IONIZING RADIATION: SOURCES AND BIOLOGICAL EFFECTS

United Nations Scientific Committee
on the Effects of Atomic Radiation

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NOTE

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ANNEX L

Biological effects of radiation in combination with other physical, chemical or biological agents

CONTENTS

Introduction

1. In man's living and working environments situations are often encountered in which different ambient factors of a physical, chemical or biological nature could conceivably combine with ionizing radiation in giving rise to undesirable effects. In this paper for the first time the Committee considers the combined action of radiation with potentially important environmental conditions. Since this paper concentrates on radiation in environmental circumstances, three important areas of combined action between radiation and chemical agents are not considered here. The first concerns the combined action of chemical agents (both chemotherapeutic compounds and sensitizers of various kinds) to enhance radiation effects in clinical radiotherapy [B28, D24, D25, H28, H29]. The second results from the restriction of this paper to radiation effects combined with agents which affect carcinogenesis, not therefore including combined effects in mutagenesis. This area may be considered by the Committee in the future. The third area not treated in detail in this paper is the effects of a combination of protective agents with acute radiation exposure [A12] because this subject is of only minor importance in estimation or modification of risk.

2. There is a great scarcity of systematic data on which an analysis of combined effects can be based, in

spite of the large number of reports where combined actions were tested and interactions claimed. Thus, this Annex must be somewhat different from others in which a large body of literature data is reviewed and systematically analysed. This Annex will be instead more hypothetical and will attempt to suggest definitions, to identify suitable methods of analysis, to select from a large amount of diffuse information the conditions and the data of importance for further consideration and to provide suggestions for future research.

3. The following review of experimental or epidemiological data should be simply taken as an illustration of some theoretical analyses using examples from the literature. These considerations point, on the one hand, to the very preliminary character of this Annex and, on the other hand, express a word of caution against hasty conclusions in view of the present state of knowledge and the large variety of situations encountered.

4. There are many instances of possible combined actions in which different agents may interact with ionizing radiation. Among the physical agents, for example, temperature should be considered. It is well known that ambient temperature in different environments may vary within a range of about 70°C although control mechanisms allow man to survive under the most extreme conditions. It is also known, however, that small changes in the temperature of cells may result in striking changes of cell survival upon irradiation. These changes are presently being investigated for their potential in cancer therapy [C1, F2, D16]. Ultraviolet light, itself carcinogenic, sound, ultrasound and vibrations are present in many living and working environments and may give rise to combined actions. The same can be said for static electromagnetic fields, and high-frequency or very high frequency (microwaves) electromagnetic radiation.

5. Man-made (xenobiotic) chemicals in the environment are a major concern for toxicologists. According to some estimates [M2] the number of identified molecules is now more than four million and every year a few hundred thousand new items are added to the list. There are some tens of thousand chemicals in common use in modern societies, not including pesticides, pharmaceuticals and food additives. The so-called "energy related pollutants" are also to be considered in this category. They include the oxydes of carbon and nitrogen, sulfur compounds, polycyclic hydrocarbons and some others. Among them 3,4-benzo(a)pyrene (BP) is frequently used as an index of polycyclic hydrocarbons with cancerogenic properties [S2]. Its yearly production is estimated to be approximately 5000 t [S31]. The time course of its production may be followed, for example, by lake sediment analysis [H11]. The concentration of BP in the air of large industrial cities may reach values of 100 ng/m^3 [B10]. BP is also one of the many chemical constituents of tobacco smoke and may be considered of importance for some sections of the population occupationally exposed to radiation. The circulation of BP and of other polycyclic aromatic compounds in the environment has been studied extensively [S2, S31].

6. The list of chemicals whose action might combine with that of radiation in the environment is very extensive. Special attention should be given to situations of practical interest where the chemical agents themselves have carcinogenic properties [H21]. For example, many industrial effluents contain trace elements such as arsenic, nickel or chromium. These substances may produce carcinogenic or mutagenic effects [T7]. The same is true for dust and fibres. Dust is a very common and widespread industrial emission and a component of many occupational environments. It has been reported that dust or fly ash from power stations may have carcinogenic properties [K9] or may serve as carriers of trace metals, radioactive nuclides or polycyclic aromatic hydrocarbons [B22]. In mines mineral dust may combine with the organic products of diesel exhausts and with radioactive radon and thoron daughters [C16]. Asbestos fibres are also often a significant component of occupational and home environments which may include ionizing radiation.

7. High levels of mutagenic chemicals have been reported in many types of food [S32]. Broiled meat and fish contain mutagenic compounds arising from the pyrolysis of proteins and aminoacids. Mutagens and co-mutagens have also been reported in derivatives of vegetable foods, such as caffeine. As mutagenicity often correlates well with carcinogenicity, the above substances may be considered potential carcinogens, both alone or in combination with radiation. According to some estimates [H2] up to 20–50% of spontaneously occurring human tumours can be attributed to diet. Some pharmaceutical substances are also known for their carcinogenic potential: depending on their use and diffusion they could also be considered as candidates for combined actions.

8. Among biological agents, viruses may be regarded as environmental factors likely to interact with radiation. It is well known that some viruses have an important role in the aetiology of some radiation-induced animal tumours as specific agents. There is a possibility that specific agents of a similar nature may be involved in the induction of tumours in the human species and non-specific associations or combined actions, even though on a purely speculative basis, may be visualized. Natural hormones could also be viewed as a special case of interaction in view of the well-known dependence on the hormone level of some forms of radiation-induced tumours in experimental animals.

9. There are two ways of carrying out an analysis of combined actions. The first is to search for any possible effect, whatever its practical significance or quantitative value might be. The second is to concentrate on those effects that may be of importance for the assessment of risk in man. The first approach is that to be followed in the present preliminary analysis. The present practice in radiation protection is that of assuming sensitivity values across the population which apply to all groups, e.g., to males and females of all ages. This practice does not deny the existence of real changes in the susceptibility between various classes of people, but recognizes the convenience that for practical purposes a single average value of the risk is desirable and sufficient.

10. In acknowledging the merits of this approach, the Committee wishes to emphasize that unless the effects to be validated as synergistic or antagonistic are extremely important (i.e., unless they might lead to changes of at least an order of magnitude in the risk estimates) and unless they also applied to substantial fractions of the population at large, they presumably may not be of relevance in assessing risk estimates in man. The above consideration applies to the estimation of risks for radiation protection purposes. It does not contradict the fact that if some synergistic or antagonistic effects can be identified under specific exposure

conditions of occupational or medical relevance, appropriate actions should be taken to change such conditions. Under such circumstances, however, the problem would not be any longer one of radiation protection philosophy, but rather one of practical occupational medicine. It would not involve basic changes in the approach to such matters but specific remedial local actions.

11. Radiation effects with particular regard to carcinogenic and to genetic and developmental consequences of irradiation were considered by the Committee in its 1977 report [U1]. Non-stochastic effects of whole- or partial-body irradiation (Annexes K and J, respectively) and genetic effects (Annex I) are also discussed in this report. When reviewing such a broad field as that of combined actions, no effects should be excluded from consideration at whatever level (subcellular, cellular, tissue, organ, whole-body) they may be manifested. This is particularly true in view of the heterogeneity of the data available and of the fact that understanding of combined effects will eventually require knowledge of the mechanisms involved. That is why effects other than those mentioned above will be discussed in this Annex. However, the main emphasis will be on stochastic effects. Where possible, epidemiological data will be considered, even though studies of this sort are rare and often statistically inconclusive.

12. Each of the possible interacting agents may act alone in producing biological effects or may only be active in conjunction with other factors, particularly radiation. Exposure to any of these agents may be acute, subacute or chronic, within a wide range of doses and dosages. The pattern of exposure may also play a role, as the contemporaneous action of the various agents or the order of their sequence and the intervals between treatments may conceivably affect the quality or the degree of the effect. Of all possible situations of combined actions the Committee chose to particularly investigate conditions where long-term exposure to low levels of the agents on large human populations may apply, because these conditions may possibly affect radiation risk estimates in man.

13. The combined action of several agents is not a new problem in medicine. As early as 1928 Loewe [L1] quantitatively reviewed the approaches to the assessment of the action of combined drugs. So-called "isobolic diagrams" were proposed in this regard. This Annex will consider this approach in detail, as well as other approaches extensively used by toxicologists [M1, T1]. Some of these ideas were adapted specifically for the needs of the Annex and illustrative material has also been derived and modified for the same purpose.

14. Nomenclature in the analysis of combined actions was a problem that was recognized very early [L1]. In order to simplify the discussion to follow, it is appropriate to provide some clear definitions and terminology. Two classes of combined effects will be considered. In the first class, both ionizing radiation and the other agent (or agents) produce the effect under discussion. The second class includes the combinations where ionizing radiation produces an effect whose nature or amount may be modified by the other agent which by itself is inactive. This classification is only made as a convenient approximation.

15. For the first class of interaction there are three types of combined actions. When the end-effect of the combined action equals the sum of effects of the two agents acting independently, the resulting situation is one of "additivity". If additivity does not apply, then there are two possibilities. When the effect of the combined action exceeds the sum of the effects produced separately by the agents, the situation is one of "synergism". Finally, when the combined action results in an effect which is less than expected from the sum of the action of the interacting agents, the situation is termed "antagonism". The precise meaning of the "sum of effects" will be expanded further in chapter I. The notion of summation of effects pre-supposes the existence of a quantity which may be meaningfully added.

16. The concept of additivity cannot be extended to the second class of combinations since radiation is here the only agent capable of producing an effect. Under these circumstances the comparison is usually between doses of radiation producing the same amount of effect in the absence or in the presence of the modifying agent. If, for a certain degree of effect, the dose of radiation required is greater in the presence of the modifying agent, the resulting action is termed "protection". Conversely, when the dose of radiation is less for the same degree of effect in the presence of a modifying agent, "sensitization" occurs.

17. The above classification is not an absolute one. For example, sensitizing substances which have been assumed to be inactive, may be able to produce some effect at high exposure levels. Also, if one considers carcinogenesis as an effect, promoters may be viewed as a special case of sensitizers and many promoters may show initiating properties. The low environmental levels of the interacting agents are mainly those of interest in this Annex. At these levels the threshold-type dose-response curves of the sensitizers and promoters may render their contribution negligible or zero. A unified approach to both classes of interaction in terms of interaction coefficient and more precise quantitative definitions of the concepts introduced in the above paragraphs will be developed in chapter I.

18. For exposure of the public the most significant man-made source of irradiation is for diagnostic medical purposes where the yearly dose equivalents may be up to the order of a few millisievert (mSv) (see Annex G). Sources of occupational exposure are much more varied and may range from exposure to radon in mines to x rays generated by electronic appliances. The yearly occupational exposure according to ICRP recommendations should not exceed 50 mSv [I1]. Average yearly exposures to natural sources of radiation are between 2 and 3 mSv (see Annex B). The actual occupational exposure in industry has average values of about 5 mSv (see Annex H). Thus the other physical, chemical or biological environmental agents would combine with ionizing radiation at levels of the latter of 1–10 mSv per year. These levels are usually referred to as low doses.

19. It is sometimes held that in view of the ubiquitous nature of background radiation all experimental or epidemiological studies on the toxicity, carcinogenicity or mutagenicity of chemicals or other agents are automatically performed to account for the concomitant radiation risk. All the relevant risk assessments would therefore be in essence assessments of combined action [S1]. This may be too broad a generalization for the following reasons. Firstly, the actual levels of exposure to ionizing radiation may be orders of magnitude higher than those cited in the preceding

paragraph, and the levels of the other agents orders of magnitude lower than those at which experimental risk assessments were performed. In view of the non-linearity of the dose-response relationships for most chemical agents, extrapolation of the risk assessments between such widely different situations would be unwarranted. On the other hand, some chemicals which are ineffective in producing detrimental changes when acting alone, may instead provide a significant modification of the radiation action, as in the case of carcinogenic promoting substances. Animal experiments are usually carried out at levels of exposure to chemical or other agents which are much higher than those found in the environment, which weakens the basis for extrapolation. Under such conditions of great uncertainty the best course of action is to reserve any judgement and to investigate the facts.

20. In summary, the scope of this Annex is:

(a) To review possible quantitative approaches to the assessment of the combined action of radiation and other environmental conditions, based on the concepts of additivity, synergism, antagonism, sensitization and protection;

(b) To explore whether and to what extent concepts in other fields of the biological sciences may be applied to the special case of interaction with radiations, particularly at very low doses of the combining agents;

(c) To consider experimental results on the combined action of radiation and other conditions, in order to elucidate possible mechanisms of action that may allow generalizations and extrapolations;

(d) To review existing epidemiological data on subgroups of populations living or working under the action of radiation and other environmental toxic agents;

(e) To identify possible areas for useful research in the field of combined effects.

I. MODES OF INTERACTION

A. GENERAL APPROACH

21. When examining the concept of combined action it is useful to start with the definition of a quantity referred to here as "exposure", X, which may apply to any environmental agent [L3, L4]. Exposure is the independent variable in exposure-effect relationships. Without exposure to the agent there can be no effect over the spontaneous level and with increasing exposure the effect appears to follow some kind of functional "exposure-response" relationship. This generalized concept of exposure is different from the notion of exposure in radiation physics (see Annex A). In the case of ionizing radiation the absorbed dose, D, is used instead of the exposure and "dose-response" relationships are established to functionally relate the energy absorbed by the irradiated object with the response observed. If radiation quality must be taken into account, the quantity defined as dose equivalent, H, may be used in place of the generalized concept of exposure, X.

22. The definition of exposure (or dose), X, is more difficult in the case of other agents [L3]. Often this notion includes the product of some intensive quantity (e.g., energy flux per unit area per unit time) multiplied by an extensive quantity (e.g., the time during which the agent acts on the biological system). It has been proposed in the case of chemical compounds [E1] to define exposure as the number of primary chemical events leading to the final effect, but at present the nature of such events is only known in rare cases and their quantification exceptional. The concentration of an agent may often meaningfully be taken as the intensive quantity, multiplied by time as the extensive one. The notion of exposure is by definition extensive and in the case of a chemical substance it could be represented by the formula

$$X = \int_0^t C(t)\,dt \qquad (1)$$

Obviously, to give exposure some biological meaning, the concentration of the agent, C(t), should be expressed at the level of the target biological structure, but this is often impossible. A useful type of exposure characterization such as the pharmacological dose (the quantity of the chemical introduced per unit weight of the organism) does not provide such information. In these cases special assumptions concerning the intake, retention, metabolism and excretion of the agent under investigation must be made [L3, W4].

23. Even the relatively simple case of a chemical acting on a culture of cells in vitro may require special consideration of the kinetics of the substances involved and of the different forms of their possible transformation [W4]. One may, for example, consider a scheme whereby a chemical A is converted into intermediate B which is in turn transformed into a cell-bound moiety C leading to the observed effect:

$$A \rightarrow B \rightarrow C \rightarrow$$

Clearly the concentration of C is the quantity to be used in equation (1) to express the exposure. It often happens, however, that the only information available is on the chemical A, the most readily measurable quantity, and this information may not be directly proportional to the values for C. Thus, there might be apparent absence of effect, in spite of a high concentration of A, on account of absence of moiety C, at least at the beginning of exposure.

24. Thus, the metabolic activation of chemicals into active forms is of great interest [M19, S33]. Chemical carcinogens are known to be subject to complex processes of enzymatic reactions in vivo. The chemical compound introduced into the body may be considered as a pre-carcinogen which, through various reaction pathways, will eventually produce proximate and ultimate carcinogenic derivatives. From a purely chemical point of view, one of the important generalizations of the recent years is that the ultimate forms of chemical carcinogens are usually electrophilic (i.e., electron-deficient) reactants. Many specialized examples of such processes are considered in the above mentioned reviews [M19, S33].

25. In some cases the binding of chemicals with cell constituents may be monitored by the use of radioactive labels. Examples of such studies in vitro with two derivatives of nitrosourea were provided in [W4]. Experiments in vivo are also available [E1, W9, P8] in which correlations are established between the administered doses of the compounds, the amount of bound moieties and the biological effects. These studies help clarify the concepts of administered versus active doses of the compounds.

26. If the exposure, X, to a given interacting agent (or to several agents) may be satisfactorily defined, the

definition of the effect, Y, should be considered. There are different ways of expressing in quantitative terms the response of a biological object. Y may be, for example, the fraction of cells showing loss of a specific function or the fraction of exposed animals affected by a given mutation or carrying a given type of tumour. In such cases Y describes the probability of induction of that given effect as a result of the exposure X. In other cases Y may describe the degree of a given effect: for example, the weight loss of an exposed animal, the mean number of tumours per animal, changes in various haematological indices. Graded effects may sometimes be reduced to probabilistic quantities by appropriate analysis, but this is not always the case and it may represent a limitation.

27. The simplest functional relationship between exposure and response, $Y = F(X)$, is the linear one:

$$Y = Y_0 + kX \qquad (2)$$

Here the term Y_0 accounts for the effect produced in the absence of exposure or of any other known cause in an apparently spontaneous fashion. The coefficient k defines the sensitivity of the biological system to the agent. When the separate action of each agent is described by equation (2), then the increment of response of the system to each agent may be written as

$$\Delta Y = Y - Y_0 = kX \qquad (3)$$

If one assumes that the increments of response to one agent are independent of the presence of the other interacting agent, the increment of response for the simultaneous action will equal the sum of increments ΔY_1, ΔY_2

$$\Delta Y = k_1X_1 + k_2X_2 \qquad (4)$$

This is the situation of additivity.

28. However, the experimental value of ΔY in case of a combined action can be higher or lower than the ΔY expected from equation (4). If $\Delta Y_{obs} > \Delta Y_{exp}$ the situation is defined as synergism. If $\Delta Y_{obs} < \Delta Y_{exp}$ the situation is one of antagonism. As a measure of the deviation of the experimental results from additivity one may introduce an interaction factor

$$\omega = \Delta Y_{obs}/\Delta Y_{exp} \qquad (5)$$

The value of $\omega = 1$ will correspond to additivity, $\omega > 1$ to synergism and $\omega < 1$ to antagonism.

29. The above concepts may be represented in a graphical form as in Figure I. Here a given level of response Y* is chosen, which level may be obtained by the action of each agent separately (X_1^* or X_2^*, respectively) or by the combined action of both agents at variable exposures X_1 or X_2. If additivity is operating and equation (4) is applicable, all points (X_1, X_2) producing the level of response Y* must lie on the middle diagonal line of Figure I. This line is called the isobolic line and the diagram is called isobolic diagram [L1]. The scale of Figure I is chosen in such a way that the co-ordinate value equals 1 for each agent acting separately, that is. $X_1/X_1^* = 1$ and $X_2/X_2^* = 1$.

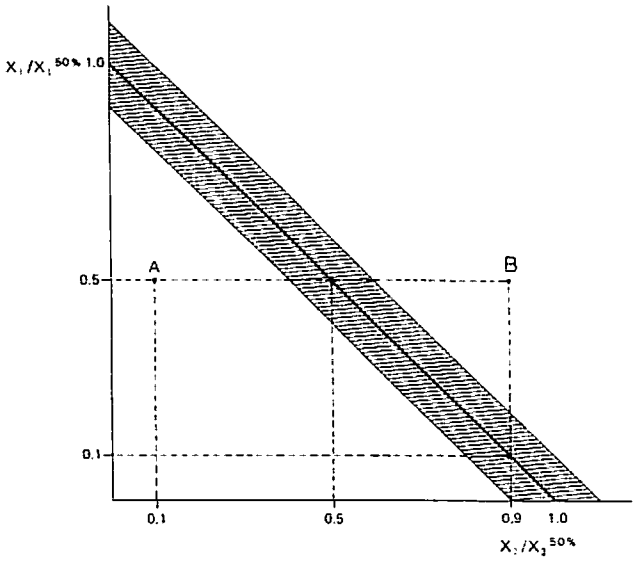

Figure I. Isobolic diagram in case of linear additive response to the action of two agents

30. The isobolic line in Figure I describes an ideal case of additivity, but all experimental exposure-response relationships are affected by errors. In real situations therefore the line of additivity expands to an area of additivity, such as that covered by the horizontal shading lines in the same figure. If the exposure-response relationships for the agents acting separately are linear, the type of interaction may be defined by simple graphical procedures. For a given level of effect, Y*, several levels of exposure to both agents are tested: if the experimental points (X_1, X_2) fall into the area of additivity, the interaction will be regarded as additive. If the points fall to the left of the area of additivity, the interaction will be one of synergism; and, conversely, one will be dealing with an antagonistic interaction when the experimental points are found on the right-hand side of the area. In Figure I the experimental point A would be regarded as confirming synergism, experimental point B as confirming an antagonistic interaction.

31. As an example of the application of this analysis, the experiments of Murthy et al. [M3] on diploid yeast BZ34 may be of interest. The cells were irradiated by ^{210}Po alpha particles or by ^{60}Co gamma rays separately or in combination. The end-point studied was reversion to arginine independence. Linear dose-response relationships were found for both radiations given separately with slopes of 25.5 ± 2.6 and 10.9 ± 0.4 reversions per 10^6 survivors per Gy applying to the alpha and to the gamma radiation, respectively. In the case of combined simultaneous treatment with both radiations (25% of the dose was by alpha radiation at 0.5 Gy/min and 75% by gamma radiation at 1.54 Gy/min) the slope of the regression line changed to 17.7 ± 0.9 reversions per 10^6 survivors per Gy. The results may be interpreted by an isobolic diagram, as in Figure II. For the level of reversion Y* = 180 rev/10^6 survivors the dose of ^{60}Co gamma would be 15 Gy and that of ^{210}Po alpha 6.4 Gy. The dashed lines parallel to the isobolic line in Figure II establish the 95% confidence limits. If one plots the points corresponding to the same level of reversions for the two agents combined, one finds the point denoted A which lies clearly to the left of the area of additivity. It is concluded that synergistic interaction of the two agents applies in this case. This is an example of isobolic diagram analysis in its most simple form.

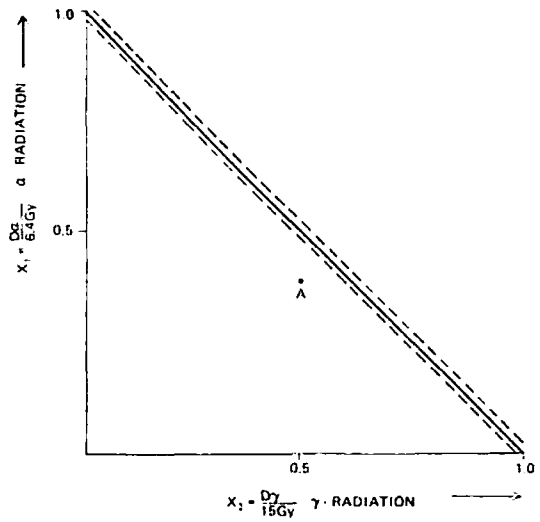

Figure II. Isobolic diagram for reversion of yeast to prototrophy (Y = 180 rev/10^6 survivors) under the action of alpha radiation from polonium-210 and gamma rays from cobalt-60 [M3]

32. In the above example the mutation frequencies could be meaningfully added because their increase with dose was linear. The same procedure is not applicable when the effects change as exponential or sigmoid functions of the dose, unless the dose-response relationships may be converted to linear or quasi-linear functions.

33. The process of additon itself may be performed in two ways. The first, takes the response to the dose A from the survival curve A and adds it arithmetically to the response to dose B from survival curve B. Both doses are counted from the origin of the co-ordinates. Loewe [L1] designates this type of addition as heteroaddition. A second process of addition, called isoaddition, is also possible. Let us assume that agent A is applied before agent B (Figure III b, e, h). Figure IIIa shows the

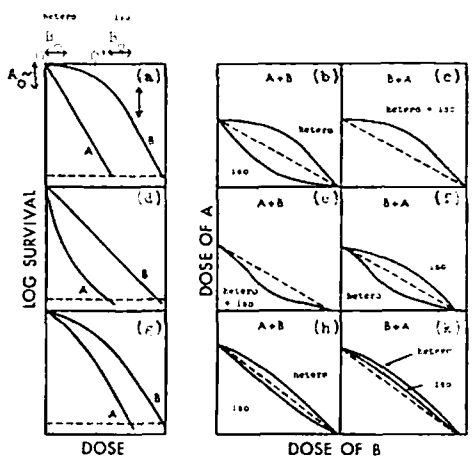

Figure III. Examples of hetero- and iso-addition for agents A and B in case of different dose-effect curves and different order of treatment by the agents [R7]

case when the dose A_o is given before B_o. In the case of heteroaddition the dose B_o would be counted from the origin of the co-ordinates. In the case of isoaddition, on the contrary, the latter dose would be counted from point O', corresponding to the survival level on curve B to which the biological system is brought by the action of agent A. It is easily appreciated that for isoaddition the response to B_o will be much greater than in the case of heteroaddition. This is the reason why the isobolic lines of iso- and hetero-addition are so different in Figure IIIb.

