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Critique of the Linear Theory of Carcinogenesis

Present data on human leukemogenesis by radiation indicate that a nonlinear relation is more probable.

Austin M. Brues

A question which has interested investigators of cancer for many years is whether various carcinogenic agents are active even in very small amounts, or whether there are amounts or concentrations of such an agent below which no effect is produced, or below which the effectiveness drops off out of proportion to the reduction of dosage. The existence of a threshold, or at least of a marked nonlinearity of effect at low dosages, is rather taken for granted in most areas of toxicology, where the degree of general physiological impairment seems to determine whether or not an end point such as death or a persistent pathologic change is produced, and where it is reasonable to assume that essentially complete repair can occur if the insult is removed or if its intensity is low.

It has been thought that a different situation might exist where cancer is the end point. The main reason for this lies in the special nature of malignant disease, which in its natural course leads inevitably to death, yet which ordinarily arises in a small anatomical focus, perhaps in a single cell. On this basis, then, it may be conceived that cancer could arise through a single randomly determined alteration in a cell of the host, which conferred on it the capacity to reproduce itself indefinitely. By analogy to the point mutation in genetics, we can see that a theory postulating a linear relation between the amount of a carcinogenic agent and the probability of a malignant response is allowable even where the respective amounts and probabilities are vanishingly low. The purpose of this article (*1*) is to examine whether such a relation has been verified (as some seem to assume) or indeed whether it seems reasonable in the light of present information.

While a generalized hypothesis of linearity of the carcinogenic response can apply equally to many types of malignant disease and to many agents, perhaps the strongest argument in favor of this hypothesis has been presented by Lewis (*2*) on the basis of several published studies of leukemia induction in man by ionizing radiation. The ensuing discussion will deal mainly with this special case, but it will be clear that many of the considerations presented apply equally well to the more general case of cancer induction by a variety of agents.

There is no question as to whether leukemia may be a result of total-body irradiation, or of irradiation of large masses of blood-forming tissue, at high dosage levels—that is, at levels at or above 10 or 20 percent of the acute lethal dose, where widespread cellular destruction occurs in both lymphoid and myeloid tissues. This has been well established in work with animals and is obvious as well from the human data to be discussed here.

The author is director of the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois.

The Human Evidence

The data on leukemia incidence in the irradiated populations of Hiroshima and Nagasaki (*3*) suffer from the fact that they have been calculated on the basis of concentric circles at 500-meter intervals from the hypocenters. Within each sector defined in this way, the range of radiation dosages is very large. For the area up to 1500 meters from the hypocenter (that is, the area where dosage to unshielded or slightly shielded persons is currently estimated as 125 rad or above), the evidence for induction of leukemia is plain, and it is clear that the incidence increases the closer a person was to the hypocenter.

In the critical area, from the point of view of this argument—that from 1500 to 2000 meters from the hypocenter—the leukemia incidence has appeared likewise to be increased, although the increment was, in absolute numbers, not more than four or five cases. Such preliminary data as were available until recently (*3*) indicated, in fact, that the increment is limited to the nearest quartile of this area—that between 1500 and 1625 meters—and that those cases seen from the area beyond 1625 meters occurred with a high probability in persons who had acute radiation symptoms (*4*). This would suggest that these individuals received substantially more than the estimated dosage, or else that other factors leading to acute aplastic anemia were of importance in the subsequent development of leukemia. It may be concluded that no increased incidence of leukemia following a smaller dose than 100 rad has been demonstrated. Publication of more recent figures (*5*) shows that the incidence of leukemia within the 1500- to 2000-meter sector equals that in the control sector within one standard devi-

ation and further emphasizes the uncertainties of present dosimetry. The present data are at least as compatible with a physiological mechanism linking leukemogenesis with initial severe hematopoietic damage, or with some other nonlinear relation, as they are with one assuming an effect that is strictly linear with dose.

