Life-style and Cancer : Rats and Humans

Author : Francis J. C. Roe
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During the last 40 or so years great strides have been made towards understanding mechanisms by which chemicals can predispose to the development of cancer. However, some of those involved in making these advances have been guilty of selectively ignoring observations which do not fit their over-simplistic theories.

The main objective of laboratory research in the field of carcinogenesis is to find ways of reducing the incidence of cancer in humans and in species of animals which serve as pets or sources of food for humans. During the 1960s studies on people who migrate from one geographical area and/or one culture to another were found to change their risks of developing different forms of cancer. Particularly striking in this regard were the decreases in risk of developing stomach cancer and increases in risk of developing colon cancer in ethnic Japanese who settled in Honolulu or on the West coast of the United States and changed from a Japanese life-style to a Western life-style (Haenszel, 1961, 1970). In the light of observations of this kind it was hypothesized that environmental, as distinct from genetic, factors are primarily responsible for some 80 to 90% of human cancers.

During the late 1960s and early 1970s studies on a wide variety of known potent animal carcinogens showed that such agents themselves, or metabolites derived from them, damage DNA and cause mutations. From this it was deduced that mutation of normal cells to cancer cells is the key step in carcinogenesis.

During the 1940s and 1950s, research by Peyton Rous and his colleagues (Rous and Kidd, 1941, Mackenzie & Rous, 1944, Fridewald & Rous, 1944) and by Berenblum and Shubik (1947a, 1947b, 1949) led to the popularization of the two-stage theory of carcinogenesis. According to this theory, the cancerous process is initiated by mutation and may thereafter be promoted by agents which stimulate cell-division such that single mutant cells become clones of altered cells. The two-stage theory was heavily reliant on findings in stereotyped skin-painting studies in mice, involving a very limited number of chemical agents to which animals were exposed, as a rule, for only relatively brief periods. Most of the tumours observed were benign warts which regressed after exposure to the, so-called, tumour-promoting agent was discontinued, or even despite continued exposure to it.

Critics of the theory, including myself (Roe, 1988, 1989) and more notably, Olav Iversen in Oslo (Iversen & Astrup, 1984; Iversen & Iversen, 1982) demonstrated that the theory was seriously over-simplistic. In particular we found that all the various non-genotoxic agents that had previously been reported to be capable of promoting tumour development but not of initiating it, are in fact complete carcinogens.

Despite the evidence that the two-stage theory is flawed, even in respect of the tissue in which it had been most studied (i.e. mouse skin), it is still being widely applied to in relation to experimental carcinogenesis studies in other tissues. Also, the conclusion that 80-90% of human cancers are environmental rather than genetic in origin was glibly translated into the simple assumption that exposure to genotoxic carcinogens with or

without the assistance of tumour-promoting agents present in the environment is responsible for these cancers. Furthermore, when it later became clear that exposure to certain non-genotoxic agents is associated with increased incidence of cancers both in laboratory animals and in man it was assumed that such agents act by promoting the multiplication of cells that had undergone mutation as a consequence of previous exposure to an initiating genotoxin.

One object in the present lecture is to show why these simplistic theories are seriously wrong and how they are misleading us in the way we lead our lives and misguiding regulators and law makers charged with the responsibility of trying to protect the public from developing avoidable cancers.

Genetic versus environmental causes of cancers

It always has been clear in the case of laboratory animals that genetically different strains (e.g. of mice or rats) differ widely both in the incidence and in the spectrum of neoplasms which they develop 'spontaneously'. [The word 'spontaneously' has to be put in parenthesis insofar as it is never possible completely to avoid exposure to carcinogens (e.g. contaminants in food, cosmic radiation, etc.)] During recent years molecular biologists have been identifying genes which influence susceptibility to particular cancers not only in laboratory animals but also in humans. Examples of genetically determined human cancerous diseases are xeroderma pigmentosum, which is characterized by increased risk of skin cancer because of the lack of the enzymes involved in the repair of uv-induced DNA damage (Cleaver, 1969), and the Li-Fraumeni Syndrome, which is characterized by a high risk of various neoplasms -

including leukaemia, sarcomata, breast and brain tumours - because of a germ-line mutation in the tumour-suppressor p53 gene. (Hollstein et al, 1991).

Although, personally, I do not dispute that environmental factors contribute more than genetic ones as determinants of cancer risk, I do feel that the contribution of inherited genetic factors tends to be under-estimated, particularly since the usual situation is that both genetic and environmental factors interact in the aetiology of most diseases - both cancerous and non-cancerous.

Testing for mutagenicity as a surrogate for testing for carcinogenicity

The realisation that environmental factors are heavily implicated as determinants of cancer risk led some investigators, quite erroneously, to assume, firstly, that most cancers are due to exposure to environmental mutagens, and secondly, that if there was no exposure to environmental mutagens the risk of developing cancer would be largely abolished. During the 1970s and 1980s, this concept of cancer causation led to major expenditure on the testing chemicals for genotoxicity and ability to cause tumours in laboratory animals. However, because of the high costs of long term animal studies, increasing emphasis was put on screening for genotoxicity using in vitro short-term tests on cultured bacteria, yeast cells or mammalian cells.

