoma ensuing, these differences being correlated with the natural life-span. If such differences exist, it would also be of great interest to know whether they are manifested equally in epithelial cell cultures overlying 3T3 feeder cells (where the stem-cell hierarchy is totally disrupted), in epithelium-only transplants (where it is partially disrupted), in full-thickness transplants (where the stem-cell hierarchy remains intact but species-specific systemic factors are absent), and in the intact donor.

than mouse DNA, why don't we all die of multiple carcinomas at an early age? Presumably some concomitant of our evolved ability to grow big and to live for threescore years and ten is involved.

What has been hinted at in this section is that perhaps the probability of a "stage" affecting a particular stem cell is small unless that cell is undergoing the chain of events preceding mitosis. Two possible mechanisms by which this might occur are:

1. Mitosis itself may cause certain forms of accidental genetic damage which otherwise would be rather unlikely to occur in the normal environment of human epithelial stem cells. (In this case, the total probability of damage to a particular stem cell might be roughly proportional to the number of mitoses it has undergone since the fertilized ovum.)
2. Mutations caused by external insults might be much more likely to become fixed, and thus inherited by a daughter cell, if they occur just before mitosis, whereas if they had occurred long before mitosis, then repair systems would have had more chance of correcting them. (In this case, mitoses occurring in a "pure" environment are not very harmful, but mitoses occurring in a mutagenic environment interact with the mutagenic processes, each one enhancing the harmfulness of the other.)

In both mechanisms, the number of divisions undergone by stem cells (since the fertilized ovum or since the start of contamination by external insults) is the critical determinant of cancer risk. If the relevant number is written as $N$, these hypotheses will not yield Equation 4 ($I(t) \propto t^k$), in which the incidence rate is a power of age, but will yield instead

$$I(t) \propto N^k \tag{7}$$

(or, more precisely, $I(t)$ proportional to an average of such terms for many stem cells). Now if stem-cell mitoses in utero are somehow almost free of risk, perhaps because of the usual cleanliness of the uterine environment, and if stem-cell mitoses which do carry some risk accumulate steadily throughout life, then $N$ is proportional to $t$ and Equations 4 and 7 agree. However, if mitoses in utero *do* count, or if, due to some subtlety of the hierarchical organization of stem cells, $N$ is not proportional to $t$, Equation 7 may differ significantly from Equation 4 in ways that, until we have a better understanding of epithelial stem cells, we cannot usefully guess at. Fundamental research into the pattern of stem-cell behavior and its relation to natural life-span might turn out to be very enlightening if the findings carry over to *human epithelial* stem cells.

As has already been mentioned, from a certain quite reasonable point of view, human epithelia could be described as being a billion times more cancer-proof than mouse epithelia. Such vast orders of magnitude of differences among species are intriguing, and they strongly suggest that if we want to get relevant answers we must study humans as well as short-lived species.

The most direct way to elucidate induction mechanisms for human carcinomas is to study the characteristics of epidemiologically determined causes of human carcinomas, and, in particular, we must look at what happens when a known cause is applied at a different dose rate or for a different time

period. The human carcinoma most easily studied by this approach is, of course, lung cancer, which is caused by smoking, by asbestos, and by many other agents.

## The Effect of Giving Up Smoking

The incidence rate of cancer is the number of cells that have suffered all but the final stage times the event rate for the final stage (times the probability that a fully transformed cell will escape control). If, therefore, we suddenly change the event rate for the *final* stage, a sudden change in the inception rate of cancers will result, which will (after allowing for the time they take to grow visible) quickly affect the incidence rate.

This is not what is observed among regular smokers who give up smoking; the extra incidence rate stays approximately constant after smoking ceases.[11] This is still good for the individual—the large increases in the lung cancer incidence rate that would have occurred later had smoking continued are avoided.

If a simple multistage model is accepted, with smoking increasing the event rate of the *penultimate* stage, this constancy of extra annual incidence after smoking has been stopped is what would be expected, and in any model it suggests that smoking cannot possibly be acting on the final stage. If a model in which partially altered cells have a selective advantage is posited, then this selective advantage must cease when smoking ends; otherwise, the number of cells ready to undergo the last stage would increase and so would the extra lung cancer incidence rate attributable to past smoking. In other words, *if* proliferation of partially altered cells occurs and affects the kinetics of lung cancer induction, *then* encouragement of this proliferation must be the way, or one of the ways, in which smoking increases the risk of cancer.

Since the lung cancer incidence rate is changed within less than 5 years of giving up smoking, we may incidentally infer that the final uncontrollable growth of the cancer to diagnosis (and a few months later to death) takes only a few years or less, and that much of the talk about "latent periods" of some decades is inappropriate terminology. Up to a time only a few years before death, the cell that is eventually going to give rise to the tumor can still in some cases be prevented from doing so by withdrawing the insult.

[11] This is one of the strongest, and hence most useful, observational restrictions on the formulation of multistage models for lung cancer. It was suggested by Pike, who also analyzed the first 17 years of Doll's data on British doctors to demonstrate that these data were compatible with it (Doll 1971). I have recently analyzed the full 20 years of Doll's data, and I find that if we take Doll's data, plus the data of Dorn (Kahn 1966) and of Hammond (1966), the same pattern emerges. Hammond's (1966) impression that lung cancer incidence rates revert to nonsmoker incidence rates 10 years after stopping appears to be wrong, perhaps due to an artifact of chance. The truth seems to be that when smokers quit, their extra lung cancer incidence rate remains remarkably constant for at least 15 years thereafter, and probably for longer. When the Dorn and Hammond studies are updated to the mid-1970's, they should hopefully provide sufficient data to demonstrate this conclusion separately for each quinquennium of age at stopping, which has not yet been possible due to the smallness of the numbers of lung cancers so far observed among ex-smokers if the ex-smokers are too finely subdivided.