The best documented evidence of increase in leukemia incidence with increase in radiation dosage is that detailed by Court-Brown and Doll (*6*). These investigators made a painstaking study of patients irradiated over the spine and elsewhere to alleviate the symptoms of ankylosing spondylitis, and the individual doses have been carefully calculated. It is noted that the calculated mean spinal marrow doses show a *curvilinear* relation to leukemia incidence. Something resembling a *linear* relation can be deduced only by discarding those cases in which extraspinal irradiation was also given. The effect of this procedure is to eliminate almost all of the cases receiving a high dosage—those which most clearly demonstrate the nonlinearity of the function. It is indicated that these cases were omitted because high doses elsewhere may have involved other marrow areas; however, since the calculated integral body doses also show a curvilinear relation to leukemia, the reason for this treatment of the data is not at all apparent. Indeed, the authors state that they cannot rule out a threshold but suggest that a linear relation without threshold constitutes a good "working hypothesis."

While the actual data indicate that this working hypothesis is a less probable one than one that assumes a threshold or a nonlinear relation, it is illuminating to make a careful study of the clinical protocols. First, of the 32 cases accepted as leukemia after careful study of pathologic material, only 22 were so certified at death, and another five cases were classed as suspicious but lacking a final positive diagnosis. Many more cases of aplastic anemia were observed within the same range of dosages. Those who have had experience with clinical leukemias will be impressed by the high proportion of equivocal cases. If one is willing to forego the statistical requirement of assigning each case to a single disease category, it is obvious that many of the cases illustrate the unfolding of a sequence of pathologic changes in which a persistent disordered or aplastic state of the marrow is a precursor rather than a consequence of leukemia. This is likewise true of other agents similarly affecting the bone marrow, notably benzol (*7*).

All of the cases of leukemia arising in this series followed doses exceeding 450 r to the spinal marrow, with one exception. In the exceptional case, the leukemia was of the lymphatic type and the patient had been more heavily irradiated in extraspinal areas.

In other series of cases where leukemia has resulted from irradiation, there is no information bearing on the question of linearity. Children receiving treatment to the thymic area have shown an incidence of about 0.5 percent (*8*). This is true in both dosage groups—above and below 200 r. There has been no further breakdown of the dosages, but it may be presumed that all irradiations were severely damaging to thymic tissue, since the purpose of treatment was to produce involution of the presumably enlarged thymus. Cancer of the thyroid is also frequently preceded by thymic irradiation above 200 r (*9*), but it is possible that complicated endocrine interrelationships may be operative in this instance.

It has been noted for some time that American radiologists are more prone to leukemia than other physicians (*10*). The average dose sustained by this group has recently been estimated as about 2000 r delivered over many years (*11*), but no particular distribution of individual dosages can be assumed, and it is certainly not justifiable to use any estimated average dose in an argument concerning linearity unless the distribution can be established. Since the average accumulated dose to radiologists seems to have been far in excess of the single midlethal dose for man, and since the increment in leukemias is much less than that observed in the most heavily exposed Hiroshima survivors, it would seem that a gradually accumulated dose is much less effective than the same dose received at one time. This is in accord with experimental data, and its significance will be discussed later.

The most impressive survey indicating that leukemia may follow rather low single doses of x-ray is that of Stewart *et al.* (*12*). In a partially complete survey of childhood leukemias in England and Wales over a 3-year period, it appears that abdominal radiography during gestation (generally radiographic pelvimetry) about doubles the probability of development of leukemia, and also of various forms of cancer, in the first 10 years of postnatal life. While the fetal radiation doses would be assumed to be small, they are quite variable even in the instances so far reported (*13*), and in a survey of an entire country, they almost certainly cover an even wider range of techniques and dosages, perhaps including a certain number of fluoroscopies. Pending the accumulation of further data (for example, regarding the variables of sex, order of birth, and domicile), it can only be said that these data reaffirm that radiation is mildly leukemogenic, but that they add little to the linear hypothesis. Certain criticisms of the study have been brought forward (*14*), particularly to the effect that radiographic procedures may be based on particular medical indications which may bear some causal relation to leukemia in children. Another recent study (*15*), while not taking into account radiographic pelvimetries, demonstrated that allergic states in the mother (including the use of antihistaminic drugs) predisposed to the development of childhood leukemias.