34. The area between two isobolic lines may be called the envelope of additivity [S3]. As a result of different sequencing of the agents this envelope may reduce to a line [R7], as shown in Figure IIIc. This occurs when one of the two interacting agents produces an exponential response. Other examples (Figure III d, e, f, g, h, k) show how the form of the response curves and their relative curvature define the form of the envelopes of additivity and the influence of a different sequence of the agents.

35. The above considerations may be generalized to any type of exposure-response relationship. Since any a priori judgement about the type of addition (iso- or hetero-addition) is impossible, both possibilities should be accounted for. The practical usefulness of the envelope of additivity lies in the fact that if the experimental points fall within the envelope, additivity is to be expected. When they fall to the left (point A in Figure IV) synergism is operating; and, conversely,

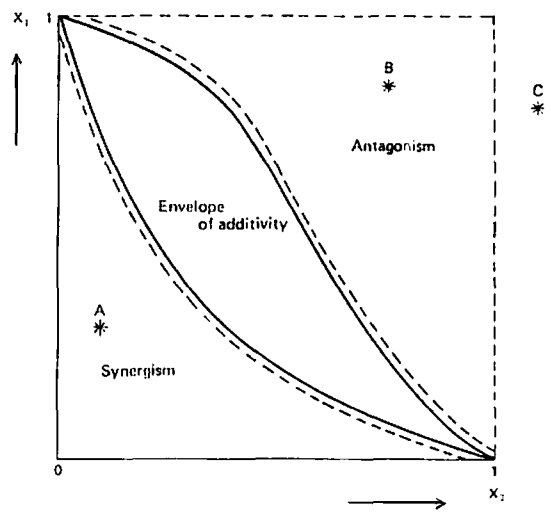

Figure IV. Envelope of additivity and areas of synergism and antagonism

antagonism will be operating if they fall to the right (point B in Figure IV). Enlargement of the envelope due to experimental errors is also shown in the same Figure IV. Attention should be drawn to the fact that although in principle the area of antagonism extends from the upper right-hand border of the additivity envelope to infinity, the straight dashed lines in the figure define the area beyond which the administration of one agent requires application of the other at levels greater than its single exposure level for the same effect. Point C in Figure IV lies in such an area where exposure X_1 requires an exposure X_2 greater than unity.

36. Discussion has so far been limited to the class of interaction where both agents may produce the effect under study. A large number of agents are however known in radiation biology which may modify the radiation response of the system without being themselves active in determining the effect. These modifying agents are called radioprotectors or radiosensitizers, without regard to their mechanism of action [M10]. The same approach as that used in the preceding paragraphs for the assessment of the interaction type may also be generalized to the modifiers. However,

since only radiation dose-response relationships are considered here, a specific approach to sensitization and protection may be developed.

37. Oxygen is one of the most important modifying agents [D1]. Its action is extremely general at all levels of biological organization in the sense that macromolecular, cellular and tissue systems irradiated under oxygen show an enhanced effect compared to that resulting from the same dose delivered under anoxia. This enhanced effect is often expressed as an oxygen enhancement ratio (OER) defined as

$$OER = D_{(non\text{-}oxygenated)}/D_{(oxygenated)} \quad (6)$$

expressing the ratio of doses D under anoxia and under oxygen to obtain a given level of effect. Other similar quantities may be used for the description of the effect of different modifiers. For example, the thermal enhancement ratio (TER) in the case of the combined action of radiation and heat, is:

$$TER = D_{(standard\ temperature)}/D_{(enhanced\ temperature)} \quad (7)$$

or the dose reduction factor (DRF) for radioprotectors

$$DRF = D_{(protector)}/D_{(no\ protector)} \quad (8)$$

For radiosensitizers, the factor in common use is the dose modifying factor (DMF)

$$DMF = D_{(no\ sensitizer)}/D_{(sensitizer)} \quad (9)$$

This quantity defined for a particular level of response is often referred to as enhancement ratio (ER) or sensitizer enhancement ratio (SER) or dose modifying ratio (DMR).

38. Also for modifying agents one may define the increment of effect in the presence of radiation alone, ΔY, and the increment in the presence of the modifier ΔY_M. The concept of an interaction factor may also be introduced, as follows

$$\omega = \Delta Y_M/\Delta Y \quad (10)$$

When linear relationships apply both in the absence and in the presence of the modifier, the value of the interaction factor ω will coincide with the value of the dose modifying factor (DMF). In Figure V the line

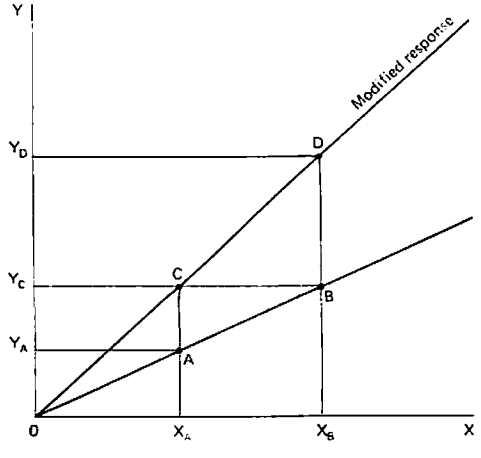

Figure V. Linear exposure-response relationships in the absence (OAB) and in the presence (OCD) of a sensitizing agent

OAB is the response in the absence of the modifier and the line OCD the response in the presence of a sensitizer. In this particular case

$$\omega = Y_C/Y_A \quad (11)$$

and

$$DMF = X_B/X_A \quad (12)$$

However, as the ratio Y_C/Y_A is equal to X_B/X_A both definitions coincide. In this special case of linearity the values of ω and of DMF will be independent of the level of exposure, because the straight line is fully defined by only one parameter (the slope at 0 exposure or the response at any specific exposure). In geometrical terms, the ratio Y_D/Y_C in Figure V is the same as Y_C/Y_A.

39. The circumstances differ of course in cases of non-linear exposure-response relationships that would most probably apply to the vast majority of the situations in practice. Figure VI illustrates one such case where the

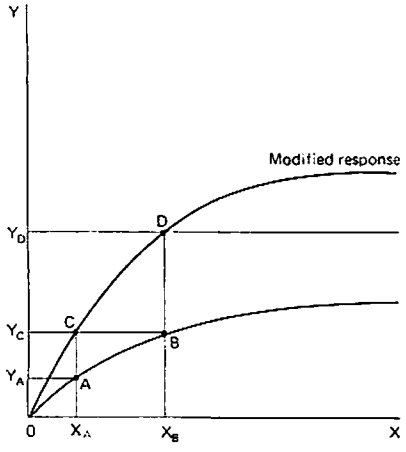

Figure VI. Non-linear exposure-response relationships in the absence (OAB) and in the presence (OCD) of a modifying agent

ratio Y_C/Y_A is not any longer equal to, but is actually much smaller than the ratio X_B/X_A. At exposure level X_B the definition of an enhancement ratio is meaningless because the line through point D parallel to the abscissa will never cross the other response curve OAB, but an interaction factor for a modified response as defined in equation (10) may still be applied. However, the value of ω will depend on the level of exposure or response.

40. The situation is further complicated when the application of a modifier significantly changes the general form of the dose-response relationship. In such cases the use of ω, that is the use of an enhancement ratio in terms of increment of effects, may not be applicable. The solution requires specifically defining a suitable quantitative measure of the modifying effect under the conditions applying to the experimental situation.

41. The concepts and approaches outlined so far are quite sufficient for a discussion of the available scientific literature on the interaction of different agents with radiation. When possible, in the text to follow the concepts of interaction factor and envelope of additivity on isobolic diagrams will be applied. However, further refinements and generalizations of the concepts outlined may be of some value, as in the

two following sections. These sections may however be omitted without significant detriment to the understanding of the experimental material reviewed in the chapters to follow.

B. SURFACE OF RESPONSE AND ISOBOLIC DIAGRAMS

42. The methodology of assessment of effects in combined exposures outlined by Loewe [L1, L5] allows a much broader approach to the problem. If, for the sake of clarity, one assumes only two interacting agents, the response to agent 1 is given by the function $F_1(X_1)$ and that to agent 2 by the function $F_2(X_2)$. The simultaneous action of the two agents will result in some new function $F(X_1,X_2)$. The functions $F_1(X_1)$ and $F_2(X_2)$ describe the response on a plane; the new function $F(X_1,X_2)$ describes the response in a three-dimensional space. This new function is called the surface of response. It may be used for any number of agents, and in these cases it will be described in multi-dimensional space. The concept of a surface of response makes the approach to the assessment of interaction geometrically clear. In this case the comparison is drawn between the surface obtained as a result of addition of responses to single agents (surface of additivity) and the surface of response for the function $F(X_1,X_2)$.

43. Let the functions $F_1(X_1)$ and $F_2(X_2)$ be linear with a simple law of addition operating for simultaneous action. Then one obtains the surface of response (and the surface of additivity at the same time) as the inclined plane in Figure VII. Cross-sections of this

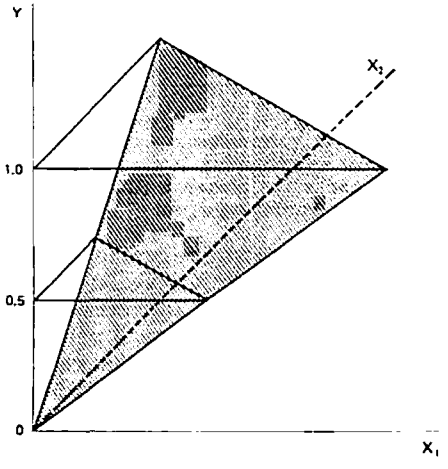

Figure VII. Surface of response in case of linearity and additivity for the combined action of two agents

plane at different levels of response (in Figure VII at Y = 0.5 and at Y = 1.0) will always produce straight lines which are isobolic lines in the sense of Figure I. By choosing the scales of the coordinate along the X_1 and X_2 axes it is possible to adjust the angle of the cross section line with the coordinate plane so as to make it equal to 45°.

44. The linearity of the functions $F_1(X_1)$ and $F_2(X_2)$ is however by no means a necessary condition for obtaining linear isobolic diagrams. The case of S-shaped functions is considered in Figure VIII. The two functions are represented by the curves in the co-ordinate planes YOX_1 and YOX_2. The surface of additivity (i.e., the dotted surface in Figure VIII) has now also a changing curvature similar to that of a tense sail, but horizontal planes at levels of response Y = 0.5 and Y = 1.0 still transect this surface by straight lines, so that

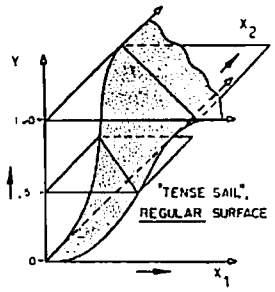

Figure VIII. Surface of response in case of additivity for curvilinear functions

again the isobolic diagrams are of the same type as in Figure I. According to this graphical representation, synergistic interaction is expressed by a deviation from the surface of additive response nearer to the OY axis. The new synergistic surface of response is presented in Figure IX and it resembles an inflated sail. The cross-

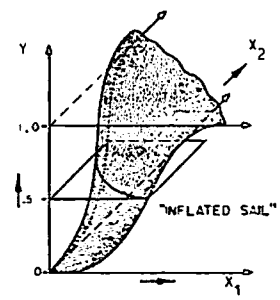

Figure IX. Synergistic surface of response

section of this surface by a horizontal plane at the level of effect Y = 0.5, for example, produces a curve which is the isobolic diagram of a synergistic interaction. The case of antagonism is exemplified in Figure X, where

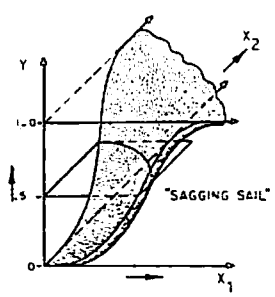

Figure X. Antagonistic surface of response

the antagonistic surface of response is further removed from the OY axis, in the form of a sagging sail. Transection of this surface by an ordinate plane (Y = 0.5) results in a curve with a concavity towards the origin of the co-ordinates, i.e., a curvature in the opposite direction than that of the synergistic action.

45. The above interactions may be represented by the isobolic diagrams of Figure XI, where the isobolic lines are the cross sections by a horizontal plane at the level of effect Y = 0.5 of the three surfaces of response in Figures VIII, IX and X. Such comparisons can be made at any level of effect, but in the case of agents present in the environment the levels would generally be low. It is therefore of interest to examine the shape of the

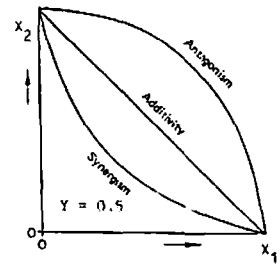

Figure XI. Isobolic diagrams obtained as the cross sections of surfaces of response in Figures VIII, IX, X at the level Y = 0.5

surfaces of response around the origin of the co-ordinate axes. It is not uncommon that the form of the surfaces might go from a synergistic type to an additive type in the region of low effects. At different levels of effect the interaction might even change from the synergistic to the antagonistic type, or vice versa.

46. Changing situations of this sort are illustrated in Figure XII, where the form of the surface of response is

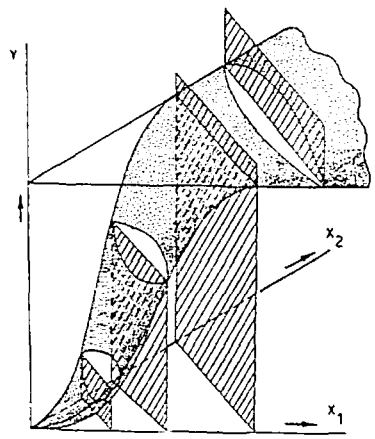

Figure XII. Cross sections of the surface of response by 45° vertical planes

made clearer through other types of cross sections. In the case shown the planes transacting the surface are diagonal, parallel to the Y axis and with an angle of 45° with respect to the X_1 and X_2 co-ordinate axes. The areas of the planes transacted by the surface and covered by the dashed lines show the extent of the difference between the real surface and an ideal surface of regular additivity. The plane nearest to the origin shows a strong antagonistic interaction, but the further the transacting plane is removed from the origin, the less important becomes the antagonism; until, at very high levels of exposure, the interaction becomes synergistic. Cross sections of this type can also help in assessing the mode of interaction and are called "interaction diagrams".

47. In Figure XII the levels of exposure are limited by the vertical planes chosen, but the levels of response may change. Such changes of response which depend on the relative contribution of X_1 and X_2 form the interaction diagram as shown in Figure XIII. The lower curve in Figure XIII shows the line of interaction (antagonistic interaction) resulting from the transaction of the surface of response by the vertical plane nearest to the origin of the co-ordinates in Figure XII. The case

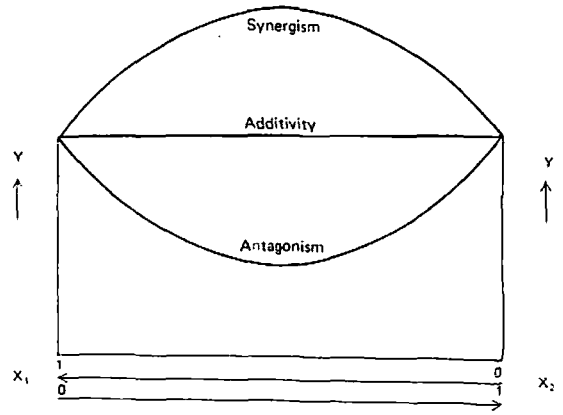

Figure XIII. Interaction diagrams in cases of additivity, synergism and antagonism

of additivity is represented by the line parallel to the exposure axis, while the case of synergism is described by the curve with upper convexity. Again, the line of additivity divides the space into two portions: an upper one, where interaction is synergistic and a lower one with an inhibitive type of effect. In essence, this method of analysis relies on the comparison between two surfaces: the actual surface of response and the surface corresponding to the presumed additivity of the effects of the two agents given separately. If the real surface of response is higher than additive, there is synergism. Antagonism would operate in the opposite case.

48. Construction of the surface of additivity is a prerequisite for all comparisons. It has however been discussed already that the addition of responses in complex biological systems represents a problem in itself because the results of iso- or hetero-addition depend on the sequence of the interacting agents. Adding to these uncertainties the experimental errors, turn the surface of additivity into a shell of additivity, corresponding to the envelope of additivity of the bi-dimensional representations. Although the actual comparisons should always be performed in relation to a shell of additivity, it is often more convenient to use the two-dimensional representations under the form of isobolic diagrams (or interaction diagrams). Elaborate methods of analysis have been developed for the interaction of several agents [C21].

49. When all the above assumptions have been dealt with, the comparison of the experimental data with the ideal case of additivity is straightforward conceptually and technically simple. For a given combination of exposures (X_1, X_2) the interaction factor ω may be calculated as the ratio of the actual ordinate of response to the ordinate of the additivity surface in the point (X_1, X_2). To this end interaction diagrams are particularly convenient. In Figure XIII the interaction factor ω for the combination X_1, X_2 as in point D will be equal to the ratio AD/BD in the case of synergism and to the ratio CD/BD in the case of antagonism. When iso- or hetero-addition give different outcomes, they should be used instead of the segment BD, and the upper and lower values of ω will be obtained, ω_u and ω_l, respectively

C. PROBABILISTIC ASSESSMENT OF THE INTERACTION

50. As any biological end-point which is expressed at a sufficiently high level of complexity may be viewed as the final outcome of a long chain of intercorrelated

events, it appears quite natural and wholly justified to express any end-effect by the probability, P, that it may occur. The dependence of this probability on the absorbed radiation dose may sometimes be one of direct proportionality but in most instances, and particularly for the most complex effects, the relationship may be more complex. Essentially the same can be said about the effects of other physical and chemical agents.

51. In probabilistic terms, if two agents act simultaneously on a biological system, one possible assumption is that the two agents act independently, which allows comparison of the results of the joint actions with the presumed outcome of the two agents acting independently. In some respects, this notion is similar to that of heteroaddition, with the difference that the probabilistic approach is conceptually much broader. The discussion to follow will again be limited to the simple case of two agents acting simultaneously but may in principle be extended to any number of agents.

52. The Committee agrees that the most important effects of radiation in man are carcinogenesis and mutagenesis, effects that are described by the ICRP [11] as stochastic in the sense that their probability of occurrence increases linearly with dose and without threshold; their severity is independent of dose; and no causal relationship with radiation exposure can empirically be established for any given case. It is well known that these effects do occur even in the absence of artificial irradiation with a frequency which is much higher than would be expected if they were induced only by natural background radiation. For the purpose of the present analysis, they may thus be viewed as stochastic derangements of physiological processes to which a probability of occurrence P_0 could be attributed.

53. If one assumes that the exposure X_1 to an agent causes a probability P_1 of a given effect, t, the overall probability that this same effect can be observed, taking into account the spontaneous level P_0 and assuming that P_0 and P_1 are independent is:

$$P_{t1} = P_0 + P_1 - P_0 P_1 \tag{13}$$

Since in biological experiments control and test groups are run concurrently, the following formulae may also be convenient:

$$P_{01} = P_{t1} - P_0 \tag{14}$$

$$R_1 = P_{t1}/P_0 \tag{15}$$

They show the absolute and relative increase of the probability to observe the given end-effect following exposure X_1. A similar set of equations can also be written for a hypothetical second agent:

$$P_{t2} = P_0 + P_2 - P_0 P_2 \tag{16}$$

$$P_{o2} = P_{t2} - P_0 \tag{17}$$

$$R_2 = P_{t2}/P_0 \tag{18}$$

54. When the action of the two agents is combined, the expected probability of observing the overall effect P_{et} may again be calculated on the hypothesis of independent action:

$$P_{et} = P_0 + P_1 + P_2 - P_0P_1 - P_0P_2 - P_1P_2 - P_0P_1P_2 \tag{19}$$

The absolute and relative increases in probability of observing the effect as a result of the joint action of the two agents will accordingly be:

$$\Delta P_{exp} = P_{et} - P_0 \tag{20}$$

and

$$R_{exp} = P_{et}/P_0 \tag{21}$$

55. When an experiment is performed on the combined action of two agents, the observed total probability of effect, P_{ot}, will in general be different from the expected probability P_{et}. One of the possible definitions of the interaction factor ω might be simply the ratio between the actual and the expected probabilities, P_{ot}/P_{et}. It is easy to see, however, that if this definition is adopted the value of the ratio will depend critically on the absolute value of P_0. When the effect under study has a high spontaneous level of occurrence, the interaction factor ω may be about 1 despite the observed absolute deviation between the experimental values and the expected value based on the hypothesis of independence. On this ground, another definition of the interaction factor, ω, is preferred, as follows

$$\omega = \Delta P_{obs}/\Delta P_{exp} \tag{22}$$

where

$$\Delta P_{obs} = P_{ot} - P_0 \tag{23}$$

The two definitions of the interaction factor will naturally coincide if $P_0 = 0$. The probabilistic definition of ω in equation (22) coincides in essence with the definition of interaction factor in equation (5).

56. The denominator of equation (22) is calculated on the basis of the independence of action of the two agents (equation (19)). Equation (20) may be rewritten by using equations (14) and (17):

$$\Delta P_{exp} = P_{o1} + P_{o2} - \frac{P_{o1}\,P_{o2}}{1 - P_0} \tag{24}$$

Accordingly, the equation for the interaction factor will assume the following form

$$\omega = (P_{ot} - P_0) \Big/ \left(P_{o1} + P_{o2} - \frac{P_{o1}\,P_{o2}}{1 - P_0}\right) \tag{25}$$

If P_{o1} and P_{o2} are small, the above equation reduces to

$$\omega = (P_{ot} - P_0)/(P_{o1} + P_{o2}) \tag{26}$$

or, changing the probabilities to the corresponding ratios for $P_0 \neq 0$, according to equations (15) and (18)

$$\omega = (R_{obs} - 1)/(R_1 + R_2 - 2) \tag{27}$$

where

$$R_{obs} = P_{ot}/P_0 \tag{28}$$

57. To give an example of such a type of treatment, the experiment on diploid yeast irradiated with alpha and gamma radiation [M3] and analysed by the method of the isobolic diagram (see section I.A.), may now be recalculated to obtain the interaction factor. For a dose of 9 Gy of mixed irradiation (25% alpha and 75% gamma) the level of reversion will be 180 per 10^6 survivors, corresponding to a $P_{ot} = 18\ 10^{-5}$. The spontaneous level of reversion $P_0 = 2\ 10^{-5}$. If one knows the alpha and gamma doses and the slopes of their regression lines, one may calculate P_{o1} and P_{o2} to be $5.7\ 10^{-5}$ and $7.4\ 10^{-5}$, respectively. The last term in equation (25) is negligible and one may use equation (26)

$$\omega = \frac{P_{ot} - P_0}{P_{o1} + P_{o2}} = \frac{18\ 10^{-5} - 2\ 10^{-5}}{5{\cdot}7\ 10^{-5} + 7{\cdot}4\ 10^{-5}} = 1.22 \qquad (29)$$

The fact that ω is greater than unity suggests a synergistic interaction.

