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RADIATION CARCINOGENESIS AT LOW DOSES

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ABSTRACT: Radiation carcinogenesis is the major contributor to the risk of small doses of ionizing radiations. Risk estimates are usually based on the assumption of a linear dose-effect relation at small and even at intermediate doses up to about 100 rad. The postulate that such linearity should generally apply is, however, in conflict with microdosimetric arguments, and experimental findings are here considered that exemplify non-linear dose dependence even at small doses.

It is pointed out that dose-effect relations are always based on a somewhat arbitrary choice of the biological endpoint, and that a well-designed experiment must therefore be aimed at more complete information. The relevant quantities in radiation carcinogenesis are the tumour rate $r(t)$, the integral tumour rate $R(t)$, or the cumulative prevalence $I(t)$.

The mathematical analysis can often be simplified if RBE is directly determined as a function of absorbed dose. This requires the comparison of the tumour incidence or prevalence in pairs of experimental groups. The largely unsolved problem of the construction of suitable statistical tests for this purpose is discussed.

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Introduction

The development of nuclear technology and the widening medical applications of ionizing radiations have brought questions of the risk of small doses to large populations into the center of public interest. It is currently believed that late somatic effects, i.e., essentially radiation induced tumours, contribute at least as much to the risk of small doses as hereditary effects. ICRP in its recent recommendations (1) bases risk estimates on the assumption of proportionality to dose equivalent. The total risk of radiation carcinogenesis is assumed to be 10^{-4} per rem as the average for both sexes and all ages. The total probability to die from cancer is in the United States roughly 0.17 (see (2)); according to the ICRP number this probability is increased by 0.3 % if an individual receives a dose equivalent of 5 rem. Small as this increment may seem, it is substantial enough that the permissible limits may have to be reconsidered if the ICRP values should turn out to be realistic.

For comparison it may be noted that ICRP estimates the risk for hereditary effects, expressed in the first two generations, as $4 \cdot 10^{-5}$ per rem. However, if all subsequent generations are included, the risk is believed to be approximately equal to that for radiation induced cancer. ICRP states, that the risk estimates are probably conservative. Other scientific bodies have made similar statements concerning their risk estimates (3,4). However, this is often disregarded.

There are also occasional claims that actual risks are much higher than any of the current estimates. A few years ago it was stated (5) that hot spots of PuO_2 particles have a vastly higher effectiveness in producing lung cancer, compared to the same activity of α -particles evenly dispersed in the lung. It was in fact stated that the limits for inhaled activity were more than 100 000 times too low. These statements were based on a misinterpretation of certain experimental data for the skin (6) that were then simply applied to the lung. A heated controversy was nevertheless created, and various assessments (see for example (7-9)) of the problem of hot spots in the lung have been attempted. The general conclusion has been that activity in particulate form is usually less effective than activity in dispersed form. The reason is, that a given activity irradiates a much smaller number of cells, if it is concentrated in hot spots (10). Experiments in various laboratories, and especially those of Lafuma et al. (11,12), have supported

the conclusion that hot spots have a reduced effectiveness in the production of lung cancer. The experiments are still in progress. Their analysis poses intriguing statistical problems that will be considered in the last part of this article.

A more recent sensational claim of unexpectedly large risk factors is made in reports of Mancuso, Stewart, and Kneale (13,14). These reports deal with radiation workers in Hanford. It is stated that workers dead of cancer in Hanford had received doses that are substantially above the average for all workers. The authors estimate from their analysis that a single dose of sparsely ionizing radiation of 0.8 rad doubles the incidence of bone marrow cancer, and that 7 and 6 rad double the chance to die of pancreas or of lung cancer.