There has been a formidable increase in leukemia incidence in the United States population in the past few decades; it more than doubled between 1925 and 1940 in all classes (white and nonwhite males and females) (*16*) and continues to increase. The increment certainly is relatively greater than that in the average population exposure to radiation in the same period. A part of this increase is no doubt due to improved diagnosis, since, as was mentioned above, a diagnosis of leukemia is not always simple or obvious. While this steady increase has been loosely attributed to an increase in human irradiation (*17*), there seems to be no doubt that many other potentially leukemogenic agents have likewise had increasing impact on man, none of which (except perhaps benzol) has been seriously considered from the standpoint of possible leukemogenic action. The suggestion has been made (*18*) that, as a result of reduced mortality from infections, persons sustaining damage to the bone marrow are increasingly likely to survive to develop leukemia.

It would appear from the foregoing discussion that, in the only series of data where linearity from zero might be demonstrated (*6*), it has indeed not been shown. It is also apparent that a critical analysis of the data fails to establish any human leukemogenic response below about 100 r, although, on further analysis in detail, the data of Stewart (*12*) might conceivably establish such a response in the special case of the fetus receiving a single, nearly instantaneous dose. Various alternative hypotheses can be constructed which conform to the existing

information as well as, or better than, this one.

There is relatively little reliable clinical information concerning other forms of malignant disease in the same context. The American radiologists, who have shown a considerable propensity to leukemia, have not shown any increased tendency to tumors of bone, although at the voltages used in diagnostic radiography, the bone-forming cells may be expected to receive several times as much ionization as do the soft tissues. Studies of juvenile cancer of the thyroid have shown that it usually occurs in persons who have had previous irradiation of the thymus, which, as mentioned above, entails a dose of about 200 r. In Stewart's studies relating childhood leukemia to diagnostic procedures on the gravid mother, a similar relation was found with other forms of malignant disease occurring before the age of ten.

Experimental Studies

In spite of the extensive work that has been carried out in experimental carcinogenesis, there is remarkably little which suggests the possibility of a linear dose-response relation—the most obvious one to look for—while there are many instances in which the response is clearly not linear. One recent review of the subject, referring to radiation carcinogenesis, states that "none of the animal experiments have indicated a linear relationship between tumour incidence and dose" (*19*).

Among the difficulties encountered in such studies are: that there is a natural "spontaneous" incidence of all or most tumor types; that there is a "latent period" which may be a significant fraction of the life span, and which appears to increase as the dose of the carcinogen is lowered; that induced tumors may continue to develop throughout life; that the spontaneous incidence usually increases with age; and that verification of a single tumor ordinarily requires careful microscopic study of well-preserved material (this is particularly true of the leukemias).

One clear instance of a nonlinear response is that of lymphoid tumors in mice induced by total-body x-ray (*20*). Here the latent period is short (about 100 days) and the response runs its course well within the life span of the species; also, the natural incidence as a function of age is well established. Under these circumstances it has been shown that the number of additional tumors increases more than tenfold as the x-ray dose is increased by a factor of three.

Further evidence of the nonlinearity of this form of leukemogenesis is seen in a study of mice irradiated by doses ranging as low as 16 rem (*21*). While the data are shown only in graph form, it is clear that incidence of thymic lymphomas rises very steeply at the higher doses and cannot be extrapolated linearly to zero. Myeloid leukemia is relatively rare, and the data (involving, apparently, less than a dozen cases at the lower and control levels) seem to allow the possibility of a linear no-threshold function but not to prove it (*22*).

There are no studies of the dose-response relation in chemical carcinogenesis that have been extensive enough to settle the point under discussion. Careful scrutiny of the data of Bryan and Shimkin (*23*) suggests a threshold for one carcinogenic hydrocarbon but not for another, but in neither case were enough animals exposed at lower doses to establish the true nature of the function. Graffi (*24*) has presented data showing a marked threshold when dimethyl-1,2-benzanthracene is painted on mouse skin, while if croton oil is also administered, a linear relation appears at daily doses of between 10 and 100 micrograms of the carcinogen.

Induction of bone tumors by divided doses of strontium-89 was demonstrated some years ago to be markedly dose-dependent (*25*). In this instance the rate of tumor development was found to be proportional to dose and to the time after the end of a latent period. I interpreted these data to indicate that each increment of radiation confers an equal probability of tumor development that is indefinite in time, *beginning after* a latent period which becomes longer as the dose rate is reduced. In a sense, this is a linear response, but in the context of the mutation theory the latent period becomes meaningless, since a simple somatic mutation theory implies equal responsiveness of any single cell receiving a certain point mutation (necessarily basic to a linear response at low doses). If one calculates numbers of tumors at any given time after the onset of irradiation, a markedly nonlinear function is seen.

