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DNA DAMAGE AND CARCINOGENESIS

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INTRODUCTION:

A number of independent lines of evidence, other than cell biology ones, support the somatic mutation theory of cancer. They indicate that damage to DNA can lead to cancer and hence one should be concerned about environmental agents that react with DNA. Nevertheless, there are arguments against this point of view (1) and, even if damage to DNA is the important element in cancer initiation, one should always keep in mind the possibilities that the switch from normal to cancer cells may arise from faulty transcription and hence translation (as seems to be the case in the death of UV-irradiated arrested human fibroblasts). Moreover, promotion steps subsequent to initiation may be of overriding importance at the initiation doses received at low exposure-rate levels.

The following are direct reasons for associating initiating events in carcinogenesis with DNA damage:

1) There is an excellent correlation among those compounds that are mutagenic, when activated appropriately, and those that are carcinogenic. (Of course, a chemical that reacts with DNA will also readily react with RNA and protein.) (2)

2) In mammalian cell cultures there is an excellent quantitative correlation between the mutagenicity of metabolites of polycyclicaromatic hydrocarbons and transformation by these metabolites and also an excellent correlation between mutation and transformation by the activated metabolites of nitrosamines (3, 4).

3) If cells are treated *in vitro* with BrdU and long wavelength UV - a process known specifically to damage DNA - the resulting neoplastic transformation is correlated with the extent of DNA damage (5).

4) The UV-irradiation of thyroid cells of the fish *Poecilia formosa* gives rise to thyroid tumors when the cells are injected into isogenic recipients. If the cells are photoreactivated before injection - a treatment known to monomerize pyrimidine dimers in the cellular DNA - the number of tumors observed decreases by greater than ten fold (6).

5) In a number of human disorders, the affected individuals are cancer prone and their cells are more sensitive than normal to exogenous mutagens (7-9) (see previous chapters in this volume). Three of these disorders (Table 1) xeroderma pigmentosum (XP), ataxia telangiectasia (AT), and Fanconi's anaemia are associated with defects in DNA repair. However, the association is weak insofar as the causes of increased cell cytotoxicity compared to normal cells are not the same for all individuals with the same clinical disorder. Moreover the correlation between cytotoxicity of UV and excision repair deficiencies is not a good one (10), indicating either that there are other repair systems of importance or that cells die for reasons than the mere existence of damage to their DNA. Certainly, the time it takes to accomplish repair, before DNA replication and transcription take place, is important, as is the fidelity of replication and transcription on damaged templates. The etiologic agent is known for only XP. The distribution of cancer types in AT is different than that observed in the population exposed to atomic bombs, indicating that the enhanced cancer risk in the AT population does not arise from ionizing radiation (11). Morevover, AT cells are hypomutable to X-irradiation (8).

6) If human cells are held in a confluent state before replating, DNA damage decreases because of excision repair, and survival increases and mutations decrease. The changes in survival and mutation correlate well with the rate of excision repair in proficient and repair deficient human cells (12, 13). Transformation in human cells also decreases as a result of such a holding procedure (14) but various methods of enhancing transformation in Syrian hamster embryo cells do not affect known DNA repair processes (15).

Thus, it is well established that DNA damage and its repair play an important role in carcinogenesis but the quantitative aspects of its role are not clear. What is needed is a thorough understanding of the types of DNA damages that may result in the initiation of the carcinogenic process and the relative

Table I. Repair Deficient Diseases

	Xeroderma pigmentosum	Ataxia telangiectasi	Fanconi's anemia
frequency homozygotes heterozygotes	1/300,000 1/300	1/40,000 1/100	1,300,000 1/300
cancer probability* homozygotes less than age 20 yr.	skin cancer: > 0.5 (melanoma > 0.1)	0.1 (lymphoreticular:0.06, leukemia: 0.02)	> 10 fold normal
heterozygotes	5-fold normal in South	5-fold normal < 45 yr, 50% greater mortality than average.	like normal
etiologic agent	sunlight	?	?
cell sensitivity	UV and mimetics	x-rays, alkylating agents	cross linking agents
repair deficiencies	one or more of excision, photoreactivation, and postreplication ($\geq$ 7 groups)	some cell strains defective defective in "X-ray" repair ($\geq$ 3 groups)	some cell strains defective in crosslink repair

*Approximate average cancer probabilities
skin cancer prevalence: 0.005
melanoma incidence: 6×10^{-5}/yr
lymphoreticular cancer: 13×10^{-5}/yr
leukemia (t_{max} ~4 yr.): 42×10^{-6}/yr.