It was widely recognised from the start that chemicals which are themselves not genotoxic or carcinogenic might be metabolized to more reactive chemicals which possess these types of activity. Consequently, it become customary to add to the culture media a concoction of microsomal liver

enzymes derived from rats. The assumption was made that if inactive chemicals could be converted to carcinogenic metabolites, the enzymes necessary for this so-called metabolic activation would be present in rat liver. Even today the extent to which this latter assumption is true is not really known.

The discovery of naturally-occurring mutagens

At first the majority of tests for genotoxicity were on man-made as distinct from naturally-occurring chemicals because there was a widely held belief that the Almighty in His infinite wisdom, would not have been so foolish as to allow naturally-occurring chemicals to be genotoxic. Only when it became clear that evolution did not preclude the natural occurrence of mutagens was a broader approach taken in the selection of chemicals for testing.

Today it is clear that numerous, naturally-occurring chemicals are genotoxic. Moreover, this is not merely an accident of evolution. Many species of plant rely for their survival on their ability to produce toxins, many of which are genotoxic, in order to avoid elimination by potential predators. Bruce Ames (1989) coined the term 'Nature's Pesticides' to describe such toxins.

Non-genotoxic carcinogens as distinct from non-genotoxic carcinogenicity

It is only during the last decade that the existence and importance of non-genotoxic carcinogens has begun to be recognised. Recognition was delayed because of the ease with which many known potent animal carcinogens had been shown to damage DNA and cause mutations. Also, it was

usually easy to show that cancer cells differed genetically from the normal cells from which they were derived. These two bits of evidence argued against there being any such phenomenon as non-genotoxic carcinogenesis. Although this may well be true, it should not be inferred that only agents which are themselves directly genotoxic, or which can be converted to genotoxins in the body (i.e. by metabolic activation) can act to increase the risk of cancer development. We now know of many non-genotoxic substances which are indirectly but not directly genotoxic, and it is to these that the term "non-genotoxic carcinogen" applies.

Some non-genotoxic carcinogens : just the tip of an iceberg

As soon as the term "non-genotoxic carcinogen" achieved respectability, examples became easy to find. Table 1 lists a random selection of examples derived from laboratory studies on rodents with suggestions as to the mechanisms involved and references to the relevant literature.

A feature of many of the examples is that they involve unnaturally high levels of exposure (e.g. lactose) and a feature of all of them is that they involve major disturbances of physiological, nutritional and/or hormonal status and long-standing hormone-induced or irritation-induced cellular hyperplasia prior to the onset of neoplasia.

Some of the examples appear to be species-specific and some sex-specific. Thus croton oil and 12-O-tetradeconoylphorbol-13-acetate (TPA) only produce skin tumours in mice and lactose, sorbitol and chemically-modified starches only produce adrenal medullary

tumours in rats. In the case of the d-limonene induced globulin nephropathy, which predisposes to kidney tumours in rats, only males are susceptible.

There are probably thousands of different mechanisms by which non-genotoxins can predispose to cancer and many of these await discovery. The most important aspect of the situation, however, is not the multiplicity of possible mechanisms, but the features which the different examples have in common. In particular, a prolonged state of hyperplasia - either hormone-induced or a manifestation of repair following cell death - is associated with all the examples listed in Table 1.

A possible common mechanism in carcinogenesis by non-genotoxic agents

There is now abundant evidence that genotoxins are produced during the normal processes by which body cells convert food substances to energy. According to Ames (1983, 1989, Totter, 1980), four endogenous metabolic processes are likely to lead to significant DNA damage. These are oxidation, methylation, deamination and depurination (see also Saul and Ames, 1986 and Totter, 1980). Of these, oxidative DNA damage is probably the most important. Oxidants are produced as by products, particularly during the peroxidation of lipids. Fortunately, body cells are well equipped with enzymes that are capable of repairing most forms of DNA damage. And where damage cannot be repaired, the chances are that the affected cells are soon replaced. Nevertheless, under ordinary life conditions some cells with unrepaired DNA and/or with damaged cell proteins persist and, maybe, even multiply. Thus, there would appear to exist a mechanism whereby in the absence of exposure to

any environmental mutagen, cells with damaged proteins and/or DNA can build up within the body.

It has been calculated that, on average, the DNA of each cell in the human body suffers damage at 10^4 points each day (N.B. the figure for rats is 10 times higher at 10^5 points each day) (Ames and Gold, 1990) and that almost all this damage is efficiently repaired. However, there is one weak brief period during the cell cycle when the process of DNA repair is impaired. This is while the cell is replicating, and DNA exists in the form of single strands. Consequently, agents - ie irritants or hormones - which stimulate cell-replication are likely to increase the risk that unrepaired DNA-damage - ie mutant DNA - will be passed on to daughter cells. This is the basis of the theory that mitogenesis predisposes to mutagenesis (Cohen and Ellwein, 1990; Ames and Gold, 1990).

This explanation of how non-genotoxic agents can give rise to cancer also enables one to understand how caloric restriction protects against the development of cancer. There is now accumulating evidence that caloric restriction is associated with a slower rate of cell turnover (see Lok et al, 1990) as well as, no doubt, with a slower rate of production of DNA-damaging electrophiles.

Factors which influence cancer risk in man

Wynder and Gori (1977), Higginson and Muir (1979) and Doll and Peto (1981), after reviewing available epidemiological data in the light of other knowledge concerning causation, listed their best estimates of the contributions of different factors to the causation of human cancers. Table 2 summarizes the conclusions reached by Doll

and Peto (1981). It is interesting to consider some of their conclusions in more detail.