In judging these assertions one must note that the statements refer only to certain cancer types and to very few cases, and that it is difficult to ascertain that these were not random fluctuations. However, even if all proper corrections, including age corrections, are applied, and if a correlation between cancer incidence and radiation exposure could actually be established among the Hanford workers, this need not be proof for a causal relation. For example, lung cancer rates have been increasing in recent years in the United States. At the same time the total doses accumulated by workers in Hanford must have been increasing and must now be higher than those of people dying earlier after a shorter employment in Hanford. Lung cancer rate and average doses have accordingly both been higher in workers dying recently. This is an example of a spurious correlation without causal connection. Various other spurious correlations may well play a role, and the Hanford study must therefore be examined very critically. Doubtlessly it contains all the difficulties of an assessment of the effects of low doses on human populations. Nevertheless studies of human data are of greatest importance. They have to be performed, even if it is often exceedingly difficult to estimate doses. Perhaps the most important set of observations is that made on the Japanese atomic bomb survivors. H.H. Rossi has, as others have done, analysed these data and in particular the leukemia data. Unlike others he has stressed the fact that the observations, in agreement with general biophysical considerations, contradict the proportionality to dose for *both* sparsely and densely ionizing radiations (15). He has also made the important point that the RBE-dose relation is more fundamental than dose-effect relations. These matters are being discussed in this symposium by

Rossi (16) and particular emphasis is given to the human data. These are, therefore, not considered in the present article. Animal experiments are less relevant to risk estimates. However, they will remain the basis of any attempt to understand the biophysical basis of radiation carcinogenesis, and they will here be considered under this aspect.

Arguments for the Linear Hypothesis

Because radiation energy is transferred in discrete events, all radiation effects must ultimately be linear at small doses. At small doses most cells will receive no energy; the number of cells subject to an event will be proportional to dose. If radiation carcinogenesis were due solely to the transformation of individual cells one would therefore have to find a linear dose-effect relation at doses of fractions of a rad for sparsely ionizing radiations and at considerably larger doses for neutrons and other densely ionizing radiations.

In vitro experiments indicate, in fact, that a linear component exists. It exists for cell inactivation and it exists for chromosome aberrations, and it is often surprisingly large. Brown (17) has recently made a strong point for the general existence of a linear component up to about 100 rad. He argues well for the postulate of the linear-quadratic dose-effect relation that is based on biophysical considerations and on microdosimetric data, and he concludes that extrapolations from high doses and high dose rates are conservative. However, he also states that the safety factor is probably not larger than roughly 2. He concludes this principally from a survey of chromosome aberration data that indicate a value of α_1/α_2 in the linear-quadratic dose relation of about 100 rad.

Brown's arguments are well taken, but, they rest, as he states, on the assumption that carcinogenesis reflects the action of radiation on individual cells. It is difficult to establish that this should be so.

It is obvious that the yield of tumours is not equal to the yield of transformed cells. In experiments of Borek and Hall (18) on the *in vitro* transformation of mammalian cells the transformation rate is larger than 10^{-5} per rad for x-rays. Even if one were to assume that only a minor fraction of the tissues in the human body is sensitive, one would still obtain several transformed cells every minute due to background radiation alone if the same or similar transformation rates were to apply *in vivo*. Obviously a

transformed cell must have a rather infinitesimal chance to produce a cancer.

One can nevertheless argue that each transformed cell may have a small but finite probability to produce a tumour. The number of radiation induced tumours would then be proportional to the number of transformed cells. But even in those cases where a mono-clonal origin of a tumour is established this will not necessarily imply a linear dose relation. The probability of a transformed cell to grow into a macroscopic tumour may well depend on immunological factors or on the mutual influence between damaged cells (19). Although their nature is not, at present, determined, it is known that inter-cellular factors exist which can lead to non-linear dose relations at small doses. This has been demonstrated for the induction of mammary tumours in the Sprague-Dawley rat.

Induction of Mammary Tumours in the Rat; An Example for Infra-Linearity at Small Doses of Density Ionizing Radiations

