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MATHEMATICAL THEORY OF THE LETHAL ACTION OF RADIATION ON YEAST CELLS

Robert Arthur Wijsman

December 11, 1952

Berkeley, California

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Robert Arthur Wijsman*

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SUMMARY

The main part of this thesis is devoted to the derivation and study of formulas for the probability of survival of the haploid and diploid yeast cell, based on models proposed by Zirkle and Tobias. Some properties of so-called completely monotonic functions are developed, and the results are applied to the investigation of some of the more complicated formulas. Finally the theoretical expressions are compared with the experimental data obtained by Tobias. If the "sensitive sites" can be identified with the genes, and if the number of vital gene pairs in the diploid cell is n, then the comparison between theory and experiment leads to the following conclusions:

1) n is between 2 and 30;
2) at least some of the genes in the diploid cell must be close together.

Furthermore some of the data suggest that homologous chromosomes are paired, so that homologous genes are close together.

It is emphasized that more data are necessary to make better conclusions, and that a more complete theory is necessary to explain better the response of yeast cells to radiation.

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I. INTRODUCTION

The material presented in this thesis is based on radiation experiments on yeast cells, performed by Dr. Cornelius A. Tobias and co-workers in Donner Laboratory of Medical Physics, University of California, Berkeley, California, and by Dr. Raymond E. Zirkle, Chicago, who collaborated with Dr. Tobias during his visit to Berkeley. A joint paper by Zirkle and Tobias is in preparation, but has unfortunately not been published yet. In spite of this both investigators will be quoted freely throughout this thesis.

The experiments by Zirkle and Tobias were designed to study the action of high energy radiation on single cells, in particular with respect to survival of the cells, in an attempt to understand the mechanism by which high energy radiation affects living organisms. Yeast cells were chosen for various reasons. One of the reasons is that there exists an artificially produced haploid strain (see: LINDEGREN (1949)) derived from the naturally occurring diploid strain, so that it is possible to compare the two strains in their response to radiation. Since the experimental results have shown a great difference between the diploid and haploid cells in their response to radiation, they strongly suggest that the damage caused by the radiation is primarily done to the genes or chromosomes, rather than to the cytoplasm. It is for this reason that the various mathematical models proposed to describe the action of radiation on yeast cells, are only concerned with what happens to the genetic material.

This thesis will be concerned mainly with the derivation and study of formulas describing the probability of survival of the haploid and the diploid cell as a function of the radiation dose, on the basis of various mathematical models. Secondly the dependence of these survival curves upon the type of radiation will be studied. Finally the theoretical results will be compared with the experimental results, in order to check whether the theory presented may have some validity. In addition, the comparison may lead to a choice between the various mathematical models, which, in turn, would furnish us with some information about the geometrical arrangement of the chromosomes and/or genes relative to each other. However, it must be emphasized that due to the tremendous experimental difficulties there are only few data available so that any conclusions drawn from comparison of theory and experiment should be regarded as preliminary, pending future work. Moreover, all experiments indicate that either of the mathematical models proposed so-far is unable to explain all the observed effects, so that most certainly nature is more complicated than described by any of our mathematical models; as a result we should consider these models and their theoretical consequences only important as a basis for future developments.

The material presented in this thesis is original except when indicated otherwise, in which cases references will be given.

II. MATHEMATICAL MODELS FOR THE HAPLOID AND DIPLOID CELL

As was pointed out in Chapter I, the mathematical models are concerned with damage done to the genetic material of the cell. It is not known, though, whether this damage is done to individual genes or to chromosomes as a whole, e.g. through chromosome breakage. Zirkle and Tobias leave this question open, and speak of "sensitive sites" in the genetic material. However, we shall assume for the moment that these sensitive sites can be identified with the genes, and shall return to the question later whether this concept should be modified.

The model proposed by Zirkle and Tobias for the haploid cell in its response to high energy radiation is as follows: Among all the genes of the haploid cell there is a number of n genes vital to the functioning of the cell. If the radiation damages any of these vital genes the cell will die (it may divide a number of times, but will not form a big colony). The number n may be equal to the total number of genes, or may be less.

The diploid cell has, as is well known, a double set of chromosomes. Two corresponding chromosomes are called homologous chromosomes and two corresponding genes are called homologous genes. It is not known whether of a homologous gene pair both genes are active in the biochemical reactions, or only one of them. It is well known, though that if the homologous genes are different (heterozygotism), frequently one of the genes impresses its character upon the appearance of the organism (dominance). It seems reasonable to assume that in those cases only the dominant gene takes part in the biochemical reactions, so that apparently the cell can function well with only one gene of the homologous gene pair active. It then seems reasonable to assume that if one gene of a homologous gene pair is irreparably damaged by the radiation, the other gene will keep the cell functioning. Thus the model for the diploid cell proposed by Zirkle and Tobias is as follows: There are n homologous gene pairs, vital to the functioning of the cell (the same number n as in the haploid cell). If of any of these n pairs both genes are damaged by the radiation the cell will not survive. If of each of the n gene pairs at least one gene escapes damage the cell will survive.