Perhaps the only recent experiment in which a linear relation is more than remotely possible is that of Bond *et al.* in which mammary tumors (benign and malignant) were induced in Sprague-Dawley rats by doses of total-body x-ray of between 25 and 400 r during the first year of life (*26*). Since these tumors are very common in females of this strain at a year or more of age, and since the numbers produced in this experiment are equivocally small, linearity cannot be proved even within those limits, and the possibility of an acceleration of a normal process has not been ruled out.

Ultraviolet light is consistently effective in the production of skin cancer in man and other animals. Blum (*27*) has analyzed the results of several time- and dose-patterns of irradiation and has failed to demonstrate any responses that fit with a linear hypothesis. The response is markedly time-dependent and requires continued exposure, and its nature indicates rather conclusively that a sequence of radiation-induced changes must take place before a tumor will appear.

For a single-event theory to hold water implies that the response must be independent of dose rate. Lack of dose-rate dependence has been one of the crucial proofs of the linearity of the genetic point mutation. No cases of strict equality of the carcinogenic response at different dose rates have apparently been reported. As a rule, the response drops off with lengthening of the total time of exposure. This is seen clearly in rats exposed to external beta irradiation in single or daily doses (*28*). In the case of lymphoid tumors in mice, the induction rate appears to pass through a maximum when irradiation is distributed over a period of a few days, falling off on either side of this optimal rate (*20*).

There are many examples of the induction of malignant disease through mechanisms which are clearly indirect—that is, where irradiation of a cell can be shown not to be the critical factor. It has been found, for example, that irradiation of the mouse ovary results in ovarian cancer only when all of the ovarian tissue has been irradiated, so that pituitary gonadotrophins are evoked and stimulate this tissue to hyperplasia and, eventually, to abnormal growth (*29*).

A striking example of the indirect mechanism is seen in induction of lymphoma in mice, which, as mentioned above, is clearly not a linear response. Mice in which lymphoma is readily induced by total-body irradiation can be almost wholly protected by shielding part of the body (*30*), or by irradiating the anterior and posterior halves of the body a few days apart (*31*). Since lymphoma can also be prevented by the administration of bone marrow following total-body irradiation (*32*), it seems likely that a prolonged depression of the

whole blood-forming system is more critically necessary to its development than irradiation of the cells. It has, in fact, been noted that while lymphoma can be prevented by thymectomy in certain strains, it may develop in an unirradiated thymic transplant in an irradiated mouse (*33*).

In addition to these and other evidences of indirect physiological mechanisms intervening between tissue irradiation and malignant change, there is a large body of evidence indicating that the malignant transformation occurs after a sequence of "precancerous" stages has taken place. The most widely observed example is in the development of skin cancer, which, in whatever way it is produced, is likely to be preceded by various types of benign atrophic or hyperplastic states; in experimental studies it most often develops in a benign papilloma. It has long been known that in rabbits treated with tar, a large number of papillomas is produced but only a very occasional one proceeds to malignant change. Glücksmann (*34*), studying precancerous tissue changes, has observed that, following local irradiation, there is a long succession of destructive and proliferative changes culminating finally in cancer; this is in contrast to induction of similar tumors by hydrocarbons, where, if the treatment is intensive, the cancer may appear almost at once. It appears to be impossible, by increasing the dose of a radioactive agent, to reduce the latent period of carcinoma or sarcoma below about 6 months (*35*). Many other lines of evidence point in the same direction: the statistical studies of Blum (*27*) on ultraviolet-induced tumors; the pathologic studies of Foulds (*36*) on spontaneous breast cancer; studies on cocarcinogens (for example, croton oil) (37); Tannenbaum's observations (*38*) concerning the different effects of diet in the early and late phases of carcinogenesis; and a number of recent studies on the gradual process by which a tumor develops invasive characteristics. The entire question of the complexity and apparent multistage nature of carcinogenesis has been discussed at length by others, including Huxley (*39*) and Oberling (*40*).