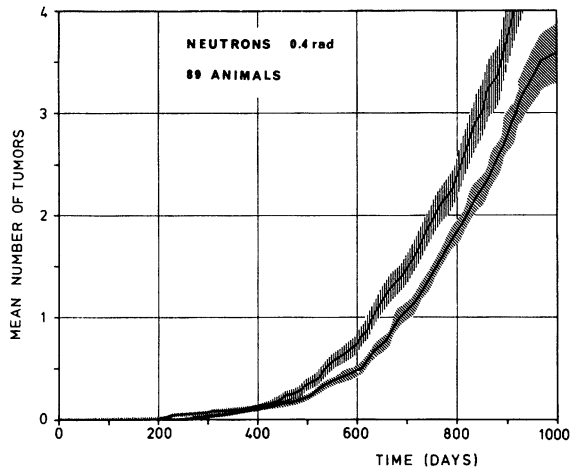
There are cases of radiation induced tumours that are non-linear in dose even at low doses of density ionizing radiations. Marshall and Groer (20) have found a quadratic dose dependence for the induction of osteosarcomas by α -rays. Mammary tumours produced in the Sprague-Dawley rat are an opposite example. In this case the dose-dependence is neither linear nor supra-linear (dose exponent >1) but instead it is infra-linear (dose exponent <1): The tumours are mostly adeno-fibromas and fibro-adenomas and they occur in the older animal with large spontaneous frequency. It is therefore often stated that this is a somewhat abnormal experimental system; on the other hand, an incidence steeply rising with age is typical for various tumours. Vogel and Zaldivar (21) and Shellabarger et al. (22) have earlier reported a dose dependence of mammary tumours in the Sprague-Dawley rat that is infra-linear for neutrons. In the meantime additional work has been performed and is still in progress. Broerse et al. (23) have presented an interim analysis of a large experiment that includes different strains of rats and that is also concerned with hormonal influences on the radiation response. The complete results of a large experiment by Shellabarger et al. will soon be published. This experiment has been specifically designed towards the determination of the effects of very small neutron doses. Some of the essential results will here be summarized, and they will then be used to indicate fundamental aspects of the mathematical assessment of tumour induction by ionizing radiations.

In the experiment rats were exposed to single doses either of 430 keV neutrons or of x-rays at age 60 days. Mammary tumours were then observed and surgically removed as they occurred. In this way several tumours could be observed in individual animals. In the conventional actuarial way a mortality corrected cumulative tumour incidence is obtained as a function of time for each irradiated group. Fig.1 gives an example, namely the curve for 0.4 rad of neutrons compared to the control group. These curves represent fibro-adenomas plus adeno-fibromas. The curves for the irradiated and the control group are nearly parallel and the same seems to apply at other doses. One can accordingly state that the effect of the irradiation is a shift forward in time of the tumour incidence.

Fig.1

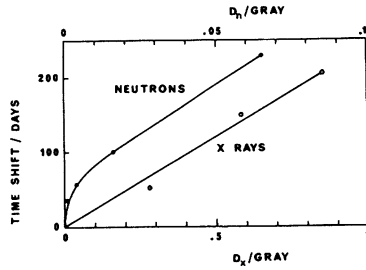
The integral tumour rate, $R(t)$ for adeno-fibromas and fibro-adenomas as a function of time after irradiation in the experiment of Shellabarger et al. on Sprague-Dawley rats.

The upper curve applies to a dose of 0.4 rad of 430 keV neutrons (89 animals) the lower curve to the control group (167 animals). The shaded areas give the standard deviations.

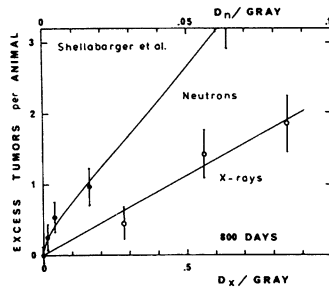


There are, in fact, many ways to construct dose-effect relations from the totality of the data obtained in the experiment. The determination of a time shift is one possibility (Fig.2a). Another possibility is the specification of the excess incidence at a selected time (Fig.2b). Still another possibility is the determination of a quantity that is perhaps more meaningful as a measure of detriment; this is the *loss of tumour-free life span*, i.e. the expected tumour-free life span of the animals in the control group minus the tumour-free life span in the irradiated group (Fig.2c). One can not *a priori* expect that the various dose-effect relations are of equal or even of similar shape, and it follows that statements on the linearity or non-linearity of dose-effect relations have little absolute meaning.

a) Forward shift in time of the occurrence of mammary tumours for different neutron and x-ray doses.



b) Excess mammary tumours per animal in the experiment of Shellabarger et al. at 800 days after irradiation.



c) Reduction of tumour-free life span in the experiment of Shellabarger et al.

