PRELIMINARY DATA ON CHROMOSOME ABERRATIONS IN SWINE
AND HUMAN LEUKOCYTES WITH A BRIEF REVIEW OF FACTORS
AFFECTING THEIR OBSERVED FREQUENCY*

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Preliminary results from a study of relative sensitivities of porcine and human leukocyte chromosomes to X-rays suggest that aberration frequencies are slightly greater in human cells. Data are also reported for ^{90}Sr - ^{90}Y irradiation of swine leukocytes but comparable results of tests with human cells are not yet available.

Radiation dose and dose rate, quality of radiation as measured by linear energy transfer, and the modifying factors, oxygen and virus, are briefly discussed for their general role in modifying the frequency of aberrations observed following radiation exposure.

INTRODUCTION

To encompass the information indicated by the title would clearly be a major undertaking and would go beyond the interests of this symposium. We will therefore restrict the discussion to effects on the cell's information system which are important in altering survival of somatic cells, i.e. effects on the chromosomes. To introduce this topic we will describe some preliminary results from a chromosome breakage study in progress in our laboratory.

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In an ongoing study of effects on miniature swine of daily ingestion of ^{90}Sr , a study to be described in more detail later in this session by Dr. Ragan, we have consistently failed to observe chromosome aberrations in frequencies appreciably above those observed in unirradiated controls, even with cumulative doses calculated to be in excess of 10-20 Krads. This raised the question of the sensitivity of swine chromosomes which this study is attempting to answer in terms of the relative sensitivity of swine and human chromosomes.

METHODS

To compare the relative radiosensitivities of swine and human chromosomes, peripheral blood samples were obtained from unexposed control animals and from a female human volunteer. Leukocyte cultures were exposed to X-rays or to radiations from ^{90}Sr - ^{90}Y .

A sample of heparinized whole blood, obtained under sterile conditions from female Pitman Moore swine and a female human volunteer, were allowed to sediment in the collection syringe at room temperature for approximately one hour. One milliliter of the plasma was placed in sterile plastic culture bottles and irradiated. Following irradiation, one milliliter of fetal calf serum and 5 milliliters of NCTC109 (Difco) containing 300 units/ml penicillin-G, 0.3 mg/ml streptomycin and phytohemagglutinin-M (Difco) were added. The cell culture was then incubated at 37° C for 45 hours at which time 0.01 mg colchicine was added, to arrest mitosis in the

metaphase stage, and four hours later the cells were prepared for observation essentially as described by Moorhead, et al.¹ The slides were quickly blaze dried and stained with 2% Giemsa in phosphate buffer, pH 6.8. Suitable metaphases were photographed under oil immersion. A 250 kVp X-ray unit operated at 15 ma and with 1 mm copper + 1 mm aluminum filtration was used as the radiation source. Air doses were determined using Victoreen dosimeters and confirmed with LiF thermoluminescent dosimeters. The one milliliter plasma volume irradiated was contained in a 30 ml plastic culture flask. The doses used were 50, 100 and 200 R delivered at a constant rate of 11 R/min.

Beta irradiations were accomplished by mixing four millicuries of carrier free ^{90}Sr - ^{90}Y (6.7 mCi/ml 0.01 N HCl) with 100 ml of the culture medium previously described and adding appropriate amounts of this solution to cell suspensions containing 1 ml of test plasma and cells, 1 ml of fetal calf serum and varying amounts of NCTC 109 such that all cultures contained 5 ml of growth medium in a total volume of 7 ml. The final cell cultures contained 7.4, 15, and 29 μCi ^{90}Sr - ^{90}Y /ml. The incubation time, colchicine treatment, cell harvest and slide preparation were the same as described for the X-irradiation.

RESULTS AND DISCUSSION

Table 1 summarizes the data now available from the studies of in vitro irradiation. As expected, the incidence of chromatid aberrations in both porcine and human cells was not significantly increased by X-irradiation.