The Somatic Mutation Theory

It has been natural to think of the cancer cell as a mutant of the normal tissue cell. What sort of mutation or combination of mutations it may represent has, however, defied intensive and prolonged study. In the last analysis, cancer is defined by its interaction with the normal tissues—by its "invasiveness" or ability to metastasize in the original host, or to transplant into a genetically nearly identical recipient or into an immunologically inert environment such as the anterior chamber of the eye (these criteria, incidentally, become satisfied only in a relatively late stage of a morphologically identified cancer). Morphologically, it is characterized by variability of cell size and by irregular mitoses; on careful cytological analysis, by aneuploidy and the presence of supernumerary chromosomes.

The unequal mitosis was first clearly recognized by von Hansemann in 1890 (*41*), and Boveri later (*42*) emphasized that this might have significance in the etiology of tumors. The concept of the somatic mutation grew, with increasing sophistication in genetics, and was first spelled out by Whitman in 1919 (*43*). Muller, in a brilliantly concise paper in 1927 (*44*), first suggested a possible relation between the mutagenic and carcinogenic actions of x-rays. In the past 30 years, workers in cancer research have kept the somatic mutation theory under scrutiny, but no definitive test of it has been achieved.

It is important to keep in mind that the somatic mutation theory is amenable to a variety of interpretations. In the sense that it is merely a restatement of well-known facts, it has little meaning to the present argument. Only if it is defined restrictively as referring to a single point mutation or similarly unique event does it imply a linear relation between cancer incidence and the amount of a mutagenic carcinogen. In the event that particular sorts of chromosome rearrangements or a certain combination or succession of mutations are necessary, a linear relation is negated. Careful consideration of the evidence has led to even broader interpretations of the theory—namely, that the critical changes occur through interaction of enzyme-determining genes, plasmogenes, and substrates (*45*). The view that cytoplasmic changes are important is supported by the fact that the chemical carcinogens appear to be fixed to cytoplasmic rather than to nuclear constituents (*46*).

Efforts have been made to find a correlation between mutagenic and carcinogenic potency of various agents and have, in general, met with exceptions in both directions (*47*). The entire matter of the somatic mutation theory has been carefully reviewed by Burdette (*48*), who finds little evidence favoring a single cell-mutation process. The remainder of this discussion, except where otherwise indicated, will refer to the point mutation in a single cell as the carcinogenic determinant, since that concept is necessary to the linear theory.

I have previously taken occasion (*49*) to direct criticism against the somatic mutation theory on the basis of the mutation rates it implies, particularly when animal species of greatly differing size are considered. While the genetic mutation affecting an individual is clearly traceable to an event occurring in a single cell, the somatic mutation which leads to a tumor in an individual would be presumed to have occurred in any of a very large number of cells of the parent tissue. A postulated rate of human leukemia development of around 10^{-6} per year per roentgen (*2*) (since there are perhaps 10^{11} proliferating myeloid cells capable of mutating) yields an estimated somatic cell mutation rate of about 10^{-17} per year (*50*). Similarly, the "spontaneous" cell mutation rate to leukemia on these assumptions must be very low. A little computation shows that if cancer is a result of a particular mutation in a single cell, a local radiation-induced perturbation in a given molecule is extremely unlikely to produce a carcinogenic somatic mutation; or, expressed in older terminology, such a mutation must occur in an infinitesimally small target area (*51*). In more modern genetic terminology, the "penetrance" of such a mutation must be so small as to require some special interpretation.

The situation is still more difficult to rationalize when we consider the implications of the fact that different species, such as mouse and man, have roughly equivalent cancer rates (spontaneous and radiation-induced) but that the number of cells from which cancer presumably can arise differs by a factor of more than 1000. This applies if mutation rates of somatic cells are properly to be calculated per generation of the species; if they were to be calculated on the basis of time, an additional factor of about 30 would be introduced. With reference, for example, to myeloid leukemia, it is apparent that the rates per mouse and per man are the same within an order of magnitude (*52*).