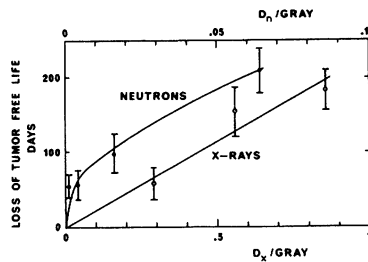


Fig.2

Various dose-effect relations for the induction of mammary tumours in the Sprague-Dawley rat.

The dose scales for neutrons and x-rays differ by a factor of 10.

However, in the present case it appears that the various dose-effect relations depicted in Fig.2 are generally of the same shape. In agreement with earlier results one finds a nearly parabolic dependence on neutron dose at doses that are so small that most cells have no energy deposition in their nucleus. The possibility of such dose relations is of obvious relevance to the problems of radiation protection. It is also important that very high RBE values of 100 or more are reached at low doses. However, these matters will not be further considered in this article. Instead the experiment will be used to exemplify basic quantities relevant to the assessment of radiation carcinogenesis.

Basic Quantities Relevant to the Assessment of Radiation Carcinogenesis

A dose-effect relation contains, as seen in the example of the mammary tumours, only limited information. The actual quantity of interest is the *tumour rate* as a function of age or of time after irradiation. The tumour rate, $r(t)$, is the probability per individual per unit time to develop a tumour. The estimate of the tumour rate for the controls in the experiment of Shellabarger et al. is given in Fig.3. It is a function that increases steeply with age for these particular tumours.

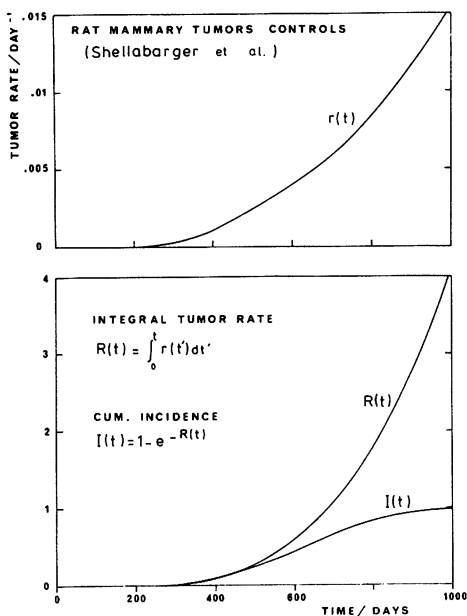


Fig.3

The three basic quantities $r(t)$, $R(t)$, and $I(t)$ for the spontaneous incidence of mammary tumours in the Sprague-Dawley rats in the experiment of Shellabarger et al.

It is often difficult to estimate tumour rates, since in most experiments an insufficient number of tumours is observed per time interval. It is then more practical to determine the *integral tumour rate*, $R(t)$. This quantity is equal to the time integral of the tumour rate up to time t , and it is also equal to the mean number of tumours per surviving animal, if multiple tumours can be scored and if mortality is unrelated to tumour incidence. The estimate of the integral tumour rate and of its standard deviation is given by the relation:

$$\hat{R}(t) = \sum_{t_i < t} \frac{1}{N(t_i)} \pm \sqrt{\sum_{t_i < t} \frac{1}{N(t_i)^2}}$$

t_i are the times of incidence of the individual tumours up to time t , and $N(t_i)$ are the numbers of animals still at risk at these times. If multiple tumours can not be observed, the integral tumour rate is obtained by the same formula. The only change is that animals are considered at risk only until they incur their first tumour.

$R(t)$ has been termed 'mean number of tumours' in Fig.1. However, in general, the term integral tumour rate is more suitable. $R(t)$ has no direct meaning if multiple tumours can not be observed, but it is closely related to the probability of an animal to have at least one tumour. The latter quantity, $I(t)$, defined by the equation in Fig.3 has been called *cumulative prevalence* by Broerse et al. (23); this appears to be a suitable term that could be generally adopted.

