New Frontiers in Cancer Causation

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Chapter 16

Epizootiology of Cancer in Rats: A Model for Oncologists to Heed

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INTRODUCTION

A rule underlying the successful promulgation of war is that one should know the enemy. This chapter draws attention to some aspects of cancer that should not be overlooked by those endeavoring to prevent it.

Below are listed a range of disparate facts that need to be taken into account in any all-embracing theory of cancer causation.

- Cancer is not one but many different diseases that run different courses.
- Cancers are only recognizable and definable at the multicellular, tissue, or whole organism level. They are not definable at the single cell, subcellular, or molecular level.
- Cancers consist of mixed populations of cells and often contain cells that show chromosomal abnormalities and are nonviable.
- Cancers are subject to progression and to spontaneous regression.
- Cancers arise in animals of all species.
- Most kinds of cancer increase in incidence with age and with manifestations of aging.
- The incidence of most forms of cancer is influenced by environmental factors, including both nongenotoxic and genotoxic agents.

- Calorie indulgence increases risk of development of most types of cancer and calorie restriction protects against such risk.
- Genotoxins are generated endogenously during the metabolism of ordinary foodstuffs, particularly fats.
- Prolonged irritation, wounding, hyperplasia, disturbed hormonal status, and increased cell-turnover predispose to cancer development in some tissues.
- Genetic constitution, immunological status, certain viruses, and oncogenes are implicated as risk factors for some cancers.

Until the mid-1960s most research in carcinogenesis relevant to cancer prevention involved studies on xenobiotic chemicals to which animals were exposed in high doses. Therefore, I decided to start testing for carcinogenicity in naturally occurring chemicals that humans eat or contact in other ways during everyday life. However, serious problems soon became apparent. First, animals needed to be observed not just for the 20 to 50 weeks that was enough in experiments using high doses of known potent carcinogens, but for periods close to the natural life span of the test species. This often proved impossible because of high rates of intercurrent disease that caused premature death. However, when the switch was made to using specified pathogen-free (SPF) animals, the even worse problem of high spontaneous tumor incidence rates was encountered.

I then learned of the experience at Imperial Chemical Industries Ltd (ICI). Over a period of 10 years after the establishment of a specified pathogen-free (SPF) animal unit, the lifetime spontaneous tumor incidence in an outbred Swiss albino mouse strain rose, in successive generations, from about 10% to about 80% (Roe & Tucker, 1974; Roe, 1981). Since careful efforts had been made to prevent genetic drift and to standardize the diet supplied, the reason for the increasing tumor incidence was puzzling. During the same 10-year period, the average mature weight of animals rose substantially and longevity was reduced. That overnutrition was at least an important part of the explanation of all three phenomena—ie, obesity, high tumor incidence, and poor longevity—was supported by the simple expedient of seeing what happened when the food intake of animals was restricted to 80% of the amount that ad libitum-fed animals eat. The results were dramatic. The avoidance of overweight by this means led to dramatically improved survival and to a dramatically reduced incidence of neoplasms.

The experience of ICI during the 1960s has subsequently been mirrored by that in experiments involving both rats and mice in many other laboratories (Rao, 1991; Abelson, 1992). Those engaged in carcinogenicity screening have shown little interest in discovering why untreated control animals develop tumors and have largely ignored nongenotoxic mechanisms. Present-day approaches to carcinogenicity testing largely fail to face up to the twin problems of how to account for the tumors that arise in untreated control animals and how to explain the mechanism by which factors such as overnutrition, natural hormones, and nongenotoxic xenobiotic chemicals can increase the risk of cancer development.

Table 1 Effects of Eating 25% More Calories on Relative Risk in Rats (95% Confidence Limits)

Outcome	Males	Females
Premature death*	2.40(1.61–3.59)	3.60(2.42–5.59)
Fatal malignant neoplasm*	4.80(2.73–8.74)	3.34(1.97–5.66)

*Before age of 133 weeks.
Data is from Roe FJC, Lee PN (1991). Carcinogenicity tests. *Lancet, 337,* 587.

The concept that cancer could be wholly avoided if there were no exposure to environmental carcinogens has led to the paradox that experimentalists seeking ways of reducing cancer incidence in humans have spent all their efforts in identifying factors that increase cancer incidence in animals. In doing this experimentalists have been ignoring the fact that untreated animals in control groups living in a protected laboratory environment are about as likely to develop cancers and die from them as humans who are not members of any high risk group (eg, smokers, persons exposed to asbestos or other industrial carcinogens, etc). Hitherto, there has been regretably little research on how to prevent cancer development in laboratory animals.