One possible way out of this dilemma would be to adopt the assumption that somatic mutation rates are much lower in man than in smaller, shorter-lived animals. In view of the great structural and functional similarities between somatic cells of the various species, and since no differences exist in the genetic mutation rates (spontaneous or in-

duced), this seems highly improbable. Another possible escape would be to assume that only a certain small number of cells (say one) in a given tissue of any species was capable of undergoing a mutation to cancer. Such circumstances as those, however unlikely, might serve to explain why certain species are able to attain greater size and age span than others. I feel, however, that the burden of proof must rest on those who are attracted to the somatic point-mutation hypothesis because of its superficial simplicity, and that they are the ones to take those difficulties into account.

If we are to accept the rather apparent fact that a large number of cells are potentially capable of mutating to cancer cells, and that therefore the somatic *cell* point-mutation rates must be enormously lower than those encountered in genetic mutations at a single locus, we are led to the conclusion that cancer is a very improbable result of a single mutation and, therefore, that other events are necessary in addition: perhaps one or more additional mutations occurring in the same or nearby cells, or other, physiological determinants. In the first instance the dose-effect relation will be a curvilinear one representing a power function of dose [the data of Court-Brown and Doll (*6*) actually fit a square-of-dose relation much better than they do a linear one]. The species discrepancy might then be explained on the basis that the functions are of a different power—that is, that different numbers of "hits" are necessary to produce cancer in different species; this has indeed been suggested to account for various spontaneous cancer rates in several species (*53*).

Considerable attention has been given recently to the natural age incidence of cancer as well as to that of gross mortality. It has been known for over a century (*54*) that human mortality rates tend to increase exponentially with age, indicating a mathematical function (the Gompertz function) of what may be called the aging rate. In recent years, with advances in medical means of combating infections and other diseases of early and middle life, this function is increasingly evident in vital statistics (*20, 55*). In animals of shorter life span, the mathematical function remains the same but the time scale is shortened, so that the doubling time of the mortality rate is about ten years in man and 100 days in the mouse.

A similar age-incidence function applies to many diseases, including the various forms of cancer (*55*). In the latter case, it has been noted that there is an early peak in childhood, the height of which varies with the tumor type (this is essentially the only component in some neuroblastic tumors and in Wilms' tumor of the kidney), suggesting that there may be two basically different mechanisms of "natural" carcinogenesis. It is apparent that the cancer curves decline from the exponential slope in extreme old age; this may be an artifact of diagnosis or it may represent an effect of senility on the process; it has also been pointed out that the curves may better be fit to a power function of time, as if a multiplicity of events is necessary (*53*).

It is difficult to explain the age incidence by a single-mutation mechanism of carcinogenesis; this certainly cannot be done if we are to assume that radiations (or similarly acting chemical agents) are totally responsible, through such a mechanism, for forms of cancer in which the exponential age incidence is observed. One possible mechanism involving mutational change has been suggested by Armitage and Doll (*56*), who postulate that an initial change gives rise to a clone of exponentially growing cells which are subject to a further, carcinogenic change. This, if applied to radiation, would imply an exceptional effectiveness of widely spaced dosages, which does not appear to have been observed. Other explanations of the natural age incidence based either on an exponential or a logarithmic curve have also postulated multiple independent events (*57*).

Alternative Hypotheses

It has already been made clear in this discussion that a linear theory resting on the somatic mutation is not valid if more than one mutation, or if some nonlinear phenomenon such as a chromosome rearrangement, is necessary. This may be generalized by saying that a linear theory is valid only if a single factor in the process is linearly responsive to the carcinogen (for example, radiation), while all others (if any) are quite independent of it. Such complications may indeed exist in radiation mutagenesis in the mouse, where neither dose nor dose rate shows a clear linear relation to genetic mutation rate (*58*).

Cocarcinogens. There is much to suggest, in experimental carcinogenesis, that "latent" tumor cells are produced by one process and brought to a malignant state by a cocarcinogen The most potent such agent is croton oil. In the mouse, this agent brings to rapid fruition many tumors that are potentially created by a chemical carcinogen (*37*). It has been found that croton oil abolishes the threshold which is observed when only the carcinogen is applied (*24*). On the other hand, repeated applications of the carcinogen have been more effective in producing malignant tumors than a single application followed by the cocarcinogen (*59*). Where croton oil treatment follows a radiation exposure, only an increase in the number of benign papillomas has been observed (*60*).