## The Effect of Delaying Starting to Smoke

This effect can be assessed when a large fraction of a whole population for which national mortality statistics are available start to smoke over a fairly short period of time. For example, consider British females. In 1953 and in 1973 the proportions of British women 55 to 59 years old who were cigarette smokers were fairly similar; but in 1953, British female smokers aged 55–59 had, on average, been smoking for only about 20 years, whereas in 1973 British female smokers aged 55–59 had, on average, been smoking for more like 35 years (Lee 1976). British female lung cancer death rates in the age range 55–59 were much higher in 1973 than in 1953, whether they were assessed by absolute annual death certification rates (0.2 and 0.5 per thousand)[12] or as the ratio of the female 55–59 certification rate to the male 55–59 certification rate (ratio 1/10 in 1951–1956 and 1/4 in 1973). (Examination of the sex ratio rather than the certified rate avoids hypothetical differences due to differences in diagnostic accuracy, since most male smokers aged 55–59 in 1953 or in 1973 had smoked throughout their adult lives.) This suggests that in the majority of people who smoke throughout adult life and die of lung cancer at age 55–59, one or more of the necessary cellular changes caused by smoking occurred more than 20 years before death.[13]

The data for ex-smokers have demonstrated that smoking increases either the rate constant for the penultimate stage or the selective advantage of partially altered cells. If the data for British females really do indicate that an earlier stage (perhaps even the first stage) is also affected by smoking, then there must be two, probably separate, roles for smoking, one early and one late.

## The Dose–Response Relationship for Smoking

In most multistage models, the age-specific incidence rate $I(t)$ is proportional to the product of the rate constants for each of the separate stages and the growth constant for any intermediate proliferative processes involved. If smoking affects one of the stages, then the simplest assumption would be that the rate of that stage would be proportional to some small background constant plus a multiple of daily cigarette consumption, i.e., approximately to

[12] Hammond (1966) suggests that the background rate among American nonsmokers aged 55–59 is 0.1 per thousand.

[13] It might be imagined that a much more direct approach to the question of the relevance of early exposure to risk at age 60 would be to take a group of people now aged 60, ask them when they started to smoke, and then compare the current incidence rates in those who started 45 years ago with the rates in those who started 35 years ago. Unfortunately, not only is recall of when regular smoking started unreliable, but also within one particular generation those who say they started to smoke at age 15 smoke throughout their lives in a manner very different from those who say they started at age 25. The earlier starters smoke more and inhale more. Differences in inhalation are particularly difficult to allow for: lung cancer usually arises in the upper bronchi, not in the peripheral airways, and a deep inhaler may actually get the carcinogen-bearing droplets past the main danger zone before they deposit. Perhaps for this reason, among *heavy* smokers, self-reported inhalers actually get *less* lung cancer than self-reported noninhalers (Doll and Peto 1976)! (Among light smokers, the pattern is opposite, and inhalers get more lung cancer than noninhalers.)

daily consumption. If two stages are affected by smoking, one might tentatively expect $I(t)$ to be proportional to the square of the daily dose rate. What is actually observed? Is the exponent of dose 1, or 2, or 3, or what? This exponent is of interest in that it will suggest (inconclusively) the number of smoking-affected stages or processes. One might imagine that epidemiology would readily yield the exponent of dose; simply see whether the incidence rate among smokers of 2 packs/day is 2×, 4×, or 8× that among smokers of 1 pack/day. Several different epidemiologic studies suggest that the incidence rate is 2×. However, before we infer that only one stage or process is caused by smoking, we must consider what biases might exist.

We want our measure of dose to be the extent of exposure to insult of the stem cells of the bronchial epithelium. In smokers, this epithelium is inflamed, deciliated, and covered in smoke-induced mucus, the mucus being more plentiful in heavier smokers. Does the excess bronchial mucus enhance carcinogenicity by helping to stop the carcinogen-bearing aerosol droplets before they are safely past the bronchi? Or does it protect the bronchi by diluting the carcinogens and clearing them when the mucus is cleared? Does a smoker of 40 cigarettes/day on the average get more or less out of each cigarette than a smoker of 20 cigarettes/day? Smokers of 40 cigarettes/day are more likely to be inhalers than are smokers of 20 cigarettes/day, and both zero inhalation and deep inhalation are apparently protective. Also, those who smoke a lot tend to have smoked for more decades than those who smoke less, and exact adjustment for this is not possible.

Thus there may well be biases, and there are certainly random errors in the estimation of the true daily dose rate—and if the insult is partly simple stimulation of unwanted mitoses in the stem cells of inflamed bronchi, then there is no reason to suppose that the true dose rate would be linearly related to carcinogenic effect. Most, but not all, of the biases would reduce the true dose rate per cigarette in the heavily exposed, thus tending to convert a quadratic dose–response relationship into a more linear one.[14] I would therefore tentatively infer from the observed linear dose–response relationship between daily cigarette consumption and lung cancer incidence rates that one *or two* rate-determining stages are strongly affected by smoking, and, in the light

[14] One particular bias with this effect deserves attention because it is purely statistical rather than biological, and hence is often overlooked. When statisticians fit the usual regression equation $y = ax + b$ (e.g., incidence = $a$•dose + $b$), they know that if there are measurement errors affecting $x$, then the estimated value of $a$ will be too low. For example, if $y = kx^2$, then a plot of log $y$ against log $x$ would have slope 2; but if log $t$ = log $x$ + error, then a plot of log $y$ against log $t$ will tend to have a slope lower than 2, and could even have slope 1 (suggesting, misleadingly, that $y$ is linearly related to $x$) if the errors in $t$ are of the same order of magnitude as $t$ itself. This would probably be the case if $x$ = true effective dose rate to stem cells and $t$ = cigarette consumption; so a quadratic truth may well underlie the apparently linear relationship between risk and stated cigarette consumption. (Any heterogeneity in susceptibility between different smokers will accentuate this bias.)

