Environmental Health Perspectives
Vol. 22, pp. 167–170, 1978

Biologic Evidence for the Existence of Thresholds in Chemical Carcinogenesis

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In a search for evidence for the existence of threshold levels below which chemical carcinogens do not induce cancer, it is essential to consider all aspects of the carcinogenic process. We should not confine our thoughts solely to the generally accepted stages of carcinogenesis: initiation, promotion, and progression.

Several factors may influence thresholds even before the proximate carcinogen reaches the target tissue. Important is the number of molecules that can react with the target tissue. Apart from the level of exposure, this number depends on several conditions. First, not all molecules with carcinogenic potential are equally well absorbed, and the degree of absorption will affect the number available. Second, not all of the molecules which enter the organism reach the target tissue. For many molecules, metabolic change may produce noncarcinogenic derivatives, while others will be activated to the proximate carcinogen. The ultimate number of molecules in this group is dependent upon the metabolic capability of the organism to perform the necessary conversions. This may be a function of the state of enzyme induction or tissue damage. Once in the active state, a large proportion of the carcinogenic molecules may be lost to the carcinogenic process by interaction with molecules other than DNA, that is, with any molecules that can be alkylated, such as a diversity of small molecules, with macromolecules, such as protein or RNA. While damage may result from these reactions, no heritable changes are produced.

From the foregoing, it is evident that several processes which have no relationship to carcinogenesis, such as absorption by the organism, metabolic change, or deactivation of the proximate carcinogen by abundantly available enzymes such as epoxide hydrase or glutathione S-transferase and reaction with molecules other than DNA, have great bearing on thresholds because of their potential capacity to reduce the number of active molecules available for the carcinogenic process.

Additional variables that influence thresholds are inherent in the reaction between DNA and the chemicals that are effective alkylating agents. Those variables that affect DNA repair are especially critical. First, the extent to which DNA damage can be repaired depends, to some extent, on the site of alkylation. Some sites are more difficult to repair than others. For example, it has been found that tumor production in some tissues is correlated with persistence of alkylation at specific locations in the base, particularly at the O^6 guanine position. Alkylation of the N^7 guanine position was not associated with cancer production, because elimination of the N^7 alkylated guanine is far more efficient and enzymes more readily available than for the elimination of an O^6-alkyl guanine base (*1*).

Second, DNA repair of succeeding alkylations is less effective. Thus, in a situation of continuous exposure, DNA repair efficiency may be reduced by subsequent entry of carcinogens into the system. A third condition, which may influence thresholds is the type of DNA–carcinogen interaction that occurs. For example, crosslinking chemicals produce DNA damage which is least amenable to repair, whereas interactions in which only a single arm of an alkylating agent is bound to DNA bases are more easily repaired. Finally, as illustrated by the "SOS repair" in bacteria, DNA repair, when incorrect, may actually lower the threshold (*2*). If repair processes are not available, cells whose DNA

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is alkylated by carcinogens undoubtedly die. However, when repair can be initiated, there is the potential for a variety of errors which may result in inappropriate reconstitution of DNA. The end result is a mutation, a case where the cure is worse than the disease. A related group of variables are those associated with the site of DNA damage as it relates to DNA function.

As an analogy, if there is a typographical error in this presentation, most of those who read it probably do not register it, since, like a point mutation, it does not significantly affect the message of which it was a part; and like a typographical mistake, most point mutations need not significantly affect the expressions of the DNA code (*3*).

The variables associated with DNA repair and with the site of alkylation then are another major set of factors which potentially influence thresholds. Successful DNA repair of a carcinogen-induced lesion raises the threshold for cancer; unsuccessful repair lowers the threshold.

As one of the illustrations in Dr. Rall's paper indicates, there may be an enormous range for thresholds: between 10^4 and 10^{20} molecules of a carcinogen. As an actual example, however, 25 μg of benzpyrene injected once subcutaneously into mice may produce some sarcomas (*4*). At this dose, the mouse will absorb in the order of 10^{16} molecules of benzpyrene. Sarcomas are induced at this dose level; therefore, the threshold for benzpyrene under the conditions specified, is below 10^{16} molecules.

In a second example, painting a different polycyclic hydrocarbon once on the skin of mice at a dose of 0.2 μg, which is of the order of 10^{14} molecules, will not produce any tumors. However, if the carcinogen treated mouse skin is painted periodically with a promoter, like croton resin or a phorbol ester, tumors will appear (*5*). In the first example then, a definite threshold exists below 10^{16} molecules of carcinogen; in the second example the threshold is above 10^{14} molecules unless a promoter is applied.

The latter example introduces the role played by promoters in modifying thresholds for carcinogen action. Experiments on the mechanism of promotion in carcinogenesis consists of exposing the skin of groups of mice to a dose of carcinogen incapable of producing tumors in the experimental group. It is preferable in this type of study to identify a dose that will produce no tumors whatsoever in the experimental population used. The promotion is then applied to the carcinogen-treated skin. Provided the promoter is applied for an adequate period, tumors will usually result.

Roe and others have made major contribution towards an understanding of the mechanism of cancer promotion. In these studies, initiation of cancer in mouse skin has been successfully accomplished with many types of carcinogens. This work has shown that long periods of treatment of up to a year or more, are required for tumor promotion (*6*). In one study, a year was allowed to elapse between tumor initiation and the beginning of treatment with a promoter, but tumors were still produced, although at a reduced incidence. The explanation for this phenomenon is that the carcinogenic process in the initiated cell remains dormant until a stimulus of as yet unknown nature is supplied by the promoter.