Role of cell division. It can be envisioned that a number of cell divisions may be necessary in order for a carcinogenic mutation to manifest itself; such phenomena have been observed in other fields of genetics. This could explain the rather high frequency with which malignant changes occur in tissues that are cultivated over a period of months (where the mass of tissue is small, but where more successive cell generations occur than in the achievement of the cell mass of an adult).

In this instance, it will be noted that massive destruction of tissue results in regeneration of cells and that this process is observed repeatedly during the "latent period" following carcinogenic irradiation of tissue. This is difficult to fit to a linear hypothesis. In the first place, Puck and Marcus have demonstrated that cell death is not linear but is better described by a two-hit curve (*61*); also, the number of cell generations required in repair would more or less be an exponential function of the relative amount of destruction.

Carcinogenic potential of a single cell. It may be observed that a single cancer cell is unlikely to give rise to a tumor. This is dramatically shown by the extraordinary frequency with which cancer cells are seen in relatively small samples of blood draining a human tumor—for example, in 10-milliliter samples from over 20 percent of patients, including many which are not showing metastases (*62*). In highly autonomous experimental tumors, under favorable conditions, single cells may transplant to give tumors, but under most conditions large numbers of cells must be inoculated for a "take."

Physiological environment. It has been generally believed that the tissue environment is important in determining cancer cell growth. This concept has many facets. The proliferation of single cells in tissue culture, for example, requires that they be closely confined, or

that they be explanted with a large number of other cells, or that they be in a "conditioned" medium in which other cells have been cultivated. Again, as was described many years ago by Willis (*63*), there is little evidence from detailed histologic work that would lead one to believe that cancer arises in a single distinguishable cellular focus, since it is usually multicentric in its earliest visible stages. Biochemical studies have led Warburg (*64*) to suggest that the tumor arises in some way adaptively to an environment in which oxidative processes are interfered with. Experimentally it has been found that small tumor inocula take more readily in altered tissue, such as in the liver damaged by chloroform (*65*), while experiments employing skin transplantation have shown the same thing in the converse: carcinogenically treated epidermis fails to produce tumors when it is transferred to a fresh site (*66*).

While these observations are somewhat varied and point in a number of directions (and there are many more such), they all point away from the simple concept of a single mutation operating free of other influences which depend on the carcinogen. To take radiation as an example, a visible disorder of tissue architecture and of its vascular supply is universally characteristic of those dosage levels at which cancer is an observed end result. It should not be forgotten that in a number of other situations where such disorder is the chief recognizable change—as, for example, following thermal burns—cancer frequently arises.

While the concept of a multiplicity of single mutation-like events leads necessarily to a nonlinear relation between dose and cancer incidence, the added concept of a state of tissue disorder as a requirement of tumor appearance implies that a true threshold can occur. There seems to be no direct evidence of any sort that can rule this out.

Enzyme deletion and similar mechanisms. Considerable evidence has been brought forth recently which indicates that cancer may very well not be due to a gene mutation mechanism at all, but to a special situation determined by cytoplasmic (that is, plasmagene-dependent) conditions leading to deletion of certain enzyme-forming systems (*67*). Evidence for this is found in the fact that many chemical carcinogens are fixed to cytoplasmic proteins; that the enzyme patterns in tumors are characterized mainly by various deficiencies; and that changes in enzyme patterns—in particular, development of new pathways—can owe their origin to shifts in the concentration of various substrates. This condition can occur through known positive and negative feedback systems. Perhaps the most important of these involves pathways, only beginning to be defined, by which the formation of thymidine is kept under control (*68*). A failure of the feedback mechanism which normally retards thymidine synthesis might be sufficient to give rise to cancer as it is clinically recognized and defined. Such a mechanism of carcinogenesis would explain the necessity for a particular substrate concentration, hence for a group of potential cancer cells or of a specially abnormal tissue milieu. It would likewise predict—unless such systems are as unique in each cell as are single genes—a multiplicity of "hits," involving in addition, perhaps, a competition between normal and abnormal plasmagenes for supremacy. Haddow (*69*) suggests a nuclear site for the development of enzymatic changes or deletions altering growth control, perhaps in the heterochromatin, and Green (*70*) has developed evidence for a theory that the loss may be of certain immunologic identifiers. While a single-event process might be consistent with some of these mechanisms, I feel that many of the considerations given above cast great doubt on this possibility.