The crossover in risk between self-reported inhalers and self-reported noninhalers as daily consumption increases means that the apparent dose–response relationship would be more quadratic among self-reported noninhalers than among self-reported inhalers, the divergence from parallelism of the two relationships being perhaps a factor of 4 (Doll and Peto 1976). This illustrates that the hypothetical biases discussed really are of sufficient magnitude to distort the data substantially, since the true relationship of risk to stem-cell exposure must be the same for both groups.

of the previous section, two stages seems a more likely hypothesis than one stage.

## The Multiplicative Effect of Asbestos Exposure

If multistage models are correct and the different stages (or the stages and the proliferative processes affecting partially transformed cells) have different causes, then we should sometimes find that the risk of lung cancer is proportional to the product of two terms, one dependent on one cause and one on the other. As is well known, this is exactly what Selikoff and Hammond (1975) have found for smoking and asbestos exposure, and it indicates that these two agents act on completely different stages. Elucidation of the age distribution of the effect of asbestos on risk is necessary before we can surmise the order of the various stages, and although the data Selikoff currently holds and will soon make available can answer this question, the data Selikoff presents in this volume are not sufficient to do so.

## Mechanisms Other Than Mutation: Are Carcinogens Mutagens?

The foregoing indicates that an incomplete, but coherent, picture is emerging from the epidemiologic study of lung cancer. When this picture is complete, the general structure, although not the specific causal agents, may well carry over to 90% of malignant human cancer (the carcinomas). There is another major unanswered question about the mechanisms of cancer which epidemiology can help to answer and that concerns the role of mutagens in human cancer induction.

Burnet (1977) has speculated that carcinogenesis involves chiefly mutations in control regions of DNA, and Ames et al. (1973) have written "carcinogens are mutagens." When we come to examine exactly what Ames meant, this turns out to be a reasonable, though possibly misleading, statement. Ames was referring chiefly to agents which are sufficient on their own to cause cancer in laboratory animals, excluding from this group most of the "promoting," "modifying," and "cocarcinogenic" agents. It is then probably fair to conclude that "initiating agents are mutagens," and indeed for such agents there is a reasonably close correlation between mutagenicity, as assayed by the Ames test, and carcinogenicity, as assessed by studies in laboratory animals given no other deliberate treatment (Meselson and Russell, this volume; C. B. Sawyer and B. Ames, unpubl.). However, even in laboratory animals, there are a host of other factors which can grossly modify the carcinogenic effects of a particular initiating agent. These range from artificial ones, such as the "promotion" of mouse skin tumors by phorbol esters, to natural ones, such as dietary restriction (Roe and Tucker [1974] have shown that if a given total daily diet for mice is taken in one meal instead of a little at a time for 24 hr, then the spontaneous tumor rate is only one-eighth as great), or wound healing (Berenblum [1974] reports that in DMBA-pretreated rabbits or mice, skin wounding can cause papillomas), or pregnancy

(McMahon et al. [1973] report that the risk of breast cancer later in life in women who had their first baby at around age 18 is only half as great as among women who first did so at around age 30), and vitamin A (Grubbs et al. [1977] have shown that after a mutagen has acted, vitamin A can greatly reduce the likelihood of cancer). *If* these acted merely by modifying the mutagenicity of some initiator, then Ames would be fundamentally correct: carcinogens are either simple mutagens or processes or agents which (although not themselves mutagenic in Ames' system) act by modifying the actions of mutagens.

But it is far from clear that this is the case. Some of the "heritable cellular changes" needed in multistage models for carcinoma induction may not be anything like the sort of oligonucleotide mutations detected by the Ames *Salmonella* test, but may instead be some larger scale genetic or epigenetic cellular changes. Alternatively, protection from cancer induction might well be offered by the hierarchical relationships between stem cells and committed daughter cells, and disturbance of these relationships might be dangerous. Also, in a multistage process, partially altered stem cells may have mitotic kinetics which differ from normal cell kinetics and which are under external control—for example, chronic irritation by cigarette smoke *could* cause partially altered cells to suffer limited proliferation, resulting in a larger population of "targets" being available for the final cellular "stage" of carcinoma induction.

It is therefore reasonable to ask whether the spirit of Ames' paper is correct. Two related questions may be posed: (1) Are there rate-determining external causes of human cancer which are not mutagens that would show up in some short-term mutagenicity test or other (and which do not act by activating or transporting such mutagens)? And, the essential question: (2) Are the causes of human cancer that we can most easily identify and control agents which will show up in some short-term mutagenicity test or other?

These are important from the point of view of grant allocations. At present, in the United States, it is being suggested that considerable resources should go immediately into the short-term testing of tens of thousands of chemicals. This has the attraction of being something that could be done now, and extensive use of short-term mutagenicity tests on large numbers of chemicals is likely to prove worthwhile, because the short-term tests themselves are really quite cheap and some of the results will almost certainly prove relevant to human cancer, germ-line mutations, or some other human disease. McCann and Ames (1976) have listed half a dozen immediate uses of such tests. But, if environmental mutagens (or their metabolic precursors) are *not* the most easily preventable causes of most human cancer, the results of a cancer research program which is too firmly fixed to the idea that nearly all carcinogens are mutagens may be less effective than the results of a program in which laboratory work is more closely governed by epidemiologic considerations. Various epidemiologic observations are pertinent to these general questions. Some of them are discussed briefly in the remainder of this paper, and for a fuller (and excellently written) review of current epidemiologic findings about the causes and mechanisms of cancer, readers are strongly encouraged to consult Doll (1977).