III. MODEL FOR THE INTERACTION BETWEEN RADIATION AND GENES

For the sake of facilitating the terminology we shall say that a gene is "hit" when it is affected by radiation in such a way that it may cause the death of the cell. There exist several theories concerning the mechanism by which the radiation produces a hit. The "direct hit" theory (see e.g.: LEA (1946)) proposes that the gene is ionized or excited directly by the incident radiation (more precisely: by the electric field of one of the charged particles present in the radiation). The direct hit theory predicts correctly the shape of the survival curves if the type of radiation is kept constant and the dose is varied, but it predicts incorrectly the relative biological effect of different types of radiation on yeast cells. Experimentally it is observed that radiation consisting of heavy particles, such as α-particles, which produce dense ionization tracks, is more effective in its lethal action on yeast cells than X-rays, which produce thin ionization tracks from the electrons emitted by the photo- or Compton effect. According to the direct hit theory, on the other hand, the lethal effect should decrease as the track density increases. This can be understood qualitatively as follows: The shape of the haploid survival curve is a simple decreasing exponential in the dose and thus indicates that a single hit is sufficient to cause a lethal effect. If the track density is increased it becomes more probable that a track passing through a gene produces more than one hit, so that some hits are wasted since one hit would have been sufficient. It is then clear that a higher dose is necessary to give the same biological effect, or, in other words, the effectiveness of the radiation decreases. In view of this disagreement with experiment the direct hit theory was abandoned in favor of an "indirect hit" theory, also called "migration" theory, proposed by Tobias. In this theory the major gene damage is done by certain chemicals which are liberated by the radiation anywhere in the cell, and which subsequently diffuse toward the gene. The increase in lethal effect with increase in track density is then theoretically obtained by proposing that there are two kinds of chemicals (or possibly more)

produced by the radiation and that the more active of the two chemicals is produced in a greater proportion relative to the less active one as the track density increases. There is some evidence that the more active chemical is the H_2O_2 molecule and the less active one the OH radical. As the track density increases, the spacing between the OH radicals at the time of their formation becomes smaller on the average, so that the probability that two OH radicals will diffuse toward each other and form an H_2O_2 molecule becomes larger. However, the exact nature of the damaging chemicals and the mechanism of their formation is not important for the theoretical developments of the following chapters, since we shall not attempt to make a quantitative theory of the relative biological effect as a function of the radiation track density. For a more detailed justification of the migration theory the reader is referred to the forthcoming paper by Zirkle and Tobias.

IV. PROBABILITY OF GENE DAMAGE FROM RADIATION TRACKS

In this and the following chapters we shall make some simplifying assumptions, which however, leave the problem in its essential form. These assumptions are:

1) each gene has the same probability of being hit;
2) all n vital genes (gene pairs) are located in the same chromosome (chromosome pair);
3) the damaging molecules are formed by the radiation in straight lines.

A remark about the assumption (2) will be made later in this chapter.

Basic for all calculations in this chapter is the calculation of the probability that a "poisonous" molecule, formed by the radiation at a distance r from a certain gene, will by diffusion eventually reach this gene and damage it. While the molecule, which we shall denote by A, diffuses through the cell, it may be removed by undergoing a chemical reaction with one of the cell constituents, other than the gene under consideration. Therefore, the medium in which A moves is both scattering and absorbing. Since it is safe to assume that all relevant distances in this problem are small compared to the dimensions of the cell, we may consider the medium in which A moves as infinite.

Suppose first that we have only one gene, which we consider as a sphere of radius r_o, embedded in an infinite scattering and absorbing medium, and suppose that a particle A is at a distance r from the center of the gene. The probability p(r) that A will eventually reach the gene is calculated in the Appendix, and is essentially given by:

$$p(r) = \frac{r_o}{r} \exp\left[-\frac{r}{d}\right] \qquad (1)$$

in which d is a constant with the dimension of a length. We shall call d the diffusion length since it is essentially the distance over which A can diffuse before being absorbed in the medium.

In reality we will have more than one gene in the cell. It is impossible to treat this case in general, because not only do we not know how the genes are spaced relative to each other, but even if we would know that, it would be a prohibitively difficult mathematical problem to calculate the probability that A reached a certain gene by diffusion. The best we can do is to consider two extreme cases which we can treat with good approximation: 1) genes far apart compared to d, and 2) genes close together to d.

