RELEVANCE OF PHENOTYPIC VARIATION IN RISK ASSESSMENT:

THE SCIENTIFIC VIEWPOINT

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INTRODUCTION

Everyone knows that people vary widely in their responses to local
environmental changes such as heat or cold, pollen, rotation, or high alti-
tudes. We know a great deal about such responses; how they depend on an
individual's previous exposures, lifestyle and age, for example. A much
more difficult problem, because of the absence of good, human data, is the
evaluation of dose-response curves for exposures to radiations or environ-
mental chemicals. If we had such data, it would be possible to estimate
risk from exposure; unfortunately, almost all the human data that exist are
not the result of chronic exposures, but come from acute, high doses. Like-
wise, animal data almost always represent effects at high doses. Thus the
problem is how to extrapolate from high doses to low doses. One can either
do the extrapolation arbitrarily or take some scientific approach to the
problem. One such approach is to understand the mechanisms at the molec-
ular level responsible for the observed effects and then to use this under-
standing to construct theories permitting the extrapolation from high to
low doses. Therefore, what we need are good biological theories. However,
superimposed on such theories must be an analysis of the variability in the
responses of the human population. These variabilities can be thought of
as subsets within rather broad categories, as indicated in Table 1. Thus,
women cannot be lumped with men because, among other things, they have a
different hormonal status and hence are more susceptible to breast cancer;
similarly, only men have prostrate cancer. We know that older individuals
usually are less able to cope with stress than younger ones and moreover,
they seem less proficient in DNA repair than are younger people[1].

Skin Cancer

Almost all skin cancer in fair-skinned people is associated with sun-
light exposure: usually the prevalence of skin cancer in pigmented indi-
viduals. is one or two orders of magnitude less because pigment in superfi-
cial layers of their skin absorbs the incident sunlight, so shielding and
protecting the underlying growing cells. As a result, fair-skinned people
will be much more affected than pigmented individuals by any depletion of
stratospheric ozone, with the attendant increase in UV radiation at the
earth's surface that may result from chemical emissions into the atmos-
phere[2]. Thus, at the level of pigmentation, it is clear that minimizing
the ozone depletion is in the best interest of Caucasians. However, the

Table 1:   Subsets with Variability in Response
Within the Human Population

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1.   Women versus men
2.   Old versus young
3.   Dark skin versus fair skin
4.   Repair proficiency versus repair deficiency
5.   Proficient carcinogen metabolizer versus
     deficient metabolizer

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white population represents a minority of the world's inhabitants, and
there is a significant ethical problem in denying the majority of people
the benefits of such chemicals because of concerns for their effects upon
the minority.

Even in the fair-skinned population there are wide variations in sen-
sitivity to UV-radiation. Genetic analysis of cancer-prone families and
studies on their cells in culture showed that several cancer-prone syn-
dromes are closely associated with deficits in the ability of cells to
repair exogenous, and possibly, endogenous damage to their DNA[3,4]. The
best understood of these syndromes is xeroderma pigmentosum (XP) a syndrome
in which homozygous individuals are extraordinarily sensitive to sunlight-
induced cancer--approximately 10,000-fold more sensitive than unaffected
people[5]. The frequency of the homozygous condition in the United States is
approximately 1 in 250,000. Hence, the condition is not an overwhelming
concern for public health. However, since the various types of XP cells
investigated seem, on the average, to be only about 20% as proficient as
normal cells in the repair of ultraviolet damage, we see that an 80% defect
in repair results in a 10,000-fold enhancement in the susceptibility to a
particular exogenous agent, ultraviolet radiation. The important question
is, What would be the effect of a 10 or a 20% defect in DNA repair? Could
such a relatively small defect, one within the range of what we would call
the normal distribution, increase susceptibility by as much as 10-fold?
Note that the variations observed in repair-deficient syndromes may far
outweigh those of differences in pigmentation, so that within each pigment-
ation group there is a large variation in repair capabilities. We know
that there are extensive variations in repair capacity, as measured by the
ability of cells in culture to repair ultraviolet damage[6]. For example,
unscheduled DNA synthesis, a measure of DNA repair, in epidermal cells
varies as much as 3-fold among individuals of similar ages from whom the
epidermal cells were obtained. In addition, the average repair decreases
almost 3-fold from age 20 to 80.

The dose-response curves for skin cancer in animals and the estimated
curves from epidemiological studies on humans are excellent. In the case
of humans, these are some of the best dose-response curves available,
because the number of new cases of skin cancer per year in the United
States is close to 500,000. The reason for this large number probably is
very high doses that sunlight provides compared to other environmental
insults.