## The Rarity of Liver Cancer

If there were lots of environmental mutagens, one would expect primary human liver cancer to be common; but, in fact, it is rather rare in developed countries.[15]

If multistage models are even approximately true, then the incidence rate for each cancer is proportional to a product of more than one event rate, each with different determinants, and it makes little sense to ask for *the* cause of a particular cancer; each has more than one distinct cause. It could be that there are low levels of "initiation" going on in all organs, all the initiating being caused by mutagenic environmental contaminants which could be identified by the Ames test and reduced, and that the variation in incidence between different organs is mediated chiefly by differences in "promotional" processes from organ to organ. However, if these putative mutagenic contaminants are agents which become proximal carcinogens when oxidatively activated by enzymes such as those of the liver microsomes, then these active species would presumably form in the liver, as well as elsewhere, and would act as initiators in the liver. The reports on the hepatomas caused by aflatoxin in Mozambique suggest that the human liver can be quite sensitive to such agents *if* they are present. It is not very plausible to suggest that liver cells are subject to far less promoting activity than other cells, for (even if no other promoters are prevalent) cirrhotic changes are quite common in developed countries, and these presumably would be quite efficient promoters.

Since there is so little primary liver cancer, I therefore conclude that liver stem cells are subject to very little initiating activity and that this suggests a limitation of what we can hope for from the widespread use of the Ames test and related assays. This inference is indirect and uncertain (especially since benzidine is a mutagen which causes bladder, not liver, cancer in humans) but it is somewhat supported by inspection of the table in Meselson and Russell (this volume), in which they list the principal animal cancers induced by agents which are positive in the Ames test and which have been adequately tested for carcinogenicity in animals. Half the sites listed are "liver," which contrasts markedly with the 0.4% of British malignant cancer certified as "primary liver."

## Various Epidemiologic Considerations

Few new leads as promising as the bacterial mutagenicity of hair dyes (Ames et al. 1975) are likely to emerge, yet an unpublished case-control study by L. J. Kinlen, using 200 breast cancer cases and 600 controls, suggests that no risk of breast cancer is associated with the regular use of hair dye for 10 or 20 years and clearly demonstrates that the risk is not doubled after these durations of exposure, although it is not yet known what really prolonged exposure would do. Kinlen is currently organizing a similar study on bladder cancer,

[15] Liver cancer is almost always fatal; moreover, there is some diagnostic confusion between primary liver cancer and metastases to the liver from other undiagnosed primary sites. Consequently, a proportion (much larger in the past than today) of deaths certified as being due to primary liver cancer may actually represent metastatic disease. Despite this, only 0.4% of British malignant cancer deaths are now certified as due to primary liver cancer, as opposed to 90% due to cancers of epithelia not in the liver.

and if this, leukemia, and liver cancer all prove negative, this will be very difficult to incorporate into a straightforward framework in which carcinogenicity is mutagenicity.

J. Cairns (pers. comm) has suggested that one observation which superficially supports the carcinogenicity-equals-mutagenicity story may ultimately tell against it. Smoking is thought to cause bladder cancer (heavy smokers have age-specific bladder cancer incidence rates which are nearly double those of nonsmokers), and recently, Yamasaki and Ames (1977) have discovered that smoking makes the urine mutagenic; their preliminary results suggest that smokers' urine may be even ten times as mutagenic as that of nonsmokers. If this is so, why don't smokers get ten times as much bladder cancer as nonsmokers (and, since these mutagenic substances are presumably present elsewhere too, ten times as much cancer of certain other sites not reached directly by the smoke)? Even when allowance has been made for smoking not starting until the age of about 20, if it is true that a large proportional increase in systemic mutagenicity produces only a small proportional increase in the total risk of nonrespiratory cancer, this suggests that most cases of cancer are not caused by systemic mutagenicity.

Some of the known correlates of human cancer—asbestos, cigarette smoke, and radiation—may act by virtue of their mutagenicity, but even for these this is far from certain.[16] However, for effects such as the promotional activity of wound healing (Berenblum 1974), the effect of parity on breast cancer (McMahon et al. 1973), or the effects of estrogens on endometrial cancer (Armstrong, this volume), a mutagenic mode of action looks distinctly unlikely. Similarly, it is difficult to see how the protective effects of dietary vitamin A and various retinyl derivatives (Grubbs et al. 1977) are connected with mutagenesis. We therefore need to see which of the epidemiologic correlates of cancer seem to act by mutagenicity. If the majority do not, then, although it remains likely that mutagens are carcinogens, we should abandon the statement that carcinogens are mutagens.