This is consistent with the general observation that circulating leukocytes from normal healthy individuals are in the interphase, G_1 , phase of the cell cycle. Thus aberrations scored as chromatid are presumed to have occurred spontaneously, not as a result of radiation exposure prior to stimulation by PHA. To minimize subjective influences of interpreting unstained gaps vs terminal chromated deletions these two categories were combined. Pooling the chromatid aberrations for the various exposures yielded spontaneous aberration rates of 0.018 aberrations/cell for swine and 0.044 aberrations/cell for humans. The value for human chromosomes is somewhat higher than the value of 0.014 aberrations/cell obtained by Bender and Gooch.² In a study on swine chromosomes, McFee et al.³ observed a much higher chromatid aberration incidence (5% and higher) but failed to comment on possible causes of such a high value.

To avoid uncertainties of interpretation, chromosome terminal deletions were combined with achromatic lesions, and the frequency of these is linearly related to dose. The average one-hit aberration coefficients calculated from the data are $0.4 \times 10^{-3} \text{ cell}^{-1} \text{ R}^{-1}$ for pig and $0.7 \times 10^{-3} \text{ cell}^{-1} \text{ R}^{-1}$ for human cells. Comparable values of $1.1 \times 10^{-3} \text{ cell}^{-1} \text{ R}^{-1}$ are reported for swine³ and $2.4 \times 10^{-3} \text{ cell}^{-1} \text{ R}^{-1}$ for humans.²

Multiple break aberrations are detected by the presence of ring configurations or multicentric chromosomes in the metaphase stage. Absence of this type aberration in both swine and human controls is consistent

with their reported low frequency¹ and the small numbers of cells observed so far in this study. The two-hit aberration coefficient for human cells was $0.46 \times 10^{-5} \text{ cell}^{-1} \text{R}^{-2}$. The apparent linearity of the swine data prevent calculation of a single value since proportionality with the square of the dose is anticipated. Our data give values ranging from $0.5 \times 10^{-5} \text{ cell}^{-1} \text{R}^{-2}$ to $0.09 \times 10^{-5} \text{ cell}^{-1} \text{R}^{-2}$. Comparable values of $0.52 \times 10^{-5} \text{ cell}^{-1} \text{R}^{-2}$ for human² and $0.092 \times 10^{-5} \text{ cell}^{-1} \text{R}^{-2}$ for swine³ have been obtained by others.

While there are notable inconsistencies within the present data which may be resolved as more data are accumulated it appears that, at comparable radiation doses, aberrations occur with a lower frequency in swine than in human cells.

At this time no comparison of swine with human chromosome breakage by ^{90}Sr - ^{90}Y beta radiation is possible since exposures of human leukocytes has not progressed to the point of providing results. At the highest dose of ^{90}Sr - ^{90}Y , 29 $\mu\text{Ci/ml}$, extensive cell death occurred yielding an insufficient number of metaphases for study. It is interesting to note that chromatid aberrations increase with radiation dose, because, in this case, the radiation is continuous with S and G_2 as well as G_1 stages irradiated. The relatively high frequency of chromatid, relative to chromosome, aberrations is similar to the in vivo results of Brooks and McClellan⁴ following injection of large doses of ^{90}Sr - ^{90}Y into Chinese hamsters.

As will be discussed later, dose rate is highly significant to the production of the multibreak aberrations most apt to be detected. From the present finding that swine chromosomes are reasonably sensitive to aberration induction it seems most probable that failure to observe aberrations in the animals chronically exposed to ^{90}Sr - ^{90}Y is more likely due to the low dose rate than to insensitivity of the chromosomes.

We have not as yet verified the appropriateness of 49 hours as the time of maximum accumulation of first division metaphases in swine and humans. In fact, the recent observation of Bender and Brewen⁵, indicating the presence of two or more dividing populations in phytohemagglutinin stimulated human leukocytes makes this evaluation of division times even more important for comparative studies such as those we have just reported.