1200-RAT BIOSURE STUDY

In a large collaborative study (Roe, 1991), groups of 50 male and 50 female Wistar rats were fed on 12 different diets/dietary regimes without any deliberate exposure to any known genotoxic carcinogen. The experiment was started when animals were weaned and was continued until they died, had to be killed for humane reasons, or reached the age of 133 weeks. During the first 13 weeks of the experiment rats were fed according to one regime and thereafter according to the same or to another regime. Included among the 12 groups were three that were provided with ad libitum access to food throughout the experiment and two that were restricted to 80% of the food consumed by ad libitum-fed animals either throughout the whole experiment or from 13 weeks onwards. (This mild level of calorie restriction did not stunt growth in terms of femur length, brain weight, etc.) These latter two calorie-restricted groups lived significantly longer and developed significantly fewer neoplasms, including fatal or potentially fatal malignant neoplasms, than the three ad libitum-fed groups. The findings expressed as relative risks (RR) are summarized in Table 1. Animals died prematurely or had to be killed for humane reasons, including malignant neoplasia and manifestations of aging-associated diseases. By eating 25% more calories than the diet-restricted animals, the ad libitum-fed males increased their chances of dying from a fatal cancer before the age of 133 weeks 4.8-fold while females did so by 3.34-fold (Roe & Lee, 1991). Table 2 lists some of the most statistically

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Table 2 Some Effects of 80% of Ad Libitum Calorie Restriction Seen in the BIOSURE Study

Organ/tissue	Males	Females
Artery (pancreatic)		
Polyarteritis		$P < 0.001$
Epidermis/adnexa		
Benign or malignant neoplasm	$P < 0.05$	$P < 0.05$
Heart		
Chronic myocarditis	$P < 0.001$	$P < 0.001$
Kidney		
Neophropathy (any)	$P < 0.001$	$P < 0.001$
Nephropathy (severe)	$P < 0.001$	$P < 0.001$
Lung		
Benign or malignant neoplasm	$P < 0.05$	$P < 0.05$
Mammary gland		
Acinar hyperplasia		$P < 0.001$
Secretory activity	$P < 0.001$	$P < 0.001$
Benign or malignant neoplasia		$P < 0.001$
Adenocarcinoma		$P < 0.1$
Pancreas (islet-cell)		
Adenoma or adenocarcinoma	$P < 0.01$	
Pituitary (anterior lobe)		
Adenoma or adenocarcinoma	$P < 0.001$	$P < 0.01$
Pituitary (intermediate lobe)		
Adenoma or adenocarcinoma		$P < 0.01$
Prostate		
Acute inflammation	$P < 0.05$	
Subcutaneous tissues		
Benign or malignant neoplasm	$P < 0.05$	$P < 0.05$

Data is from Roe FJC, Lee PN (1991). Carcinogenicity tests. *Lancet, 337,* 587.

significant effects of overnutrition on degenerative and neoplastic diseases observed in the study.

Other groups in the 1200-rat study were fed on a low-energy (high-fiber) diet or according to a regimen in which calorie restriction was attempted—but not very well achieved—by limiting access to food to 6 hours per day. These stratagems led to slightly reduced weight gain, slightly improved survival, and slightly lower incidences of neoplasms than seen in animals fed ad libitum on the standard diet.

In the study as a whole, highly significant correlations were found between, on the one hand, body-weight 6 months after the start of the study and, on the other hand, subsequent risk of premature death ($P < 0.001$) and the risk of death from malignant neoplasia before the experiment was terminated at 133 weeks (P

Table 3 Effect of Eating 25% More Calories on Mean Liver Weight as a Percentage of Body Weight

Calorie intake	Males	Females
Ad libitum	3.7	4.2
80% of ad libitum	3.3	3.6
Significance of difference	$P < 0.01$	$P < 0.01$

< 0.001; see Roe et al, 1991). These significant correlations were found despite the fact that different groups were maintained on different diets and different dietary regimens.

Reduced calorie intake was associated with effects on the organ principally involved in metabolism, namely, the liver. These included reduced liver weight as a percentage of body weight (Table 3) and lower levels of P450 enzyme production resulting in prolonged phenobarbitone sleeping time. Nearly 25 years ago Ross (1969) reported such effects of nutrition on hepatic enzyme activity patterns in the rat. Despite this, toxicologists have still been using liver enzyme levels as an end point in toxicity studies under conditions in which they make no attempt to control caloric intake.