The epidemiologic fact that lung cancer incidence rates among regular smokers approximately freeze when smoking stops indicates, as has already been discussed, that smoking does not affect the final rate-determining stage of cancer induction. This last stage and a rate-determining stage affected by smoking cannot both involve electrophile-induced mutations (or smoking would cause both or neither), and at least one rate-determining stage in human lung cancer induction must therefore involve processes other than electrophile-induced mutation. Other aspects of multistage-model theory (particularly the multiplicative relationship between smoking, asbestos, and

[16] Even the effects of X irradiation on tumors other than leukemia may be chiefly promotional rather than initiating. R. Doll and P. G. Smith (pers. comm.) have found that 10 to 20 years after therapeutic X irradiation of the adult human spine (for relief of ankylosing spondylitis), the death rate from all neoplasms together was 60% greater than would have been expected in an age-matched sample of the general population, the excess being, of course, greater for heavily irradiated than for lightly irradiated anatomical sites. The same 60% excess was found in young adults, whose "spontaneous" death rates are small, and also in older adults, whose "spontaneous" death rates are much larger. Multiplication of the whole pattern of the spontaneous rates by a constant factor is not what I would expect if X irradiation acts chiefly as an initiating agent, and it is more suggestive of a promoting action or, in the language of multistage models, acceleration not of the first stage but rather of some later stage(s).

lung cancer risk) reinforce this conclusion. In general, it seems that different stages in carcinoma induction have qualitatively different causes and are therefore themselves qualitatively different from each other.[17]

Finally, despite the vast mutagenicity of ultraviolet (UV) light for all our skins, the large majority of British people never get pathological skin cancer. UV repair is certainly relevant, but, even after allowing for this, thousands or millions of mutations per person must remain, almost all of which do not lead to cancer. This again indicates that processes other than mutation are also needed to complete neoplastic transformation.

## Disparity between the Exponents of Dose and Time

It was noted earlier that the incidence rate of lung cancer among regular smokers is proportional to (observed daily dose)$\cdot$(duration of smoking)$^4$ but that this indicates that the incidence rate might really be proportional to (true effective daily dose)$^2\cdot$(duration)$^4$. Lee and O'Neill (1971) have shown that when benzpyrene is applied regularly to mouse skin, the resultant tumor incidence rate is proportional to (dose rate)$^2\cdot$(estimated duration)$^3$. However, because the mice studied were random-bred, and therefore genetically heterogeneous, and also because the time needed for a growing tumor to emerge is not known, the estimated duration is rather uncertain, and the exponent of true duration could well be greater than 3.

Finally, in an analysis of data from over 7000 mice treated regularly throughout life with various cigarette smoke fractions, Lee et al. (1977) report incidence rates of carcinomas to be proportional to (dose rate)$^{1.8}\cdot$(duration)$^{4.4}$.

The point I wish to emphasize in all these sets of data is that if we write (incidence rate) $\alpha$ (dose rate)$^a\cdot$(duration)$^b$, then $(b+1)/a$ is more like 2 or 3 than unity, a statement which is also supported by the data of Druckrey (1967) on carcinogenesis in rats induced by nitrosamines.[18]

[17] Multiplicative relationships can also be seen in vitro when DMBA and phorbol esters or DMBA and viruses (Mishra et al. 1977) are used to transform cells in culture.

[18] It is easy to demonstrate that if incidence rates are proportional to (dose rate)$^a\cdot$(duration)$^b$, then, in the absence of other causes of death, the individuals being studied will get cancer at various different times, these times being distributed randomly according to the rule $((b+1)/a)$ log (cancer time) = (constant) $-$ log (dose rate) + (random error), the random error having mean zero and distribution independent of the dose rate, but not a normal distribution. This formula predicts that the standard deviation of the random error will be independent of dose, a fact noted by Blum (1959) in relation to murine UV carcinogenesis. Druckrey (1967) noted that for nitrosamine-induced rat hepatomas, 2.3 log (median cancer time) = constant $-$ log (dose rate); so Druckrey's observations suggest that $(b+1)/a \cong 2$ or 3.

This value of 2 or 3, however, has been misleadingly inflated due to our inevitable ignorance of the growth times from the beginning of the first microscopic malignancies until microscopic lesions were detected. First, it has been inflated because whatever the average growth time, $w$, may be, any power of $(t-w)$ is approximately proportional to some still higher power of $t$ (Peto and Lee 1973). Second, it will also have been inflated if growth times tend to be briefer at higher doses. It is not clear whether the toxic effects of high doses will accelerate or retard tumor growth, but it is clear that growth times will be shortened if, at high doses, later hepatomas arise which overtake the first one, or if at high doses smaller hepatomas are often present which cause earlier palpability of the largest hepatoma. Emmelot and Scherer (1977) have tried to allow for this last bias in Druckrey's (1967) data, but their treatment is not satisfactory. (Also,

(*continued on facing page*)

Now $a$ is probably a reasonable estimate of the number of stages strongly affected by the carcinogen being studied, and this seems to be only 1 or 2 for cigarettes or for the laboratory carcinogens with which I am familiar. If there were only two rate-determining stages in carcinoma induction, it would be difficult, although not quite impossible (see earlier sections on multistage models), to understand why carcinoma incidence rates should rise so steeply with duration of exposure. The fact that $(b + 1)$ is about two or three times as large as $a$ therefore indicates that, in addition to the mutagen-induced stages, there are some rate-determining stages in the in vivo transformation of epithelial cells which are not affected by these applied carcinogens. Whether the applied carcinogens act by their mutagenicity or not, it follows that there must be some rate-determining processes that are not caused by mutagens (see also the discussion by Armitage following Doll [1971]). The arguments in this section are slightly mathematical and for this reason are not nearly as widely appreciated as they deserve to be. The fact that the exponent of dose rate is so much lower than the exponent of time is one of the most important observations about the induction of carcinomas, and everyone should be familiar with it—and slightly puzzled by it!