58. The question arises of establishing errors and limits of confidence for the interaction factor. The theory of error transfer may be applied to this end. The value of ω as defined in equation (22) may be considered as the ratio of two stochastic quantities ΔP_{obs} and ΔP_{exp}. The mean value of this ratio is

$$\overline{\omega} = \frac{\overline{\Delta P}_{obs}}{\overline{\Delta P}_{exp}} \qquad (30)$$

The error matrix for $\overline{\Delta P}_{obs}$ and $\overline{\Delta P}_{exp}$ will be

$$\begin{bmatrix} S^2(\overline{\Delta P}_{obs}) & S(\overline{\Delta P}_{obs})\, S(\overline{\Delta P}_{exp})\, q_{12} \\ S(\overline{\Delta P}_{obs})\, S(\overline{\Delta P}_{exp})\, q_{12} & S^2(\overline{\Delta P}_{exp}) \end{bmatrix} \qquad (31)$$

where S is a symbol representing the mean quadratic error and q_{12} is the correlation coefficient. The mean quadratic error of $\overline{\omega}$ will be in this case

$$S^R(\overline{\omega}) = \frac{(\overline{\Delta P}_{obs})^2}{(\overline{\Delta P}_{exp})^2}\left[\frac{S^2(\overline{\Delta P}_{obs})}{(\overline{\Delta P}_{obs})^2} + \frac{S^2(\overline{\Delta P}_{exp})}{(\overline{\Delta P}_{exp})^2} - 2q_{12}\frac{S(\overline{\Delta P}_{obs})\, S(\overline{\Delta P}_{exp})}{\overline{\Delta P}_{obs}\,\overline{\Delta P}_{exp}}\right] \qquad (32)$$

If the distribution of $\overline{\Delta P}_{obs}$ and $\overline{\Delta P}_{exp}$ is normal and $\overline{\Delta P}_{exp}/S(\overline{\Delta P}_{exp}) > 5$, then the distribution of $\overline{\omega}$ should also be approximately Gaussian.

59. The same problem of assessing an error to $\overline{\omega}$ was considered by Rothman [R1] for the case of epidemiological investigations and he used the same definition of the interaction factor. If a log-Gaussian sampling distribution is assumed, an estimator of ω referring to a large sample interval may be written as

$$\omega_l = \exp\left[\ln\overline{\omega} - k_\alpha S(\ln\overline{\omega})\right]$$
$$\omega_u = \exp\left[\ln\overline{\omega} + k_\alpha S(\ln\overline{\omega})\right] \qquad (33)$$

where k_α is the abscissa value for a given level of significance α. The methods for the calculation of $S(\ln\overline{\omega})$ for cohort studies and case-control studies have also been given in [R1].

60. To illustrate further, the confidence limits for the experiment on yeast considered above [M3] in paragraphs 31 and 57 may now be calculated. In the example $\overline{\Delta P}_{obs} = 16\ 10^{-5}$ and $\overline{\Delta P}_{exp} = 13\ 10^{-1}$; $s(\overline{\Delta P}_{obs}) = 0.8\ 10^{-5}$; $S(\overline{\Delta P}_{exp}) = 0.9\ 10^{-5}$. In case of a synergistic interaction there cannot be a negative correlation between $\overline{\Delta P}_{obs}$ and $\overline{\Delta P}_{exp}$; equation (32) may therefore be used without the third term within brackets as an upper estimate of $S^2(\overline{\omega})$. Fitting the above values to the equation, $S^2(\overline{\omega}) = 0.01$ and $S(\overline{\omega}) = \pm 0.1$. The estimated value is therefore $\overline{\omega} = 1.22 \pm 0.10$ and, for 95% confidence limits, $\omega_l = 1.02$ and $\omega_u = 1.42$.

61. For complex biological systems a possible situation of isoaddition should be kept in mind. In probabilistic terms this means that if the action of agent 1 takes the system to probability level P^*_{o1}, then P_{o2} will depend not only on the level of exposure X_2 but also on the value of P^*_{o1}. Therefore P_{o2} becomes a conditional probability $P_{o2}(X_2/P^*_{o1})$. The same is true for a reversed order of application of the agents. In this situation different conditional probabilities depending on the sequence of the agents and on the levels of exposure should be used in equation (24). This will give finally upper and lower limits for ΔP_{exp}. Corresponding upper and lower limits for the interaction factor ω may be calculated as

$$\omega_u = \Delta P_{obs}/\Delta P^l_{exp} \qquad (34)$$

and

$$\omega_l = \Delta P_{obs}/\Delta P^u_{exp} \qquad (35)$$

These limits will be further extended by the presence of experimental errors.

D. THEORY AND PRACTICE

62. Before proceeding further to the analysis of some experimental and epidemiological data, it is necessary to discuss briefly the applicability of the concepts reviewed in the preceding section to situations involving complex biological effects. In doing so, it will immediately be realized that even problems which may appear of minor and mostly speculative importance in the analysis of the action of a single agent, are likely to become very difficult to disentangle when various agents are combined, giving rise to much uncertainty in the assessment of the type of interaction that might apply.

63. The definition of an effect is hardly ever a problem in radiation biology. The conditions under which the effect is manifested, its degree of expression or its probability of occurrence may usually be described with sufficient precision. What may be less easy to define is the dose-effect relationship at all levels of exposure, particularly at the low ones. When two agents are combined, depending on the form of the respective exposure-response relationships, more or less effect might be obtained at a given exposure regime than might be predicted on fragmentary knowledge of the relevant relationships. This points to the need to establish with sufficient precision through appropriate controls not only the response to be expected at the exposure levels of interest for the particular experiment, but to obtain a full dose-response curve for both agents under study. The ultimate aim is to establish experimentally the surface of response corresponding to the full

range of both agents. However, if the number of experimental points to establish a given exposure response curve with one agent is N, to establish the whole surface of response with the same number of experimental points in a given sequence of administration requires N^2 experimental points. Reversing the order of administration will in turn double the number of experimental points to $2N^2$. Such an increase in the size of the experiments is often not feasible and complete series of the type envisaged are almost never reported or conducted.

64. The definition of the level at which a supposed synergistic or inhibiting action may take place is extremely important in the analysis of such actions. Here the need for operational definitions of practical significance and the need of resolving mechanisms in biological experiments may often be at variance or even incompatible. If, for example, one takes a very complex biological short-term end-point, such as the death of an animal (but the loss of reproductive integrity of a cell may be sufficiently complex, depending on the level at which the mechanisms of action may be resolved), exposure to any toxic agent in sufficient amounts could produce such an effect. This of course will happen at times and with mechanisms differing from one agent to another and mostly specific to each agent. The combined application of two agents may in principle produce apparently antagonistic or synergistic effects when some of the pathways of action of the two agents happen to interfere with each other. But at this level of complexity, even though the end-point might be of practical significance, the real existence of combined actions may be difficult to assess. Only when the mechanisms of action of the two agents are reasonably well defined will there be any merit in making use of the concepts of synergism or antagonism, in order to avoid misuse of the terms. Within this framework it may also be discussed how the presence of one may enhance the detectability of another interacting agent, when both produce the same effect.

65. Confusion of iso- with hetero-addition could result in the false identification of synergism. For example, one could visualize two agents, both toxic to the bone marrow and both inducing leukopaenia with a very curvilinear relationship to exposure, as is usually the case. It is easy to imagine that the action of the combined treatments might produce more effect than expected by the same doses of the two agents separately, simply because of the curvilinearity of the relationships and of the isoadditive character of the combined effect. It is also easy to understand that death of the animals might ensue at levels of the combined agents which are much below those of the two agents acting separately. If leukopaenia and death were the end-points of reference, in the absence of any other information one might be tempted to think of a synergistic action. Yet, to call such an effect synergistic would be unjustified because isoaddition would be operating in this case. Clearly, without knowledge of the whole range of responses, it would be impossible to clarify the issue. It should be realized that too often the cases of synergism claimed in the literature have been insufficiently analysed in this respect and there is ground to doubt that they might stand up to more refined investigations.

66. As to long-term effects, it is usually thought that tumour induction is a sufficiently well-defined phenomenon to be taken as an end-point, as though all tumours have the same aetiology and pathogenesis and there are not great variations in the incidence of various tumours between species, strains and experiments. This assumption is imprecise when different doses of the same agent are administered, because expressing the response as overall tumour induction may mask important effects on some tumour classes. The assumption is however particularly dangerous in studies of combined actions because under these conditions changes in the tumour spectrum would certainly be expected. It is essential therefore that the end-point of the studies be specific and extremely well defined. The same reasoning applies to the genetic and developmental effects.

67. Changes in the state of the biological system may be brought about by sequential treatment. For example, a large body of evidence on mammalian cells indicates that dose fractionation in radiobiology is a difficult subject to investigate. Usually the first dose produces partial synchrony of the irradiated population, so that the response of the surviving cells to the second dose fraction is altered with respect to that of a non-exposed undisturbed population. It would be very easy but totally unjustified to think of antagonistic or synergistic effects in the absence of information on the survival curve of the overall population and of its constituent sub-populations and in the absence of data on the amount and time sequence of synchrony induced by the first treatment. There is every reason to believe that such cases may occur also in respect to other chemical treatments and it should in fact be pointed out that treatments with chemicals (BUdR, hydroxyurea, for example) are often used to obtain experimentally synchronized cell populations. The amount of information available in respect to such effects by the various agents discussed in the following parts of this Annex is lacking or extremely limited. Efforts to clarify the situations occurring in practice through experimental analysis might help to avoid misconceptions.

68. Another point calling for great caution concerns the time parameters of the combined action. Two types of treatment may be visualized, contemporaneous and sequential. Partial overlapping and fractionation of the exposure to each of the agents could increase the complexity of the temporal patterns of exposure. Contemporaneity of the treatment time does not necessarily imply a simultaneous action at the level of the target structures. For example, in the case of chemical or pharmacological substances, variable time for metabolic processing of the agents might be required and it would temporally displace the action on the biological structures of interest. If hetero-addition is assumed to operate, administration of one agent before another, or vice versa, should not in principle lead to a change in the end-result. But, on the other hand, if reversing the order of administration does produce a change (qualitative or quantitative) of the response, the conclusion should not necessarily be drawn that some interaction differing from additivity applies. This all points to the relativity of the definitions and to the difficulties of translating into sensible biological terms the precise statements of the theory.

69. There are biological effects for which the timing and the sequence of the actions is all-important. According to one hypothesis, for example, tumour induction may be regarded as the result of two independent phenomena, initiation and promotion. Initiation is visualized as a fast irreversible process

acting on normal cells and conferring upon them the character of neoplastic ones. It precedes promotion but without the latter could not result in a growing tumour. Promotion, which on the other hand is ineffective if not preceded by initiation, takes place during fairly long times and may be reversible. Many agents share the properties of initiators and promoters in different degrees at different doses. Thus, reversing the order or altering the time pattern of administration of two carcinogenic agents is bound to produce changes in the qualitative or quantitative expression of their final action. This should be kept in mind when designing experiments on combined action.

70. The issues discussed in the preceding paragraph are further complicated by the fact that the time for tumour appearance is important, as is the final tumour yield. The rate of appearance of tumours in time (once this rate is referred to a given tumour type and is corrected for competing risks) is an important parameter since, in principle, it is related to the promotive action of a treatment; while the final tumour incidence is related to the initiation action. When agents possessing both properties are administered in combined experiments the precise nature of the interaction and its influence on the combined end-point would not normally be resolved without detailed information of the mechanisms involved.

71. The decisive importance of the temporal pattern of exposure to ionizing radiation vis-à-vis practically all biological end-points is documented for a variety of biological effects in the specialized sections of the previous report (see Annexes H, I, J of [U1]) and in Annexes I, J and K of the present report. In general, fractionation or protraction of the exposure lead to a decrease of the final effect, although in some cases deviations from this general pattern are reported [H19]. It is not unreasonable to expect that changes in the yield of effect may also occur by altering the pattern of exposure to other agents interplaying with radiation, so that the final effect of the combined treatment cannot be predicted, particularly in the region of the low doses which are of major concern in the present context. Precise information about the temporal distribution of the exposure is therefore required in evaluating the combined effects.

72. In conclusion, the notions of synergism, additivity and antagonism which may be defined in theory and evaluated by appropriate statistical analyses, are seen to lose some of their clarity when confronted with the complexity of biological organization and the variability of experimental conditions. They may, on the other hand, acquire important practical connotations. Normally the assessment of combined actions requires clear understanding of the nature of the biological effect under study; precise knowledge of the pattern of its manifestation in time for the combining agents; reasonable definition of the exposure-effect relationships for each of the interacting agents, particularly when effects must be analysed over a range of exposures; control experiments to check for the applicability of the effect to different conditions of exposure. Without the detailed information described above, such notions will probably remain confined to the realm of theory and the subject of disbelief or overestimation, as the case might be. Only studies of mechanisms might eventually solve these uncertainties.

II. PHYSICAL AGENTS

A. COMBINATIONS OF VARIOUS TYPES OF IONIZING RADIATION

73. The simplest type of interaction, where most of the reservations raised in the preceding sections do not apply due to the similarity of the underlying mechanisms, is that between two different types of ionizing radiation. Mixtures of high- and low-LET radiations have repeatedly been tested for the presence of synergistic or inhibiting effects due to the combination of two beams, since current understanding of radiation action is not sufficiently advanced to allow prediction of possible interactions. In other studies external irradiation was combined with internal or the effects of mixtures of radionuclides were tested.

74. Studies on the combined action of fast neutrons, heavy ions and x rays were stimulated by possible radiotherapeutical applications [N2, N4, N5, B16, D13, F5]. Interaction of sublethal reparable lesions produced by neutrons and x rays was shown in experiments where x-irradiation was delivered at different intervals after neutrons [N4]. The actual survival curves of cells in vitro lay between those to be expected on the basis of iso- and hetero-addition. Similar experiments with results in the same direction were performed with neon ions [N5]. Cells irradiated with ions, incubated for three hours and then exposed to x rays showed a partial restoration of the shoulder of the survival curve. However, the results of Durand and Olive [D13] are in disagreement with those reported above because they showed no recovery after combinations of neutron-neutron, x ray-neutron and neutron-x-rays. It should be pointed out that these experiments were not confirmed. A theoretical description of the interaction of high- and low-LET radiation based on the theory of dual radiation action was provided by Zaider and Rossi [Z3]. Within the frame of definitions accepted in this report their interaction would be confined to the envelope of additivity.

75. Some insight into the nature of the underlying processes may be provided by studies of repair. When tested at the tissue level, the rate of recovery from sublethal damage appeared to be independent of the radiation causing it [H12]. It was suggested that recovery from sublethal damage does apply to the low-LET component of the damage, whatever the radiation producing this damage [G3, H13]. Naturally, in the case of neutrons which cause relatively more lethal than sublethal damage the final effect will not be clearly determined until one of the two components, the sublethal, has been repaired at sufficiently long fractionation times [H15]. Further evidence shows [H14, F3] that tissues treated with neutrons or with x rays to similar levels of biological damage and then submitted to an x ray course appear to be more radiosensitive when neutrons had been delivered in the conditioning treatment. Thus, the presence of different components in the LET spectrum and the presence of different types of damage to be repaired (sublethal, potentially lethal) each with characteristic time parameters make the picture rather complex.

76. New studies on combined radiation treatments were reported on Chinese hamster cells in culture irradiated first by neon ions (LET = 180 keV/μm) and subsequently by 225 kVp x rays. Cell survival was the end-point analysed [N9, N10, N11]. The results for the three levels of survival presented in Figure XIV show

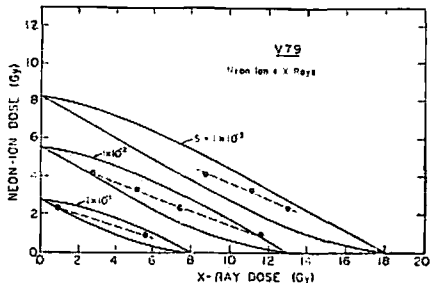

Figure XIV. Isobolic diagrams at three levels of survival for Chinese hamster V79 cells after irradiation with neon ions followed by x rays [N10]

that the experimental points fall in the middle of an envelope of additivity formed by application of hetero-addition (upper curve) and isoaddition (lower curve). When the order of application is reversed (Figure XV)

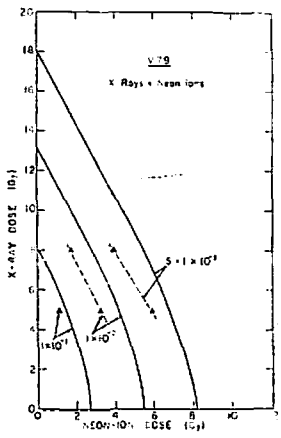

Figure XV. Isobolic diagrams at three levels of survival for Chinese hamster V79 cells after irradiation with x rays followed by neon ions [N10]

the envelope of additivity is reduced to a line, a situation illustrated earlier in Figure III c and e. Under these circumstances one would conclude for the presence of synergism, if not for the fact that the actual levels of survival do not change after the two sequences of treatment. This apparent paradox is explained by the fact that radiation may be considered to be synergistic with itself when survival is not exponential with dose.

77. The above examples suggest that in cases where the sequence of treatments does not change the final outcome, the isobolic diagrams should be constructed with the order of treatment that gives the greatest possible area of the envelope of additivity. As to the interaction of ions and x rays, this does not appear in general to exceed the isoaddition limits (interaction of x rays with itself) and only in few experiments true synergism may be suspected. Further work on the mechanisms at the cell kinetics and molecular level could clarify the precise conditions of the interactions.

78. Moskalev et al. [M21], modelling the effects of nuclear fallout, gave ^{131}I orally (0.3 kBq/g) to rats and irradiated them at the same time externally with gamma ($\sim$ 6 Gy) or beta (surface dose $\sim$24 Gy) radiation. Other animals were only irradiated externally. Lethality at 90 days was about five times lower in the combined treatment group, which was attributed to changes in the hormonal state in the course of acute radiation sickness. Other studies were also reported on the yield of mammary tumours in rats following ^{131}I and external irradiation with x or gamma rays [M22]. For low iodine exposure (0.04–0.08 kBq/g body weight) an increased yield of tumours was seen in the combined treatment group; for high iodine exposure the reverse was true. Other experiments [V4, V5] tested tumorigenesis in rats with ^{131}I (3.7 kBq/g) and external thyroid irradiation (up to 3 Gy). No significant effects of the combination were reported. The interaction in these cases is attributed to hormonal disturbances, about which more data will be provided in chapter IV. Luz et al. [L14] reported on an enhanced osteosarcoma induction in mice by the joint action of two radionuclides, a short-lived alpha-emitter (^{227}Th at 190 kBq/kg) and a beta emitter (^{227}Ac at 1.9 kBq/kg). A higher than additive osteosarcoma incidence was reported at 700 days post-exposure, amounting to interaction factors of about 1.7 in terms of final tumour incidence and of about 1.3 in terms of the time for 50% tumour appearance, as compared to the effects of the two doses given individually. The authors attributed the interaction to the stimulation of osteogenic cell division due to the protracted action of the low level ^{227}Th formed from ^{227}Ac or to the continuing activation of a virus by the same cause. It is clear however that without kinetic studies and accurate physical dosimetry at the level of the sensitive bone cells it would be difficult to validate the phenomenon as belonging to the class of synergistic effects.

B. UV AND IONIZING RADIATION

79. The combined action of UV and ionizing radiation has been examined repeatedly in various experimental systems of micro-organisms [H10, Y4]. The experiments of Haynes in E. coli B/r [H10] may be examined as a good quantitative example. Pre-irradiation of the bacteria with different UV exposures increases the final slope of the x ray survival curve. Changing the sequence of the agents leads to a disappearance of the synergistic interaction, but only if the cells are irradiated in rich medium. If cells are irradiated in buffer the order of irradiation is not so important. This suggests that post-irradiation events may affect the interaction mechanisms. Many experimental data point to these events in relation to repair mechanisms [M24]. It appears that the repair of single-strand DNA breaks induced by x rays may be inhibited by prior UV irradiation. For some recent reviews of DNA repair mechanisms and their genetic control see references [D10, M23, S34].

80. Experiments are also available on mammalian cells in culture [H16]. Synchronized Chinese hamster cells were irradiated in mid-S phase with fixed doses of UV and then exposed to graded doses of x rays (case I); alternatively, fixed doses of x rays were followed by graded doses of UV (case II). In case I the resultant survival curve may be obtained by isoaddition showing that, despite the different nature of the molecular lesions, the damage by UV is fully additive with that of x rays. The UV survival curves in case II are higher than the theoretical curves obtained by isoaddition, but lower than those obtained by hetero-addition. The size of the shoulder on the combined action curves in case II is less than that of the pure UV survival curve. Thus, the damage produced by x-ray pre-irradiation is only partially additive with the subsequent UV damage. In mammalian cells, according to these data, the situation

of survival additivity seems to prevail. These data have been analysed by others [L28] according to the molecular theory of cell survival.

81. Transformation of mammalian cells in vitro is also a relevant end-point. DiPaolo and Donovan [D17] tested the morphological transformation of Syrian hamster cells with UV and x rays. Irradiation by UV alone (254 nm) gave a yield of transformants linearly increasing with dose. X-irradiation produced no transformation at all. X-irradiation (2.5 Gy) followed by UV (1.5 J/m^2) at 24, 48 or 72 hours resulted in a greatly increased yield of transformants. An interaction factor of 3, 11 and 2.2 may be calculated at the above time intervals, showing the interaction to be very time dependent. Increasing the UV dose to 3 J/m^2 led to a decrease of the 48-hour interaction factor, thus showing its dose dependence. The relevant biological mechanisms remain unclear owing to the lack of understanding of the phenomenon of transformation.

82. A synergistic interaction of UV and x rays was found by Holmberg and Jonasson [H22] for chromosomal aberrations in human lymphocytes. G_0 cells were irradiated first by 254 nm UV (5 – 10 J/m^2) and then by scalar doses of 260 kVp x rays (1.25–2.0 Gy). UV alone gave a very small yield of dicentrics; UV followed by x rays doubled the yield of x rays alone. The interval between the treatments was less than half of a minute. Reversing the order of administration did not change the interaction factor of about 2. When phytohaemoagglutinin-stimulated cells entering stage G_1 were used in the same experiments no synergism was observed [H23].

83. Experiments with chronic exposure to UV light of different spectral composition and parallel chronic or acute exposure to ionizing radiation were also reported. They involved complex biological end-points such as LD_{50} or life span. Galanin et al. [G2] studied in mice and guinea-pigs the haemopoietic functions and the life span under conditions of combined chronic irradiation by UV and gamma rays (dose rate 0.5 Gy/day). The experiments showed that animals receiving the combined treatment lived longer and had haematological values closer to normal than controls receiving only the gamma treatment. Acute damage was also influenced in the same favourable way by the combination of chronic UV irradiation with lethal and sublethal doses of gamma radiation [L15]. In experiments by Yatzula [Y3] on rats the treatment by UV preceded or was made concurrently with x-irradiation or internal irradiation by ^{32}P. Again, a decrease in the $LD_{50/30}$ was seen after the joint treatment. Animals under combined irradiation had a better recovery of the body weight and showed less severe skin reactions. Physiological adaptation mechanisms could be invoked to explain such effects.

84. A comparison of the carcinogenic action on rat skin of UV and ionizing radiation, was made by Burns and Albert [B11]. The predominant tumor type observed following UV irradiation was a keratoacanthoma; after electron irradiation epidermal tumours were mostly seen. The yield of keratoacanthoma in rats irradiated at four weeks of age by different doses of ionizing radiation up to 30 Gy and then exposed for different periods to high and low fluences of UV was not influenced by the ionizing radiation dose and depended primarily on UV exposure. Absence of interaction was also seen in the case of epithelial skin tumours. Only one UV treatment schedule (high-fluence, 25.2 10^4 J/m^2, from 5 to 16 weeks of age) enhanced the yield of epithelial tumours for lower doses (5.5 and 11 Gy) but not for higher doses of electrons (17 Gy). However, some of this increase was also observed in the zero-dose group and neither of these increases was statistically significant at $P = 0.05$. The absence of oncogenic interaction between the two radiations is a particularly good illustration of the fact that there may be a difference in the targets specific to the two radiations.

85. The examples reviewed in this section illustrate several important points. They show that the type of interaction depends on the biological end-point studied, on the level of exposure of the agents applied, on the order of their administration, on the stage of cell cycle, state of growth of the cells, etc. Under these circumstances it is not surprising that no general conclusion about the character of the UV and ionizing radiation interaction may be drawn.