RELATIONSHIP BETWEEN AGING AND INCREASED CANCER RISK

Until very recently the disciplines of nutrition, toxicology, and gerontology have, for the main part, kept their distance from each other. Nutritionists have seemingly been obsessed with ensuring the avoidance of dietary deficiencies and have assumed that maximum and rapid growth is indicative of good nutrition. Furthermore, the farming community has succored this assumption in relation to the production of meat for human consumption. Most nutritionists have failed to study the relationship between food intake throughout life and the incidence of diseases that mostly occur late in life, including the degenerative diseases of old age and neoplastic diseases. On the other hand, toxicologists have taken far too little interest in the diets they have fed to animals in toxicity tests. They have been content to assume first that the diets formulated for laboratory animals by suppliers have been fully researched and, second, that, since they were giving the same diets to animals in treated and control groups, the precise nature of the diet was not important. Third, although it has been known for many years that dietary restriction is associated with reduced tumor incidence in laboratory rodents, (McCay et al, 1935; Ross and Bras, 1965) and that, irrespective of dietary composition, calorie restriction reduces cancer risk (Masoro, 1983), little thought

has been given to the concept that overnutrition per se is carcinogenic. Instead most investigators have assumed that by simply eliminating genotoxic carcinogens from food irrespective of calorie intake, they could find ways of reducing cancer risk in humans.

The book by Weindruch and Walford (1988) constituted a landmark by bringing together the three fields of nutrition, gerontology, and carcinogenesis. From the research they review emerges the theory that damage to DNA or to cell proteins by free radicals, glycosylation, or other cross-linking mechanisms may be involved both in aging and in carcinogenesis.

Increasing interest in the existence of such an association has, unfortunately, so far had little impact on approaches to cancer prevention or on the design and interpretation of the results of tests for carcinogenicity in laboratory rodents.

NONGENOTOXIC MECHANISMS OF RELEVANCE TO THE EFFECTS OF CALORIE RESTRICTION ON CANCER RISK AND DEGENERATIVE DISEASES

A nongenotoxic carcinogen may be defined as an agent that does not primarily damage DNA or chromosomal structure, but which enhances the risk of cancer development by some other means. The most currently favored theory is that the nongenotoxic agent increases the risks of DNA damage and other cellular damage from endogenously generated electrophiles either by increasing their production or by reducing the time available for repair of DNA damage prior to cells dividing to form daughter cells.

Oxygen radicals can reversibly or irreversibly damage all biochemical classes including nucleic acids, proteins, free amino acids, lipids, lipoproteins, carbohydrates, and connective tissue macromolecules. They may be generated endogenously in the course of the mitochondrial, microsomal, and chloroplast electron transport chains. In addition, certain endogenous enzymes generate free radicals as also do phagocytic cells. These endogenous sources supplement exogenous sources such as ionizing radiation, tobacco smoke, sunlight, redox-cycling substances, and drugs. In humans, oxygen radicals are thought to be implicated in the causation of a wide variety of conditions including aging, arteriosclerosis, senile dementia, rheumatoid arthritis, and various other degenerative conditions as well as cancers (Cross, 1987; Gensler & Bernstein, 1981; Johnson et al, 1986).

Cumulative cancer risk increases as approximately the fourth power of age and, irrespective of the usual lifespan of the species, the accumulated cancer risk is approximately 30% by the end of the life span (Ames & Saul, 1987). Broadly speaking, there is an inverse relationship between metabolic rate on the one hand

and body size, longevity, and age-standardized cancer risk on the other. In the 1200-rat study, a highly significant correlation was seen between (a) body weight, (b) longevity, (c) age-standardized incidence of degenerative diseases, and (d) age-standardized cancer mortality. Unfortunately, it is not known whether the diet-restricted animals had a lower basal metabolic rate than the ad libitum-fed animals.

Sacher (1977) postulated that diet restriction does lead to lower basal metabolic rate. However, Weindruch and Walford (1988) were critical of their calculations and Masoro et al 1982) found that in Fischer 344 rats, within 6 weeks of starting a food restriction regime, the Kcal value of food ingested per unit of body mass was higher in diet-restricted rats than in ad libitum-fed rats. McCarter et al (1985) and McCarter and McGee (1989) also reported that the daily metabolic rate per unit of lean body mass was the same in food-restricted rats as that in ad libitum-fed rats within 6 weeks of starting restriction.