Occupation and cancer risk in humans

Historically, there have been several outbreaks of cancer among workers in particular industries as a consequence of their exposure to particular chemicals. Well nown examples are scrotal and skin cancers among chimney sweepers and cotton workers exposed, respectively, to coal tar and unrefined mineral oils; bladder cancers in chemical and rubber workers exposed to antioxidant aromatic amines, such as -naphthylamine; cancers of various sites in radiographers and radiotherapists etc. exposed to ionizing radiation, etc. Continuing to the present day are cancers of the lung and mesothelium due to the inhalation of non-genotoxic asbestos fibres and lung cancer in persons involved in ion-exchange resin manufacture and chloromethylation processes because of exposure to the highly genotoxic chemical bis-chloromethyl ether (BCME). In the case of BCME, deaths from lung cancer in men aged around 40 suggest that its carcinogenic potency is orders of magnitude higher than that of cigarette smoke (Roe 1985; Van Duuren and Van Duuren 1988). In fact, most occupation-related cancers can be avoided if appropriate occupational hygiene standards are applied. Furthermore, tragic though all such examples are, only a small minority of cancers are, nowadays, attributable to exposure to carcinogens at work.

Doll and Peto (1981) estimated that only 4% of human cancer deaths are occupational in origin, though the figure might be as high as 8% or as low as 2%. Earlier, Wynder and Gori (1977), referring to clinically-diagnosed cases of

cancer, as distinct from deaths from the disease, estimated that 4% of cancers in man and 2% in women are attributable to occupational exposure. Similarly, Higginson and Muir (1979), referring to cases of cancer in the Birmingham area of England, estimated that 6% of cancers in men and 2% in women are occupational in origin.

It should not be assumed, however, that all occupation-associated cancers are due to exposure to chemicals, dusts or fumes etc. at work. Nearly 300 years ago Ramazzini (1700) observed that nuns are at higher risk of developing breast cancer than child-bearing women in the greater population. Today over two and a half centuries later we have some insight into why this is so. MacMahon et al (1973) found that the bearing of a child before the age of 30 protects a woman against developing cancer of the breast compared with bearing a first baby after the age of 30 or with remaining nulliparous. This observation indicates that the higher risk of breast cancer in nuns is attributable not to what they are exposed to but to what they are not exposed to!

Environmental pollution, pesticides, medicines, etc. versus life-style factors as determinants of cancer risk in humans

According to Doll and Peto (1981),these factors, between them, contribute less than 9% of all cancer deaths. By comparison tobacco, alcohol, diet and reproductive and sexual behaviour contribute 75%, i.e. more than eight times as many. It is convenient from the viewpoint of this lecture to bracket these four factors together as constituting life-style.

Reproductive and sexual behaviour

The contribution of reproductive and sexual behaviour mainly concerns women firstly because the risk of dying from cancer of the uterine cervix is increased by multiplicity of sexual partners and secondly, because the risks of dying from cancers of the breast, ovary and endometrium are higher if they bear no children, or delay doing so until after they reach the age of 30. The increased risk of cervical cancer probably arises because venereally-transmitted viruses are implicated in the aetiology of the disease. The risks of ovarian, endometrial and breast cancer are related to complex differences in hormonal status between nulliparous women and women who have first borne children whilst still reasonably young.

Smoking and alcohol

The association between smoking cigarettes and increased risk of various cancers including lung, larynx, oral cavity, oesophagus and urinary bladder, is too well documented for me to dwell on here. In non-smokers, light or moderate alcohol consumption has relatively little effect on cancer risk and, in any case, it is difficult to distinguish between effects of alcohol *per se* and other chemicals present in alcoholic beverages. Heavy alcohol consumption, however, is associated with increased risk of cirrhosis of the liver and this, many believe, is in turn associated with increased risk of liver cancer. In the case of cancer of the oesophagus, there is persuasive evidence of synergism in relation to smoking and alcohol consumption on risk.

Diet

Doll and Peto (1981), Wynder and Gori (1977) and Higginson and Muir (1979) all concluded that dietary factors have an even larger influence on cancer mortality than smoking and alcohol consumption together.

A large part of the human brain is concerned with the sense of smell. It is understandable that this should be so because it is initially by this sense that one is able to distinguish between edibility and non-edibility. However, as food processing and mass marketing of prepared foods has burgeoned, Westernized man is in danger of ignoring both what his olfactory sense tells him and what his eyes or even his taste buds tell him. Instead, he is all too apt to rely on the 'sell-by date' and other information on the label. Not surprisingly, therefore, a mythology has grown up concerning the dangers associated with food additives and food contaminants including pesticide residues. In these areas widespread phobia has been fostered, not only by ambitious, but largely ignorant, reporters vying to grab headlines, but also by scientists blinkered to all but their own narrow areas of research.

The fact that huge amounts of money are spent both nationally and internationally on the safety testing, monitoring and regulating of the use of food additives further encourages the general public to believe that their health, including their risk of developing cancer, is heavily influenced by man-made chemicals present in the food they eat. Of course it may be that cancer risk from food additives and contaminants is only very low because of the effectiveness of measures taken by governments and international regulatory bodies. However, it is also

arguable that at the present time the money spent on this continuing activity could be better spent in other ways.