C. ELECTROMAGNETIC AND IONIZING RADIATION

1. Experimental data

86. Many industrial, scientific, military and domestic appliances produce microwaves, electromagnetic radiation having frequencies of from approximately 10 to 10^5 MHz. In some cases the same apparatus may produce very soft x radiation, as well as microwaves; in other instances ionizing radiation from other sources may be present in an occupational environment together with microwaves. The assessment of a possible combined action of these two agents is very difficult because exposure parameters for microwaves equivalent to the absorbed dose of ionizing radiation are absent [B23]. Even such a simple characteristic as the density of energy flux (DEF) is lacking in some experimental work. The quantitative expression and the underlying mechanisms of effects are far from clear. Differing views have been expressed on the nature of these mechanisms. Some authors consider the effects of microwaves to result from the dielectric heating of the tissues [M13]; others place the main importance on specific actions of the microwaves, particularly on the central nervous system [P9, G4]. A combination of these views has also been considered [B12].

87. In several early experiments [P18, M14, T3] the changes induced in the lethal action of radiation by microwaves were studied. The results consistently showed an antagonistic type of interaction on the lethality induced by ionizing radiation following pretreatment of rats [P18], dogs [M14] and mice [T3] with microwaves. Later, interaction in the sense of additivity was reported for the same biological end-point [B13]. In more recent experiments Davydov et al. [D14] studied the lethality to mice after high exposure rates of microwaves prior to acute gamma irradiation. Curves of the Rashevsky type describing the relationship between mean survival time and radiation dose were reported, and a shift to shorter survival times after combined treatment was observed. The animals were irradiated for 10 consecutive days with microwaves at a frequency of 2400 MHz with density of energy flux (DEF) 10, 20, 40 and 100 mW/cm^2, and exposure times of 40, 20, 10 and 4 minutes, respectively. It is interesting to point out that the degree of interaction was highest for DEF = 100 mW/cm^2 and depended rather on the intensive factor (DEF) than on the extensive one (energy admin-

istered). An approximately linear decrease in the $LD_{50/30}$ with increasing DEF was observed. Extrapolation of this relationship to zero gamma dose would give a DEF value of about 325 mW/cm². The authors argue that at this level of DEF death might be brought about by microwave irradiation alone. If so, this would imply additivity of the two agents for a very complex end-point.

88. The state of the haemopoietic system of the animals in the course of the microwave pre-treatment described above was studied by Tichontchuk [T4] after 31 day irradiation at 100 mW/cm². Gamma radiation at 4 Gy was given after the last microwave treatment. The haematological parameters that were followed in the course of these experiments included the weight of spleen and thymus, and the number of cells in the circulating blood. An inhibitory action of the microwave irradiation alone on the haemopoietic system was noted, with a pronounced leukopaenia. The subsequent gamma treatment added further injury to the blood-forming organs, which could explain the additive type of interaction observed in [D14]. It is however fair to point out that other data on an inhibitory interaction of the same two agents have been reported in the literature [L19, F4, R8].

89. Rotkovska and Vacek [R8] exposed mice under conditions similar to those in [D14]. Lethality following low-LET radiation was again the experimental end-point tested. A definite decrease of lethality was observed if the mice were exposed after the x-ray treatment for five minutes to microwaves (2450 MHz, 1000 mW/cm²). Animals treated with microwaves showed also an increased number of haemopoietic stem-cells surviving and increased values of erythro- and myelo-poiesis. Differences of these results from those in [D14] could possibly be due to the reverse order of application of the interacting agents. A more recent paper by Rotkovska et al. [R14] provided further details of the therapeutic effect of microwaves on short-term mouse survival following whole-body x-irradiation and attributed the antagonistic effect of the two agents to an increased survival of the stem cells in the bone marrow.

90. For the purpose of the present document most interesting are the studies where clearly non-thermal, down to environmental, levels of microwaves were tested. Sakovskaya et al. [S25, S26] modelled in the animal a situation of chronic irradiation by microwaves and low-energy x rays. Female mice were irradiated in 31 or in 82 sessions, each delivered every second day and including 20 minutes microwave irradiation at DEF 2.5 and 5.0 mW/cm², and x irradiation (effective energy 10 keV) up to doses of 0.15 or 0.3 Gy. The end-points studied comprised body weight and weight of several organs (i); number of mice producing litters (ii); fertility (iii); weight of the litters at one month of age (iv); fraction of bone-marrow cells carrying chromosomal aberrations (v); lysozyme content of the blood serum (vi). Control groups and groups irradiated with only microwaves or x rays were also included in the experiments.

91. Statistically significant ($P < 0.05$) changes of the control values were obtained for the end-points (ii), (iii), (v) and (vi). A decrease of the lysozyme content of blood serum was observed, approximately to the same degree, for both the experimental groups irradiated with microwaves alone or with x rays alone. In the group receiving the combined irradiation the decrease of the enzyme was slightly greater, but the corresponding point on an isobolic diagram would fall into an envelope of additivity. For the chromosomal aberrations again an increased yield was seen in the groups receiving the separate treatment and again to approximately the same extent. The group under combined treatment showed a slightly higher incidence of aberrations, but the corresponding experimental points were not outside the envelope of additivity. Microwaves alone seemed to show a slight stimulating action on the fertility of the mice and on the fraction of mice producing litters; but in the combined treatment group a decrease of both end-points was observed. An additive type of interaction would appear more likely to apply in general to these experiments.

2. Epidemiological evidence

92. The combination of ionizing radiation and high- or low-frequency electromagnetic radiation is characteristic for a number of occupational environments in electronic and radiotechnical plants. Increased ambient temperature, constant electric or magnetic fields, sound pollution and vibration may also be part of these environments [O2]. Wolfovskaya et al. [W3] studied the health of female workers employed in assembling, testing and vacuum pumping of high-voltage electronic equipment. They were exposed to electromagnetic fields of different frequencies (electric field strength 600–2500 V/m and magnetic field strength 50–320 a/m) and to x irradiation at dose rates of up to 25 μGy/hour. The effects studied included frequency of functional disturbances of the nervous system, blood pressure, dysmenorrhoea, changes in the sedimentation rate of erythrocytes, thrombo- and leuko-cytopenia. It was claimed that the important determinant of the symptoms was x irradiation. However, a high percentage of disturbances of the nervous system found among these workers was attributed to microwave exposure. The nature of the end-points, their variability between groups and the lack of any precise dosimetry and statistical analysis make it difficult to validate such conclusions.

93. Similar comments may be made with respect to other epidemiological studies on the clinical effects of combined exposure to microwaves and ionizing radiation. A survey was reported of workers testing microwave generators [B9]. Four groups of people (200 subjects in total) were included in the survey: two groups worked under combined exposure to microwaves and ionizing radiation; one was exposed to microwaves only and the last one was the non-exposed control. A group exposed to gamma radiation alone was not included in the study. Asthenia and migraine were characteristic complaints in all exposed groups. For the first two of them the symptoms were 20–50% more frequent than for the third group and between 2 and 2.5 times more frequent than in the fourth. Dysfunctions of the autonomic nervous system were 2 times more common in the groups with combined exposure than in the third group and 3–4 times higher than in controls. The combined action of ionizing radiation and microwaves was also investigated in workers by Lysina [L17] but loosely defined conditions of exposure render any judgement of the type of interaction impossible.

D. SUBOPTIMAL TEMPERATURE AND IONIZING RADIATION

1. High temperature

94. Broad quantitative studies on the effect of heat on cells and the interaction between heat and ionizing radiation started in the 1960s, stimulated by the possible application in the treatment of cancer. Several conferences and symposia have by now taken place on this subject [C17, C18, P12, D20] and good reviews are available [D18, F2]. A full discussion of this subject is beyond the scope of this Annex, which will only briefly cover the most basic aspects. Heat alone may damage mammalian cells and tissues at temperatures of 42°C given for a sufficiently long time [F8]. Thermal inactivation curves as a function of the temperature or of the treatment time at a given temperature may be produced, having characteristics similar to those of the radiation inactivation curves. There are reasons to consider that the target for cell killing by heat may be plasma membranes [D18] but other targets such as lysosomal membranes or macromolecules cannot be excluded.

95. Treatment of cell cultures with heat increases their sensitivity to radiation in the sense that the final slope of the x ray survival curves becomes steeper after pre-heating [F8]. Thermal enhancement ratios as defined by equation (7) may be used to quantify the effect and for different cell lines the values of this ratio seem to correlate well with the sensitivity of the cells to heat [R9]. After pre-heating for 1 hour at 42.5°C the TER may reach values higher than 2 [R9]. These values seem to increase for irradiation at low dose rates [B24] because recovery from sublethal damage is sharply reduced by treatment with heat [L18]. A delay of rejoining of strand breaks in DNA [C19] and inhibition of DNA synthesis, including repair synthesis [S37], were observed after heat treatment. The targets for enhancement of radiation sensitivity by heat are different from the targets for simple heat inactivation and include all the repair systems and the chromatin [W10, D18, D26, S49].

96. The temporal pattern of treatment is very important for the synergistic interaction of radiation and heat [S35, D19, S36, O3]. Maximum interaction is usually observed with the simultaneous presence of the two agents and it declines as the interval between treatments increases. Figure XVI [F2] illustrates the time course of the decay of heat potentiation of x-ray damage in a variety of normal mammalian tissues for hyperthermia given either before or after irradiation.

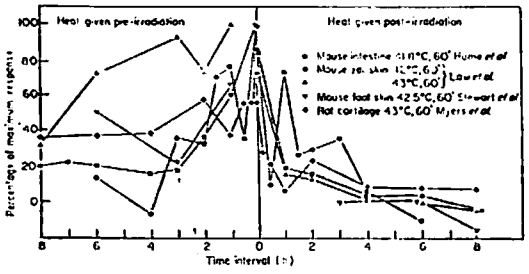

Figure XVI. The time course of the decay of heat potentiation of x-ray damage in normal tissues for hyperthermia given at different times before or after irradiation. The tissue responses are normalized to the percentage of the maximum response which occurs, for each curve, at the shortest time intervals. Data from [F2]

97. Data on the life span of animals irradiated for the duration of their life under conditions of high environmental temperature are reviewed in Annex K. A study with pre-implantation mouse embryos exposed in vitro to 39°C immediately after x irradiation [V8] showed a great increase in the number of micronuclei in the cultured cells, indicating an enhanced chromosomal damage after combined treatment.

98. For animals, and for mammals in particular, it is difficult to foresee under what conditions a direct sensitizing action of high temperature in the environment might come about, since normally healthy mammals maintain a fine regulation of their body temperature. However, Dobrovolsky [D11] reported experiments on rats where the effects of chronic irradiation due to the daily intake of ^{35}S (0.55 MBq/kg), ^{45}Ca (1.3 and 2.8 MBq/kg) and ^{32}P (0.37 MBq/kg) in the course of a year were studied in combination with daily exposure for two hours to a temperature of 40°C. Survival, body weight, fertility (mean number of litters per female), haematological parameters and histology of the ovaries were the end-points studied. During the first period of treatment the changes characteristic of a chronic radiation injury appeared to be aggravated by the combined treatments. During the second half of the treatment, however, the combination of irradiation and high temperature appeared to increase and accelerate repair processes. Fertility of the female animals depended on the mating time, but in general the combined action group had enhanced fertility in comparison with the control groups [D12].

99. Epidemiological investigations of combined actions were made on workers at metallurgical plants who were exposed to ionizing radiation and also received periodically high temperature exposure [M12]. Primary functional disturbances of the nervous system were seen with higher frequency in this group as compared to other groups of workers. People having a shorter occupational history were reported to show vascular dysfunctions attributable to dystonicity of the autonomic nervous system. For longer occupational times asthenia also accompanied the above symptoms in a higher percentage of workers. Owing to the obvious difficulties in the quantification of such subjective symptoms any judgement about this type of interaction should be reserved.

2. Low temperature

100. Cold-blooded animals are good experimental material for studies of the influence of low temperature on radiation sensitivity. Much relevant information was published in a specialized symposium [R10]. In very general terms, regeneration of tissues [H24], lethality after fractionated irradiation [E7] and recovery from radiation injury in self-renewing tissues [E8, E9] in fish are considerably inhibited when the animals are kept at suboptimal temperatures. A detailed discussion of these data is beyond the scope of this report which is essentially centred on mammalian systems and on end-points of practical importance for man.

101. Trujillo et al. [T5] reported that RF/Un female mice showed a linear decrease of their ability to withstand a standard cold stress (6°C – 7°C for 14 days), as a function of increasing age. Mice exposed to protracted ^{60}Co gamma exposures at 0.5 Gy/day and then allowed to recover for 90 days showed a similar linear decrease with increasing radiation exposure in

their ability to survive the same stress. This radiation induced effect was considered similar to life shortening through natural aging and was estimated to be equivalent to 9.3 days/Gy. Other data on the combined effect of duration of life irradiation in animals under conditions of low ambient temperature are discussed in Annex K.

102. In experiments by Gambino et al. [G5] rats were irradiated whole-body or on the adrenals only with a standard exposure of 5 Gy and then were exposed for 3 hours daily to 0°C. Reduced longevity, growth retardation, cataract, greying of the fur, and induction of tumours were the long-term effects seen in animals that had been whole-body irradiated, while animals irradiated only on the adrenals did not show such phenomena. The treatment at low temperature did not modify the incidence of these effects, with the exception of a slight reduction of the accelerated onset of tumours seen in whole-body irradiated animals. Since the treatment with low temperature as such gave rise to a reduction of the life span and to differential effects in the incidence of inflammatory and neoplastic conditions, the experiments are not easily interpreted [H17].

103. In many of the experiments described the temperature could not act as such, but as a condition producing physiological adaptive changes. Some information on the influence of miscellaneous physical treatments (permanent or transitory high altitude, high or low ambient temperature, mechanical damage, severe metabolic or physical stress) in respect to tumour induction in animals were already reviewed by the Committee in its 1977 report (Annex I) [U1]. The findings were on the whole negative. When interaction effects were reported they were not very large and explanations in terms of physiological adaptation mechanisms to the exposure conditions could readily be produced. In respect to life shortening, which at the low doses and dose rates of interest in radiation protection is mostly associated with tumour induction, some data are reported in Annex K. They concern low and high environmental temperature and specific and non-specific stress. Here again, the effects reported were marginal and often of antagonistic character, i.e., leading to an increased life span by the joint treatments. These effects could be explained on the ground that suboptimal living conditions frequently act by decreasing, rather than by enhancing, the susceptibility of the animals to the effects of radiation. However, even though the impression is in favour of the lack of positive synergistic evidence, the data are few, the effects unspecific and the underlying mechanisms obscure so that no definitive statement can be made.

E. MAGNETIC FIELDS AND ULTRASOUND

104. A fairly extensive body of literature exists on the effects of magnetic fields in biological systems [P14] but studies of their combined action with radiation are relatively few. A review is to be found in [N6]. This problem may conceivably be of practical significance for workers in thermonuclear fusion devices. It should also be recalled that the use of transversal magnetic fields to improve the dose distribution of high-energy electrons in radiation therapy has recently been envisaged.

105. Sikov [S38] tested various combinations of high-intensity magnetic fields with gamma-irradiation in mice. Of the various end-points considered (lethality, developmental changes, biochemical effects) only two appeared to be susceptible to the action of magnetic fields applied alone (2 to 4 10^8 Tesla, T): audiogenic seizure and the level of tryptophan pyrrolase in liver. In both cases radiation alone had little effect and the results of the combined treatments could be attributed to the action of the magnetic field. A decrease in the slope of the probit line of mortality (gamma rays, 5.8, 7.5, 8.6 and 10 Gy) without change of the LD_{50} value following the contemporaneous exposure to the 4 10^8T field indicated a decrease of spectrum of radiosensitivity values induced by the joint treatment. Fields of 2 10^8 T were inactive to this end.

106. Although some indications of synergism were reported for biochemical indices following localized liver irradiation [W11], experiments on the survival of cell cultures in vitro were negative in this respect [R11, N6]. Uniform magnetic fields of 1.4 10^7 T in combination with radiation produced no changes in the form of the survival curves of cells in vitro or in the pattern of recovery from sublethal damage, as compared with radiation alone [R11]. Higher intensities of the magnetic field (2 10^8 T) or non-uniform fields were also without effect for similar end-points in other experiments [N6]. There is therefore on the basis of presently available evidence little ground to expect an enhancement of the effects of radiation by the joint application with magnetic fields.

107. Ultrasound is widely used for diagnostic and therapeutic purposes, as well as in many industrial appliances. Some experiments considered its possible interaction with ionizing radiation. Harkanyi et al. [H18] irradiated mice by ultrasound (800 kHz, exposures of 0.1, 0.5 and 1.0 W/cm^2) followed two hours later by 0.5 Gy of x rays. Single-treatment groups were also set up at the same time. The yield of chromosomal aberrations in the bone marrow of the animals was taken as the end-point. None of the ultrasound exposures produced any significant increase over the spontaneous level, while the effect of the ionizing radiation dose was easily assessed. No change in this level of effect was found in the group of mice undergoing the combined treatment.

F. DUSTS AND FIBRES

108. For many industrial environments the combination of radiation exposure and exposure to dusts is quite usual, as, for example, in mining, metallurgical industries, power plants and construction works. Many dusts and fibres have been shown to be carcinogenic or pathogenic by themselves. Direct experiments on mammals about the action of dusts are available [C3, C16, K9, P2, P10, P11]; concerning fibres, asbestos and other minerals have been given particular attention [W12, B25]. Since dusts may be soluble or insoluble, according to the different types of materials, studies of their combined action with radiation could be covered under the chemical or under the physical section, respectively. In the first instance the chemical compounds dissolved from dust particles would be the actual agents taking place in any combined action; in the latter the size and the distribution of the dust particles would be the parameters of relevance.

109. Panov et al. [P10] studied the respiratory and renal systems of rats after intra-tracheal instillation of a neutral ^{210}Po solution (37 kBq/rat) and quartz dust (50 mg in saline suspension). Lung fibrosis was found to be

more pronounced in the combined treatment group. Malignant tumours of the respiratory tract were also said to be observed more frequently in this group, although no precise description of all the histological and statistical aspects of these tumours was presented in the work. Similarly, in kidneys glomerulo-tubular lesions were found more often in the group under the combined action of the two agents.

110. Ponomareva et al. [P11, P2] used different types of mineral dust with admixture of highly active thorium oxide. Rats were made to inhale or were instilled intratracheally for periods of time up to one year. The chronic action of these agents gave rise to inflammatory lung processes and to fibrosis. Tumours of the lung were also observed after 1.5 to 2 years. When an additional chronic whole-body irradiation course was given to the animals (gamma rays, 20 mGy/day, total dose 2.5 Gy) the lung tumour yield was increased by a factor of two, in comparison with the group under combined treatment and a group receiving external irradiation only. There were no experiments performed to define the specific role of dust in combination with internal or external radiation treatment. The data obtained from the group combining internal irradiation and dust were used to standardize conditions of occupational exposure including a combination of these agents [B15].

111. Experiments on the combined action of internal alpha irradiation ($^{239}PuO_2$) and chrysotile asbestos fibres (mean fibre length 1–10 μm) were performed by Sanders [S12, S24, S13]. Insoluble particles were administered to rats by intra-tracheal instillation. Animals receiving only the PuO_2 had a more homogeneous distribution of plutonium particles in the lung, while the combination of treatments led to a concentration of the radioactive particles within the asbestos-induced scars in the peribronchiolar regions of the lung. In groups receiving plutonium alone the pulmonary retention half-time of the nuclide was about 200 days; in the combined-treatment group it was 450 days. Correspondingly, the cumulative absorbed doses to the lung two years after instillation were 4 and 12 Gy. The incidence of pulmonary carcinoma was 4.5% in rats given the asbestos, 32% in rats receiving plutonium alone and 21% in the combined-treatment group. Thus, per Gy of absorbed dose, the incidence was about four times greater in the plutonium group than in the combined-treatment group. An explanation for the finding could be that by a reduction of the number of epithelial cells receiving alpha dose a reduction of the resulting yield of tumours could come about. In another series [S12] the two agents were injected intra-abdominally. The agents both tended to concentrate in the fibrous adhesions of the peritoneum and the omentum, inducing sarcomas and mesotheliomas to a final incidence which was not appreciably different from an expected sum of effects.

112. Lafuma et al. [L16] reported preliminary results of experiments with rats where internal or external irradiation were combined with intrapleural injection of crysotile asbestos. In a first series 8 rats were exposed to 3000 WLM (see definition of WLM in Annex D) of radon-222 over 1 month and they received about 70 days after the beginning of exposure, 2 mg of crysotile in suspension intra-pleurally. As in the case of previous experiments with radon inhalation [L8] a very small proportion of animals developed lung tumours after radiation exposure and no mesotheliomas were observed at all. Exposure to crysotile only resulted in a very low incidence of mesotheliomas. However, the combined treatment led to the appearance of lung cancer in all rats, 7 of them being mesotheliomas. A clear synergism is here obtained. The same type of results was obtained in a second experimental series where whole-body mixed gamma-neutron reactor irradiation was given (2.3 Gy of 0.5 MeV neutrons with a gamma component of 0.75 Gy). The animals were injected with the same amount of crysotile 125 days after the radiation exposure. The results on lung tumor induction are given in Table 1 and show that, in addition to an increase in total tumours, mesotheliomas only appear in the irradiated group given crysotile intrapleurally. These preliminary data should be confirmed in larger experiments.

113. Sanders et al. [S22] studied the effects of beryllium oxide aerosol inhalation in combination with plutonium oxide aerosol on more than 600 rats. Aerosol particles were of micron and submicron sizes. Exposures up to initial alveolar depositions of 1 to 91 μg beryllium and 0.15 to 6.7 kBq of ^{239}Pu were performed. The results obtained by the two agents given separately and by their combination (beryllium aerosol being introduced prior to plutonium aerosol) as total incidence of pulmonary tumours show that the changes in lung tumour incidence due to the combination of the agents were insignificant. This in spite of the fact that the alveolar clearance of plutonium was decreased by exposure to beryllium and the translocation of plutonium to the thoracic nodes was increased.

III. CHEMICAL AGENTS

A. INORGANIC COMPOUNDS

114. Changes in the physical and chemical characteristics of the water matrix of biological systems may bring about changes in radiosensitivity. Chinese hamster cells were exposed to media containing deuterium oxide (D_2O) following ^{60}Co gamma irradiation and cell survival was scored as the end-point [B17]. Under these conditions the cell response to radiation was greatly enhanced. Depending on the concentration and the treatment time of D_2O, dose modification factors of up to 4.5 could be found. Pre-irradiation incubation had, on the contrary, a very slight effect on the radiation response. The sensitizing effect of D_2O depended clearly on the conditions of cell metabolism, since it was influenced by the type of media and by the temperature. It was found that the radiation damage capable of interacting with the deuterium oxide was repaired by the cells when they were kept for three hours at 37°C in the growth medium and split-dose experiments suggested that the sublethal damage repair capacity was reduced in the presence of D_2O. The heat sensitivity of the cells was unaffected by D_2O and the enhancement of radiation response induced by heat was also independent of the presence of D_2O.

115. Some natural mineral components of the diet may change the radiation response of the animals [K10]. Rats were kept on diets with low (50 mg/d Ca and 0.2 mg/d F) or high (150 mg/d Ca and 3 mg/d F) content of calcium and fluorine and after 5 weeks of such diet were given radioactive ^{90}Sr. As a result of the combined treatments the haemopoietic system of the first group of animals was more severely damaged and their mean life span shortened by 50–70 days, as

compared with the group with high Ca and F in the diet. The protective action of the high Ca and F diet is achieved at intakes of the two minerals not higher than the upper limits of physiological intake for humans. Similar results were obtained if external gamma irradiation was added to the internal ^{90}Sr irradiation. In other experiments rats were subjected only to gamma irradiation and to diet changes. In all cases the survival at short term and the life span proved to be higher in groups with high calcium and fluorine intakes.