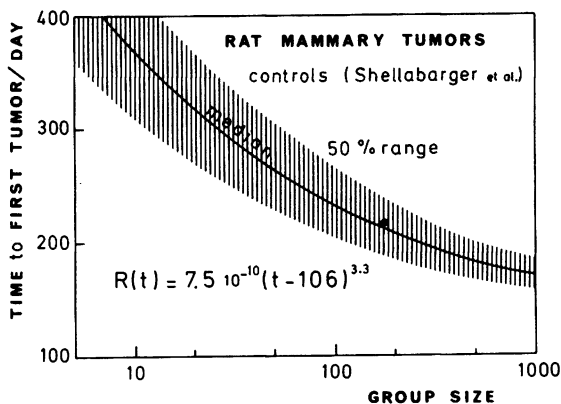
Determination of $r(t)$, $R(t)$, or $I(t)$ should ideally be the aim of a well designed experiment. However, one may frequently be unable to use sufficiently large experimental groups. Then simpler quantities and approximate concepts are desirable. It is still very common that quantities such as *fraction of animals with at least one tumour* or *mean number of tumours per animal* are quoted with reference either to total life time or to a specified observation period but without any mortality correction. In fact, these may still be the most commonly quoted quantities. The mortality correction is of no importance at low doses. However, low dose studies will always be performed in the context of experiments that include also higher doses where mortality corrections are essential. The uncorrected quantities are therefore of limited usefulness, and may, in fact, be misleading.

Another simplified concept that is commonly applied, although it is not always meaningful, is the so called *latent period*. It would be difficult to define this concept rigorously, but in practice it is most often equated with the time to the appearance of the first tumour in an experimental group. Obviously this is unsatisfactory since the result will depend on the size of the experimental group. For example, the incidence of the mammary tumours increases steeply with age, and this suggests that the concept of a latent period may indeed apply. The true 'latent period' according to the estimate for the controls in Fig.3 is about 100 days after the beginning of the observation. However, the time to the first tumour will generally be considerably larger than the theoretical value; this is the case even for extremely large experimental groups. In Fig.4 the median of the time to the first tu-

mour is plotted as a function of the size of the experimental group and it is apparent that no realistic estimate of the true value is possible. The latent period is therefore a somewhat misleading concept; it should be used with great caution, and whenever possible it should be substituted by other, modified parameters.

Fig.4

The median (and the 50 % range) for the time from the beginning of the observation (age 60 days) to the occurrence of the first tumour in groups containing different numbers of animals. The dot represents the actual occurrence of the first tumour in the control groups of the experiment by Shellabarger et al.



It has been stated earlier that the determination of RBE is often more fundamental than the determination of the dose dependence of various biological endpoints. It is then not necessary to establish dose-effect relations, and it is sufficient to perform simple comparisons between irradiated groups. Accordingly one needs a suitable statistical test that permits the comparison of the tumour incidence in two groups, such as a group of animal exposed to an x-ray dose and a group of animals exposed to a neutron dose. If such a test is available one can determine the RBE dose dependence in the direct non-parametric way which has been described earlier (24).

Purpose of the statistical test is the examination of the null-hypothesis, i.e. the assumption that the tumour rates are equal in the two groups that are to be compared. In order to obtain a test with sufficient power one will avoid an approximate treatment where the observation period is subdivided into several finite intervals, and where then the χ^2 -test or the Fisher exact probability test is applied. Instead a test is desirable that is based on the full experimental observations. The Mann-Whitney rank order test could be used if all, or nearly all, animals survived until they developed a tumour. Animals could then be ranked according to their tumour free life time and application of the Mann-Whitney test would be straightforward.

However, in all experimental groups there is a substantial number of animals that die without developing a mammary tumour. The Mann-Whitney test is therefore not applicable.

However, a non-parametric test has been developed that has nearly the same power-function as the Mann-Whitney test (25) while it is applicable even if loss of elements from the two observed groups occurs. The test has earlier been applied in order to obtain the RBE dose dependence for the neutrons in the experiment of Shellabarger et al. (22). Its details need not be discussed in the present context; however, it is felt that reference to its existence is useful, since the test may be suitable in various experimental situations where radiation carcinogenesis in two groups is to be compared.