Ionizing Radiation

Ataxia telangiectasia (AT) is a complicated homozygous recessive con-
dition in which the affected individuals and their cells are extraordi-
narily sensitive to the cytotoxic effects of ionizing radiation. The
nature of the repair defect in AT is not known; the disease is more commmon
than XP, affecting in the neighborhood of 1 person per 40,000 in the United

Table 2. Estimated DNA Damage Rates and Repair
Rates in Human Cells at $37^\circ C$[1]

Damage

Endogenous	No./hr	Exogenous	No./hr
Depurination	580	Pyrimidine dimers (noon Texas sun)	5×10^4
Single strand breaks	2300		
O^6-methylguanine	130	Single strand breaks (background radiation)	10^{-4}
Thymine glycol	13	Thymine glycol (background radiation)	$10^{-5} - 10^{-4}$

Repair

	No./hr
Single strand breaks	2×10^5
Thymine glycols	10^5
Pyrimidine dimers	
Normal cells	5×10^4
XP C cells	5×10^3
O^6-methyguanine	$\sim 10^4$

States and hence, the number of heterozygotes in the population is approximately 1%. A significant fraction of the latter group may be at increased risk, especially when one considers the observations in several studies that the cells of AT heterozygotes are more sensitive than normal to ionizing radiation and to chemicals that cause DNA strand breaks[8,9].

Lacking a direct measure of DNA repair in AT, a convenient measure is the cytotoxic sensitivity given by D_0, the reciprocal of the slope of the survival curve (a graph of logarithm of survival versus radiation dose). Values of D_0 have been measured for a large number of fibroblast strains obtained from normal individuals, from AT homozygotes and AT heterozygotes. The results from a number of surveys show that there is a large difference between normal cells and AT cells, that in normal cells there is a wide distribution in sensitivities, and that, on the average, AT heterozygotes fall somewhere on the low side of the normal distribution[6,8,9]. If we had confidence in using such data to predict dose-response curves for people, we could construct the curves associated with each segment of the population and then estimate risks for these individual segments.

Endogenous vs. Exogenous Damage

When most of us think of hazards or risks, we think of bombs, nuclear reactors, food additives and toxic waste dumps. However, the biggest source of damage to the DNA of our cells results from endogenous chemical reactions at 37°. Table 2 compares the amount of DNA damage per hour per mammalian cell resulting from endogenous reactions and from exogenous

ones[1]. The rate of introduction of endogenous damage into DNA is so large that if it were not repaired, approximately 10% of the DNA bases per cell would be damaged in a 70-year lifetime. One would conclude from such a number either that DNA is not the genetic material, nor does it form the basis for transcription, or that there are potent DNA repair mechanisms. The latter is the case. Table 2 also indicates some of the rates of repair of damage. A comparison of these numbers indicates that endogenous damage is much more important than exogenous damage, except for that produced by exposure to ultraviolet radiation. The amount of damage resulting from background ionizing radiation is negligible compared to the damage resulting from endogenous reactions. Indeed it is these reactions that are probably responsible for a large fraction of the background rates of cancer in the human population.

A special case in which the repair reaction is much faster than the reaction introducing the damage is the production and repair of O^6-methylguanine. This mutagenic, and presumably carcinogenic, adduct is introduced into DNA as a by-product of an essential metabolic reaction. Its repair is intriguing, in that the repair reaction is a stochiometric one. The repair protein can repair only one adduct, and upon effecting this, it is inactivated[4]. Although this system works rapidly, it is soon used up and there is no further repair until new protein is made. On this basis one would expect that the dose-response curve for effects arising from this particular adduct have a concave upwards shape. At low doses repair is very effective, and little damage persists. At high doses, the repair system becomes saturated and the crucial adduct can persist long enough to cause mutations in dividing cells. Indeed, this seems to be the case for mutations induced in male mice treated with the alkylating agent ethylnitrosourea[10]. The induction of mutants is a function of injected concentration and the response curve is concave upward, and appears as if it has a threshold that would not be detectable by extrapolation from high doses.

Smoke contains alkylating agents derived from tobacco[11]: presumably the DNA adducts from such alkylating agents are repaired effectively. However, at high levels of O^6-alkylguanine, the repair system may be saturated and, indeed, it was observed that cells washed out of the lungs of smokers have a significantly lower repair capacity for O^6-methylguanine than cells washed out of the lungs of nonsmokers[12]. The decreased repair observed in smokers could either be the result of the inactivation of repair protein during the repair reaction itself, or could result from the fact that smoke contains oxidizing agents which oxidize the crucial SH-group responsible for the activity of the repair protein. In either event, in smokers we have a situation in which smoke contains nitrosamines, both alkylating agents and carcinogens, and the lungs of smokers have less repair activity. Here we have an environmental agent that not only reacts with DNA but tends to destroy the repair activity that would ameliorate the effects of DNA damage.

CONCLUSION

A number of examples have been presented indicating the types of variations that may be expected in the responses of the human population to deleterious agents of an endogenous or exogenous nature. If one assumes that the variations in repair in the normal population are reflected in large variations in carcinogenic risk per unit of exposure, then the dose-response curves at low doses cannot be extrapolated from high doses without knowing the distribution of sensitivities among humans. The probability of determining this range by epidemiological studies on a random population by small. On the other hand, the probability of determining the range by careful genetic and molecular studies appears high enough so that such

experiments now are being carried out. They cannot be carried out on real populations, using chronic exposures. Hence, the ability to estimate dose-response relations in the low dose region on human populations can only be by making theoretical constructs that, in turn, are dependent on fundamental research.

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