Here we want to insert a remark about the assumption (2), mentioned in the beginning of this chapter. Let us consider the haploid cell, and suppose that the n genes are located in several chromosomes. It is reasonable to assume that these chromosomes are far apart. If the genes in each chromosome are far apart (case 1), it obviously does not matter whether the genes are in one or in more than one chromosome. On the other hand, if the genes in a chromosome are close together (case 2), we have groups of genes, two genes in one group being close together, but two genes in different groups being far apart. However, we can consider this case as a mixture of the cases 1 and 2 for one group of genes (one chromosome), so that it makes sense to treat these two cases separately for one chromosome. The reasoning goes in the same way for the diploid cell.

In case 1 the genes do not disturb each other (approximately), so that the probability that A reaches a gene is still given by (1), in which r is the initial distance between A and the gene under consideration.

In case 2 the gene complex may be considered as one unit, and distances large compared to the size of the complex may be measured from any point within the complex. The probability that a particle A, being a distance r from the gene complex, will diffuse toward any of the genes in the complex is approximately a function of r only, and given by (1) except for a multiplicative constant. To see this we may imagine a sphere of radius R, large compared to, and concentric with the gene complex. If $p_n(r)$ is the probability that A will reach the gene complex

consisting of n genes, and P(r, R) is the probability that A will at some time pass through the sphere R, then

$$p_n(r) = P(r, R)\, p_n(R),$$

If the gene complex consists of one gene only, then the corresponding equation is

$$p(r) = P(r, R)\, p(R),$$

with the same function P. By dividing the first equation by the second one we obtain $\frac{p_n(r)}{p(r)} = \frac{p_n(R)}{p(R)}$ = constant. If the gene complex consists of n genes, $n > 1$, then it is obvious that $p_n(r) > p(r)$. Since we treat all the genes in the complex on equal footing the probability per gene is equal to $\frac{1}{n}\ p_n(r)$. It is also clear that this probability per gene should be less than if only one gene were present i.e. $\frac{1}{n}\ p_n(r) < p(r)$, since some particles A which would otherwise have reached a particular gene may now be caught by neighboring genes. We may now put the probability per gene equal to $a_n\, p(r)$, with $a_n < 1$. The probability that A hits any of the n genes is then $n\, a_n\, p(r)$, and the probability that any gene out of a subgroup of k genes is hit ($k \leq n$) is

$$p_{n,k}(r) = k\, a_n\, p(r) \qquad (2)$$

Since from physical considerations the probability for a whole gene complex $p_n(r) = n\, a_n\, p(r)$ increases with n, whereas the probability per gene $\frac{1}{n}\ p_n(r) = a_n\, p(r)$ decreases with n, we have for a_n the two relations

$$a_1 = 1 > a_2 > a_3 > \ldots\ldots \qquad (A_1)$$

$$a_1 = 1 < 2\, a_2 < 3\, a_3 < \ldots\ldots \qquad (A_2)$$

The incident radiation produces ionization tracks, which we consider as straight and parallel. If they are not parallel, we may pretend they are without changing the results of the calculations. We shall ignore δ-rays. The number of ion pairs per unit length along the track will be denoted by n_x. According to the model described in Chapter III these ion pairs will be converted into at least two kinds of damaging

molecules A and B. We shall assume that there are only these two kinds of molecules; a generalization to more than two kinds of molecules is easily made. Suppose that the density of A along the track is $f_1 n_x$ and of B $f_2 n_x$ in which f_1 and f_2 are functions of n_x, and $0 \leq f_1 \leq 1$, $0 \leq f_2 \leq 1$. For our purpose the particles A and B differ only in their diffusion lengths, which we denote by d_1 and d_2 respectively. We assume B to be much more effective in its damaging action, so that we have $d_2 >> d_1$. The two extreme cases we shall treat in the following are: 1) genes far apart compared to d_2, 2) genes close together compared to d_1.

If two particles from the same track each hit a gene (the same or different ones) the probabilities for these hits to occur are not statistically independent due to the special correlation of the damaging particles. On the other hand particles from two different tracks produce independent hits, since the tracks are formed at random.