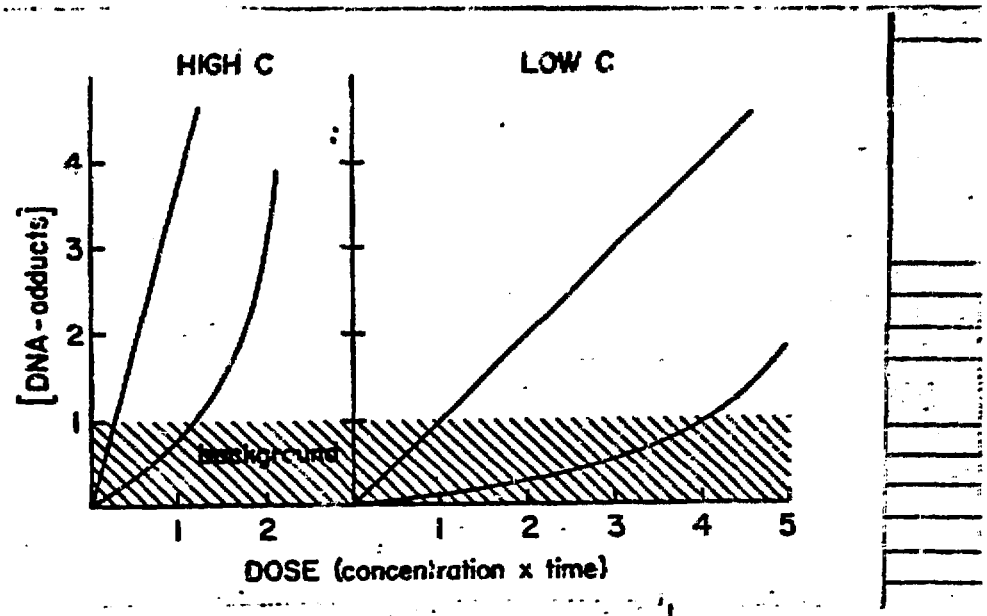

Fig. 1. Possible dose-response curves for DNA adducts caused by physical or chemical agents at high or low dose rates or concentrations.

probability of initiation for one product versus another. Sometimes DNA repair measurements themselves are used to identify a crucial DNA adduct. For example, in new born rats exposed in utero to ethylnitrosourea the repair of O^6 ethylguanine in neuronal tissue is very low compared to that in other tissues, but the major alkylation damage, N-7 ethylguanine seems to be affected equally in all tissues(16). Since neuronal tumors are the principal ones observed, these data are evidence for the importance of the O^6 ethylguanine in carcinogenesis and the unimportance of the N-7 ethylguanine. A crude measurement of DNA repair such as unscheduled DNA synthesis, or its equivalent, would measure primarily the repair of the N-7 product.

QUANTITATIVE ASPECTS:

An attractive feature of the strong implication that DNA damages act as initiating events in carcinogenesis is the possibility of using the implication to obtain good dosimetry at the level of cellular DNA. Thus, if one could identify biological damages to DNA and develop ways of analyzing for them in small numbers, as is now being done by the use of specific antibodies or nucleases (17-19), one could measure the accumulation of such products in experimental animals or people exposed experimentally or environmentally. It should be possible to determine the relations between physical dose or chemical dose

(concentration x time) and the level of DNA adducts in terms of adducts per unit length of DNA. It is important to determine the dose response curve for such adducts - whether it is linear or curvilinear - and how the relation depends on the concentration or dose rate (see Fig. 1). The existence of activating and deactivating enzyme systems for most chemical carcinogens, and the existence of repair mechanisms for chemical damages, as well as for physical damage, implies that the dose response relations probably will depend on dose rate and will not be linear. The determination of such dose response relations is within the realm of technical capability. Of course, one of the first steps in such determinations is the identification of the proper adduct to measure.

Fig. 1 illustrates a second important consideration in extrapolating experimental results on such simple systems to humans. What is the background level of DNA adducts in the absence of any man made contribution. An important question for chemical carcinogenesis, as it is for radiation carcinogenesis, is what is the level of man made adducts compared to the background level? Even if one can do the dosimetry indicated in Fig. 1, there is the near impossibility of using such data plus carcinogenesis data on animal systems to extrapolate to low dose

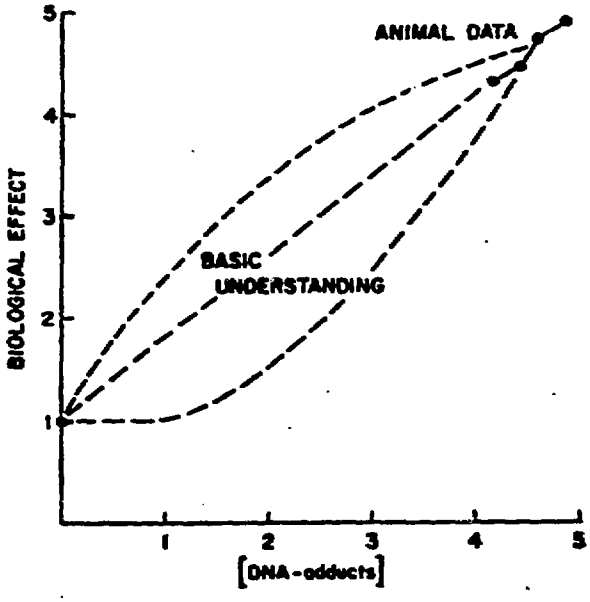

Fig. 2 Possible relations between DNA adducts and biological effects. The effects at zero adduct is deliberately shown as finite to emphasize that the effect could, in part, be independent of DNA or depend on other, not measured, adducts.

effects since most animal data are obtained at relatively high levels of DNA adducts. The extrapolations to low levels, levels that the general population might be exposed to, can only come from a knowledge at the fundamental level relating adducts to biological effects. Fig. 2 illustrates some of these possible extrapolations. It is meant to emphasize the fact that human epidemiological data at low levels of adducts do not exist and if they did, might be confounded by genetic or physiological variability in the population as well as the necessity for considering steps in carcinogenesis other than initiation. Nevertheless, a scientific goal should be the understanding of the biological systems and their variabilities so as to make the extrapolation based on sound biological theory. Part of this theory has to do with the lifetime of adducts and the relations between lifetime and other internal and external processes such as replication, transcription, and promotion.