In parallel with this there has been a succession of preachers of different gospels providing the public with often quite conflicting advice on what it is healthy for them to eat and what is not. It may well be true that people who eat less fat, more fibre and more fresh vegetables, etc. are less at risk of developing diseases such as atherosclerosis, colon cancer, appendicitis and diverticulitis than they would otherwise be. However, the benefits of switching to a putatively healthier diet late in life are difficult to prove or quantify. Almost certainly the effects of inappropriate diets build up throughout life from childhood onwards. Also, and most importantly, it is now becoming clear that overall caloric intake, as distinct from dietary composition, is the factor of paramount importance. This has been shown to be true time and again in laboratory studies, but has been more difficult to demonstrate in man, because people have poor recall when it comes to remembering what they ate a week ago, let alone what they ate years ago or when they were children. Furthermore, it is virtually impossible to obtain reliable information on how much people eat, or have eaten in the past. 'Fat pigs' and 'bean poles' alike claim to eat 'just normal-sized portions'. But one has only to observe them closely at social functions to see that 'normality of size of portion' covers a very wide range!

Dietary fat and breast cancer in humans

The relationship between intake of saturated fat and/or meat has been much studied in relation to breast

cancer. According to Williams (1993), the findings in 18 case:control studies of this relationship had been published up to 1990. Of these, 6 indicated increased risk in association with higher intake of saturated fat, and 2 with higher intake of meat. By contrast, 6 found no association with intake of any dietary constituent.

An editorial in the Lancet of February 1993 asks, in relation to breast cancer: 'Have we lost our way?' The article points out that, despite claims by Cancer Charities and despite much media hype, the mortality rate from cancer of the breast remains static. This suggests that the many qualitative changes in what people eat which have taken place during the past two or so decades have had no impact on breast cancer mortality. Perhaps there needs to be more emphasis on reducing caloric intake and on diet during the first 50 years of life rather than solely on dietary composition during later life.

Dietary fat, fibre, body weight and energy value of the diet in relation to risk of colon cancer in humans

Although comparisons of populations suggest that high consumption of fat and animal protein is a feature of populations with high incidences of colon cancer (Drasar and Irving, 1973; Carroll and Khor, 1975; Miller et al, 1983), it is not clear whether the association is causal in nature. The possibility that reduced consumption of foodstuffs which protect against the development of cancer, such as fibre and polyunsaturated fish oils, is responsible for the high risk has not been disproved (Stemmermann et al, 1984). However, Bingham et al (1979) did not find fibre to be protective. Finally the ingenious theory that the consumption of saturated fats leads to changes in the gut flora such that

carcinogens are produced in the gut lumen from bile acids (Hill et al, 1971) remains of uncertain importance.

Wynder and Shigematsu (1967) found a weak association between obesity and colon cancer in men, but no clear association in women. Bristol et al (1985) found that the habitual diet of patients with bowel cancer led them to consume, on average, 16% more energy than control subjects without bowel cancer.

Alas, probably the most important truth of the situation was stated by Wynder and Shigematsu (1967) when they wrote "Dietary information on specific food items as obtained in retrospective histories is of little value in determining the influence of diet on cancer of the large bowel".

Association between food intake, body weight and cancer in general in humans

Perhaps the best evidence of a beneficial effect of caloric restriction in humans comes from a comparison of food intake and death rates from heart disease, cerebro-vascular disease and cancers in Okinawan Japanese and Japanese in general, between whom there are no genetic differences (see Kagawa, 1978). In Japan food consumption data are obtained annually from randomly chosen households in hundreds of districts of Japan. This collection of data involves detailed personal interviews and the direct weighing of the foods eaten during a period of 3 consecutive days. Insofar as the lower calorie intake begins in childhood, it is not surprising that the Okinawans are shorter and lighter in body weight than other Japanese. As shown in Tables 3 & 4 the lower intake of calories is

associated with increased longevity and reduced incidence of degenerative and neoplastic disease in much the same way as occurs in laboratory rodents.

In a large study sponsored by the American Cancer Society (Lew and Garfinkel, 1979), in both men and women, excessive body weight was found to be associated with increased mortality from certain cancers (see Tables 5 & 6).

Influence of life-style on longevity, ageing and cancer incidence in laboratory rodents

One may think that there is little scope for studying the influence of life-style variables in laboratory rodents that spend their lives confined to cages located closely controlled environments. However, the limitation in the number of variables involved facilitates interpretation of any observations that are made. Thus one does not have to standardize data in respect of smoking, drinking or sexual habits and one can closely control what and how much animals eat. Additionally, one can, by providing exercise wheels, study the effects of physicial exertion.

I have been involved in two small studies and one very large study of the influence of life-style factors in rodents. These merit brief description here.

Experiment 1 (Salmon et al, 1990)

In this experiment 4 groups of 20 male Wistar rats and 3 groups of 20 female Wistar rats were used to study the following 4 variables:-

(i) The effect of limiting access to a standard laboratory chow to 6.5 hours/day instead of making it available for 24 hours/day

(ii) Housing males in a single-sex room instead of in a mixed sex room

(iii) Life-long enforced celibacy for males compared with access to one fresh virgin female for 5 days during each alternate week

(iv) Uniparity versus life long virginity in females

Table 7 summarizes the design of the study and Table 8 the main findings. Restriction of access to food to 6.5 hours/day resulted in reduced food intake (80% of that consumed by animals given continuous access), proportionately reduced body weight gain, improved survival and significant reduction in relative liver weight, various ageing-related diseases and benign and malignant neoplasia. Housing animals in a unisex room instead of a mixed sex room was without significant effects. Uniparity reduced the prolactin-mediated mammary gland hyperplasia associated with nulliparity, and the opportunity for sexual activity increased food consumption and longevity in males despite significantly increasing the incidence of Leydig-cell hyperplasia and neoplasia in the testis.