REVIEW OF FACTORS AFFECTING ABERRATION FREQUENCY

Chromosomes Critical to Cell Survival

To comply with the symposium assignment, the following discussion will briefly identify some of the factors which modify the amount of chromosomal damage expected in studies such as those described above. Several reviews are available which cover topics relating to chromosome aberration more extensively than is appropriate here.^{6,7,8,9}

Before proceeding it may be useful to direct attention to early findings of Zirkle and Bloom¹⁰ and Rogers and von Borstel¹¹ that chromosomes are the

structures critical to radiation induced cell death. This evidence has been repeatedly supplemented by a wide variety of cell survival data such as that of Puck et al.^{12,13} and Skarsgard et al.¹⁴ showing strong correlation of survival with aberration frequencies. While it is true that radiation induced lethality in cells such as the nondividing oocyte¹⁵ of young mammals does not appear to be explainable by chromosome breakage, most cell death following radiation correlates well with the frequency of chromosome aberration. From a variety of observations it is evident that induction of breakage and the resulting new chromosomal associations are not of themselves lethal but become lethal when cells containing such aberrations undergo cell division. During the division process information transfer to daughter cells is imprecise when aberrations lead to loss of acentric pieces, dicentric chromosomes with internuclear bridges, duplications, etc. As the magnitude of such irregularity increases the probability of losing or comprising information critical to survival of subsequent cell generations also increases.

Aberration Types

Chromosome aberrations have been produced by ionizing radiation both to provide material for genetic studies and to determine the manner by which aberrations are produced. If breakage occurs while the chromosomes are single, deletion type aberrations may be present in both chromatids and potentially present in both daughter cells. If the breakage occurs subsequent to the S phase, when the chromosomes are split and called chromatids, the deletion is observed in only one of the two chromatids and would be expected

to affect only one of the daughter cells.

Chromosomal aberrations resulting from two breaks may involve a single chromosome in which case inversions or rings and deletions are produced. With techniques now available, inversions are not readily identified in mammalian cells since only the order, not the amount, of genetic material in each chromosome changes. Ring shapes are readily identified and are categorized as a double break type. Also easily identified as a double break type are the chromosomes having two centromeres. These result from breaks in two different chromosomes with rejoining in such a way that one of the portions has both centromeres, the other none. Equally likely are rejoinings with one centromere on each of the newly associated pieces. Recombinations involving more than one chromosome are termed translocations and, as with inversions, those combinations which result in all chromosomal materials attached to one and only one centromere have little effect on cell survival and are not readily identified. Rings and dicentrics in the metaphase stage are easily identified and signify multiple breakage. If the events causing the breakage are independent, the frequency of these two break aberrations typically approaches proportionality to the square of the dose.

Dose and Dose Rate

Neary et al.¹⁶ report that breakage can be induced by as little as 60 eV, while earlier work indicated a somewhat higher energy requirement.¹⁷

Sixty electron volts is roughly equivalent to the energy involved in producing two ion pairs and this amount of energy, or slightly more, is found in the ion clusters along the tracks of γ , x, or energetic β rays. Thus one typically observes a linear response of terminal deletions (caused by a single break) to increasing absorbed dose.¹⁸ In the data reported, frequencies of both swine and human chromosome deletions were approximately proportional to the X-ray dose. There was no effect of X-ray dose on chromatid deletions since the leukocytes were in the G_1 (interdivisinal) stage of the cell cycle. The expected proportionality of rings and dicentrics, i.e. two break aberrations, with the square of the dose was observed with our human data but the response was linear with the swine chromosomes. Presumably this is merely the result of the small sample involved and we presume that with more data the swine response will fit more closely to a two break curve.

Not all breaks result in a change in the chromosomes. Following breakage the two broken ends are in close proximity and a vast majority of breaks rejoin¹⁹ with no residual damage or prejudice to cell survival. The importance of the proximity of the broken ends is well established^{16,20} as is also the factor of time involved in restitution.²¹ Since breaks remain open for limited times, it is to be expected that at lower dose rates or with fractionated doses a break may reconstitute before a second break occurs within the volume critical for exchanges, i.e. within 0.4-0.6 μ m of each other.^{16,21} This is a factor of considerable importance

with metabolized isotopes such as ^{90}Sr - ^{90}Y which may deliver large cumulative doses but at relatively low dose rates.

Quality of Radiation, LET

The rate at which energy is imparted to the biological system linearly along the track of a penetrating radiation is referred to as linear energy transfer (LET) and is expressed in terms of kilovolts/ μm .²² Generally X, gamma, and hard beta radiations are considered to be low LET radiations while protons, alphas, accelerated nuclei, etc. are considered high LET radiations. While two break aberrations typically are proportional to the square of the dose with low LET radiations, they are directly proportional to dose when high LET radiations are involved. In the region of the high LET track the energy released is so great that multiple chromosome breakage is highly probable for chromosomes lying within the zone in which recombinations can occur -- i.e. a volume having a diameter of 0.4-0.6 μm . Since the simultaneous production of two breaks with four broken ends in close juxtaposition encourages the production of two-hit aberrations and tends to minimize restitution this accounts for some of the higher cell killing efficiency of the high LET radiations.