116. The different trace metals found in the air, food and water of some parts of the industrialized world [T7] may alone induce adverse health effects, including malignancies and teratological effects, at sufficiently high concentrations. They may also conceivably combine with the action of ionizing radiation at the background level or under special conditions of exposure. The universal spread of these metallic contaminants make studies of their possible combined action particularly important. Data on the combined action of silver ions and radiation in bacterial systems (spore or vegetative stage) have been provided by Richmond and Powers [R12] and Held and Powers [H25].

117. Lead chloride ($PbCl_2$) in concentrations of 0.1 and 1 $\mu g/cm^3$ was studied in combination with radiation (doses of approximately 1 Gy) for its ability to induce various effects in vitro on embryonic systems [S15]. The number of nucleated cells per mouse embryo, the labelling and mitotic indices and the number of micronuclei per cell were among the effects scored. At both concentrations a synergistic increase of the micronuclei was found, accompanied by an inhibition of embryonic development. For cadmium, the combined effects with radiation were found to be additive in the same system [M26]. Lead was studied by Kudrizkaya [K3] for its capacity to damage spermatogenesis in the mouse. Exposure was given chronically over a period of about six months up to cumulated concentrations of 0.3 mg/g of lead chloride and 81 kBq/g of ^{90}Sr, administered in drinking water. Testis weight or the number of spermatocytes were unaffected by lead alone, while ^{90}Sr significantly decreased the control values of both end-points. Combination of the treatments produced a final effect which was lower than that caused by radiation alone, an antagonistic type of interaction. Lappenbush [L25] injected adult male rats with cadmium chloride (125 to 250 mg) intraperitoneally for 30 days twice per week and subsequently irradiated them with x rays. The 60-day survival was unaffected by doses of the contaminant lower than 125 µg. The radiation $LD_{50/30}$ was found to decrease linearly with increasing exposure to cadmium. The numbers of red and white cells in the peripheral blood were affected by the combined treatment in a complex way.

118. Platinum (cis-dichloro-bis platinum, DBCP) and radiation affected the survival of ovarian-derived Chinese hamster cells in culture according to a synergistic type of interation [C8]. Chromatide aberrations were also induced in higher percentages. In order to observe synergism the chemical had to be administered between four hours before and two hours after irradiation. Two or three days elapsing between the chemical and the radiation treatment abolished the interaction. The synergistic effect was considered to result from radiation-induced single-strand breaks in the DNA which occurred in linear proportion to dose, opposite to a single platinum complex intra-strand cross-link which occurred linearly with respect to platinum concentration. The combination of the two lesions led to lethality. A simple mathematical model to describe the experimental data was developed.

119. The nitrocompounds, especially the oxides, are rather common pollutants of the air. Sensitization of anoxic bacterial spores was reported when they were irradiated in NO_2-saturated water [P15]. A study is available in mammals [K4] where inhaled plutonium-239 under the form of plutonium pentacarbonate ammonium (69 kBq/kg of lung tissue) was administered to rats, after which the animals were also made to inhale nitrogen oxide (0.09 mg/l) or chlorine (0.05 mg/l) for 15 minutes. After the combined treatments the incidence of lung cancer was almost doubled as compared with the irradiation treatment alone. Tumours were multifocal and different types of tumours were seen in the combined than in the single treatment. Pneumosclerosis was also enhanced in the combined treatment group.

120. Occupational situations where exposure to ionizing radiation may be accompanied by exposure to other detrimental chemicals should not be uncommon in industrial practice, but epidemiological data in this field are very rare. In one case observations were carried out on workers exposed to gamma rays for industrial radiography and also to vapours of hydrofluoric acid (HF) [S21]. The changes investigated (levels of T-lymphocytes, C-reactive protein and auto-antibodies) were mainly immunological. The group under the combined influence of radiation and the toxic chemical was reported to have lower levels of T-lymphocytes and higher levels of C-reactive protein and auto-antibodies than the groups exposed to only one of the agents.

B. ORGANIC RADIOSENSITIZING COMPOUNDS

121. The present section includes what is essentially a review of substances which may enhance the radiation response of biological systems, and are called radiosensitizers. Compounds inhibiting the radiation response are called radioprotectors. In many cases these substances were specifically developed for their protective or sensitizing properties. The study of radioprotective chemicals has been strongly pursued [R2, M7, M29, B29]. More recently, the application of such compounds in clinical tumour therapy has been discussed [Y7]. Also, a new field has grown and is still rapidly expanding, that of the radiosensitizing compounds [M10, R6], whose potential in clinical radiotherapy is being tested.

122. The relevant data will be reviewed briefly because it seems unlikely that situations will arise in which these substances may pose significant problems of public or occupational health. For a review of biological effects, mostly lethal, of the combined action of acute irradiation with other common industrial poisons (at high toxic levels) the reader is referred to Tiunov et al. [T2]. Annexes J and I of the 1977 report [U1] reviewed the action of radioprotective and radiosensitizing chemicals in respect to the production of embryonic and foetal damage by radiation and of tumour induction, respectively. The available information on the action of chemical radioprotective drugs for life-shortening effects in animals is reviewed in Annex K of this report.

123. Several classifications of radiosensitizing substances have been proposed [M10, P16, S39], based on their mechanisms of action. Keeping in mind that in some cases the molecular mechanisms are still unknown and that some agents may act through more than one mechanism, one classification may be as follows: 1. Agents modifying the primary radiation chemical processes, including (a) electroaffinic agents and (b) iodine compounds; 2. Agents interacting with DNA metabolism (DNA-base analogues); 3. Antibiotics and other agents interfering with repair processes (see section III C); 4. Agents reacting with nucleophilic groups (SH groups); 5. Other radiosensitizing agents.

124. The best known example of the first class of agents is oxygen whose level in biological systems at the time of irradiation greatly influences the yield of radiation effects. A massive body of literature exists on the action of oxygen and the interested reader is referred to [A11, P1]. A large number of electroaffinic compounds or hypoxic cell sensitizers is also known but their detailed discussion is beyond the scope of this Annex. Such compounds may contain one of the following chemical groups: the carbonyl (CO), the aldehyde (CHO), the nitro (NO_2), the cyano (CN) groups, homo- and hetero-cyclic rings. Stable free radicals are also electro-affinic agents. The radiosensitive properties of such compounds are manifest when they are present in biological systems at the time of irradiation or if they are irradiated separately and then immediately added to the biological system.

125. The same is true of the iodine compounds which may also change the concentration of radiation-induced free radicals. If cells are exposed to irradiated iodoacetamide within milliseconds after irradiation cell killing takes place, which is not observed if irradiated cells are exposed to non-irradiated iodoacetamide, thus showing the role in sensitization of short-lived transient compounds [D9]. Radiosensitization takes place also with other iodine compounds: iodide, iodoacetic acid, iodopropionic acid, methyliodide, p-iodophenol, iodobenzoic acid and others. Reactions with -SH groups may account for part of the sensitizing effect of some of these compounds [M7].

126. Attention has recently been given to the radiosensitizing properties of iodine contrast media used in radiodiagnostics [S43, N7, A2, M25]. Sensitizing effects on bacterial killing were first reported [S43] and then an increased yield of chromosomal aberrations in peripheral lymphoctyes of children undergoing x-ray angiocardiography with contrast media [A2, N7]. It has also been reported that sensitization of mammalian cell killing by iodine compounds would occur for x but not for gamma rays [M25]. These data are explained by the difference in doses due to photoelectric effect in the case of x rays. An accurate physical dosimetry should clarify this issue.

127. Quinones are unsaturated carbonyl compounds with conjugated structures and electron affinic properties. Several quinones and their derivatives have been found to sensitize bacterial and yeast cells under oxygenated and anoxic conditions [A4, M11, S16, S17]. It has been postulated that the sensitization of E.coli B/r by vitamin K5 is mediated by radiolytically produced hydroxyl radicals [S16]. Diphenylquinone was found to enhance the action of radiation in mice [A4]. In some bacterial systems under anoxia the value of DMF could be about 3 (10^{-3} M indanetrion monohydrate [B7]) or even 4 (100 ppm vitamin K5, [S16]). Newly synthesized isoindole quinones showed promising characteristics when tested in vivo on soft tissue sarcomas transplanted into mice [C13].

128. Electroaffinic compounds containing nitro groups can specifically increase the radiosensitivity of anoxic cells, leaving that of oxygenated cells unchanged or even decreased. These properties would be advantageous for tumour radiotherapy [A5, D8, H4, H8, P17, Y5]. The radiosensitiziation by mizonidazole was proved to occur for hypoxic mammalian cells in vitro and in vivo [A12]. Under aerobic conditions no sensitizing effect of the compound at any stage of the cell cycle was observed [P17] and under anoxia the strongest effect occurred in middle-S. Toxicity of the agent under anoxia requires low exposures to the agent.

129. Yuhas and Li [Y5] studied the effects of the compound at a concentration of 6 mM in combination with the radioprotective compound cysteine (8 mM) on mammalian cells in culture, showing protection under conditions of oxygenated irradiation and sensitization under anoxia. Hall et al. [H8] tested eight different nitro compounds: for all of them the DMF was an increasing function of the concentration and for some it reached a value of about 3.5, equalling the average value of the OER in the cells tested. In general, 2-nitroimidazoles were more effective sensitizers than 5-nitroimidazoles. Other nitrocompounds, the nitrofurans, may be even more effective, specifically under anoxia [R3, R6]. Sensitization by nitrocompounds was greater when they were administered prior to irradiation [D8]. Radiosensitizing properties were also described for nitrogen-containing stable free radicals such as triacetone-amide-N-oxyl (TAN) [E5, B21] and 2,2,6,6,-tetramethyl-4-piperidinol-N-oxyl (TMPN) [P6].

130. DNA base analogues belong to the second class of radiosensitizers. Extensive studies were made especially on halogenated DNA base analogues such as 5-fluorouracyl (5-FU), 5-bromouracyl (5-BU) or 5-bromo-2-deoxyuridine (5-BUdR) [K13]. Significant enhancement of killing was shown for viruses, bacterial and mammalian cells [S18]. Some attempts for a clinical application of these substances have also been reported. For a review of the relevant studies see [M7, M10].

131. The third class of radiosensitizers will be considered in section III.C. Here various substances capable of modifying the biochemical cellular processes should be mentioned, belonging to classes 4 and 5. Several organic chemicals capable of enhancing radiation damage share the property of being -SH reactive. Since -SH compounds are known to be radioprotectors, the correlation has been investigated between the ability to bind -SH groups and the capacity to sensitize the cells to the action of radiation. Bruce et al. [B8] found that the capacity to sensitize was well correlated to the amount of p-hydroxymercuribenzoate bound to cells.

132. Sensitization of anoxic cells is an important goal for tumour radiotherapy [A13, R6]. Radiosensitization of bacterial cells under anoxia by N-ethyl-maleimide (NEM) was shown as early as 1960 by Bridges [B6]. Other data on bacterial and mammalian cells are also available [L12, M18, K11]. A DMF of 1.5 with human cells in vitro irradiated with x rays was reported by Klimek [K11]. The known property of NEM to bind -SH groups led to the hypothesis [L12] that NEM could bind the free non-protein -SH groups, thus preventing DNA repair through donation of hydrogen from these

groups. Other experiments by Klimek and Zemanova [K12] showed that under concentrations of NEM too low to inhibit DNA synthesis a high proportion of the original free thiol groups was still present, thus implying other mechanisms for NEM sensitization. However, the role of intracellular thiol groups would also be supported by experiments of Sinclair on oxygenated [S40] and anoxic [K17] Chinese hamster cells exposed to NEM and radiation. Repair of lethal damage is inhibited by the presence of NEM but the mechanisms of such an inhibition are still unknown.

133. Another organic compound that may produce cytological changes is carbon tetrachloride (CCl_4). Its administration to animals induces, for example, liver cell proliferation [A6] similar to that induced by partial hepatectomy. Cole and Nowell [C20] examined the effect of CCl_4 on the induction of hepatomas in fast neutron irradiated mice with doses of 1.7 to 3.1 Gy. At various times after irradiation some animals received the compound subcutaneously. Sixty-one percent of animals receiving the combined treatment developed hepatomas, as compared to 19% of the mice irradiated only. Since CCl_4 alone produced no hepatomas, the interaction factor is approximately 3. Histologically the tumours were similar in both groups but tumours of larger size were more frequent in the combined modalities group. The authors concluded for a promoting effect of CCl_4 in liver cancerogenesis. Procaine hydrochloride, a local anaesthetic acting on cell membranes, has been shown to sensitize bacterial and mammalian cells to the action of radiation [S19, S20].

134. Alkylating agents may react with DNA bases and thus directly influence the radiosensitivity of cells. The alkylating agent spirohydantoin mustard (SHM) was tested in combination with x-irradiation on brain tumour cells in vitro [D15]. The enhancement of cell killing was greatest when the cells were irradiated four hours before the drug treatment. The doses ranged from zero to 20 Gy and the subsequent chemical treatment with SHM lasted one hour at concentrations of 0, 2, 3, 4 and 5 μg/ml. The results were normalized and the corresponding isobolic diagrams were built. At levels of cell killing down to 10% a synergistic interaction was apparent, although for lower levels of survival down to 0.1% the interaction turned into an additive one. This and another paper [D2] by the same authors are some of the rare examples where the analysis of the interaction type was carried out according to the approach outlined in chapter I of this Annex, involving the use of isobolic diagrams.

135. The same brain tumor cells cultured in vitro were exposed for one hour to 1, 3, 5, 7.5 μg/ml of 1,3-bis(chloroethyl)-1-nitrosourea (BCNU), followed 15 hours later by a series of x-ray doses of up to 20 Gy [D2]. Survival curves for the x rays alone, the BCNU alone and for the combination of both agents were obtained and on their basis isobolic diagrams for survival levels of 1, 2 and 3 log cell kill were constructed as in Figure XVII. The figure shows the experimental points for the combined treatment connected with a dashed line; all the points except one fall into the envelope of additivity applying at each survival level. The point at the lowest level of survival (7.5 μg/ml of BCNU, 4 Gy of x rays) falls outside the respective envelope, although the displacement is not so great that it might not be explained by experimental uncertainties.

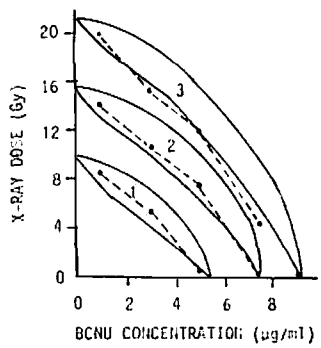

Figure XVII. Isobolic diagram for the combined action of BCNU and x rays on 9L rat brain tumour cells [D2]

136. The scope of this brief overview of chemicals capable of modifying radiation sensitivity is simply that of illustrating the very wide range of processes whose alteration may in turn lead to a synergistic or antagonistic interaction in irradiated biological systems. The data reviewed are as such of little relevance for the main scope of this Annex, because the doses of radiation used are usually very high (up to several Gy, depending on the susceptibility of the systems tested) and the concentrations of the chemicals often toxic. It is however appropriate that they should be mentioned because the processes governing different aspects of cell radiosensitivity might also be relevant at lower levels of exposure. No practical situation where the above mechanisms would be significant can currently be envisaged at the low doses of interest for the purpose of the present Annex.

C. CARCINOGENIC CHEMICALS

137. Organic substances which are known to have carcinogenic properties should be discussed separately. Some, such as the alkylating agents, have already been mentioned in section III. B. Carcinogenic agents are usually divided roughly into initiators and promoters, following the two-stage theory of carcinogenesis [B18]. It is known however that such a subdivision is not rigid because many agents share the properties of both classes. In combination experiments it may be expected that the final tumour yield may depend on the properties of the interacting agents, as well as on the order and time pattern of their administration. A potent initiator followed by an active promoter might be expected to give the highest carcinogenic response and reversal of their order of administration a drastic reduction of this response. Another important trait is the spectrum of the tumours induced, as some agents may be extremely specific in this respect and their interaction with radiation might change this selectivity.

138. Precise quantitative data were provided by DiPaolo et al. [D21, D17, D22] and Kennedy et al. [K14] on the morphological transformation of mammalian cells in culture in regard to the interaction between ionizing radiation and the carcinogen benzo(a)pyrene or the promoting agent phorbol ester. The experiments elucidated the dose-time relationships for an effect of special significance for practical purposes, showing enhancement factors of up to 9 fold, depending on the conditions of exposure and on the doses of the agents interacting. This series of experiments also attempted to elucidate the mechanisms of interaction. For the same biological end-point, the

promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) administered after x-ray or neutron irradiation to C3H/10 T 1/2 cells in culture was shown to act synergistically, with complex relationships as a function of the radiation type and dose [H27].

139. A study showing an increased yield of leukaemia in mice pre-irradiated with x rays and subsequently treated with methylcholanthrene was published by Furth and Boom [F9]. An increased yield of leukaemia in mice induced by x rays, methylcholanthrene or oestrogens was also shown by Kawamoto et al. [K6] when the animals were simultaneously treated by urethan. After Berenblum investigated the interaction of x rays and urethan in mouse leukaemogenesis in great detail and showed that the order of administration of the combined agents was of decisive importance [B2, B26] many other authors reported enhancement of leukaemia under the same agents [D4, L22, V6, G1] particularly in young animals [B1, L23] where enhancement is especially pronounced. This may be on account of differences in the drug distribution or catabolism as a function of age [C2]. Data have also been reported for croton oil [I2], myleran [U5] and novoembicyn [A8] in conjunction with radiation.

140. Combined treatments of pre-implantation mouse embryos in vitro with x rays or phenols (which are promoting and mutagenic chemicals in some test systems) showed that the effects were, at most, additive [M30]. Schmahl and Kriegel irradiated mouse embryos in utero at 11–13 days p.c. (1 Gy at each time) and injected the pregnant mothers at 17 days p.c. with 0.5 mM/kg of ethylnitrosourea [S42]. Tumour development was followed post-natally up to 18 months. Results from this series are shown in Table 2, with the interaction factor calculated according to equation (5). If one considers total tumours as the end-effect, interaction appears to be antagonistic ($\omega = 0.44$). If one takes each category of tumours separately, one may conclude for at least one clear case of synergism and one of antagonism for leukaemia and hepatomas, respectively. This appears to be a good example of a change in the tumour spectrum brought about by the combined treatment. However, for more definitive statements exposure-response curves within a broader range of values for both the single and the combined actions would be required.

141. Much work has been carried out on the skin, the tissue where the two-stage mechanism of carcinogenesis was originally identified [B18] and can be more easily tested. Electrons or UV light in association with other carcinogens usually result in a higher yield of tumours than any of the agents administered alone. This applies to methylcholanthrene [C14] and to 7,12-dimethylbenz(a)anthracene (DMBA) [E6, S28]. However, a recent report [B19] on this latter substance in association with 0.8 MeV electrons (5–25 Gy) in respect to carcinogenesis of rat skin showed that the tumour yields were approximately equal to the sum of the yields induced by the separate treatments, so that prior irradiation did not appear to alter the susceptibility of rat skin to DMBA carcinogenesis.

142. The case of 4-nitroquinoline-1-oxide (4NQO) has been particularly well analysed. When applied in combination after beta rays from ^{90}Sr-^{90}Y (both agents at doses that did not separately induce tumours) it appeared to have a synergistic effect for skin tumour induction in mice. Reversing the order of administration of the treatments led to a much smaller yield of tumours by about an order of magnitude [H5]. When the interval between beta irradiation and subsequent chemical treatment was made to vary between 11 and 408 d, the tumour induction rate was found to be almost at the same level for all treatment times, indicating that the latent carcinogenic change induced by skin irradiation may persist for a very long time and remain available for subsequent interaction with the 4NQO [H6]. Finally, caffeine was found to further increase the incidence of malignant tumours in mouse skin when painted after beta rays and 4NQO treatment [H7].

143. Croton oil, a typical promoter of skin neoplasia from which TPA is extracted, gives uncertain results when combined with radiation: enhanced effects with UV [E2] and electrons [S29] or absence of any enhancement [G6, B20] have in fact been reported. It may be said in very general terms that the concepts of initiation and promotion may be verified on the skin also in the case of drug-radiation interactions. However, the results of combined treatments on the skin could also be interpreted on different grounds and some of the previously mentioned experiments [H5, E2, E6] would in fact be regarded by others [N3] as clear examples of co-carcinogenesis by chemical and physical agents.

144. The situation with respect to other tumours or to systemic leukaemogenesis is definitely more difficult to interpret. In the case of the lung, urethane (which specifically induces adenomas in mice) has been used in association with x rays at various doses and dosages. A reduction in the incidence of tumours (both as percentage incidence and as tumours/animals) has been obtained in one experimental series [F6]: cell killing by the high radiation dose in the urethane-induced tumours was held responsible for the effect. Recalculation of these data by others [L21] led however to a different interpretation. Additive effects of radiation and urethane were reported in another series, and the final outcome of the treatments was deemed to depend on two competing phenomena, cell killing and cell transformation, whereby, depending on the dose of the two agents, any effect may become possible. Immunological phenomena might also interfere in this case to make the picture very complex [C15].

145. Procarbazine (PCB), a drug used frequently in the treatment of the Hodgkin's disease, is a known carcinogen in experimental animals since it gives rise to pulmonary adenoma and leukaemia in mice, mammary tumours in rats and acute myelogenous leukaemia in primates. Hybrid (BALB/c × DBA/2) F_1 mice were given this drug and ionizing radiation at different times to test for possible synergistic effects [A7]. Single-treatment groups received 300 mg/kg PCB weekly for four weeks, a dose effective for induction of pulmonary adenoma and leukaemia; or 0.6 Gy/d of 300 kVp x rays for five d, a dose which did not result in tumours of the lung. Combined-treatment groups received radiation three days or three weeks before PCB or PCB three days before irradiation at the above dosages. The experiments were terminated within 12 weeks with killing of the surviving animals. Pulmonary adenomas in mice receiving both agents were significantly increased over the level of induction by PCB alone. Thymomas were also increased significantly in the animals given the drug three days before or after irradiation. The authors concluded for a synergistic effect of the combination and hypothesized that an increase of the normal tendency of mice to develop pulmonary adenoma

would be at the origin of the interaction. Immunosuppression combined with direct cellular damage might also be responsible for the effect.

146. Among studies where combinations of chemical carcinogens and radiation were tested, the experiments of Metivier [M6] regarded the action of PuO_2 given by inhalation, in combination with benzo(a)pyrene (BP) or dimethylnitrosamine (DMNA), compounds which are widespread environmental pollutants. Both carcinogens were given after the exposure to the PuO_2: BP (2 × 5 mg) was administered intra-tracheally in association with haematite 2–3 weeks after the nuclide; DMNA (2 or 20 ppm) was given orally, added to the drinking water. Tumours of the lung and of other sites, histological types of tumours, invasiveness and survival time were the principal end-points investigated.

147. BP alone led to a small increase of tumour incidence above the control level. PuO_2 (0.63 kBq) produced similarly a slightly increased incidence. Both agents combined produced an appreciable increase in the number of tumours with an increased invasiveness. Survival time reflected closely the results on tumour incidence, being essentially unchanged for the two agents given alone and practically halved by their combination. At least on qualitative grounds, a synergistic interaction was operating in these experiments, the latency period of the tumours in the combined treatment group being evidently shorter. For higher levels of PuO_2 (6.3 kBq) a synergistic action might also be present, but its expression (particularly with regard to survival time) is much less clear. In the case of DMNA no synergistic action with respect to alpha radiation alone was found. At high concentrations (20 ppm) the latter drug produced a subacute intoxication and no synergistic effect. It was reported however that inhalation of PuO_2 in association with DMNA did result in an increased tendency of liver tumours to metastasize into the lungs.

148. In the experiments of Little et al. [L10] the interaction between benzo(a)pyrene (BP) and alpha radiation of ^{210}Po, was examined. The experiments were performed on hamsters and the two agents were administered by intratracheal instillation, absorbed on haematite particles or dissolved into physiological saline. In a first series of experiments the two agents were administered simultaneously in 15 weekly instillations (0.3 mg BP + 0.2 kBq ^{210}Po/treatment). Under these conditions the results were compatible with an additive interaction of the two agents.