Experiment 2 (Conybeare, 1988)

In this study groups of 32 male and 32 female CD-1 and B6C3F1 hybrid mice were fed on a standard diet either ad libitum or restricted to 75% of ad libitum. In parallel

similarly sized ad libitum-fed and restricted groups were provided with an exercise wheel - one wheel per cage of 4 mice.

In each of the boxes with wheels mice queued up to get on the wheel, but only achieving their goal when the mouse ahead of them fell off from giddiness or exhaustion. Whilst on the wheel, the eyes of mice glazed over and their little faces assumed the expression of ecstatic blankness that one associates with obsessional jogging. By multiplying the inner circumference of the wheel by the number of revolutions clocked up it was possible to calculate the mean distance run by each mouse in each cage per day. These means ranged up to 5.02 kilometers per 24 hours (see Table 9).

As expected, dietary restriction reduced body weight gain in both sexes and both mouse strains. By contrast, exercise had virtually no effect on body weight in either strain or either sex. In CD-1 mice, restriction either alone or in combination with exercise, reduced survival up to two years reduced life span in males, while a combination of restriction and exercise increased survival in females. In CD-1 mice, restriction alone or in combination with exercise, was associated with reduced tumour incidence, whereas exercise alone had little effect. Too few of the hybrid mice had died before the termination of the experiment at 2 years to judge whether either restriction or exercise had affected either survival or tumour incidence.

All in all the findings provided little encouragement to those who imagine that exercise can counter the adverse effects of overeating.

Experiment 3 : The 1200 rat Biosure study

A few years ago my colleagues and I embarked on a huge study in Wistar rats involving 12 different groups, each of 50 males and 50 females, plus several sub groups. It was already clear from numerous other studies that simple dietary restriction confers benefits to rats in terms of survival, incidence of ageing-related diseases and incidence of neoplasia of any kind. However, as pointed out in a prelimary account of the study (Roe, 1991), several important questions remained to be answered. The list included:-

(i) Does restriction reduce the incidence of malignant, potentially fatal, neoplasms as well as benign tumours?

(ii) Can diet restriction be reliably achieved by restricting access to food to 6 hours per day?

(iii) Can diet restriction be achieved by limiting the energy value of the diet?

(iv) Does restriction confined to the first 4 months of life during which rats are growing rapidly confer lasting benefits in terms of survival, incidence of ageing diseases and neoplasia?

(v) Is there a relationship between body weight early in life and (a) survival and (b) tumour incidence?

(vi) Are in-life serum chemistry, haematological, urinalysis or circulating hormone levels of any value in predicting the long term fate of animals?

The design of the study is too complex and the findings too numerous for me to present more than just a few of the highlights here in tabular form (see Tables 10, 11, 12).

Clearly simple caloric restriction to 80% profounding affected the age-standardized incidence of fatal malignant tumours as well as the incidence of benign tumours. Furthermore, the beneficial effect on tumour incidence appeared to be a general one affecting all sites including, specifically, the epidermis and adnexa, connective tissues, mammary gland, anterior and intermediate lobes of the pituitary, pancreas islet cells and exocrine cells and the lung (see Table 10).

For reasons which are not understood in the light of the findings in Experiment 1, restricting access to food to 6 hours per day did not effectively reduce caloric intake and offered very few of the benefits seen in the animals restricted by rationing.

If the findings for malignant tumours are presented in the same way as epidemiologists nowadays usually present human cancer mortality data, i.e. by calculating relative risks (RR) with upper and lower 95% confidence limits, we see that simple dietary restriction is associated with very big, and highly significantly, lower RR in both sexes of rat (see Table 11 and Roe and Lee, 1991).

Although a variety of diets and regimes was used in the study, it was of interest to see whether, notwithstanding this variation, there was any relationship between body weight in animal early in life, and their

eventual fate. Accordingly, 600 animals of each sex were divided into quintiles based on their body weights 6 months after the start of the study. As shown in Table 12 there was a highly significant association between body weight at 6 months and RR of death before week 133, and between body weight at 6 months and the subsequent development of benign or malignant neoplasia (see Roe et al)

An unexpected finding in the study was that although the ad libitum provision of a high-fibre low-energy diet had many of the same effects as simple rationing to 80% of ad libitum of a normal diet, it dramatically increased the incidence of three kinds of neoplasm, namely uterine adenocarcinomas, haemangiomas and haemangiosarcomas of the mesenteric lymph node, and Leydig cell tumours of the testis (Table 13). We suspect that one or more unsuspected genetic factors contributed to the aetiology of these tumours (see Deerberg et al, 1980, 1982 and Deerberg & Kaspareit, 1987). Nevertheless, the fact that the high fibre diet led to their occurring in increased incidence suggests that we have probably discovered another non-genotoxic carcinogen.

