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NEUTRON CARCINOGENESIS

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SUMMARY OF THE DISCUSSION ON MAMMARY CARCINOGENESIS IN THE RAT

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The discussion left no doubt that, even in this specialized field of investigations, there is no lack of experiments still to be performed, and no shortage of controversional interpretation and application of those results that are available.

The first part was focussed on technical aspects, although the technical questions did largely reflect essential differences in approach and interpretation. Major topics were the problem of multiplicity of mammary neoplasms and the decision to score first tumors only or all tumors in an animal. Closely related is the choice of the strategy either to remove all palpable lesions or to leave them in the animal for later pathological assessment at necropsy. A further related point concerned the not uncommon observation of partial and even complete regression of mammary neoplasms. This, in turn, led to the question, whether the pathological classifications have distinct individuality or whether they are mere labels affixed to largely transitional types of neoplasms.

The second part of the discussion started out with considerations of terminology and of technicalities in the mathematical analysis. It then addressed the wider question of the relevance of animal data to considerations of breast cancer in humans. The considerations were focussed on the relative biological effectiveness of neutrons at different dose levels and in rat strains of different spontaneous incidence. Particular attention was given to the possibility that low incidences are associated with low neutron RBE and high spontaneous incidences with high RBE. The subsequent résumé aims at those points that found particular interest.

First part: The discussion started on an appropriate keynote with brief inquiries exemplifying the nearly unlimited range of parameters to be varied, and ultimately to be understood, in radiation induced mammary neoplasia. Broerse pointed at the possible effect of a temporal reversal of DES-implantation and irradiation; Shellabarger reported that such reversal has indeed been studied in ACI rats and that certain effects - although no dramatic changes - have been seen. Paretzke emphazised the desirability of information on the temporal dependence of tumor-growth rates and the interest in any variations with dose level or radiation quality; van Zwieten responded that the importance of the question had been appreciated in the experiment at TNO, but added that the analysis of the extensive data on tumor volumes was far from complete.

A familiar and recurrent topic in the study of mammary tumorigenesis in the rat was then considered. Gould posed the question whether first tumors only should be scored, in view of the common observation that animals with a previous neoplasm have a higher probability for mammary tumors than animals without previous neoplasms. Van Zwieten concurred that the group at TNO derives actuarian incidences based on animals with tumor, regardless of the number of tumors. He added that multiple tumors, too, are registered and that their numbers are evaluated. There appeared to be agreement with the further statement, that the increased probability for subsequent tumors need not be caused by the first tumor; variations of inherent tumor rates within the strain, due for example to different hormonal status, would lead to the same result. If, however, the non-Poissonian distribution of mammary fibromas or carcinomas is a reflection of biological variability among the strain, accounting for first tumors only would bias the investigation towards the lower tumor rates in the less susceptible individuals. Both the incidence based on first tumors only, and the cumulative tumor rate based on all tumors are therefore quantities of interest.

This led to deliberations on the merits or disadvantages of removing palpable lesions or leaving them in the animal until they reach a certain size or the animal dies. There was agreement that neither approach could provide all information. But there is also evident disagreement, as the large laboratories have chosen opposite approaches.

Van Zwieten commented on the procedure adopted at TNO not to remove all palpable lesions. This conservative procedure leads doubtlessly to a certain increase in the complexity of bookkeeping in a large experiment. The advantage is added information on tumor growth, and several participants of the discussion were intrigued by the not uncommonly observed regression or disappearance of mammary carcinomas. In the current radiation experiments little hard information on this point is obtained, and most of the regressing lesions could well be cysts. However, as Shellabarger emphasized, there is well established evidence for regression of mammary adenocarcinomas after chemical carcinogenesis in the rat. Adenocarcinomas tend to appear earlier then adenofibromas, although averaged over total life time, their frequency is low. In pregnancy carcinomas tend to grow faster, but they frequently stop after lactation.

Barendsen raised the related point whether observed mammary neoplasms are pathologically distinct, or whether they tend to be transitional in type. He added the more general question whether transforming events are to be thought of as specific. Van Zwieten commented that there were certainly predominant patterns, but no clear-cut,stable types. Anaplastic tumors remain anaplastic, but most tumors dedifferentiate after repeated transplantation.

A final point of interest was uniformity or non-uniformity of the frequency of tumors in different parts of the mammary tissue. With chemically induced adenocarcinomas there can be a substantially higher incidence in the anterior part of the mammary tissue. No similar observation has been made after uniform irradiation. Shellabarger had earlier demonstrated the lack of an abscopal effect; an increased tumor incidence was found only in the irradiated mammary tissue of Sprague-Dawley rats, no change of the spontaneous incidences was observed in the shielded areas. Montour emphasized the desirability and the potential of studies that would utilize heavy ions of properly chosen range and stopping power. Such studies could elucidate a number of factors that are not controlled in experiments with x-rays or neutrons.

Second part: The discussion continued with considerations on terminology in the analysis of radiation carcinogenesis. There was consensus that competing risk corrected quantities had to be employed, but there appeared to be less

agreement on terminology. One proposal was to use the term *nominal incidence* for the competing risk corrected cumulative incidence up to a specified time. Another proposal, approved by several participants, was to utilize the term *actuarian incidence*. The considerations then turned to the more general aspects of statistical methodology.