149. In a second series of experiments BP was given 15–18 weeks after the administration of a single dose of 1.5 kBq ^{210}Po. While BP alone (2.4 mg in eight weekly instillations of 0.3 mg) or ^{210}Po alone produced practically no lung tumours, the combinations of both agents resulted in a clear synergistic effect, with 17% of the animals developing frank tumours of the lung. Physiological saline and gelatine were mostly used as carriers. When the administration of BP preceded the ^{210}Po treatment no increase of tumour induction was seen. It is remarkable that when the second treatment consisted of saline alone, without BP, a sharp increase of the tumour yield was seen, compared to the ^{210}Po treatment alone. The instillation of isotonic saline could act as a non-specific stimulus to cell proliferation [L11] and subsequent experimental work [L24] appeared to lend support to this hypothesis. Autoradiographic experiments showed that after treatment by BP or by saline the epithelial cells of the hamster lung undergo a wave of mitoses. This enhanced proliferation would be essential for the expression of the radiation-transformed cells.

150. A biochemical approach to the study of mechanisms of interaction between radiation and chemicals in the case of lung tumour induction was followed by Queval and Beaumatin [Q1]. These authors studied the correlation between the capacity by various substances of inducing pulmonary enzymes and their ability to shorten the latency period of the lung tumours in rats, following inhalation of radon daughters. The research established that compounds such as benzoflavone, methylcholanthrene and benzopyrene are highly effective in enzyme induction and capable, at the same time, to shorten the latent period of tumour appearance.

151. Large experimental series were carried out on the combined effects of radiation and inhalation of uranium ore dust and diesel oil exhaust fumes at the Pacific Northwest Laboratory [C16]. The experiments on hamsters involved about 600 animals non-exposed or exposed to radon and radon daughters, uranium ore dust and diesel engine exhaust, alone or in various combinations. Squamous cell carcinomas developed in only a few of the animals exposed to radiation and they were always preceded by a squamous metaplasia of the alveolar epithelium. In general, however, the hamster lung was found to be rather refractory to the malignant transformation and did not even develop lesions that could be classified as pre-cancerous when exposed to levels of the above agents which were regarded as realistic for life exposure regimes. Thus, the hamster lung under these conditions may not be a useful model for pulmonary cancerogenesis in man.

152. Knizhnikov et al. [K9] modelled another case of industrial exposure by a combination of shistose ash, benzo(a)pyrene and ^{210}Po. The mice were exposed to ash alone, ash with BP or with ^{210}Po, and to the triple combination of the agents together. The yield of lung tumours and their latency period were studied and at the levels used the yield was reported to increase from 35% (ash only) to 61% (triple combination). The latency period decreased in the same two groups from 300 to 200 days. An interaction factor may be calculated from these data of about 1.3, indicating some synergistic interaction. Other control groups were included in this series.

153. The intragastric administration of 3-methylcholanthrene followed by x rays [S30] or fission neutrons [S4] produced no more than additive effects for induction of mammary adenocarcinoma. However, the same chemical applied locally on the brain, in association with beta irradiation resulted in an antagonism which was proportional to radiation dose [M15].

154. There are many different experiments concerning a variety of other tumours. X rays alone or in combination with benzo(a)pyrene produced the same incidence of neoplasia [K7]. Dibutylnitrosamine (DBNA) or 4-ethylsulphonyl-naphtalene-1-sulphonamide (ENS) combined with x rays showed no effect on tumours of the urinary bladder but a reduction of the mammary tumour incidence [F7]. A synergistic action on the production of liver and gastric carcinoma by fission neutrons in combination with N,N'-2,7-fluorenylenebisacetamide (2,7-FAA) was reported, but no interaction for intestinal tumours was found [V7].

Localized x-irradiation in association with the same drug administered in the diet accelerated the induction of hepatomas [N1] and similar effects were reported with the association of x rays and o-aminoazotoluene [K8] and of ^{144}Ce and dimetylaminoazobenzene (DBA) [M16]. Experiments on additive carcinogenic effects of 9,10-dimethyl-1,2-benzanthracene or 1,2,5,6-dibenzanthracene in association with chronic internal irradiation from ^{90}Sr were also reported [Z1, Z2].

155. The mutagenic substance N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was tested in combination with whole-body fission neutron irradiation for its carcinogenic properties on the gastrointestinal tract of rats. A high incidence of gastric and duodenal carcinomas was found after the MNNG treatment but the neutrons did not produce any tumour. Combining the two treatments did not change the effect of MNNG [V3]. Survival and tumour induction were tested in three strains of rat following x-irradiation in association with urethane. In spite of some interesting differences observed between strains, the overall effect of the joint treatment was not greater than the sum of the separate effects at the dosage level studied [M17].

156. In work by Sanders [S12], besides the combined action of $^{239}PuO_2$ and asbestos, the combined action of $^{239}PuO_2$ with benzo(a)pyrene was also studied, both agents being administered intra-peritoneally. The action of BP alone produced mostly abdominal sarcomas. The combination of BP and 13.3 kBq of $^{239}PuO_2$ resulted in an approximately additive yield of sarcomas. Other tumours which are characteristic of the plutonium action were also produced. The administration of BP increased the translocation of plutonium to liver and lung, which points to the need that possible metabolic effects leading to different dose patterns in various organs upon the joint administration of two substances should be taken into account when discussing the results of combined actions.

157. In conclusion, it appears that the evidence reviewed is very conflicting. The number of substances tested is large and the amount of information relating to each of them very little. The pathogenesis of the tumour systems tested is complex and the conceptual distinctions between induction and promotion cannot be held in many instances. In some cases the application of chemicals after irradiation may enhance the tumour yield by comparison with the opposite order of application. In other cases, the association of treatments may actually decrease rather than enhance the induction of neoplasia when the toxicity of the combined agents outweighs their additive carcinogenic properties [U2]. No definite conclusions with respect to any class of tumours may therefore be drawn before the dose, the dosage schedule, the order of administration and modalities of the combined treatments are properly and thoroughly explored, which is very rarely the case in the contributions that have been reviewed.

D. THE SPECIAL CASE OF TOBACCO SMOKE

1. General

158. Tobacco smoking is a widespread habit of many human populations in spite of a well documented association between smoking and lung tumour incidence. The relationship between annual death rate from this cause and number of cigarettes smoked per day is reported to be linear with slope of about 10^{-4} [D5, D23] and with incidences of lung cancer rising from about 0.07 10^{-3} per year in non-smoking males to 3 10^{-3} per year for male persons smoking 35 or more cigarettes per day. The chemical composition of tobacco smoke is very complex and includes more than 1000 identified compounds [S9], a number of which are aromatic hydrocarbons that have been shown to act as carcinogens. Smoke and tobacco tar also contain a number of tumour promoting and co-carcinogenic agents.

159. The two-stage nature of the carcinogenic action of tobacco smoke was shown by a classical experiment on mouse skin by van Duuren et al. [V1]. The initiator, 1,2-dimethylbenz(a)anthracene (DMBA) acted in this case as an initiator and cigarette smoke condensate (CSC) as the promoter. Five weekly applications of CSC after a single application of DMBA greatly increased the rate of tumour appearance, by shifting the latency period from 450 d (for DMBA alone) to approximately 100 d. The initiating action of the tar components is relatively low compared to that of DMBA. In this particular case the initiator was a chemical substance, but any other carcinogenic agent, ionizing radiation in particular, could be effective in combination with the promoters contained in the smoke concentrate. This point was proven experimentally by McGregor [M27, M28] who treated rat skin with beta radiation and subsequently applied CSC. Rats treated with CSC only produced no tumours. A two- to three-fold increase in the numbers of skin tumours was observed in the groups under combined action, as compared with the animals exposed to beta radiation alone. It should, however, be realized that only few agents can be considered as pure initiators or promoters, the rule being that many carcinogenic agents have the properties of both classes of substances and sometimes to various degrees, according to the different animal models tested.

2. Experimental data

160. Various examples of interaction between radiation and tobacco smoke have been reported in animals [C5, C6, C16]. In experiments by Chameaud et al. [C5, C6] rats were exposed to radon inhalation in special chambers. They developed respiratory cancers as a function of exposure and exposure rate, starting from a control background incidence of practically zero. Similarities could be shown histologically between these tumours and human lung tumours. Inhalation of cigarette smoke in these animals did not result in malignant transformation of the respiratory cells but only in benign lesions of the bronchial epithelium and lung parenchyma [C7]. In interaction experiments the exposures to radon daughters was chosen to be 100, 500 and 4000 WLM, because it was shown in previous tests that the incidence of lung cancers of respectively 1–2, 5–10 and 30–40% would result from them [L8]. Cigarette smoke inhalation was carried out under standardized conditions for periods of 15 min ten times per day, four days per week, for one year. No change in the animals' life span was seen after this treatment. An elaborate classification of the pathology was set up to follow the spread of tumours at death.

161. For the highest radiation exposure (4000 WLM) the incidence of lung cancer was 34% and it increased to 68% in animals also exposed to smoke. At 500 WLM,

7% and 28% were the corresponding figures and at 100 WLM, 0% and 3.3%. Since smoking was without effect, equation (22) in a simplified form may be used to analyse the data. Accordingly, the values of the interaction factors for the above groups are 2, 4 and ∞. Pathologically, tumours appeared to be more advanced in animals receiving the combined treatments, indicating that neoplastic lesions developed earlier in these animals. Microscopically, the same tumour histotypes were found in the irradiated group and in the group with combined exposure. The authors pointed out the similarity between these findings and those in uranium miners and proposed their rat tumour system as a good model system for the human situation [C7, L8].

162. Another interesting aspect of the laboratory experiments with rats which is in accordance with some results from epidemiological studies on uranium miners is the complex dependence of the lung tumour yield in the animals on the exposure rate and on the level of exposure. Human data strongly suggest that lung cancer may be produced more efficiently at low than at high exposure rates [L6, K1], in the sense that per unit dose higher incidences of tumours are produced at low than at high dose levels. It should be pointed out that low doses are usually obtained at low dose rates. In the experiments of a French laboratory the incidence of lung cancer in the rat per 10^6 WLM changed from over 200 at cumulative exposures of around 175 WLM to 46 at 8000 WLM [L8].

163. The above mentioned French group investigated in further experiments the temporal aspects of a combined treatment in rats of radon daughters and tobacco smoke, by reversing the order of administration of radiation and tobacco smoke with respect to the previously cited experiments [C7, C8]. In this case radon exposure followed exposure to smoke [L9], without any enhancement of carcinogenesis. This observation is in keeping with the notion that tobacco smoke has a promoting action. It was not possible to examine the relationship between the level of exposure to smoke and tumour incidence, since higher levels of exposure led to a toxic action of some tobacco constituents and, on the other hand, lower exposures required an excessive number of animals for statistical validation of the data.

164. The effect of grading the exposure to tobacco smoke may to some extent be studied by the use of chemicals which are constitutents of tobacco smoke or tar, although it should be kept in mind that in this case the mechanism of action could be rather different. Morin et al. [M5] examined the effect of inhaled radon daughters in combination with the I.P. administration (25 mg/kg/week for 13 weeks) of benzo-5, 6-flavone (BF), a substance which is not in itself a carcinogen. Treatment with BF was started at three months after the end of radon exposure at 6000 WLM during about two months. One hundred percent of the animals developed lung tumours (multifocal, invasive epidermoid type with a latency period of 3 months), as compared with an expected 50% within 15 months after radiation exposure given alone. When BF administration was started 16 months after radon exposure, no difference was seen with respect to the group receiving only radon. This was taken as evidence that the promoting action of BF was exerted during the period of latency of the radon induced malignancies.

165. Grading the exposure to BF (25, 9, 3 mg/kg/week) and to radon daughters (6000, 3000, 500 and 100 WLM) gave 12 possible combination groups [L9]. Preliminary data showed that the reduction of the latent period was dependent on the product of the parameters characterizing exposure to each agent, as though a lower dose of one could be compensated by a higher exposure to the other in a multiplicative manner. Such a dependence resembles to some extent the "relative risk model" proposed by Lundin et al. [L6] to account for epidemiological data in uranium miners.

166. Modelling of chronic inhalation of radon daughters and tobacco smoke simultaneously was carried out on experimental animals at the Battelle Northwest Laboratories [C16]. The temporal aspect of the administration of the combining agents differed from the experiments of the French group already reviewed [C5, C6], where exposure to smoke followed the radon treatment. The experiments comprised seventy beagle dogs: twenty of them were exposed to radon, uranium ore dust and cigarette smoke; twenty to smoke only; and twenty to radon plus uranium ore dust. The other animals served as the controls. Exposure to tobacco smoke was performed through special masks during several daily sessions.

167. Animals that developed lung tumours had in general cumulative exposures to radiation in excess of 13 000 WLM. This dose level is about two orders of magnitude higher than that reported to cause lung cancer in man. The possibility was therefore considered that the longer life span of the human species might allow more tumours to appear while, for the same tumour incidence, much of the exposure in dogs would be "wasted", i.e., ineffective in producing additional tumours. Differences in histotype between human and dog respiratory neoplasms were also noted. Cigarette smoke had a reducing effect on the radiation lung cancers (2 cases out of 20 animals) as compared to animals non-exposed to smoke (8/20). It was suggested —but in the absence of direct experimental evidence— that smoke through an increased production of mucus might result in a lower dose of radiation to the target cells; alternatively, smoke might stimulate mucociliary clearance. Changes in the lung that were associated to tobacco smoke were emphysema, chronic bronchitis and bronchiolitis, lung fibrosis. The antagonistic effect of tobacco smoke on lung tumors induced by radon daughters was confirmed in a very recent report of these experiments [C22].

168. It may thus be concluded that reasonable dose-response relationships for lung tumour induction in experimental animals may be obtained for exposure to ionizing radiation. The separate effects of tobacco smoke may also be studied, but testing their combined action poses serious problems. The temporal sequence of administration is very important; there are probably differences in target cells with respect to the two agents; there may be other unknown factors complicating the picture; the mechanisms of induction have not been sufficiently clarified. It may be tentatively proposed that a common feature of many experiments in animals (and of some epidemiological series in man) is a promoting action of the smoke (or some of its constituents), leading to a shortening of the latency in tumour appearance. Whether this might be due to a non-specific stimulating action on the proliferation of the respiratory epithelia or to a specific effect of some smoke constituents is impossible to say at present.

3. Epidemiological evidence

169. Uranium miners are exposed to radon and radon daughters. They represent the first occupational group on which extensive epidemiological surveys were made of the effects of radiation in combination with tobacco smoke. The exposure levels for this group of workers are usually expressed in WLM: for the equivalence of this operational unit with other radiation units, see Annex D. In an epidemiological survey [L2] 3414 miners exposed to up to 10^4 WLM from the year 1950 were followed up to September 1967. Against 251 deaths expected during this interval of time, 398 deaths were actually observed, the main causes for the excess being violent deaths (120 observed versus 51 expected) and malignant tumours of the respiratory tract occurring ten or more years after beginning of work in the uranium mines (62 observed versus 10 expected). The time relationship and the increase in cancer mortality as a function of radiation exposure indicate a causal relationship between the two variables.

170. Information about the smoking habits of the miners were collected during the survey and also in an annual census of uranium miners which was started in 1963. Standardized mortality ratios of lung cancer by smoking categories [H3] were used for calculation of the expected death rate for respiratory cancer. The reference population was a random sample of adult males from the United States and the ten expected cases mentioned above were calculated according to these data. It was found instead that the cases expected would be 16 for the same total population of 3414 miners if the lung tumour incidence among males of four Colorado plateau states in the United States would be taken as the reference control. Table 3 shows the distribution of observed and expected respiratory cancer deaths between smokers and non-smokers. The increase in the number of cancer cases is attributable to irradiation by inhaled radon daughters. The relative excess of risk between smokers and non-smokers is the same (3.9 against 4.0 for the two categories, respectively). If one calculates the increase in cancer incidence due to irradiation per person year at risk, one finds $1.7\ 10^{-3}$ for smokers and $1.7\ 10^{-4}$ for non-smokers, the difference being attributed by some to a 10-fold synergistic increase of the risk for the smoking miners.

171. A more accurate analysis shows however that this could be a misleading argument. It should be realized that the statistical significance to be attached to the number of tumours observed in the non-smoking group is very low, owing to the small number of cases observed. The estimate of the probability of tumour induction obtained from this number is therefore affected by a large error. A statement such as the preceding one of a ten-fold increase in risk in the smoking population, would be equivalent to using for the assessment of the interaction factor the formula

$$\omega = (P_{o1} - P_{11}) / P_{o2} \qquad (36)$$

where the signs 1 and 2 refer to smoking and radiation, respectively. In fact, this formula cannot be used under the circumstances, because of the mentioned low statistical significance of the term P_{o2} (the probability of respiratory cancer death in the non-smokers) and of the absence of the term P_{o1} from the numerator and denominator.

172. According to the reasoning presented in chapter I of this Annex, when P_{o1} and P_{o2} are small, one calculates the interaction factor ω by the formula

$$\omega = \frac{P_{o1} - P_0}{P_{o1} + P_{o2}} \qquad (37)$$

Table 4 shows the results of separately analyzing the data for the period 1950–1967 [L2] (A: top line) and for the last four years of the same period, 1964–1967 [A1] (B: bottom line). As may be expected, risk estimates based on the most recent period of observation are higher, excluding re-evaluated estimates of spontaneous risk and risk of smoking. The interaction factors are however close enough to each other and indicate a synergistic interaction. In view of the low statistical significance of the results, other indirect evidence may be of great value.

173. Archer and collaborators [A1, A3, L6] point out some of this evidence. In a larger group of uranium miners 207 lung cancers were identified; all of these individuals except three were cigarette smokers [A1]. Since it is known that 71% of miners are smoking, it is clear that in the above group smokers are over-represented. Another observation relates to the age at diagnosis: in the 207 people mentioned, 17 stopped smoking eight or more years before diagnosis; 16 stopped between four and eight years; 19 smoked less than 15 cigarettes/day (light smokers). Controls were chosen to match as closely as possible the exposed individuals in relation to age at the start of mining, cumulative radiation exposure, years of hard rock non-uranium mining. All of them smoked 20 or more cigarettes per day and none stopped smoking more than one year before diagnosis. The comparison showed that non-smokers or those who stopped smoking eight or more years before developing lung tumours had a mean age at diagnosis three years greater than smoking controls. Light smokers differed from controls by a year and a half, and those who stopped smoking between four and eight years before diagnosis differed from controls by less than one year. The results support the hypothesis that cigarette smoking acts in these miners as a promoting agent [V1] by decreasing the length of the latent period. These conclusions were strongly supported by an update of the earlier uranium miners mortality studies in the United States [A3]. The incidences of lung cancer between different categories of smokers are shown in Figure XVIII [A3].

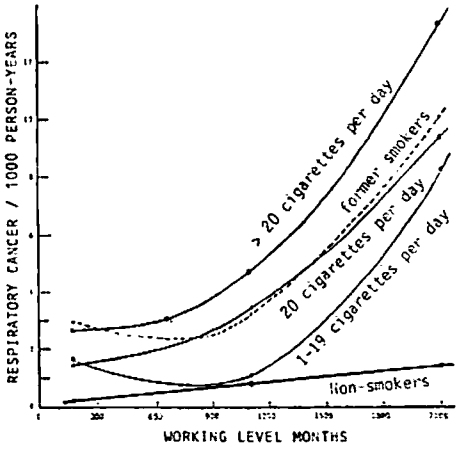

Figure XVIII. Mortality from respiratory cancer as related to radon daughter exposure in different smoking groups [A3]

174. High rates of lung cancer in smoking persons are also observed in workers of industries that use known carcinogenic substances such as chromates [O1, L7] and asbestos [S10]. In addition, among persons developing lung cancers in the same groups of workers, smokers were over-represented. These data may be taken to show a non-specific promotive influence of tobacco smoking. At the present time there is by no means a full understanding of these mechanisms in smoking individuals. It could be that tobacco smoke contains enough initiators and promoters to give the observed yield of respiratory cancer. Alternatively, in case of smoking acting apparently alone, some environmental factors may provide the initiating stimulus and the role of smoking might be essentially promotive. The chemical composition of smoke itself might even not be the decisive factor in the promoting action of this agent. As shown by experiments of Little et al. [L10] reviewed previously, the irritation of the respiratory epithelia by non-specific physical or chemical agents (instillation of saline solutions, for example) could have a promoting effect.

175. It could of course be debated if promotion as such is an effect to be included under the general heading of synergistic. Two extreme situations may be visualized in this respect. There could be, on the one hand, a forward displacement in time of the tumours appearing, but with a final yield of tumours not different from the situation in which promotion is not operating. Alternatively, a continuously increasing rate of tumour appearance might take place, leading finally to an incidence higher than that in the absence of promotion. A variety of intermediate situations could also operate between these two extremes. Clearly, if the final tumour incidence would be taken as the reference end-point, the first of the two situations depicted would not come under the definition of synergism, while the latter would. But if instead, more correctly, the length of tumour-free life lost is taken to be the reference parameter and it is assumed that smoking alone could cause cancer, both situations, as well as all the intermediate ones, would be rightly described as synergistic interactions.

176. In their 1979 paper Lundin et al. [L6] gave a more elaborate quantitative treatment of respiratory cancer death in uranium miners. A log-normal distribution of the time elapsed from exposure to diagnosis based on other experimental and theoretical evidence [M4] was assumed to apply. It was also assumed that this distribution would have a standard deviation of 0.17609 in log t units, where t is the number of years elapsed after the beginning of exposure. The choice of the standard deviation was rather arbitrary and, according to criteria developed in reference [M4], somewhat below the range expected usually. The parameter describing exposure (when the risk from earlier was added to that of later exposures) was the Eff WLM (k) for the year k, defined as

$$\text{Eff WLM (k)} = \sum_{j} w\,(k - j)\ \text{WLM (j)} \qquad (38)$$

where $0<j<k$; w(k–j) is the proportion of the area under the log-normal distribution density curve which is bounded by the interval from (k – j – 1/2) years to (k – j + 1/2) years; and WLM(j) is the exposure in WLM during the year j.

177. Two alternative hypotheses were examined: that the increase in absolute risk might be proportional to radiation exposure, in which case the risk increase would be independent of the rate associated with cigarette smoking, aging or other environmental factors (a); that the increase in relative risk may be proportional to radiation exposure (b). In this case the increase in risk should be proportional to that risk which would have affected the miners in the absence of radiation. The analysis of both models was carried out based on the form of the temporal distribution of the respiratory cancer deaths.

178. Three temporal parameters were used, namely, the age of the miners, the calendar year and the years after the beginning of exposure. Computations were as follows: (a) for the absolute risk hypothesis

$$n_x = \alpha_A\, X_{rad} \qquad (39)$$

where X_{rad} = av (Eff WLM_x). In the above formula n_x is the predicted excess of lung cancer deaths among miners in stratum x and the av (Eff WLM_x) is averaged over all the person-years at risk in the stratum; (b) for the relative risk hypothesis

$$n_x = \alpha_R\, E_x\, X_{rad} \qquad (40)$$

in which n_x is proportional to the product of the expected number of lung cancers E_x in the stratum, multiplied by the exposure X_{rad}. The symbols α_A and α_R in the above equations are coefficients applying to the two situations postulated. The sum of $n_x + E_x$ gives the total predicted number of lung tumours for each particular set of parameters.

179. Calculations were made of the number of deaths according to the age category and with assumptions of mean latency times of 5, 10 and 15 years. The relative risk model gives results which are closer to observations and latency times of 10 to 15 years fit the data best. One of the parameters which is most strongly influencing the expected number E_x is the smoking category (Table 5). It may be assumed that E_x is proportional to smoking exposure X_{sm}. Then the predicted excess of respiratory tumour deaths will be proportional to both smoke and radiation exposure as

$$n_x \simeq X_{rad}\, X_{sm} \qquad (41)$$

The probability of developing lung cancer per person per year is shown in Table 5 for four smoking categories. The last column of the table gives the corresponding interaction factors and shows that the highest value is for former smokers and the lowest for heavy smokers, ω being intermediate for light smokers. This result comes about through an insufficient increase in P_{ot} by comparison with P_{tt} for heavy smokers, an observation which contradicts the previous conclusion about the applicability of the relative risk model. The authors interpret this observation as evidence against a possible "synergism" defined as an increase in the total radiation risk of lung tumour development. Such a risk they consider to be approximately the same for all categories of smokers and somewhat higher than for non-smokers. They classify the observed increase in the lung tumour death as promotion. However, as already discussed before, there is good ground to describe it as synergism (see paragraph 175).