The virus theory. Brief mention may be made of the theory, for which there is some experimental evidence, that there is a provirus which may, through action of a carcinogenic agent, be altered to produce a tumor agent in the cytoplasm. Such viruslike agents include the milk factor and the leukemia agent (*71*). The possibly analogous production of infective agents from lysogenic bacteria has been shown by Marcovich (*72*) to be linear with a large range of radiation dosages. Again, the great rarity of carcinogenesis as a cellular change appears to be strong evidence against accepting this as a single-hit cause of cancer; also, the type of leukemia in which this sort of agent has been demonstrated, the lymphatic leukemia of the mouse, is the most notorious instance of a nonlinear radiation response.

Discussion

It has been suggested that strontium-90 from fallout might be linearly responsible for a very low (but in absolute numbers, appreciable) incidence of leukemia. It has been further suggested that very low-level increments of carbon-14 might (due to its 5000-year half-life) result, in many thousands of years, in calculable, if not determinable, numbers of leukemias. From the foregoing discussion it is deduced that this seems most improbable. Moreover, strontium-90 can, in man, affect only a very localized part of the marrow, and at a dose rate which is extremely low.

This review has necessarily included only a small part of the literature pertinent to this subject; the evidence offered against linearity at low doses must be taken only as illustrative, while the evidence in its favor has been discussed rather completely. With present experimental evidence failing to demonstrate linearity even in genetic mutations in the mammal, it would not seem reasonable to give undue credence to linearity in the much more complex matter of cancer production. The reader is encouraged to examine some recent thoughtful reviews on the subject (73) before accepting a simple theory of carcinogenesis.

Summary

1) Present data on human leukemogenesis by radiation fail to indicate a linear relation between dose and effect. Because data are scanty, such a hypothesis cannot be ruled out statistically, but it is less probable than a nonlinear or threshold relation.

2) Other instances in which carcinogenic agents have been examined from the standpoint of dose and dose-rate relations show many clear instances where the relation is nonlinear and none in which linearity is unquestionably demonstrated.

3) Theoretical consideration of the probability that a single critical molecular event, such as a mutation, will give rise to cancer indicate that a malignant change must be an extraordinarily improbable result of such a perturbation. It is also very difficult to reconcile this mechanism with the rather comparable spontaneous and induced-cancer incidences in species with greatly different numbers of cells.

4) Any scheme in which multiple events caused by the carcinogen are required to produce a tumor is incompatible with a linear relation, while, if a disordered state of tissue is an important factor, a true threshold may even occur. There is much evidence, from cancer research of all sorts, indicating that one or both of these conditions is involved in the carcinogenic process.

References and Notes

1. Much of this material was originally proposed as a letter to the editor; I have elected to expand it in view of the importance of the subject and the accumulation of new documentary material. This work was performed under the auspices of the U.S. Atomic Energy Commission. I wish to emphasize that I alone am responsible for the opinions herein expressed.
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CURRENT PROBLEMS IN RESEARCH

Muscle Research

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Albert Szent-Györgyi

If science is the art of measuring, then muscle has no equal as a material in the study of life, for there is no other tissue whose function is connected with equally extensive and intensive changes in chemistry, physical state, energy, and dimensions. This is why physiology, up to the turn of the century, was mainly muscle physiology. After muscle had been pushed into the background by enzymes and hormones for a while, the development of modern physical methods once more turned attention toward it with its macromolecular organization and its "mechanochemical coupling" (the conversion of chemical bond energy into work).

Muscle also has a strong appeal to the medically minded. The heart and the uterus both are, in a way, but bags of muscle, and our blood pressure is regulated by muscles that determine the lumen of our smaller blood vessels.

The function of muscle is to create motion. There are many sorts of motion, and thus there are many sorts of muscles, even if the basic principles on which they are built may be identical. A muscle cell or fiber is a very complex system, and the unit of its function, the twitch, is a very complex cycle. Hence, "muscle research" covers a wide field of

The author is director of the Institute for Muscle Research at the Marine Biological Laboratory, Woods Hole, Mass.