The ongoing study of mammary neoplasms at TNO permits, at present, only a partial analysis, because the rats have longer tumor free life spans than, for example, the Sprague-Dawley rats used by Shellabarger or by Vogel in the US. The attempt to draw meaningful conclusions from partial results necessitates the use of joint fits to the various dose groups. As reported by Broerse, the notion of a forward shift in time of the tumor incidences with increasing dose agrees well with the data. This is in line with previous findings of Shellabarger in Sprague-Dawley rats. Adequate fits were obtained by postulating power function in time for the tumor rates, and it was emphasized that even such comparatively simple parametric solutions require computer analysis rather than visual fits.

There is little evidence from available observations that variation of more than one parameter is needed to represent the change in temporal dependencies of tumor rates after different doses of different radiations. Evidently this may be a matter of insufficient data. The question is therefore open. That it has importance beyond the mere technicalities of curve fitting was exemplified by two further considerations in the discussion:

- Mole stated that RBE values may well depend on the method of analysis, and could differ whether one asked for equal cumulative incidence up to a certain time, or for equality of the temporal distribution of tumors. If the change in temporal patterns after irradiation is described by one single parameter modifying the response curves, equality of the parameter would imply equality of the entire response. One would then expect the same RBE values, regardless of the criterion chosen. If, on the other hand, the radiation induced change is more complex, and requires the description by several parameters that may be affected differently by different radiation qualities, the very concept of RBE would begin to be of doubtful applicability.

- Gould emphasized that one must distinguish between mere changes in latency time, on the one hand, and actual increases of incidences, on the other hand.

It appears that better information will be required to judge whether an acceleration in time, a forward shift in time, or a time independent increase of tumor rates is the most suitable approximation to experimental observations. It was, furthermore, pointed out that tumor rates may vary as a power of time and that, under this particular condition, an acceleration in time can not be distinguished from an increase of the tumor rates by a time independent constant factor.

Paretzke pointed out that a reasonably modest approach would focus on tumor incidences integrated over the life span. However, he added, in agreement with Dennis and Mole, that the loss of life time or of cancer free life time is ultimately the more meaningful parameter for radiation protection. The more sophisticated considerations aimed at a quantification of detriment are given particular attention in present investigations at GSF.

Experimental studies in the rat and the mouse can, as reiterated by several participants, not be directly relevant to radiation protection and risk determination in the sense of providing actual numerical values. Their potential value, as that of all animal studies, lies in the identification of recurrent traits and common characteristics of responses to different types of radiation and to different absorbed doses. The comparative study of different strains or stocks of animals was therefore an obvious focus of the discussion.

No conclusion was reached on the cause of the striking difference in appearance times of tumors for the Sprague-Dawley rats used in the United States and in the Netherlands. A comparative study that included keeping the Dutch Sprague-Dawley rats at Brookhaven, resulted in the same late appearance of tumors as observed at TNO. Van Bekkum pointed out that the later appearance of the mammary tumors is in line with a somewhat longer life span of the Dutch stock.

Greatly different values of the RBE of neutrons are found in different strains and even in the same strain with and without estrogen treatment. Various participants expressed the opinion that high values of the RBE of neutrons are observed for adenofibromas, and generally for tumors with high spontaneous incidence. With low incidence of tumors - this was presumed to be the 'physiological' condition - the RBE of neutrons tends to be low.

There was agreement that this is a question - if not *the* question - of interest in the studies of mammary neoplasia in the rat. But it was pointed out that the association of high incidences with high neutron RBE and of low incidences with low RBE need not be inherent, although it is commonly encountered. Animals with low incidence are generally less sensitive and require higher doses of any ionizing radiation for statistically meaningful results. At high neutron doses, however, the RBE is low, for fundamental biophysical reasons emphasized by Rossi. In animals with high incidence one can investigate small neutron doses, and the high RBE values reported by Vogel and by Shellabarger were obtained under these conditions.

In a recent experiment by Shellabarger on ACI rats, preimplanted with a relatively high dose of DES, high incidences of adenocarcinomas were observed and the RBE of 430 keV neutrons was in excess of 100 at 0.01 Gy. At larger doses the RBE decreases, but at 0.09 Gy it is still substantially larger than 10. On the other hand, the Sprague-Dawley rats with their high spontaneous incidence and their high RBE values at low doses show the same RBE of about 10 at doses close to 0.1 Gy as the non-DES treated ACI rats and other strains with low spontaneous incidence.

Rossi expressed the opinion that meaningful differences in RBE for different strains can only be established on the basis of additional data. Many of the data reported at this conference were of preliminary nature, without the adequate error analysis that is required for definitive conclusions. Based on available evidence the differences of RBE for mammary carcinogenesis at specified neutron energy and dose may well be within a factor of 2 in different strains. He made the further point, that radiobiological observations are generally obtained at doses and at incidences beyond the acceptable levels in radiation protection. There is therefore every reason to expect that RBE values relevant to radiation protection will be at least as large, and possibly much larger, than the values obtained in animal experiments with neutrons.

Shellabarger concluded with the comment that the rat may, in many ways, be a poor model for man, but that differences between humans are equally striking. As Metalli cautioned, we do not know how relevant the studies on the rat are for risk estimates, but we know that comparative research is indispensable, and that it must include animal experiments as well as human epidemiology.