Ovary Tumours in the Mouse; An Example of a Quadratic Dose
Dependence for Sparsely Ionizing Radiation

Yunas has reported (26) a large experiment on the radiation induction of ovary tumours in the mouse. The tumours are in most cases lethal and are therefore readily discovered. Groups of 200 mice each were exposed to various doses of γ -rays at various durations T of the exposure. The data are represented in Fig.5. The spontaneous incidence which is quite small is sub-

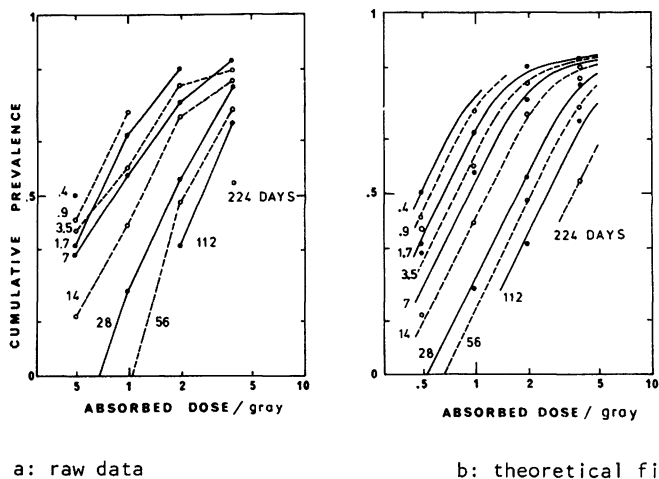


Fig.5

Cumulative prevalence of mouse ovary tumours (-control value) versus absorbed dose at various durations of exposure to γ -rays.

tracted. Yuhas has concluded from these data that the dose dependence is nearly quadratic. Lam and Rossi (27) have, more recently, reanalysed the results. They have applied an intriguing analysis, that assumes that the cumulative prevalence follows an equation:

$$I(t) = f(\alpha \cdot D + q(T) \cdot D^2)$$

where the form of the function $f(\cdot)$ is left open and where a recovery of the quadratic component is assumed and accounted for by the reduction factor $q(T)$. On the basis of recovery factors that correspond to an exponential decay of sublesions a decay time of 15 hours has been obtained for an optimal fit to the data. Furthermore the value $\alpha=5$ rad has been deduced. This is a much smaller linear component than the value of roughly 100 rad that has been postulated for human data in general, and that is implicitly utilized in the deduction of various risk factors. The result is therefore of importance and deserves further consideration.

It is of particular interest whether the somewhat arbitrary assumption of an exponential decay of sublesions is critical for the magnitude of the linear component that is obtained in the analysis. To answer this question one must ask simultaneously for the optimal function $f(\cdot)$, the optimal value α , and the optimal repair function $q(T)$. There are numerical proce-

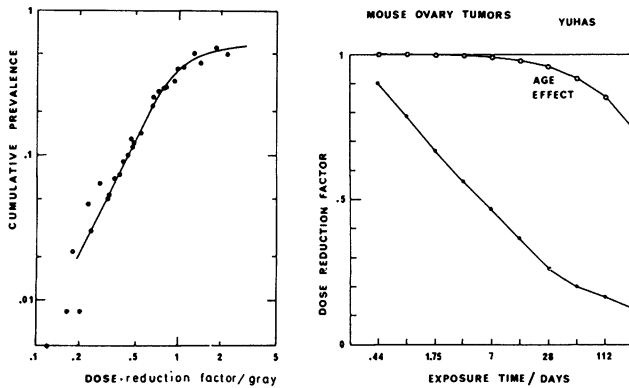


Fig.6

a) Cumulative prevalence of mouse ovary tumours (-control value) vs. absorbed dose times dose reduction factor

b) Dose reduction factor as a function of exposure time. The reduction due to loss of sensitivity at increasing age (26) plays only a minor role, as indicated by the upper curve.

dures that can provide the results with suitable constraints that ensure smooth functions $f(\dots)$ and $q(T)$. No analytical expressions are invoked, i.e. the functions are represented by arrays of numerical values. The mathematical technique is the non-linear optimization by the method of the generalized reduced gradient (28).