180. Lundin et al. [L6] do not exclude the possibility that for longer time intervals the yield of lung tumours in non-smokers might be the same as that of the smokers exposed to radon daughters. One preliminary

report on lung cancer in Swedish iron miners seems to support this possibility [R15]. In an other epidemiological study of metal and iron-ore miners in Sweden carried out by Axelson and Sundell [A9] the risk for the non-smokers was claimed to be higher. However, the size of the groups analysed was rather small and the statistical significance of the observed effects correspondingly low. Also, the methodology of the case-control study was not fully described in the publication [A9] and it raises some questions in the form presented.

181. Long latency time for lung cancer and the incidence dependence on the dose rate could also obscure the final picture. Uncertainties in the distribution of miners between exposure categories could lead to distortions in the estimates of risk. The lower risk of the American uranium miners could be justified to some degree by their possible misclassification into higher exposure categories [S11, K1]. It should also be mentioned that for these miners the observation time elapsed from the beginning of work in the uranium mines is not much longer than 20–30 years, which might be insufficient for the development of lung cancer among non-smoking individuals. Another important factor could be the exposure rate which was lower for the Swedish than for the miners in the United States. It has already been mentioned that lower exposure rates may bring about a higher total yield of potential tumours. Different exposure rates can be met in epidemiological studies with Czechoslovak [S11, K1] and Canadian [H20] miners.

182. Enhanced mortality for chronic respiratory diseases other than cancer resulting in pulmonary insufficiency (pneumoconiosis, pulmonary fibrosis, emphysema) and for acute conditions (pneumonia, asthma) could also be the result of combined radiation and tobacco smoke exposure [A3]. An increase in the rate of mortality from these diseases for uranium miners in the United States was clearly observed which might be related to radiation exposure. It is interesting to note that the rate is highest for light smokers, so that at high exposure levels mortality is twice as high as for heavy smokers. Some possible interaction between radiation and smoke is also evident for these diseases but, at this point, the contribution of other ambient conditions like siliceous dust or diesel fumes should also be considered, for which data are very scarce.

183. The epidemiological data discussed point to a synergistic interaction between tobacco smoke and radiation exposure in the sense discussed under paragraph 175. Non-specific effects induced by some component of tobacco smoke could be responsible for the results described. Thus, changes in the production of mucus, a slower rate of clearance of radioactive particles by the ciliary action and metaplasia of the epithelia might result in a higher dose delivered to the target cells in smokers than in non-smokers. Against this general proposition is however the observation that promotion by tobacco smoke is still found when smoke is applied long after radiation exposure. Clearly, these questions cannot be settled now with the limited information available. Quantitation of the degree of synergism is also impossible with the necessary degree of precision and significance, owing to the low number of tumours observed, particularly among the non-smoking individuals, and to the complex temporal pattern of lung cancer development.

E. OTHER DRUGS

184. It may appear somewhat artificial to separate in this section substances which are utilized for their pharmacological properties in clinical medicine from other organic substances mostly developed for their radiosensitizing actions. The separation may be made on the ground that interaction with radiation may be incidental in the former case but is pursued as a specific goal in the latter. In spite of a widespread and increasing use of many drugs in modern societies, it is difficult to visualize situations where the combined effects of any of them with radiation may pose significant problems in public health. The cases of interaction in the treatment of specific diseases where the combined use of radiation and drugs might increase the risk of undesirable effects on the patient may be more important. However, in most of the work reviewed radiation doses were very high and, irrespective of the nature of the interaction (synergistic or antagonistic), extrapolating the findings to lower levels may be very difficult or impossible in view of the modification of the form of the dose-effect relationships that might occur at low doses.

185. Antibiotics are widely used in clinical medicine and some of them are also used in combination with radiation for cancer chemotherapy [P3, P4, P5, P16]. Among them, actinomycin D was shown to have a synergistic interaction with radiation on Chinese hamster cells in culture. Elkind et al. [E3] related this effect to the ability of the drug to impair recovery of sublethal damage, as shown by a reduction of the shoulder of the survival curves at low doses (2–5 10^{-3} μg/ml). Ten-fold higher doses given before irradiation increased the exponential slope of the survival curves. Time was also an important parameter in these experiments because no synergism was observed with treatments by actinomycin later than 10–12 h post-irradiation or when the drug was applied more than six hours before irradiation [E4].

186. The molecular basis for the action of actinomycin D on cells is due to its proven ability to bind to DNA and thus to create a steric hindrance to the synthesis of RNA. Interesting studies on the combined action of this drug and of another drug, cordycepin, were reported on two cell lines in culture by Robertson et al. [R13]. For actinomycin D interaction factors of 1.2 and 1.3 in the two cell lines were reported with x rays. The survival parameters affected were both the exponential slope and the shoulder of the survival curve, but mostly the former. With cordycepin the interaction factors were 1.1 and 2.2, respectively, and the main parameter affected was the extrapolation number. The nature of the differences described led the authors to some hypothesis on their molecular basis.

187. Actinomycin D was also tested in preimplantation mouse embryos in tissue culture for its combined effect with radiation [S15]. The concentration of the drug was here several orders of magnitude lower (10^{-4} μg/ml) than in previously reported experiments by Robertson [R13] and the drug alone was ineffective at these concentrations in retarding the development of the embryos to the blastocyst stage. Combining the drug with tritiated water led to a higher effect than that of tritiated water alone, with interaction factors between 2 and 4, depending on the tritium concentration in the culture medium. The lower values were observed at the highest concentrations. This observation could be explained by the more effective inhibition of the repair

processes at low radiation doses. A re-analysis of these data in terms of isobolic diagrams [S48] showed that the results of combined treatment fell clearly outside of the envelope of additivity in the direction of synergism. The possibility that the shape of the dose-response relationships may be changed by the combined treatments has been discussed in this context [S39]. Other experiments showing a radiosensitizing action of actinomycin D have also been reported [M7].

188. In humans, Wara et al. [W2] studied the effect of actinomycin D on the induction of radiation pneumonitis occurring 1–3 months after the irradiation of lung metastases in 41 patients. Doses of the order of 7.7 Gy were necessary to elicit pneumonitis in 5% of the patients and these doses were reduced to 5.5 Gy (DMF = 1.4) when an actinomycin treatment was given along with radiation. Similar DMF were obtained for radiation-induced intestinal injury, oesophageal lethality and pulmonary lethality in LAF_1 mice treated with this drug at the same time [P5]. The values of the DMF obtained for a variety of chemotherapeutic drugs in the above experimental series are given in Table 6 and a detailed review of experimental and therapeutic findings related to this topic has been written by Phillips and Fu [P3]. In patients treated for erythema of the skin d'Angio et al. [D6] reported a DMF of 3.4 by combining x irradiation and actinomycin D.

189. Dritschilo et al. [D7] investigated the mechanisms of the combined action with radiation of actinomycin D and adriamycin. Non-toxic levels of actinomycin and minimally toxic levels of adriamycin produced suppression of potentially lethal damage repair in plateau-phase Chinese hamster cells in culture. For actinomycin this suppression persisted as long as the drug was present in the culture medium, but as soon as it was removed prompt repair took place. This suggested that suppression did not act through fixation of injury to a non-repairable state. Adriamycin was different because cells exposed to it could eventually proceed to repair potentially lethal injury even in the presence of the drug, after an initial delay of the repair processes.

190. Redpath et al. [R4] studied the effect of combining adriamycin (2 or 1 mg/kg, 5 daily fractions) and x irradiation (10 Gy/fraction, 5 daily fractions) on mouse tissues. Enhancement of damage was seen for lung and foot skin damage, when the interval between the beginning of the radiation and of the drug course was within two to seven days. In another series the radiation sensitivity was studied after the single dose of 1 mg/kg intraperitoneally. No effect was found in this case. Experiments were performed in the same laboratory on the radiosensitivity of ICR male mice irradiated whole-body with fast neutrons (mean energy 25 MeV) or photons (6 MeV), in combination with a single dose of adriamycin (10 mg/kg) [C9]. The $LD_{50/6}$ for photons was reduced from 13 to 10 Gy; that of neutrons from 5.6 to 4.3 Gy. The RBE for gut damage was unaltered by the addition of adriamycin. The data indicated that for drug administration 16 hours before or after the radiation exposure the interaction will be the same.

191. A cell cycle dependence of the synergistic interaction of a drug with radiation was shown for dihydroxyanthraquinone (DHAQ), a potential cancer chemotherapeutic agent similar to adriamycin and actinomycin D [K5]. The survival of x-irradiated Chinese hamster cells in combination with different exposures to DHAQ was the end-point of this study. DHAQ had a toxicity which was more pronounced during the early phases of the cell cycle. After combined treatment a synergistic effect was noted for cells in the S phase, but in all other phases of the cycle additivity prevailed. In asynchronous populations DHAQ enhanced the radiation-induced cell lethality primarily by increasing the slope of the radiation dose-survival curve.

192. Lucanthone (Miracyl D) has long been used in the treatment of schistosomiasis. The drug has a heterocyclic ring structure resembling that of actinomycin D. A synergistic type of interaction of this drug with ionizing radiation has been shown for HeLa cells. This effect decreased with the time lag allowed between radiation and the treatment with lucanthone [B4]. The same publication refers also to increased 30-day lethality in mice given 4 Gy of total body radiation and a simultaneous injection of 180 mg/kg of the drug.

193. The influence of lucanthone in combination with x-irradiation was also studied on V-79 cells and on spheroids [D3]. The treatment of asynchronous cells with 5 μg/ml of the drug led to a progressive decrease in the proportion of cells in G_1 and to an accumulation of S-phase cells. The toxicity of the drug was noted only during this latter phase of the cell cycle. In general, the survival of the single cells after the combined treatment was lower, owing to a reduced capacity of the cells to accumulate and repair sublethal damage. For equal levels of drug toxicity, the radiation-modifying effect of the drug was greater in the spheroids, pointing to a larger interaction in the system which has greater capacity for accumulation and repair of the sublethal radiation damage.

194. Lucanthone has also been shown to be active in respect to induction of developmental defects in mice. Pregnant animals (8 days p.c.) were given 70 mg/kg of the drug and treated one hour later with 0.5 Gy of x-radiation. The treatment resulted in a distinct synergistic increase of the eye abnormalities of the embryos [M8]. The above studies were further developed with a decrease of the x-ray dose down to 0.01 Gy [M9]. Pregnant mice of the strains F/A and NMRI were irradiated at eight days p.c. with 140 kVp x rays, with or without treatment with lucanthone. The foetuses were observed 4–5 d after irradiation for the presence of macro- and microscopic developmental defects (post-implantation loss, growth retardation, eye abnormalties, exencephaly, cleft palate and limb defects). There was a strain specificity with respect to the sensitivity to lucanthone given alone, the NMRI mice being more susceptible to lower doses of the drug. A dose of 0.01 Gy was reported to produce a statistically significant increase of the abnormalities and combination of the two treatments gave rise to a synergistic interaction. Some strain specificity was also found for the combined effects, because the F/A mice were more susceptible to the joint action. Higher doses of radiation (0.5 Gy) producing an approximately 4-fold increase of the control abnormalities were also reported to produce synergism. Other data on the enhancement of radiation effects by antibiotics (ledermycin, reverine) were also reported [M9].

195. Bleomycin was reported to potentiate the radiation damage in rat brain tumour cells of the line 9L [H9]. The drug enhanced cell lethality mostly through an increase of the slope of the radiation dose-survival curve, its D_o decreasing from 3.7 to 2.1 Gy in

the presence of the drug. There was also a more modest decrease in the capacity for accumulation of sublethal damage, shown by a decrease of the D_q from 3.2 Gy in the absence to 2.9 Gy in the presence of the drug. This was evidence for an inhibition of repair of the sublethal radiation damage. Other authors observed an additive effect of bleomycin and x rays with small doses of the antibiotic and a synergistic action with high doses [B30].

196. In experiments by Lin et al. [L20] Chinese hamster cells in culture were treated with the drug diethyldithiocarbamate (DDC), a substance that has been shown to inhibit the enzyme superoxide dismutase responsible for eliminating the O_2^- radical from the cells. The killing effect of the drug depended on its concentration and exposure time. Eight to ten days of incubation at concentrations of 10^{-9} M produced no changes; while 10^{-4} M was a definitely toxic level. DDC-treated cells later undergoing gamma irradiation survived less well than cells treated with the same doses of the two agents separately. The drug also significantly enhanced the heat sensitivity of the cells.

197. Antimalarial drugs such as quinacrine and chloroquine or their derivatives are now also used for diseases requiring long-lasting treatments such as rheumatoid arthritis. Sensitization by these drugs in combination with radiation was shown on a variety of systems. E.coli K-12 rec$^+$ incubated after irradiation with quinacrine up to 0.4 mM showed a killing increase [F1]. Chinese hamster cells [V2] as well as tumour cells in vitro [K2] were also reported to show similar effects. In vivo Utley et al. [U3] showed a distinct effect on skin sensitivity, as judged by the hair loss in irradiated rats treated intraperitoneally with daily doses of hydrochloroquine (52 mg/kg) for one week prior to irradiation. A case of skin sensitization in a woman who had been taking hydroxychloroquine daily (200–600 mg/day) for years prior to and during radiotherapy for a breast tumour was reported in the same paper.

198. Enhancement of x-ray induced cell killing by caffeine treatment for 16–20 hours post-irradiation was shown on a number of rodent and human cells in culture. The effect was brought about by concentrations of the drug causing less than 15% killing. It consisted mainly in a removal of the shoulder of the survival curve, without much alteration of its slope. The findings indicated the existence in mammalian cells of processes of repair of radiation damage that are inhibited by exposure to the drug [W5]. Enhancement of the killing effect of single doses of ^{60}Co by caffeine was also confirmed on human and hamster cells in culture at concentrations of 2.0 – 2.5 mM in the culture medium for two days after irradiation [S23]. In split-dose experiments exposure to caffeine of the cells for 4 hours between dose fractions did not result in any effect and it was therefore concluded that the sensitization by caffeine was brought about through a modification of expression of the potentially lethal, rather than of the sublethal, damage. The possible role of caffeine as a sensitizer of the single-hit potentially lethal damage was further confirmed experimentally and through an analysis of the existing literature [S27].

199. The sensitization by caffeine on x-irradiated HeLa cells was found to depend on the drug concentration [T6]. At post-irradiation levels of caffeine of 1 mM a synergistic effect was observed mainly on cells in the G_2 phase, irrespective of where in the cell cycle they had been irradiated. Increasing the caffeine level to a range of 7 to 10 mM brought about not only a higher response of the G_2 cells, but also some response of the G_1 cells. Treatment at higher concentrations resulted in dose-survival curves having smaller shoulders and steeper terminal slopes. It is interesting to point out that the shapes of the time-survival curves measured for the G_1 and G_2 cells differed. The authors interpreted this difference to reflect two operationally distinct modes of interaction with the drug.

IV. BIOLOGICAL AGENTS

A. GENERAL

200. Many biological conditions may influence the state of health of a human population. Viral and bacterial infections, eating habits and the state of nutrition, the conditions of living and working, the use of biologically active substances or drugs are known to affect to various degrees the incidence and pattern of diseases in humans and therefore to alter the actuarial characteristics of populations. There seems to be little hard evidence that conditions adversely influencing the survival and disease incidence could also substantially change man's sensitivity with respect to late radiation effects. This notion cannot however be dismissed or excluded because the complex pathogenesis of the effects of major interest in human radiation biology (tumour induction, genetic changes, developmental abnormalities) leaves scope for combined actions in both directions.

201. The Committee wishes to stress that the above general notion is much easier to be entertained academically than to be experimentally demonstrated. The agents that may be considered as possible candidates for interaction with radiation are very many and diversified; their influence, already in the absence of radiation, is often little known in respect to the effects described above; and the data available are fragmentary. Therefore, to attempt a systematic discussion is almost impossible. In spite of such limitations, the Committee has decided to gather the available evidence on the effects of hormones and the effects of infections. Reference to existing epidemiological studies will also be made.

B. HORMONES

202. The influence of hormones on the radiation sensitivity of human populations with respect to cancer induction can be predicted on the general notion that many experimental and human tumours are known to be variously susceptible to the action of hormones. Mammary gland, prostate and thyroid tumours are very hormone-dependent, while for other malignancies a certain degree of dependency may be postulated, for example, on the notion of a different susceptibility between sexes or on the effect of castration. As to the practical significance of a combined action of hormones and radiation, changes of the hormonal state take place during physiological conditions (menarche, pregnancy, menopause, stress). Treatment of many diseases requires prolonged use of hormonal preparations and an increasingly large part of the female population use hormonal treatments (essentially oestrogens) for contraceptive purposes. Oestrogens are also contained in commercially available cosmetic preparations and some drugs used rather extensively (derivatives of Rauwolfia, phenothiazine, chlorinated

hydrocarbons) have hormonomimetic activities. Finally, hormones themselves could be to some degree carcinogenic [C4]. There appears to be therefore sufficient ground for some analysis of their combined actions with ionizing radiations.

203. Much general evidence about tumour induction in animals has been discussed in the 1977 report of the Committee, Annex I [U1]. It was concluded that various radiation-induced tumours are differently affected by the animals' hormonal balance during the course of the carcinogenic process. The effects reported seemed to be tumour-, strain- and sex-specific and it appeared likely that the mechanisms of action (which are at present almost unknown at the molecular or even at the cellular level) might have been very different under the various conditions tested. Annex K to this report contains some discussion of the influence of the animal's sex on the life shortening action of ionizing radiation. This appears mainly as a higher susceptibility to sex-specific tumours, particularly the mammary neoplasms and the tumours of the genital tract in the female.

204. Segaloff and Maxfield [S7] studied specifically the influence of oestrogens on mammary carcinogenesis in the rat. Pellets containing 5 mg diethylstilbestrol (DES) and 15 mg cholesterol were implanted subcutaneously into 8-week old A × C rats. The animals were hysterectomized to prevent fatal oestrogen-induced uterine infections. X-radiation was delivered only to the left mammary chain by shielding the opposite one. Spontaneous mammary tumours in this strain of rat are essentially nil. Radiation alone (about 8 Gy) produced only a small number of tumours (1.1 per chain at risk) appearing late (median 80 weeks at the appearance of the first tumour). DES alone gave 1.7 tumours per chain with median appearance times of 33 weeks. Combined treatments resulted in an earlier appearance of the tumours (26 weeks) and in an increased incidence (5.6 tumours/chain). Even a crude estimate based on final incidence would lead to an interaction factor ω of the order of 2, an estimate which (apart from its unknown statistical value) fails to take account of the appearance time which is shortened by the hormonal treatment.

205. Shellabarger et al. [S8] irradiated with 0.43 MeV neutrons rats of the strain A × C in doses of 0.096 Gy. The carcinogenic response to irradiation was insignificant (3 adenocarcinomas in 33 rats); DES, on the other hand, produced some effect (182/25 rats). Combining the treatments led to an earlier appearance of tumours in much greater number (842/35 rats). There were therefore strong indications of a synergistic interaction and a crude estimation of ω is in the range of about 3. However, on Sprague-Dawley rats the same combined treatment produced a negligible incidence of tumours (2/31 rats) in comparison with the action of radiation alone (11/31 rats). DES in this case had no synergistic but rather an antagonistic effect. The experiment is a good example that, depending on the strain used, the same type of treatment may give rise to antagonistic or synergistic actions.

206. Most recent experiments by the same group [H26] confirmed the effect of DES and showed, in addition, a synergistic action of 17-ethinyl-estradiol (EE2) in rats. The complex of these data would imply that the synergistic interaction is not with the hormones examined but rather with their oestrogenic activity. In other studies by Segaloff and Pettigrew [S5] graded doses of radiation of 0.5, 1.5 and 4.5 Gy were used. Radiation given alone increased the incidence of benign tumours in proportion to dose, but the increase of malignant tumours did not follow a statistically significant proportionality and resulted in a rather low number. Combining radiation and DES produced a synergistic interaction at 0.5 Gy ($\omega \simeq 1.4$), but the increase in tumour incidence was most pronounced at 1.5 Gy (crude $\omega = 2.0$) and somewhat less at 4.5 Gy (crude $\omega = 1.6$). The combined treatment led to an earlier tumour development.

207. The role of prolactin in combination with radiation and with the chemical carcinogen N-nitroso-N-buthylurea was studied by Yokoro et al. [Y2] in W/Fu rats. Prolactin was produced by grafting a mammotropic pituitary tumour. Prolactin alone was ineffective in inducing mammary tumours. After doses of 2 Gy of x rays two fibroadenomas were seen among 27 animals with mean appearance times of about 6 months. Prolactin in combination with irradiation accelerated tumour appearance and induced tumours in 60% of the animals at the dose of 2 Gy. There were statistically significant differences in the tumour incidence between animals receiving 0.5 or 2.0 Gy and a similar synergistic interaction of prolactin and radiation was also found in respect to 14 MeV neutrons. Two interesting observations were made in these experiments. First, delaying the pituitary graft as long as seven months after irradiation still produced an enhanced effect, showing that the transforming lesions induced by radiation could remain available for hormonal interaction for a very long time. Secondly, most of the tumours produced by the interaction were adenocarcinomas, while most of the spontaneously occurring ones in this strain of rat are late appearing fibroadenomas.

208. In a more recent study by the same laboratory [Y6] fission neutrons (2 MeV mean energy) mixed with gamma rays were given to W/Fu rats. Only 2% of the animals developed mammary tumours after irradiation alone (up to 0.2 Gy) but 42% did when prolactin was given shortly after irradiation by grafting the prolactin-secreting pituitary tumour. Delaying the prolactin treatment up to 12 months produced 24% tumours, which observation supports the one previously reported [Y2]. A similar synergistic interaction of diethylstilbestrol (DES) and neutron irradiation in the production of mammary, pituitary and hepatic tumours was observed in castrated male W/Fu rats [S41].

209. The above results suggested to Yokoro [Y2] that in the previous studies the synergism between DES and radiation [S5, S7] could act via an increased production of prolactin. At the same time, Shellabarger [S6] was able to show that A × C rats (in which interaction with hormones was found) carried prolactin-secreting pituitary tumours; on the contrary, the Sprague-Dawley rats which did not show any synergism between radiation and DES carried no such tumours. In recent experiments by the group of Shellabarger [S44, H26] on A × C rats a strong dependence was shown between the interaction factor and the dose of DES and radiation. The dependence on the DES dose appeared to be mediated via the oestrogenic stimulation of prolactin secretion. The higher and the earlier the levels of prolactin in plasma, the greater was the yield of individual and multiple mammary adenocarcinomas.

210. Another oestrogenic hormone (polyestradiol phosphate) and a corticosteroid (methyl-prednisolone) were tested in combination with internal irradiation by

Nilsson et al. [N8] on CBA mice. Three doses of ^{90}Sr (0.925, 1.850 and 7.400 kBq/g) were applied, which led to a maximum of 2% animals with pituitary tumours. Polyestradiol alone produced 10% of such tumours. Combining the treatments resulted in 44 and 37% of animals with tumours, for the first and the second dose of ^{90}Sr, respectively, an increase corresponding to an interaction factor of approximately 4. Combined treatment also led to a decrease of the tumour induction time with respect to the groups given the radionuclide alone, close to that of the animals receiving only the hormone. Prednisolone in combination with radiation was ineffective in increasing the incidence or decreasing the induction time in comparison with groups receiving strontium alone.