Oxygen

As with most biological damage, oxygen increases the frequency of chromosome breakage induced by low LET radiations. Typical data are reported by Deschner and Gray²³ who evaluated chromosome abnormalities

in ascites cells exposed both in vivo and in vitro. Apparently the presence of oxygen during the exposure period encourages production of free radicals in the suspending solution, radicals themselves capable of producing chromosome breaks. Aberrations induced by high LET radiations are not influenced or are less influenced by the presence or absence of oxygen. Seemingly there is ample energy available along each high LET particle track to break any chromosomes within the rejoinable distance and additional free radicals are of no consequence. In any event, high LET radiations typically show neither an oxygen effect nor a dose rate effect.

The role of oxygen in affecting aberration frequency is not a simple one since, in addition to enhancing the frequency of break induction by low LET radiations, oxygen, through the normally functioning oxidative metabolic systems of the cell, enhances the fusion of broken ends.^{24,19} Until recently it was assumed that rejoining concerned the structural back bone of DNA. However, Wolff and Scott²⁵ find that inhibitors of protein synthesis keep breaks open while known DNA repair mechanisms have little affect on the fusion of broken ends, emphasizing the importance of the protein moiety of the nucleoprotein complex which makes up the chromosomes.

The observation that high LET radiations are unaffected by oxygen poses an attractive possibility to radiologists treating solid tumors whose central zones remain relatively anoxic despite all efforts to raise

their oxygen tension. Thus, in typical X or gamma therapy, the cells in the center of solid tumors are anoxic and thus less damaged than are the more highly oxygenated cells on the surface of the tumor and the surrounding normal tissues. High LET radiations capable of releasing their energy in the tumor region would overcome this problem.

Other Factors

Other features such as mitotic delay²⁶, selection against aberrations²⁷, etc. might well be discussed but instead we will briefly refer to the observation of a persistent factor in plasma capable of increasing aberration rates in cells from normal individuals. Goh and Sumner²⁸ observed such a factor in plasma of individuals accidentally irradiated several years previously and Littlefield et al.²⁹ obtained similar results using plasma of patients receiving therapeutic irradiations. Such a factor was sought when aberration frequencies remained high over much longer post irradiation times than expected. It is difficult to imagine an effective free radical persisting over such long times. One possibility might be that viruses, were involved since they are known to induce at least simple breakage.³⁰ Inclusion of viruses as factors important to genetic considerations is logical since knowledge of transduction clearly implicates them as part of the cell's potential information system. The extent to which they may modify aberration data has, as yet, not been evaluated but we are not aware of any evidence that multibreak aberrations such as rings and dicentric are increased by the presence of viruses. Neither have we seen data which

would force rejection of the possibility that viruses were the persistent agents in plasma inducing aberrations frequencies above those observed in controls. The interaction of radiations, viruses, and host information are subjects of intense interest and speculation is well warranted if it leads to informative experimentation.

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TABLE 1
CHROMOSOMAL ABERRATIONS OCCURRING AFTER IN VITRO IRRADIATION

	<u>No. of Cells Scored</u>		<u>CTD* + CTG</u>		<u>CD* + AL</u>		<u>Rings + Dicentrics</u>	
	P**	H**	P	H	P	H	P	H
X-Ray								
Control	160	94	3	4	0	0	0	0
50R	168	139	2	5	4	6	2	2
100 R	171	186	2	10	5	10	4	8
200 R	165	171	5	7	17	26	6	25
⁹⁰ Sr- ⁹⁰ Y								
Control	133		3		1		0	
7.4 µCi/ml	152		6		5		2	
15 µCi/ml	169		40		11		1(1)***	

* CTD, chromatid deletions, CTG, chromatid gaps. CD, chromosome deletions, AL, achromatic lesions on both arms.

** P, porcine. H, human.

*** The number in parenthesis refers to a quadricentric chromosome.