## Identification of Nonmutagenic Carcinogens

If nonmutagens typically act synergistically with mutagens in the production of carcinomas, then laboratory studies should try to identify both types of agents and should try to discover the mechanism whereby agents such as phorbol ester and saccharin exert their promotional effects. Saccharin is an agent which only just achieves any measurable carcinogenic effect when fed to rats that receive no other treatment but which has a gross carcinogenic effect when rats pretreated with the mutagen methylnitrosourea are exposed to it (Hicks et al. 1973). It is perhaps unfortunate that animal feeding experiments routinely use an untreated control group but do not also routinely feed the test substance to a group of animals that have been exposed to a moderate dose of some well-known mutagen. If this had been standard practice for the past few years, we would perhaps by now know of as many nonmutagenic cocarcinogens as we do mutagenic carcinogens, and we might be some way towards classifying them[19] and developing various short-term tests of cocarcinogenic activity.

(*Footnote 18 continued*)
their mechanistic incidences from Druckrey's uniquely high time exponent of about 12 are perhaps naive, in view both of the biases I have discussed earlier and of the lack of other experimental systems yielding such values.

[19] The agents and processes thus uncovered might be quite surprising and quite heterogeneous. For example, we know that before tumors can grow efficiently, they must secrete a factor which causes normal blood vessels to grow toward them and to vascularize them (Folkman 1976): Does the development of this angiogenesis factor have any externally varying determinants? It appears that vitamin A and its chemical analogs can exert a considerable inhibitory effect on the process of cancer induction (Grubbs et al. 1977). What is the basis of this inhibition and what other classes of inhibitors exist?

Griseofulvin causes errors in chromosome segregation at mitosis and could make a diploid cell which had suffered a recessive mutation on one of its chromosomes yield a daughter cell with that mutation on both chromosomes. If this is the basis of its cocarcinogenic action (Barich et al. 1962), this will have no bacterial analog. More generally

(*continued on following page*)

## Conclusions about Short-term Tests

Short-term mutagenicity tests probably represent a real breakthrough; they are practicable, where animal testing never was, and are many orders of magnitude more sensitive to the effects which they monitor than animal tests could ever be. They could be really valuable, not only for preventing some cases of cancer, but also for preventing germ-line mutations (and maybe even atherosclerotic plaques!). Short-term tests are not yet sufficiently widely used, and I suspect that they will lead to discoveries which would otherwise have been delayed for many years.

I have used various arguments against the simplistic carcinogenesis-is-mutagenesis viewpoint (expressed, for example, in Boyland 1977) which sometimes goes with enthusiasm for mutagenicity tests, not against these tests themselves. The observation by Bruce et al. (this volume) of the mutagenicity of some human feces is an obvious example of the promise of such tests. However, animal tests may also detect carcinogens that are not mutagenic, such as saccharin, and rather than arranging tests in some kind of hierarchy of supposedly increasing relevance to man (Bridges 1976), we should accept that different tests may be independently valid.

## Difficulties with Multistage Models

Even if interest is restricted to the carcinomas, where multistage models hold the most promise of being a useful framework for describing the process of neoplastic transformation, there are various observations which do not appear to fit naturally into the multistage formulation. These difficulties have for the most part been glossed over in this paper and some attention should be drawn to them:

1. If two genetically distinct mouse embryos are removed from their mothers early in fetal life and then mixed together and reimplanted in one mother,

(*Footnote 19 continued*)
(and very speculatively!), is transformation of diploid epithelial cells in vivo usually rate-limited by the process of conversion of a recessive mutant to a homozygous mutant and, if so, how is this usually effected—by mitotic recombination, by nondisjunction, or by a second independent mutation on the homologous chromosome? The first two of these yield as a possible cancer precursor a cell with a substantial region of exact identity on two homologous chromosomes. (If, in this region, there were a "recessive lethal" genetic variant, then such a precursor cell would not be viable. This might actually confer an evolutionary advantage on organisms with a number of different recessive lethals scattered about their genomes!)

It might be possible in principle to discover whether a population of cancer cells had arisen from a precursor cell containing a region of exact identity on two homologous chromosomes if only the individual was heterozygous for electrophoretic variants of certain proteins coded for by this region, for homogenates of normal cells would then contain both variants, whereas homogenates of the tumor would, if uncontaminated, contain only one variant of each such protein. Unfortunately, it would be enormously difficult to demonstrate this phenomenon experimentally. Most people are heterozygous for very few known electrophoretic variants, and for most of these proteins one would not expect the one-variant phenomenon to be caused by the existence of a region of identity (because a particular protein is not a priori likely to lie in a particular region of identity on a particular chromosome), whereas the one-variant phenomenon could easily be caused by the haphazard loss of, or damage to, various chromosomes, which occurs in carcinomas and, probably, in the cells from which they arise (Spriggs 1974).

a single "allophenic" mouse composed of an intimate mixture of genetically distinct cells may be born. Most tumors reported in allophenic mice are exclusively of one genotype or another, but Condamine et al. (1971), in a study which has unfortunately never been repeated, have described two allophenic mice which developed mosaic hepatomas composed of some cells of one genotype and some of the other. It is possible that a monoclonal tumor could pick up some normal cells and, by putting them in the strange environment of a proliferating tumor, make the normal cells behave abnormally; but this is speculation designed to relieve multistage models of the embarrassment of these mouse tumors in allophenic mice, rather than established fact.

2. Prehn (1975) took a group of inbred mice and implanted four carcinogen-impregnated pellets into each mouse in four standard, well-separated positions. After a time, local tumors began to develop. Whenever a mouse developed its first tumor, an animal that had not yet done so was selected as a control for it. Then the tumor and the pellet which had caused it were excised, and so was the corresponding pellet in the control. The animal that had had the tumor then usually developed a second tumor at one of its remaining three pellets before its control developed a tumor at its remaining pellets. Why? What systemic differences existed between these inbred mice, and how can they be accommodated in multistage models?
3. Likewise, in an experiment (Peto et al. 1975) in which benzpyrene was applied twice weekly to the shaved backs of mice, it was found that among animals which already had one or more tumors, further tumors appeared with an incidence rate approximately double that of first tumors among similarly treated mice that had not yet developed any tumors. Why? It is possible that genetic heterogeneity could account for this, although the animals had been random-bred in a closed colony for many generations. (Some skin-painting experiments on *inbred* mice have been done over the past 10 or 20 years, and, if records still exist, it would be interesting to know what they show in this respect.)