All in all, there is not at present any convincing evidence that within species variations in metabolic rate are associated with differences in incidence of degenerative diseases or cancer.

By contrast, interest is growing in the association between increased cell turnover and increased cancer risk and in the possibility that endogenous electrophiles contribute to the proportion of DNA damage that remains unrepaired in circumstances where cell replication rates are increased (Cohen & Ellwein, 1990; Preston-Martin et al, 1990; Ames, 1989; Ames & Gold, 1990).

According to Weinstein (1991), however, Ames and Gold (1990) went too far when they suggested that endogenous electrophiles are mainly responsible for the mutations involved in carcinogenesis and that exposure to environmental xenobiotic chemicals adds negligibly to the overall risk. He pointed out first that whatever else, carcinogenesis is a complex multistage phenomenon involving, inter alia, sequential genetic changes, cell proliferation and clonal expansion, activating mutations in proto-oncogenes, inactivating mutations in putative growth suppressor genes, and gross chromosomal aberrations. This leaves a place for agents that stimulate cell proliferation to contribute to the carcinogenic process by stimulating clonal expansion of mutant cells rather than by impeding effective DNA repair. Furthermore, Weinstein (1991) was not convinced that DNA damage produced by endogenous mutagens has the same deleterious consequences as that produced by some exogenous mutagens.

Weinstein's warning against oversimplification is timely. Nevertheless, the evidence that overnutrition substantially increases cancer risk and that calorie restriction reduces it is strongly supportive of the view that endogenous mutagens contribute significantly to the risk of cancer in laboratory rodents. It is implausible that a mere 25% increase in intake of dietary genotoxins could explain the effects seen on the incidence of cancers, degenerative diseases, and longevity in the BIOSURE Study. Some other explanation is needed. A clue to what this

may be is supplied by evidence that dietary restriction is associated with reduced cellular proliferation rates in various tissues (Heller et al, 1990; Lok et al, 1990).

RELEVANCE OF LABORATORY EVIDENCE OF BENEFICIAL EFFECTS OF CALORIE RESTRICTION TO PREVENTION OF CANCER IN HUMANS

It is difficult to categorize people in terms of what they eat and virtually impossible to do so in terms of how much they eat. Comparisons of populations in rich and poor countries are of limited value because poverty determines what rather than how much people eat, and in any case one cannot compare "like" with "like" with respect to intake of dietary carcinogens and of factors other than diet that are known to affect longevity and cancer incidence. For these reasons it is difficult to obtain hard evidence that calorie restriction, per se, has as much effect on longevity and cancer incidence in human beings as it does in laboratory animals. Nevertheless, it would be very surprising in view of the strength and consistency of the laboratory animal data if overnutrition were not an important contributor to cancer risk in humans.

Most published human studies in this area have been concerned with the association between dietary fat intake and incidence of cancers at particular body sites such as the breast and colon. In light of the results of such research, it is widely accepted that high dietary fat intake is an important cancer risk factor. Furthermore, this theory is plausible insofar as the peroxidation of fats is especially productive of oxidative damage to DNA and other cellular structures. However, laboratory research strongly points to calorie intake, as distinct from any aspect of dietary composition, as being the more important cancer risk factor.

Another problem in research aimed at defining the contribution of dietary factors to human cancer risk is that animal studies indicate that the beneficial effects of dietary restriction on cancer risk increase with the period of restriction, with the most marked effects being seen where calorie restriction dates back to weaning or adolescence. Unfortunately, most epidemiological studies are only of short duration (eg, less than 10 years) and case:control studies that rely on memory of past food intake are extremely unreliable, particularly with regard to the quantity of food consumed.

A cradle-to-grave prospective study that includes some objective measurement of calorie intake as well as detailed information on exposure to known cancer risk factors is clearly needed. According to Cathcart et al (1984), urinary levels of thymine glycol and thymidine glycol provide an index of repair of oxidative damage to DNA. Also, Loft et al (1992) found significantly ($P < 0.001$) higher levels of the DNA-repair product, 8-hydroxy-deoxyguanosine, in the urine of smokers as compared with nonsmokers and significantly lower levels in the urine of persons with a 23% below average energy intake. Could measurements of the levels of thymine and thymidine glycols and/or of 8-hydroxy-

deoxyguanosine from regularly collected urine samples be used as a surrogate for assessing overnutrition over a period of many years?

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