211. Modelling of situations in animals that may operate in women taking contraceptive oestrogens was undertaken in the Netherlands [B3, B5]. The complete outline of these experiments calls for three different strains of rat (Sprague-Dawley, Wistar Wag/Rij, Brown Norway); four types of radiation (300 kV x rays and 0.5, 4 and 15 MeV neutrons); a range of different doses (from 0.1 to 2 Gy, according to the radiation employed); and various types of female animals (intact or hysterectomized, respectively with or without hestradiol-17-beta). The results of this series are still incomplete, but some preliminary conclusions may be drawn. For WAG/Rij rats the proportion of animals surviving without tumours abruptly decreased starting from nine months of age after irradiation with 4 Gy x rays and hormonal treatment. For animals receiving only irradiation or hormonal treatment a 50% decrease was observed after 22 months. The total yield of tumours for the combined treatment group was also higher. Considerable differences in the susceptibility to tumour induction were found between strains. Brown Norway rats having the lowest spontaneous incidence of mammary tumours had an intermediate susceptibility to the radiation-induced ones. Pathological data showed that malignant tumours were relatively rare in the Brown Norway and in the Sprague-Dawley strains, but were instead quite common in the Wag/Rij rats, amounting in the latter strain to nearly one-half of all tumours. A synergistic interaction of radiation with the oestrogen treatment was manifested not only through an increased proportion of rats with malignant tumours (from 0.43 to 0.83 in the Wag/Rij rats) but also through an increased absolute incidence of neoplasia in Wag/Rij and Sprague-Dawley rats. The minimum latency period in untreated control animals could be in excess of 22 months; in irradiated animals without hormones this period decreased to 10–12 months and a decreased latency in the hormone-treated rats in comparison with untreated groups was observed as a rule. The synergistic action of the hestradiol-17-beta is of the same type as the interaction between radiation and DES.

212. Kennedy and Weichselbaum [K15] reported a synergistic interaction between cortisone and x rays for transformation of C3H 10 T 1/2 cells in culture. The end-point scored is of great significance since it relates to tumour induction in vivo and the synergistic effect was statistically significant at $P < 0.001$. However, the transformation mechanisms in this particular cell line are still little understood [K16] and it is not possible to quantitate the results in terms of transformation frequency per surviving cell. It seems thus more prudent to test for effects in vivo before accepting the conclusions as generally valid.

213. Although the most informative data on the subject of combined action of radiations and hormones can only come from epidemiological surveys, data in this area are only indirect. It is known for breast cancer induction that age at exposure is a major determinant in all series available [T8, B27, S47]. Taken together, the data suggest that when the most profound hormonal changes occur (menarche, menopause) the risk per unit dose deviates most significantly from the mean risk for the whole life.

C. INFECTIOUS AGENTS

1. Viral infections

214. Viruses have a very important role in the pathogenesis of some radiation-induced experimental tumours like the thymic lymphoma, the myeloid leukaemia and the osteogenic tumours of different strains of mice. The Committee has reviewed the relevant evidence in Annex I of its 1977 report. It is difficult in fact in many instances to separate the action of the virus from that of radiation, because the interplay of the biological and of the physical factors is in these cases so intimate that it would not be possible to elicit the effect without the presence of the two agents combined. To think of a synergistic effect under the circumstances would be inappropriate because none of the agents alone may be active for the specific end-point. Moreover, the vertical transmission of the viruses through successive animal generations makes it a normal constituent of their genome, which is exactly the reason why some tumours are specific to some strains.

215. Radiation enhancement of in vitro cell transformation by viruses has long been reported [P13, S45]. An example was given of a combined treatment of Wistar/Furth rats with radiation and Gross mouse leukaemia virus [Y1]. Animals aged 7–8 weeks were intraperitoneally inoculated with a standard dose of virus (0.4 ml of leukaemic filtrate) and none of the 15 animals injected developed leukaemia. Whole-body x-irradiation (four doses of 1.5 Gy given at five days interval) produced also no tumours in 12 irradiated animals. The combination of both treatments gave rise to more than 50% leukaemias in 20 treated animals. In view of the lack of effects by the separate treatments, the interaction factor would in this particular case be equal to infinity. It was suggested that radiation might have acted through a modification of the physiological state of the target cells by rendering them susceptible to the action of the virus or through a modification of the immunological response of the host.

2. Bacterial infections

216. Environmental conditions have often been reported to influence the induction of specific tumour types in irradiated animals through their action on the microflora. It is conceivable that the response to any carcinogenic stimulus, including radiation, may interfere with expression of the carcinogenic damage by modifying the number, susceptibility or turnover rate of the target cells or by altering the immunological response against transformed cells. The most extreme conditions under which to test such hypotheses are provided by the study of germ-free as opposed to gnotobiotic or conventional animals.

217. Following irradiation of RF/Un mice myeloid leukaemia is decreased in the absence of microbial flora [W6], an effect which has been attributed to the reduced myelopoietic cell proliferation in germ-free animals [W7, W8]. Radiation-induced lymphatic leukaemia is, on the contrary, unaltered by germ-free conditions in many other strains of mice [P7, W1, W6]. Gnotobiotic and conventional animals show no qualitative differences with regard to virus particles found with the electron microscope [P7]. Induction of other solid tumours in irradiated mice gives variable results [A10, W7] and radiation-induced malignant or benign tumours are unaffected in germ-free rats. Thus, the data essentially show that the pathogenesis of radiation-induced cancer is similar in conventionally reared or in gnotobiotic animals. It should be concluded that the microbial flora as such has only a minor role in the development of haemopoietic neoplasms, perhaps via a modification of the immune system.

V. CONCLUSIONS

218. The interaction between ionizing radiation and other agents represents a field of great potential importance in view of the ubiquitous nature of radiation and of the many situations of interaction that might occur in modern life with a variety of physical, chemical or biological agents. Yet, it is very difficult to define and substantiate the notion of interaction with even a moderate degree of refinement. Many reports have claimed some kind of interaction but comprehensive analysis does not show a sufficiently good conceptual basis for the nature of the interactions. There is a lack of systematic treatment of any given case, particularly with regard to the mechanisms of action. There is further a need to apply existing methodologies of analysis from other fields of the biological sciences to the study of these problems.

219. The Committee has carried out a preliminary analysis of the combined actions in the radiobiological field, centered mainly around situations that may possibly be of importance for risk assessments in man and may therefore reflect on the present foundations of radiation protection. Available information on tumour induction, genetic defects and developmental effects was therefore scrutinized in the course of this analysis for any evidence of combined actions. The conditions of long-term exposure to low levels of the interacting agents were reviewed in detail, although in most of the reports the levels of exposure were much higher than the environmental. Where possible, the accent was on the results of epidemiological studies in humans, although the bulk of the information relates to animals.

220. The Committee proposes that two types of interaction may be considered. The first is one where both the ionizing radiation and the other interacting agent(s) are capable of producing some effect. Additivity, synergism and antagonism are the three possible conditions of interaction. The second type of combined action is that between ionizing radiation and other agents which are, when given alone, inactive. Protection or sensitization are the terms that apply in these cases, when reduction or enhancement, respectively, of the radiation effect are the end-results of the interactions. Such classification is not an absolute one because the doses of the interacting agents and the types of effect may influence profoundly the nature and degree of the interaction.

221. The concepts of exposure, dose and response may be applied to the special case of the combined action with ionizing radiation. The existing methodologies of analysis (isobolic diagram, envelope of additivity, surface of response) allow the assessment, at least on a semi-quantitative basis, of the results of combined treatments. These analyses may be further extended to generalized probabilistic treatments of the experimental results, taking into account the variability of the biological systems under study and leading to a more quantitative and satisfactory description of the interaction factors.

222. The applicability of these rather abstract notions to practical situations, particularly in the presence of complex biological effects, has been discussed. The need to define the effects with precision and to explore the full exposure-response ranges to all agents, acting separately or jointly, is a necessary prerequisite to meaningful studies. Also, pitfalls have been identified which may simulate conditions of interaction. In relation to important biological end-points such as the induction of tumours, the need to combine pathological and actuarial observations for a complete description of the phenomena has been underlined.

223. The temporal pattern of the exposure (contemporaneous or sequential, chronic or acute, single or fractionated) as well as the order of administration appear of decisive importance in respect to the production of a given type or degree of effect and have also been examined in the Annex. All these conditions relate to practical situations, even though they may tend to blur the clearly defined notions of additivity, synergism and antagonism. A detailed knowledge of the nature of the effects, their relationships to time and to the full range of doses of the interacting agents, including the zero-dose condition, is important. In many papers these basic conditions were imperfectly described. In other cases, the statistical significance of the results was too low for a complete assessment of interaction. Thus, the present conclusions should only be considered as preliminary.

224. An instance of interaction could be that between two different types of ionizing radiation, usually a combination of high- and low-LET radiation. Uncertainties exist as to the degree of interaction, owing to the essentially unknown nature of the primary radiation lesions and their repair systems. Even in cases where the yield of effect per unit dose of the two radiations differs by an order of magnitude, the interaction is within the limits of hetero- and iso-additivity. The study of the combined action of UV and ionizing radiation may be very valuable for the analysis of primary lesions and repair mechanisms. Experiments on survival of mammalian cells point to simple additivity. The important practical case of skin cancer induction, when tested in the animal, produced no evidence of interaction.

225. Examples of synergistic effects have apparently been reported in workers exposed jointly to ionizing radiation and microwaves in the radiotechnical industry. Functional disturbances of the nervous system and subjective symptoms of discomfort were mainly found in these workers. The nature of the symptoms, the difficulties of their quantification, the frequently uncontrollable conditions of exposure and the unsatisfactory dosimetry, the incomplete statistical evaluation, are all reasons for which these reports should be regarded with some reservation.

226. The combined action of suboptimal temperatures and radiation has given evidence of interaction in both directions, synergistic or antagonistic, depending perhaps on the type of effect, order of administration and level of exposure to the interacting agents. It would not be expected that any such effect would normally play any important role in higher animals, in view of their highly developed system of body temperature regulation. High altitude, metabolic or physical stress, mechanical damage, magnetic fields and ultrasound were also considered for a possible interaction with radiation: the results were variable but there was no evidence of significant synergistic interaction. In all these fields the data are very few, the effects non-specific and the mechanisms too obscure to allow any definitive statement.

227. The combined action of radiation, given internally or externally, with various types of dust shows under repeated testing, particularly with regard to tumour induction in the respiratory system, synergistic, additive or antagonistic effects. Considering the uncertainties and limitations of the data, the synergistic effect of the combined treatment did not exceed a factor of about two and the inhibitory effects a factor of about four, compared to situations where radiation was administered alone.

228. A variety of inorganic chemical compounds containing lead, silver, cadmium, calcium, beryllium, platinum, chlorine and fluorine, were also tested in experimental animals in conjunction with radiation for their carcinogenic, developmental or generally toxic properties. The results were once more extremely variable. In many cases the experience was so superficial, the effects so varied and the biological systems so different that no conclusions could be offered. Some of these interactions may be of significance in working situations and could profitably be explored further.

229. In this review, radioprotective and radiosensitizing substances were not examined in detail, since conditions relevant to the exposure of the population were the main object of the Annex. High levels of radiation and nearly toxic levels of these substances have been used in the relevant studies. A great variety of underlying mechanisms, complex relationships to the dose, to the radiation type, to the presence of oxygen were described for these chemical compounds. Since these substances are only utilized in the clinical field, none of them would be expected to pose significant problems of public or occupational health.

230. The possible combined action of radiation with compounds known for their carcinogenic properties has been the object of special attention. The substances examined include many initiators and promoters but the systematic information collected for each one of these substances is very incomplete. The evidence reviewed is conflicting and no final statement may be offered in regard to any substance or to any class of tumours before the dose, the schedule of administration and the treatment modalities are analysed to a greater depth, which is seldom the case in the experiments available.

231. Regarding benzo(a)pyrene and dimethylnitrosamine, two compounds having a widespread diffusion in the environment, experiments on lung tumour induction provided some evidence of a synergistic interaction (expressed mostly through a shorter latency time) for the former, but not for the latter substance. Fairly elaborate experiments in the hamster on the combined effect of radiation, uranium ore dust and diesel oil exhaust fumes yielded no evidence of synergistic effects, but the animal tested could be rather refractory to lung tumour induction. These studies should be extended in view of their practical implications.

232. Experimental data in animals and epidemiological experience on occupationally exposed human populations is available concerning the combined action of radiation and tobacco smoke. Tumours and inflammatory diseases of the respiratory system have been studied in this respect. In humans it appears that smoke may act by shortening the time of appearance of the radiation-induced lung tumours. It is not yet clear if such an action may be the result of promotion by some component of the tobacco smoke or due to a non-specific effect of the smoke on the respiratory epithelia. The experience in animals is still insufficient for a firm conclusion.

233. The precise evaluation of an interaction factor in humans critically depends on the length of the observation period as well as on the age structure and exposure history of the populations under study. It is impossible to say if the displacement in time of the tumour appearance will eventually result in an increased final yield of tumours in the smoking as compared to the non-smoking irradiated population. However, even if the final incidence of tumours between smoking and non-smoking irradiated individuals were the same, the effect should still be regarded as a synergistic one, since it would effectively lead to a reduction of the tumour-free life of the smokers developing tumours. This appears to be the only well documented case of a synergistic interaction in humans and in this sense it is a special case.

234. Antibiotics and other drugs were also considered for their possible interaction with radiation. Variable degrees of synergistic interaction were described for effects ranging from cell survival in vitro to tumour induction in animals. The relevance of these findings to individuals outside the clinical field is however difficult to evaluate, particularly in view of the limited diffusion of these substances in the general environment and of the high doses usually involved in the above interactions.

235. Possible cases of interactions with biological agents which were considered included those with hormones and with infectious agents. Regarding hormones, there is evidence that a variety of tumours of the experimental animal may be sensitive to their action. Diethylstilbestrol and oestradiol-17-beta were shown to have synergistic interaction for the production of mammary tumours in various strains of rat, with interaction factors in the range of 1.5 to 4. This type of synergism is also expressed through a shortening of the time for tumour induction. There is a large variability between strains, such that the same treatment schedule could produce potentiation in some strains and inhibition in others. There is also variability in relation to the tumour type. Epidemiological information in the human species is scanty and only indirect.

236. It is difficult for many animal tumours which are known to have a viral etiology (thymic lymphoma, myeloid leukaemia, osteogenic tumours) to consider their induction as the result of a synergistic interaction,

because the effect could not be elicited in the absence of either the virus or radiation. There is also no evidence that bacterial infection may play a major role in combination with ionizing radiation in modifying the yield of tumors.

237. For humans in environmental circumstances the Committee has been unable to document any clear case of synergistic interaction between radiation and other agents, which could lead to substantial modifications of the risk estimates for significant sections of the population. Presumably this is due to the fact that most of the agents likely to act synergistically with radiation, as judged by the results of animal experiments, are not found in sufficient concentration in nature. A specific exception is the case of tobacco smoke, which raises essentially problems of industrial hygiene in some working environments. Further research in the field of the combined effects is desirable because this area of study is still in an early stage of development and could profitably be pursued in a systematic way.

VI. RESEARCH NEEDS

238. An eminently practical research need is that of modelling experimentally situations encountered in living or working environments to test for undesirable effects. A second important and more basic research need is the identification of interaction mechanisms. The first need is essentially descriptive, the second essentially interpretative and both may interrelate to mutual advantage. There is also a third research need for the monitoring of possible effects in human populations by epidemiological studies. This latter is the most valuable for risk estimates in man.

239. Experiments of the first type are usually to study in experimental animals situations of practical interest for humans. It should be recalled that results obtained in a given animal species are not easily extrapolated to other species. In designing these experiments, exposure levels should be kept as similar as possible to the modelled situation. In combined action work, the assumption that effects showing at a given dose may exist to a lower degree at lower doses may not be true. Numerous examples of changes in the interaction with changing dose levels of the combining agents exist. The order and rate of administration of the agents should ideally mimic the real situation, although this may be impossible for chronic exposures of interest in practice. Long-term chronic rather than acute end-points should be focused upon. Tumour induction, effects on pre- and post-natal development after exposure in utero and genetic effects are the most significant classes of radiobiological end-points for further studies.

240. In the more basic studies, frequently involving experiments at the cellular and sub-cellular levels, there is considerably more latitude for research because the range of end-points is wider and the experiments financially less demanding. Good planning requires the careful choice of experimental end-points and of exposure level.

241. Epidemiological studies should have high priority under the existing circumstances. The inherent lack of control over many of the exposure variables should be compensated by the best possible definition of the exposure conditions, by the quantitation of the responses and by adequate statistical treatment of the observations. A conceptual and practical distinction should be made between interactions of relevance under special working environments involving possible problems of occupational medicine and large-scale exposure situations which could change risk estimates and could pose therefore more difficult problems of public health.

242. The use of a standardized nomenclature in the field of combined effects is highly desirable, because too often misconceptions are made possible by inaccurate terminology.

243. Considering the main technical requirements for experimental investigation of combined effects:

(a) Efforts should be made to report biological data as some function of the exposures in the target structures. For radiation, this problem is relatively simple and studies of energy deposition are reasonably advanced. In other cases (physical agents) this may simply require development of better dosimetric techniques and apparatus, but in most cases (particularly for chemical substances) it will imply detailed studies of the intake, metabolism, concentration and excretion of the interacting substances when a direct measure of their concentration at the level of the target structures is not possible;

(b) There is a need to define clearly and specifically the effects to be studied, especially when they are complex ones. For example, overall tumour induction may not in itself be a sufficient indication of a combined action because, even in the absence of significant changes in the overall interaction factor, changes in the spectrum of different tumour classes could take place. In the case of tumours it is important to study the rate of appearance, together with the final incidence, because shifts of the occurrence in time might reveal synergistic actions which would not be apparent otherwise. Also, actuarial and pathological observations should be combined and data corrections for competing risks should be applied;

(c) The variable "time" in the combined actions should be given proper attention, in the sense that contemporaneous and sequential treatments and reversal in the order of application should be examined. These studies are particularly important when the agents under examination have initiating or promoting characteristics and the sequence of their action is therefore decisive. Fractionated and chronic treatments could also be profitably examined, depending on the specific model situation and on the time characteristics of the agents combining;

(d) It is essential that appropriate methodologies of analysis of the interactions be used to avoid mistakes in the interpretation or inaccurate reports of the data. It is only through such objective analyses that precise statements and quantitative evaluations may be drawn. There is, more specifically, a need to refer any given interaction to conditions of iso- and hetero-addition;

(e) Appropriate control series should be set up to test exposure-reponse curves not only around the exposure levels of interest for the particular experiment but also for an extended range of exposures, including the zero values. Different combinations of exposures of the interacting agents should also be tested;

(f) It is important that the nature of the interaction should be as much as possible resolved through an analysis of the effects at various levels of

biological complexity, from the population level, through the whole-body, tissue, cellular and molecular levels. These studies allow generalizations and avoid misrepresentations of the interaction.

244. The specific areas of work identified by the Committee as particularly important for their basic or practical implications are:

(a) At the molecular and chromosomal levels, studies on the interaction of chemical, physical and viral agents on constitutive and induced processes related to DNA replication and repair of radiation damage (in simple as well as complex organisms) and relevant to the understanding of the mechanism of mutagenesis and to the estimations of genetic risks to man. These studies should concentrate whenever possible on low doses of radiation and of exposure to other agents and be correlated to relevant biological end-points like gene mutations and chromosome abnormalities as well as to cell differentiation (e.g., the immune system, developing organisms) or carcinogenesis;

(b) Studies of the interaction of different types of radiation, particularly for end-points which are of significance for practical purposes;

(c) At the systemic and whole-body level studies of combinations of tobacco smoke, fibres and dusts, organic and inorganic carcinogens and pollutants with radiation would be of great value;

(d) In human populations, further surveys of smoking and non-smoking workers professionally exposed to internal lung irradiations should be pursued. Under special working conditions the study of interaction of radiation with chemicals and microwaves would also be appropriate;

(e) For the population at large, the possible interaction of hormones and radiation, particularly in human females, should be tested, provided suitable groups might be identified. The increasingly widespread use of contraceptive hormones is of particular importance.

(f) Studies of combined effects in the treatment of patients (for cancer and other diseases) by combined treatment with radiation and chemotherapy and hormones, leading to carcinogenesis and to non-stochastic effects which may be "recalled".

Table 1

Lung tumours following neutron irradiation and crysotile treatment
[L16]

Group	Number of rats	Number of rats with lung tumours	
		Carcinomas	Mesotheliomas
Irradiated	20	1	0
Irradiated + Crysotile	9	4	3

Table 2

Effects on tumour development of prenatal exposure of mice to x rays and ethylnitrosourea (ENU) or to either treatment alone
[S42]

Type of tumour	Number of affected animals a/				Interaction factor ω (when applicable)
	x-irradiation (3 x 1 Gy)	ENU treatment (0.5 mMl/kg)	x rays + ENU	Control	
Leukaemia	3 (5.3)	3 (2.4)	10 (12.6)	2 (2.3)	3.4
Lung tumours	8 (14.3)	22 (17.8)	6 (7.6)	11 (12.8)	
Hepatomas	2 (3.5)	6 (4.9)	2 (2.5)	1 (1.1)	0.23
Pancreatic adenomas	0 (0)	1 (0.8)	2 (2.5)	0 (0)	
Intestinal tumours	0 (0)	2 (1.6)	0 (0)	0 (0)	
Ovarian tumours	6 (7.0)	0 (0)	10 (11.2)	0 (0)	1.6
Total tumour incidence	19 (33.9)	34 (27.6)	30 (38.0)	14 (16.6)	0.44
Tumour multiplicity (affected organs per animal)	1.0	1.0	1.5	1.0	
Number of tumours standardized	34	28	57	17	1.1

a/ The percentage of affected animals is shown in parentheses.

Table 3

Respiratory cancer deaths (RCD) in Colorado plateau uranium miners in relation to smoking [L2]

Smoking category	Person-years at risk (PYR)	Observed RCD (O)	Expected RCD (E)	O/E	$\frac{O-E}{PYR}$
Smokers	26392	60	15.5	3.9	$1.7\ 10^{-3}$
Non-smokers	9047	2	0.5	4.0	$1.7\ 10^{-4}$

Table 4

Interaction factors and probabilities ($\times 10^{-4}$) of respiratory cancer deaths per one person-year at risk for single and combined action of smoking and irradiation

Data base	Spontaneous	Smoking		Irradiation		Combined action			
	P_0	P_{t1}	$P_{o1} = P_{t1} - P_0$	P_{t2}	$P_{o2} = P_{t2} - P_0$	P_{ot}	$\Delta P_{obs} = P_{ot} - P_0$	$\Delta P_{exp} = P_{o1} + P_{o2}$	$\omega = \frac{\Delta P_{obs}}{\Delta P_{exp}}$
A	0.6	5.9	5.3	2.2	1.6	23	22	6.9	3.2
B	1.1	4.4	3.3	7.1	6.0	42.2	41.1	9.3	4.4

A: Derived from the 1950-1967 data base [L2].
B: Derived from the 1964-1967 data base [A1].

Table 5

Interaction factors and probabilities ($\times 10^{-4}$) of respiratory cancer deaths per one person-year at risk for uranium miners of different smoking categories [L6]

	Spontaneous	Smoking		Irradiation		Combined action			
	P_0	P_{t1}	$P_{o1} = P_{t1} - P_0$	P_{t2}	$P_{o2} = P_{t2} - P_0$	P_{ot}	$\Delta P_{obs} = P_{ot} - P_0$	$\Delta P_{exp} = P_{o1} + P_{o2}$	$\omega = \frac{\Delta P_{obs}}{\Delta P_{exp}}$
A	1.7	1.7	0	6.5	4.8				1
B	1.9	2.6	0.7			42.1	40.2	5.5	7.3
C	1.3	7.0	5.7			41.0	39.7	10.5	3.8
D	1.1	27.7	26.6			51.2	50.1	31.4	1.6

A: Non-smokers.
B: Former smokers.
C: Light smokers.
D: Heavy smokers.

Table 6

Dose modifying factors (DMF) for combined treatment by some chemotherapeutic drugs and radiation [P5]

Drug	Drug dose mg/kg	Dose modifying factor (DMF)		
		Intestine injury	Oesophageal lethality (from $LD_{50/28}$)	Pulmonary lethality (from $LD_{50/160}$)
Actinomycin D	0.75	1.3	1.6	1.6
Adriamycin	15	1.7	-	-
BCNU	8.25	1.1	0.9	0.74
Bleomycin	3	1.1	1.14	0.98
Cyclophosphamide	75	1.0	0.86	1.3
Hydroxyurea	500			1.03
Vincristine	0.5			1.18
Prednisolone	10			0.84

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back to first page