Fig.5b represents the result. The optimal value of α is zero, with a dependence of the sum of least squares on α that appears to exclude values of α larger than roughly 4 rad. The curves in Fig.5b are shifted parallel to the axis in accordance with the dose reduction factors. For a clearer comparison of the data and the theoretical curves a plot of the cumulative incidence versus the 'effective dose' $q(T)^{0.5} \cdot D$ is given in Fig.6a. This brings all theoretical curves into coincidence and bunches the observed incidences around the theoretical curve. In view of the expected fluctuations of observed tumours in the low dose range the agreement between observations and theoretical curve is excellent. The essential result is, that no linear component is found and that simple proportionality between incidence and the quadratic component results over a wide dose-range. In Fig.6b the time factors, i.e. the dose modifying factors are given as a function of duration of exposure T . It is of interest to note how precisely recovery can be determined in a well designed experiment.

The two examples of the ovary and the mammary tumours make it quite obvious that no universally valid dose dependence can be expected for radiation induced tumours. It is therefore required that detailed and rigorously analysed studies be performed on as many experimental systems as possible, both with sparsely and with densely ionizing radiations. Such studies are relatively simple in those cases where tumours are readily discovered, and a direct estimate of tumour incidence is therefore possible. In other experimental systems where tumours are not readily discovered the statistical problems are much more serious. One is then forced to estimate prevalence instead of incidence. This will be considered by reference to recent studies of Lafuma et al.

Statistical Problems in the Determination of Tumour Prevalence

Inhalation studies with different α - and β -emitters in various chemical forms and applied in various temporal patterns have in recent years been performed in a number of laboratories. These studies pose various dif-

difficult statistical problems, as the radiation induced lung tumours are even in their malign forms usually not lethal. The tumours can therefore only be observed when the animals die for other causes or are sacrificed. An example from studies by Lafuma et al. may serve to illustrate the situation.

Fig.7 refers to studies with inhaled radon. These studies do not involve low doses; however, they are aimed at a clarification of the effects of low doses that can not be directly analysed. Studies with high doses are relevant because the microscopic pattern of energy deposition by α -particles or by α -emitting hot spots is the same for high and for low doses. However, difficulties arise due to radiation induced mortality. It is obviously meaningless to compare different modes of irradiation on the basis of tumour prevalence at the death of the animals because a higher dose or a more effective temporal pattern of the application of the activity may shorten the life span of the animals considerably. Tumour prevalence in the dead animals may be reduced significantly because animals die too early. Methods are therefore required to compare tumour prevalences in a way that is independent of radiation induced mortality.

Fig.7 refers to two groups of mice that have been exposed to radon at the level of 7000 WLM (Working Level Months) and 1470 WLM. While lung tumours are classified into various stages, only animals with malignancies are, for the purpose of this analysis, discriminated from the other animals. The observed data are indicated by the two panels. The upper dots in these panels symbolize animals dying with malignancies; the lower dots symbolize animals dying without malignancies. The animals in group 1 are dying considerable earlier than the animals in group 2. It is therefore difficult to judge how strongly prevalence is increased in group 1 in comparison to group 2.

No general solution of the problem has apparently been developed; however, a relatively simple method will be described to estimate a time shift in tumour prevalence between the two groups.

The method is based on the notion of *inversions*. The term inversion applies to a pair of animals not in the same group. The pair forms an inversion if the animal that dies earlier has a malignancy, while the animal that dies later has none. By considering all pairs of animals one can determine the total number of inversions. If the number of inversions is high this may

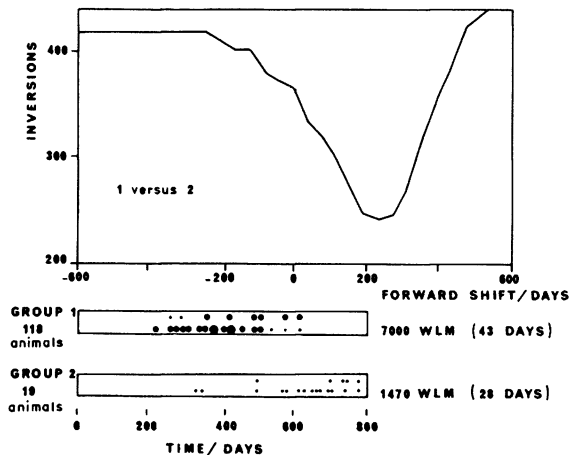


Fig.7

Comparison of two groups of radon exposed rats from experiments by Lafuma et al.