   If, as may well be the case, carcinomas do not arise independently on one animal, multistage-model formulation will be seriously incomplete until the basis for this tendency toward multiplicity is understood. Study of X-linked mosaicism, either in rabbits with multiple tumors or in black female smokers with several "carcinomas in situ" in their lungs, could be done to indicate whether multiple tumors tend to derive from closely related cells. Studies in which one side of an animal is implanted with a carcinogen-bearing pellet for a few months could show whether this makes the other side of the animal more cancer-prone when it is later implanted. If neither of these explanations proves to be true, multistage models may have to coexist uneasily with Prehn's (1975) hypothesis of systemic predisposing factors which differ substantially and at random in genetically identical mice.
4. Pathologists claim that many human breast tumors are of mixed cellularity —part carcinoma, part sarcoma. Are they right? And, if so, why are these tumors mosaic? Is it just that some connective tissue has been caught up in a growing carcinoma and induced to proliferate with it? (If so, why doesn't this always happen?) Pathologists also sometimes claim that human bladder cancer is polyfocal and that these separate foci cannot be derived

from one tumor which has spread around. In both these cases, G6PD typing of the lesions concerned in X-linked heterozygotes would test the correctness of the pathologists' opinions and, if the pathologists are correct, would be of interest.

## SUMMARY

If a synthesis of several different lines of evidence relating to the mechanisms of cancer is to emerge, then, at least for carcinomas, it seems that such a synthesis will only be achieved in the framework of one or another of the possible multistage models. All of these models indicate that the final incidence rate of cancer is the arithmetic product of more than one term, the different terms each being dependent on different causative agents or processes. If this is so, it may be misleading to ask what *the* cause of a certain type of cancer is, as if there was one fundamental cause and all else was ancillary. For example, if environmental mutagens can be identified and manipulated to halve the net mutation rate in a certain tissue, then the cancer rate could be correspondingly reduced, but an equivalent improvement might be equally achievable by manipulating the environmental determinants of some other, qualitatively different, necessary cause in the sequence of changes which culminates in malignancy. It is still an open question, to be answered separately for each different type of cancer, as to which class of causes can most easily be identified and reduced, even if, thanks to recent improvements in methods for determining mutagenicity, the most rapid progress in determining causes over the next few years results from the study of mutagens.

In reviewing multistage models, the epidemiology of lung cancer, and the suggestion that almost all carcinogens are mutagens, it is concluded that:

1. Multistage models do not necessarily require such a large number (about six) of distinct rate-limiting cellular changes as is often inferred to take place during the transformation of an epithelial cell.
2. While the mechanisms of induction of most carcinomas may all be rather similar, they probably differ fundamentally from the mechanisms of induction of leukemias, sarcomas, etc., and much of the research into leukemia viruses or sarcoma viruses may be irrelevant to that 90% of human tumors that are carcinomas.
3. Some of the extrinsic rate-determining causes of human lung cancer do not act, either directly or indirectly, by being, transporting, or activating the sort of agent detected by in vitro mutagenicity tests, and this is probably true for other carcinomas as well.

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I have been helped in the preparation of this paper by discussion with and ideas from Bruce Armstrong, Richard Doll, David Oppenheimer, and, most of all, John Cairns. Gale Mead typed the manuscript until midnight. I am grateful to Leo Kinlen, Charles Sawyer, Richard Doll, and Peter Smith for permission to quote unpublished results, and to Sarah Parish for correcting

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# Origins of Human Cancer

## BOOK C
## Human Risk Assessment

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## Peto, R (1977). Epidemiology, Multistage Models, and Short-term Mutagenicity Tests

Pp 1403-28 in Hiatt HH, Watson JD, Winsten JA (eds), *Origins of Human Cancer:* New York, Cold Spring Harbor Laboratory, 1977. (These changes to the proofs were requested, but arrived too late: although I don't feel the paper as it stands is actually wrong, it would be better for anybody who wishes to read it carefully to do so in conjunction with the following list of minor textural emendations. R Peto, 1977)

*P1404, paragraph 3*

The essential multistage hypothesis is that a few distinct changes (each heritable when cells carrying them divide) are necessary to alter a normal cell into a malignant cell, and that human cancer usually arises from the proliferation of a clone derived from a single unlucky cell out of the many at risk in a tissue that suffered all the necessary changes and then started to proliferate malignantly. Although the proliferation of such a clone might in principle induce gross proliferation of other classes of cells, thus yielding a mosaic tumour, G6PD studies (Fialkow 1976) show that tumours usually consist predominantly of cells derived just from a single such clone.

*P1408, footnote 5, last sentence*

The ability of epithelial cells to divide is strongly influenced by contact with the basement membrane, and by the nonepithelial cells below it.

*P1409, footnote 7a, last sentence*

Heterogeneity of susceptibility will only cause appreciable deviations, however, if *most* of the highly susceptible individuals develop the relevant cancer before they die.