The point here is that, at least in mice, there are levels of carcinogen exposure which will produce only dormant, initiated cells unless adequate promotion is supplied. This can be interpreted to mean that the promoter in effect lowers the threshold for production of observable tumors.

The promotion phenomenon is even a political issue, since in one country promotion of carcinogenesis has been declared to be only a laboratory curiosity and without significance for human health. This official position is based on the high doses of promoter required. I agree that practically all promotion experiments done on mice used large amounts of croton oil and that humans do not have their skins painted repeatedly with croton oil. On this basis, promoter studies are indeed laboratory curiosities. On the other hand, we all know people who do chronic experiments on themselves with cigarette smoke inhalation. This is a similar situation.

A related situation and one of many that has been documented involves the occupational exposure of the skin of workers to coal tar and oil fractions, which frequently yield unexpectedly high cancer incidences (*7*).

Little information is available on interactions between structurally related chemicals, particularly between those which are either carcinogens of different potency or inactive. There is only fragmentary knowledge of the types of related compounds to which the human population may be exposed concomitantly with exposure to known carcinogens. For instance, the identity of all of the components of coal tar which may enter the body after washing the skin with coal tar soap is unknown as are the components of the soot that is inhaled by most of the human inhabitants of the world.

The results of an interaction between such chemicals in experimental animals depend on the dose and the type of treatment. Every possible response has been reported. In the case of interaction between two "strong" carcinogens or between two "weak" carcinogens, there are, as anticipated, additive effects. On the other hand, combining a

"strong" and a "weak" carcinogen results in some inhibition of carcinogenesis; the effect of the "strong" carcinogen is blocked while only that of the "weak" carcinogen, introduced at higher dose levels, is expressed (*8*).

Inhibition of carcinogenesis has an obvious bearing on cancer thresholds (*9*). Initial studies on inhibition date to the 1930's and represent some of the earliest experimental findings in carcinogenesis. Some observations can now be explained on the basis of metabolic detoxification. For example, the puzzling finding in 1941 that liver cancer induced by an azo dye could be prevented by high doses of Vitamin B_2 (riboflavin) (*10*) has later on been explained in experiments which showed that the azo linkage is hydrogenated through an enzymatic reaction which requires a coenzyme containing riboflavin (*11*). The azo dye is thus detoxified, since the molecule is split into two inactive amines. This observation has engendered innumerable studies. A possible human correlation would be that individuals who are deficient in riboflavin might be less able to deactivate an azo dye.

The complex role of nutrition in carcinogenesis is yet another factor which may influence thresholds for carcinogenesis. Overeating, especially a high fat intake, appears to be a factor in carcinogenesis. This condition is based on the finding that a high incidence of skin (*12*) and breast tumors (*13*) was produced in experiments on rodents first given a polycyclic hydrocarbon initiator and then fed a high fat diet. A specific component or contaminant of fat has not been identified as a causal factor; only the intake of excess fat has been implicated.

The foregoing examples illustrate some of the many conditions, most of them complex and only in the early stages of scientific exploration, that may influence thresholds for carcinogenesis. However, there are several additional generalizations which are pertinent to an argument in favor of thresholds. First, it is obvious, as pointed out in Dr. Rall's paper, that the threshold may be moved upward and downward by a number of factors that are temporal in nature. In other words, thresholds at any given point in time may be different due to a variety of factors such as intercurrent disease, dietary splurges, exposures to other chemicals, etc. The latter may for instance preempt detoxification of a carcinogen because of the abundance of the other xenobiotics.

Second, it is important to realize that there are situations where a recognized chemical carcinogen is an essential constituent of the mammalian system. For example, estrogen at the levels normally present in the human body does not constitute a carcinogenic risk despite the evidence that this hormone in large amounts may be a carcinogen. Even if estrogens were carcinogenic at physiologic levels, any attempt to eliminate them from the body by whatever means would be disastrous to human development and function. It is also important, in any consideration of thresholds, to understand that this situation is not unique for sex hormones. Other essential chemical constituents of the mammalian organism may be carcinogens at high doses. These also include some trace elements, like nickel or arsenic, which are present in every tissue and organ.

The fact that a number of essential chemical constitutents of the body are carcinogens is major evidence for the reality of thresholds in carcinogenesis. The concentrations of these chemicals which are absolutely necessary for normal physiologic activity are obviously below their carcinogenic concentrations since no cancer is induced despite their presence throughout life.

The evidence I have presented for the existence of thresholds in carcinogenesis has been limited to the first phases of carcinogenesis. I have left in the dark the lengthy chain of events that must occur after the carcinogen enters the cell and reacts with the DNA and before the initiated cells start to multiply and spread throughout the organism.

However, even within this limited view of carcinogenesis, there is ample reason to believe that thresholds do exist for cancer. The major support for this conclusion stems from the considerations I have outlined, including the small percentage of exogenous carcinogenic molecules that ultimately express their activity once in the organism, the variables that affect DNA repair and mutation, the action of promoters, the interactions between carcinogens and related compounds, the several known processes inhibitory to carcinogenesis, and the fact that a number of carcinogenic molecules in low concentrations are essential constituents of the mammalian system throughout life without causing cancer.

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