Conclusions

* Most cancers in untreated laboratory rats and mice and most cancers in humans arise not because of exposure to environmental mutagens, but as a consequence of (a) their genetic make-up and (b) what they eat and other life-style factors. Many of the latter are not genotoxic, but increase the risk that endogenously produced genotoxins give rise to frequently. Substances which cause cell death following by reparative hyperplasia and substances which disturb hormonal status in such a way that cell turnover rates are increased in endocrine or other tissues are apt to

predispose to cancer development. The hyperplasia of the respiratory epithelium associated with smoking may be more important in relation to lung cancer risk than the genotoxic chemicals present in the smoke. Formaldehyde, which is genotoxic, only gives rise to nasal tumours in rats if the level of exposure is high enough to cause necrosis of the nasal mucosa which is followed by reparative hyperplasia.

* Electrophiles are produced during the normal metabolism of food stuffs, especially of fats. These electrophiles damage both cell proteins and DNA. Repair of this damage is impeded under conditions of accelerated cell replication so that tissues age and unrepaired mutations accumulate. Dietary restriction reduces the rate of cell turnover and protects both rats and humans from both ageing diseases and cancer.

* People are much more likely to develop cancers as a consequence of what they eat, drink and smoke than because of exposure to carcinogens in factories, in offies or on farms. etc.

* Epidemiologists looking for cancer risk factors - particularly weak risk factors - are likely to be wasting resources unless they make every effort to control for lifestyle factors.

* The adverse effect of overeating on cancer risk in laboratory rodents is only marginally influenced by exercise.

* Experimentalists who fail to take caloric intake into account in carcinogenesis studies are guilty of conducting inadequately controlled experiments.

* The administration of unrealistically high doses of non-genotoxic chemicals may produce tumours by non specific mechanisms (eg irritation, hormonal disturbance, overwhelming of detoxification pathway) and thereby give rise to misleadingly false positive results in carcinogenicity tests in animals.

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O/CANTAB

Table 1 : Some examples of non-genotoxic carcinogens in rodents

Substance, etc.	Target	Mechanism	Reference
Raw soy protein	Pancreas	Anti-proteolytic effect leading to increased cholecystokinin	McGuinnis et al (1980)
Limonene, 2,2,4-pentane and many other substances	Kidney	Accumulation of -globulin due to anti-enzymic activity	Alden (1986)
Lactose, sorbitol, chemically modified starches	Adrenal medulla Testis (Leydig cell)	Increased absorption of calcium	Roe & Baer (1985)
Chronic nephropathy	Adrenal medulla	Hypercalcaemia	Roe (1993)
Cadmium	Testis (Leydig cell)	Tubular atrophy blocks negative feedback	Roe et al (1964)
Inducers of P450 enzymes in liver	Thyroid	Elimination of thyroid hormones leads to TSH drive	Oppenheimer et al (1968)
Croton oil, TPA	Skin	Persistent hyperplasia	Iversen (1988)
Implantation of ovary into spleen	Ovary Pituitary	Interference with negative feedback leads to FSH drive	Biskind et al (1944)
Glass beads implanted into bladder	Bladder	Persistent hyperplasia	Ball et al (1964)
Overnutrition	All sites	Increased cell turnover	Roe (1991)

30CAN2-TAB

Table 2 : Proportions of cancer deaths attributable to different factors : Best estimates of Doll and Peto (1981)

	Best Estimate	Range Low	Range High
Tobacco	30	25	40
Alcohol	3	2	4
Diet	35	10	70
Food additives	<1	-5	2
Reproductive and sexual behaviour	7	1	13
Occupation	4	2	8
Pollution	2	<1	5
Industrial Products	<1	<1	2
Medicines, medical procedures	1	0.5	3
uv light, ionizing radiation	3	2	4
Infection	?10	?1	?

3.CAN3.TAB

Table 3 : Okinawa (O) vs Rest of Japan (J)

	J	O
Sugar	100	75
Cereals	100	75
Yellow/Green veg	100	300
Meat/Fish	100	200
Total Protein	100	100
Energy consumed		
children	100	62
adults	100	80

from Kagawa (1978)

3 I CANS. TAB

Table 4 : Okinawa (O) vs Rest of Japan (J)

	J	O
Death rate from		
- heart disease	100	59
- cerebral vascular disease	100	59
- cancer	100	69
Deaths per 100,000 pa		
- ages 60-64	2181	1280 (59%)

from Kagawa, (1978)

3CAN2.TAB

Table 5 : Relationship between body weight index and mortality from certain cancers in MEN*

% of Average weight	85	100	125	>139
Colon/ rectum	0.86	1.00	1.23	1.73
Prostate	0.92	1.00	1.37	1.29
Pancreas	0.82	1.00	0.88	1.62
Stomach	0.61	1.00	0.97	1.88
All cancers	1.13	1.00	1.09	1.33

from Lew & Garfinkel (1979)

*Standardized for age and tobacco usage

Table 6 : Relationship between body weight index and Mortality from certain cancers in WOMEN*

% of Average weight	85	100	125	>139
Endometrium	1.04	1.00	1.85	5.42
Uterine cervix	0.77	1.00	1.51	2.39
Gall bladder	0.74	1.00	1.74	3.58
Kidney	0.70	1.00	1.30	2.03
Stomach	0.95	1.00	1.28	1.03
Colon/rectum	0.84	1.00	1.10	1.22
Breast	0.86	1.00	1.16	1.53
All cancers	0.92	1.00	1.19	1.55

from Lew & Garfinkel (1979)