*P1410, footnote g replacement for the whole footnote*

g. Cellular event rates not all small -- Hakama (1971) and Moolgavkar (1977)note that even for the simplest multistage model an exact log/log relationship with slope *n*-1 between incidence and age only follows if all *n* event rates are small in comparison with the inverse of the lifespan, but that if not then incidence rates "must gradually fall below this approximation as age increases" (Armitage and Doll 1961). This shortfall will be even greater if the stages are less strictly ordered than Armitage and Doll (1961) supposed. (Call the product of the age times the average of the *n* event rates per cell *e.* Ignoring higher order terms, log incidence will fall below its predicted straight line relationship with log age by some multiple of *e.* This multiple is (*n*+1)/2 if no restrictions on the order of stages exist, and Moolgavkar (pers. comm.) has shown that it is unity if the stages all have to occur in a certain fixed sequence. Such a progressive shortfall may still yield a fairly straight line, but with a slope somewhat shallower than *n*-1.)

*P1410, paragraph 1, last sentence*

For example, carcinoma of the uterine cervix, which is related to some aspect of sexual intercourse, depends on age rather as carcinoma of the lung would if people smoked only from early adult life to middle age and then gave up smoking (see the section below on effects of giving up smoking).

*P1412, last sentence*

This suggestion is supported by the fact that whereas some of the rare human tumors show little percentage geographic variation, all tumors presently common in one country are rare in some other country (Doll 1977).

*P1413, footnote 10, halfway into paragraph*

The species differences in penetration of the mutagen through the impermeable overlying keratinized cells, the species differences in enzymatic activation (Schwartz·1975) and the species differences in DNA repair (indicated by Hart and Setlow 1974) would be of interest, but I doubt whether they alone could account for the whole of the billion-fold differences between mice and men.

*P1413, footnote 10, last sentence*

If such differences exist, it would also be of great interest to know whether they are manifested equally in epithelial cell cultures overlying 3T3 feeder cells (where the stem-cell hierarchy may be disrupted totally [Sun and Green 1976] or partially [Steele et al 1977]), in epithelium-only transplants (where it is partially disrupted), in full-thickness transplants (where the stem-cell hierarchy remains intact but species-specific systemic factors are absent), and in the intact donor.

*P1415, paragraph 3, beginning*

If simple multistage model is accepted, with smoking increasing the event rate of the *penultimate* stage, this constancy of extra annual incidence after smoking has been stopped is what would be expected, and in any model it suggests (unless long latency of pre-existing carcinomas distorts ex-smokers' risks) that smoking cannot possibly be acting on the final stage.

*P1415, footnote 11, beginning*

This, if confirmed, will be one of the most useful observational restrictions on the formulation of multistage models for lung cancer.

*P1417, line 8*

Several different epidemiologic studies suggest that the incidence rate is 2*x* or 3*x*. (3*x* comes from my unpublished analysis of the "cleanest" subgroup of smokers in the most accurate epidemiological data available -- cigarettes only,

up to 40/day, constant habits to within 5/day, age 40-79, started age 16-25, incidence data 1951-71 for British doctors.) However, before we infer that only one stage or process is caused by smoking, we must consider what biases might exist.

*P1417, footnote 14, first paragraph, last sentence*
This would probably be the case if *x*=true effective dose rate to stem cells and *t*=cigarette consumption; so a quadratic truth may well underlie the apparently linear or 1.5 power relationship between risk and stated cigarette consumption.

*P1420, last paragraph, first sentence*
Hair dye study now published (Kinlen et al, 1977).

*P1422, lines 1-3*
Asbestos review now published (Saracci, 1977).

*P1422, line 6*
UV repair is certainly relevant, but, even after allowing for this, thousands or millions of mutations per person presumably remain, almost all of which do not lead to cancer. This again suggests that processes other than mutation are also needed to complete neoplastic transformation.

*P1422, paragraph 2*
It was noted earlier that the death rate from lung cancer is usually reported to be proportional to (observed daily dose) • (duration of smoking)$^4$ but that despite this the actual incidence rate might really be proportional to (true effective daily dose)$^2$ • (duration)$^4$

*P1423, follows second paragraph*
Substantial progress is already taking place in the understanding of what the rather peculiar class of phorbol esters known as "promoters" actually do (see, for example, Weinstein and Wigler 1977), although it is not yet clear whether or not the normal mechanism of induction of human carcinomas involves any processes analogous to the peculiar effects of the phorbol esters on mouse skin.

*P1425, No. 3, middle of paragraph*
It is possible that genetic heterogeneity could account for this, although the animals had been random-bred in a closed colony for many generations, and hundred-fold differences in susceptibility would be needed to explain away the effects we observed, which is quite extreme. However, such differences may really exist, for Boutwell(l964)has shown that it is simple to breed out, in just five or ten generations from random-bred mice, sublines which differ by more than an order of magnitude in their susceptibility to skin carcinogenesis. (Some skin-painting experiments on *inbred* mice have been done over the past 10 or 20 years, and, if records still exist, it would be interesting to know what they show in this respect.)

If, as may possibly be the case, carcinomas do not arise independently on one animal ...

*P1426, add after first 4 lines*
5. Ease of transformation in vitro. Cells which grow in vitro often have to adapt themselves considerably in order to allow them to survive in culture, and so they may be quite poor models for cells in the normal environment of the mammalian body. In particular, the one-hit kinetics of transformation in vitro reported by Huberman et al (1976) may obviously be a poor model for the kinetics of transformation in vivo, since the adaptations which allow growth in vitro may constitute partial transformation. Despite this, it is surprising that Huberman et al (1976) and others have found that, when mutagens are applied to cells in vitro, it is actually an order of magnitude easier to cause "neoplastic" transformation than to cause gene mutation. This was a puzzling observation, until Holliday's unpublished suggestion that the correct repair of mutagen-induced lesions may reset epigenetic switches, possibly producing a transformed phenotype.

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