The dots in the two panels represent deaths of animals at the indicated times. Upper dots: animals with malignancies; lower dots: animal without malignancies; small dots: 1 animal; intermediate dots: 5 animals; large dots: 15 animals.

The estimate of the relative time shift of prevalence is obtained from the location of the minimum of the number of inversions (see text).

be an indication that tumours occur earlier in one of the two groups. The number of inversions for the two groups represented in Fig.6 is 365.

One may now consider the possibility that the prevalence is shifted forward by a time Δt in group 1 compared to group 2. It will be readily seen that a reverse shift by Δt of the observed patterns leads to a configuration with a decreased number of inversions. Fig.7 represents the number of inversions for different time shifts. The minimum number of inversions is obtained when group 1 is shifted with respect to group 2 by roughly 230 days. One can prove that this provides a suitable estimate of the time shift of prevalence. The method is readily applied, and can be utilized even with small groups. However, it poses a number of intricate statistical problems which will require further study.

Whenever an experiment involves large number of animals and a considerable number of experimental groups, one can obtain an actual estimate of the age

dependence of prevalence. To this purpose the maximum likelihood method is combined with the non-linear optimization method, that has been mentioned earlier in the context of the ovary tumours. Fig.8 represents the result for the radon experiment. One common prevalence curve is here obtained for all groups. But the curve is shifted in time in the various groups which have been subjected to different levels of radon inhalation. It is of interest to note that for the two groups considered in Fig.7 the shift obtained by the two methods is the same.

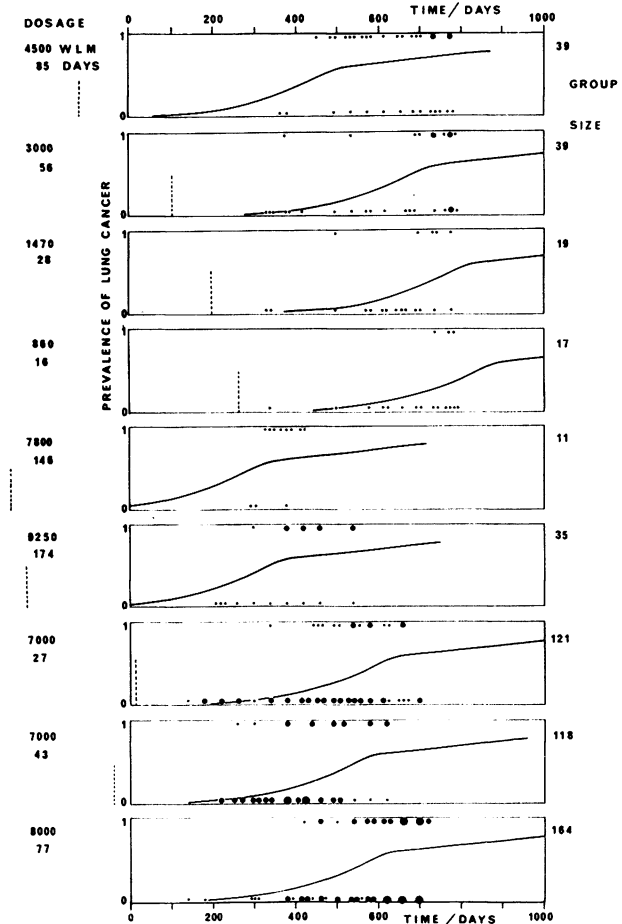
Fig.8

Maximum likelihood estimate of lung cancer prevalence in mice after radon inhalation (Experiments by Lafuma et al.).

The same curve that is merely shifted in time is assumed for all groups. The shape of the curve results from non-linear optimization.

The dots represent deaths of animals and are to be understood in the same way as in Fig.7.

The vertical broken lines are markers to indicate the time shift.



Certain solutions of the problem of estimation of incidence or prevalence of radiation induced tumours have been indicated in this article. However,

there is an obvious need for further development of suitable statistical concepts and methods. A coherent conceptual framework and improved statistical methods will be required in any comprehensive effort to arrive at better risk estimates and ultimately at a deeper understanding of radiation carcinogenesis.

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