*Standardized for age and tobacco usage

Table 7 : Design of Experiment 1
(20 rats/group)

Group	Sex	Access to food (hr/day)	Room type	Sexual Activity
1	M	24	M + F	0
3	M	6.5	M only	0
4	F	6.5	M + F	0
5	M	24	M only	0
6	M	24	M + F	+++
7	F	24	M + F	1 litter

from Salmon et al (1990)

Table 8 : Main results of Experiment 1

Diet Restriction

- Survival ↑
- Food consumption 20% ↓
- Body weight gain 20% ↓
- Ageing diseases ↓
  - mycocarditis
  - nephropathy
  - polyarteritis
  - radiculo-neuropathy
  - testicular atrophy
  - prolactin-mediated mammary gland changes
- Neoplasia ↓
  - any benign/malignant
  - any malignant
  - multiple
- Organ/Body weight ↓
  - liver
  - kidney

Sexual Activity

- Survival ↑
- Food consumption 5-10% ↑
- Leydig cell hyperplasia/neoplasia ↑

Parity

- Prolactin-medited mammary gland changes ↓

Housing in unisex versus mixed sex room

- No effects

Table 9 : Experiment 2 : Mean distances run per 24 hours

Strain	Sex	Feeding	Kilometers/day	
			at 8 months	at 12 months
CDI	M	AL	2.4	1.8
		R	4.4	2.2
	F	AL	3.3	5.0
		R	3.3	2.0
B6C3FI	M	AD	2.6	1.3
		R	2.2	2.3
	F	AD	7.3	4.1
		R	4.9	4.4

from Conybeare (1988)

Table 10 : Some effects of restricting calorie intake to 80% of ad libitum in 1200-rat Biosure Study

	Males	Females
Mature body weight	Down 20%	Down 20%
Survival to age of 133 weeks	Up from 41% to 69%	Up from 34% to 76%
Non neoplastic ageing-associated disease*		
Polyarteritis		---
Chronic myocarditis	---	---
Prostatitis	-	
Neoplastic disease (benign and/or malignant)* incidence)		
Any site	---	---
Epidermis and/or adnexa	-	-
Subcutaneous	-	-
Mammary	(-)	---
Pituitary - anterior lobe	---	---
Pituitary - intermediate lobe	--	--
Pancreas - islet-cell	--	
Pancreas - exocrine	-	
Lung	-	-

Key

*	=	age-standardized incidence
(-)	=	$p<0.1$
-	=	$p<0.05$
--	=	$p<0.01$
---	=	$p<0.001$

Table 11 : Effect of ad libitum feeding compared with that of restriction to 80% of ad libitum on relative risk of development of a fatal or potentially fatal malignant neoplasms

Relative risk (95% confidence limits)	Males	Females
of premature death	2.40 (1.61-3.59)	3.60 (2.42-5.59)
of developing fatal or potentially fatal malignant neoplasms	4.80 (2.73-8.74)	3.34 (1.97-5.66)

(see Roe & Lee, 1991)

Table ~~10~~ 16 : Relative risks (RR) of (a) dying prematurely and (b) developing one or more benign or malignant neoplasms before the age of 133 weeks in relation to body weight at the age of 29 weeks (M = male; F = female)

	Sex	Body weight quintiles at 29 weeks					
		Very Low	Low	Medium	High	Very High	Trend
Number of rats	M	120	120	120	120	118	
	F	120	121	118	122	116	
Relative risks of:-							
Premature death	M	1.00	1.23	1.56	1.85	2.56	p<0.0001
	F	1.00	1.49	1.36	1.97	2.11	p<0.0001
Benign or malignant tumour at any site	M	1.00	1.69	2.43	2.78	3.22	p<0.0001
	F	1.00	1.39	1.33	1.96	1.69	p<0.01
Malignant tumour at any site	M&F	1.00	1.36	1.76	2.71	2.93	p<0.0001

(see Roe et al, 1991)

Table 13 : Response of rats to a high-fibre low-energy diet

	Males	Females
SURVIVAL	NS	+++
BODY WEIGHT	---	---
LIVER:BODY WEIGHT	+	++
AGEING DISEASES		
- Mycarditis/Fibrosis	-	---
- Nephropathy	---	---
- Radiculo-neuropathy	NS	---
NEOPLASMS		
- Mammary	NS	---
- Pituitary	-	---
- Pancreas islets	--	NS
- Testis - Leydig cell	+++	
- Uterus adenocarcinoma		++
- Mesenteric lymph node	+++	NS
- Any site	NS	---

+++ or ---	p<0.001
++ or --	p<0.01
+ or +	p<0.05

How may diet influence cancer risk in humans?