SKIN CANCER:

The experimental and epidemiological evidence indicates that non-melanoma skin cancer arises from the cumulative exposure to UV in sunlight (20). In the United States there are reasonable data on skin cancer incidence over a wide range of latitudes which, because of changes in light path through the stratospheric ozone with latitude, means that there are data of skin cancer incidence versus the average yearly UV exposure. At all ages investigated, skin cancer incidence increases exponentially with average yearly exposure (20, 21). I assume this rapid increase means that most individuals are exposed to sufficient UV to bring them well above any threshold level. (This would probably not be the case for ambient chemicals.) In the United States, skin cancer is the most common of all cancer. There are approximately 400,000 new cases per year (21).

The rate of increase of cancer incidence with UV exposure depends critically on the wavelength band one considers to be the effective one. Since the action spectrum for cytotoxicity for normal and XP cells follows that for the production of pyrimidine dimers in DNA (22) and such dimers are implicated in the lethal, mutagenic, and tumorigenic effects of UV, and are poorly repaired in most XP cells, it is reasonable to suppose that the effective wavelengths in sunlight are those that affect DNA. Hence, one should multiply the sun's spectrum by the DNA action spectrum in mammalian cells, so as to obtain the average yearly UV exposure. In arbitrary units of UV dose, the average exposure in the United States is approximately 0.3 units per year, and the doubling dose is 0.15 per year (20). Thus, a 50% increase in yearly dose would

double the cancer incidence rate*.

How do we extrapolate these data to assess the role of DNA repair in minimizing the effects of environmental agents in initiating carcinogenesis. Recall from Table 1 that XP individuals have a risk approximating unity of getting skin cancer before the age of 20. On the other hand, the average white person in the United Staes may be estimated, by a long extrapolation, to have a risk of somewhat less than 10^4 of getting skin cancer before that age (23). If one ascribes the differences between the average and XP individuals as solely the result of DNA repair, one must compare the doses that would give the same cancer incidence. Thus, the question is what UV dose would increase the average cancer prevelance before the age of 20 by a factor of 10^4? Since a two-fold increase arises from 0.15 units of dose, a 10^4 fold increase ($2^{13.3}$) would require 0.15 x 13.3 = 2 units of dose. Since the average exposure in the United States is approximately 0.3 units per year, these data indicate that there would have to be an approximately 7-fold increase in UV dose per year to give a skin cancer incidence in the average population equal to that observed in XP individuals at the present low level of flux. The 7-fold estimated increase obviously has large theoretical errors in it, as well as the practical one that most XP individuals tend to avoid direct sunlight, because of its more immediate deleterious effects, and hence are probably not exposed to more than 0.1 units of dose per year. If the latter number were correct, a twenty-fold increase in dose would be required to bring the average population up to the incidence observed in XP individuals. Thus, from these data on skin cancer one may conclude that in normal individuals repair processes act to remove between 6/7ths to 19/20ths of the UV damage and as a result decrease the skin cancer incidence rate by 10^4. Repair certainly seems to remove greater than 85% of the UV damage.

The population of XP individuals is very heterogeneous. Their repair deficiencies are not absolute for excision repair. They range from greater than 95% to 40% (24). If one considered the average deficiency, as approximately 85% one would conclude that this defect in excision repair leads to a 10^4 fold increase in skin cancer. Hence, one would also conclude that relatively small changes in DNA repair among the population could have large effects on initiation events for cancer. This conclusion is

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*If a weighting factor similar to the erythemal spectrum were used, a two-fold increase in cancer incidence would arise from a 30% increase in UV dose (21). This number is lower than that obtained with a DNA spectrum because the longer wavelengths effective in erythema production are not effective in DNA damage.

reinforced by the observations that there is a rough correlation between the severity of the clinical disease and the magnitude of the excision repair defect observed on cells in culture.

CONCLUSIONS:

Although cancer may arise as a result of many different types of molecular changes, there is little reason to doubt that changes to DNA are one of the more important ones in cancer initiation. Although DNA repair mechanisms seem able to eliminate a very large fraction of deleterious changes to DNA, we not only have little insight into the molecular mechanisms involved in such repair, but have a negligible amount of information to permit us to estimate the shape of dose response relations at low doses. The case of skin cancer is a special one, in that the average population is exposed to sufficient solar UV so that the effects of small increments in UV dose may be estimated. An approximate 85% reduction in DNA repair increases skin cancer incidence 10^4 fold .

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