Food additives/contaminants – negligible influence

– ? because of regulation

Food composition – important in relation to ethnic differences

– influence starts in childhood

Total caloric intake – probably most important but difficult to prove

Breast cancer and fat/meat intake

Results of 18 case:control studies

Positive relationship – 8
Possible relationship – 4
No relationship – 6

6 – year prospective study on 90,000 women

Non – significant negative trend

"Dietary information on special food items as obtained in retrospective histories is of little value in determining the influence of diet on cancer of the large bowel"

Wynder and Shigematsu, 1967

Okinawa (O) vs Rest of Japan (J)

	J	O
Sugar	100	75
Cereals	100	75
Yellow/Green veg	100	300
Meat/Fish	100	200
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*Standardized for age and tobacco usage

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5	M	24	M only	0
6	M	24	M + F	+ + +
7	F	24	M + F	1 litter

from Salmon et al (1990)

Main results of Experiment 1

Diet Restriction

Survival ↑

Food consumption 20% ↓

Body weight gain 20% ↓

Ageing diseases
- mycocarditis
- nephropathy
- polyarteritis
- radiculo – neuropathy
- testicular atrophy
- prolactin – mediated mammary gland changes

Neoplasia
- Any benign/malignant
- Any malignant
- Multiple

Organ/Body weight
- Liver
- Kidney

Main results of Experiment 1 – contd.

Sexual Activity	Survival ↑
	Food consumption ↑ 5 – 10%
	Leydig cell hyperplasia/ neoplasia ↑
Parity	Prolactin – mediated mammary gland changes ↓
Housing in unisex versus mixed sex room	No effects

Experiment 2 : Mean distances run per 24 hours

Strain	Sex	Feeding	Kilometers/day at 8 months	Kilometers/day at 12 months
CDI	M	AL	2.4	1.8
		R	4.4	2.2
	F	AL	3.3	5.0
		R	3.3	2.0
B6C3FI	M	AD	2.6	1.3
		R	2.2	2.3
	F	AD	7.3	4.1
		R	4.9	4.4

from Conybeare (1988)

Experiment 2 : Main findings – MALES

	CD – 1			B6C3F1		
	BW	S	T	BW	S	T
Restriction	↓	↓	↓	↓	–	–
Exercise	0	0	0	0	–	–
Restriction & Exercise	↓	↓↓	↓	↓	–	–

Experiment 2 : Main findings – FEMALES

	CD1			B6C3F1		
	BW	S	T	BW	S	T
Restriction	↓	0	↓	↓	–	–
Exercise	0	↓	0	0	–	–
Restriction & Exercise	↓	↑	↓↓	↓	–	–

1200 Rat Biosure Study : Questions addressed

* Relation between diet and malignant tumours?

* Can restriction be achieved by
  - 6 hours/day feeding?
  - low-energy diet?

* Does restriction early in life have long term benefits?

* Is body weight early in life predictive for
  - survival?
  - neoplasia?

* Are in-life measurements of predictive value?

DIET RESTRICTION did not stunt growth

- Femur length
- Mandible length
- Absolute brain weight

Effects of eating 25% more calories on relative risk (RR) in rats (95% confidence limits)

	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

[from Roe & Lee (1991)]

Body weight early in life and risk of premature death in rats (sexes combined)

	Trend across bodyweight quintiles
Premature death	
– all causes	$p < 0.0001$
– malignant neoplasia	$p < 0.0001$

[from Roe et al (1991)]

Effect of eating 25% more calories on degenerative diseases in the BIOSURE rat study

	Males	Females
Polyarteritis		+ + +
Chronic myocarditis	+ + +	+ + +
Prostatitis	+	
Nephropathy	+ + +	+ + +

+ + + = $p < 0.001$

\+ = $p < 0.05$

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Effects of eating 25% more calories on incidence of neoplasia at particular sites in the BIOSURE rat study

	Males	Females
Epidermis/Adnexa	+	+
Lung	+	+
Mammary gland		
Pituitary		
– anterior lobe	+ + +	+ +
– intermediate lobe		+ +
Pancreas		
– islet cell	+ +	
Subcutaneous tissues	+	+

+ + + = $p < 0.001$

+ + = $p < 0.01$

+ = $p < 0.05$

Response of rats to a high-fibre low-energy diet

	Males	Females
SURVIVAL	NS	+ + +
BODY WEIGHT	− − −	− − −
LIVER:BODY WEIGHT	+	+ +
AGEING DISEASES		
− Mycarditis/Fibrosis	−	− − −
− Nephropathy	− − −	− − −
− Radiculo-neuropathy	NS	− − −
NEOPLASMS		
− Mammary	NS	− − −
− Pituitary	−	− − −
− Pancreas islets	− −	NS
− Testis − Leydig cell	+ + +	
− Uterus adenocarcinoma		+ +
− Mesenteric lymph node	+ + +	NS
− Any site	NS	− − −

+ + + or − − − $p < 0.001$

+ + or − − $p < 0.01$

+ or + $p < 0.05$

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	$P < 0.01$	$p < 0.01$

EFFECT OF CALORIE RESTRICTION (to 75% of Ad LIB) ON CELL TURNOVER IN THE MOUSE (Lok et al, 1990)

	% INHIBITION of [^{3}H] THYMIDINE LABELLING INDEX
Mammary Gland	72**
Bladder Epithelium	43*
Dermis	57**
Oesophageal Epithelium	49**
Colon – Crypt cells	54**

* $p < 0.05$

** $p < 0.01$

Main conclusions

* Genetic determinants of cancer susceptibility merit more attention

* Non – genotoxic carcinogens probably contribute more than environmental mutagens to cancer risk

* Overnutrition → Increased cell turnover → increased unrepaired DNA damage from endogenous mutagens

* Epidemiologists looking for environmental carcinogens must control for life – style

* Experimentalists testing for carcinogenicity must

  (a) study realistic dose levels
  (b) control for caloric intake