Suppose a track passes a gene complex, consisting of n genes, at a distance s. We pick out a subgroup of k genes and consider the probability $P_{n,k}(s)$ that any of the particles A or B hits at least one of the genes of the subgroup. We shall say, in this case, that the subgroup receives a track hit. $P_{n,k}(s)$ can be calculated as follows: The average number of particles A reaching the subgroup is $f_1 n_x \int_{-\infty}^{\infty} p_{n,k}(r)\, dx$, where $p_{n,k}(r)$ is given by (2) and (1) with d replaced by d_1, and x is a coordinate along the track such that on the positive axis $x = \sqrt{r^2 - s^2}$. After the substitution $x = \text{Sinh } t$ the integral is transformed into $k a_n f_1 n_x r_o \int_{-\infty}^{\infty} \exp\left[-(s/d_1) \text{ Cosh } t\right] dt = k a_n 2 f_1 n_x r_o K_o(s/d_1)$ (see: WATSON (1922)), in which K_o is the second Bessel function of imaginary argument. For the particles B we have a similar expression. For the sake of abbreviation we introduce

$$f(s) = 2 n_x r_o (f_1 K_o(s/d_1) + f_2 K_o(s/d_2)) \qquad (3)$$

so that the average number of either kind reaching the subgroup is $k a_n f(s)$. It can be argued that, due to the randomness of distribution

of A and B along the track, the number of particles reaching the subgroup follows a Poisson distribution*). Thus the probability of no hit from the track is $e^{-ka_n f(s)}$, and therefore:

$$P_{n,k}(s) = 1 - e^{-ka_n f(s)} \tag{4}$$

Next we want to average (4) over s in order to find the probability $Q_{n,k}$ that the subgroup of k genes out of the complex of n genes is not hit. We imagine a plane perpendicular to the tracks, consider the points of intersection of the tracks with this plane, and observe that due to the nature of the radiation these points are distributed at random over the plane, with an average density of D/n_x per unit area. As a result the number of track hits follows a Poisson distribution with average $D/n_x \int_0^\infty P_{n,k}(s)\, 2\pi s\, ds$, so that the probability of no track hit in the subgroup is $\exp\left[-(D/n_x) \int_0^\infty P_{n,k}(s)\, 2\pi s\, ds \right]$, and, using (4):

$$Q_{n,k} = \exp\left[-\frac{D}{n_x} \int_0^\infty (1 - e^{-ka_n f(s)})\, 2\pi s\, ds \right] \tag{5}$$

It should be noted that the integration over s has been extended to s = 0, i.e. into a region in which (3) strictly does not hold. However, we may make this simplifying approximation in view of the fact that d_1 is supposed to be large compared to the size of the gene complex.

We shall introduce the following notation:

$$c_{n,k} = \frac{1}{n_x} \int_0^\infty (1 - e^{-ka_n f(s)})\, 2\pi s\, ds \tag{6}$$

which will be useful for the following chapters. In the special case n = k = 1 we shall write simply c_1. Since $a_1 = 1$, we have:

$$c_1 = \frac{1}{n_x} \int_0^\infty (1 - e^{-f(s)})\, 2\pi s\, ds \tag{6a}$$

With help of (6), equation (5) can be written shorter as:

$$Q_{n,k} = e^{-c_{n,k} D} \tag{7}$$

and in the special case k = n = 1:

$$Q_1 = e^{-c_1 D} \tag{7a}$$

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* A Poisson distribution P(n) is given by the formula $P(n) = e^{-x} x^n / n!$, in which $x = \bar{n}$ is the average value of n. The probability that n = 0 is $P(0) = e^{-x}$.

The coefficients $c_{n,k}$ depend, besides on the indices n and k, on the track density n_x (f(s) depends on n_x not only through a factor n_x, but also through the functions f_1 and f_2, see (3)). If it is necessary, we shall label the $c_{n,k}$ with the type of radiation under consideration, e.g. with X for X-radiation.

We may consider X-rays as radiation tracks in the limit $n_x \to 0$. In that case we can evaluate the integral in (6). Using (3), and assuming that with X-rays only the particles A are formed, so that $f_1 = 1$, $f_2 = 0$, we obtain $(c_{n,k})_X = \frac{1}{n_x}\int_0^\infty k a_n f(s)\, 2\pi s\, ds = k a_n\, 4\pi r_o d_1^2 \cdot \int_0^\infty K_o(x)\, x\, dx$, and since the latter integral is equal to unity, we have:

$$(c_{n,k})_X = k\, a_n\, 4\pi r_o\, d_1^2, \qquad (6b)$$

a result which can be obtained more easily by integrating over the whole volume at once, instead of first over a track. It is seen from (6b) that in the case of X-rays:

$$(c_{n,k})_X = k\, (c_{n,1})_X. \qquad (8)$$

a relationship which will be used in Chapter VIII.

V. PROBABILITY OF SURVIVAL OF THE HAPLOID AND DIPLOID CELL

Survival of the haploid cell. We shall treat the extreme cases 1 and 2. Case 1, genes far apart compared to d_2. The probability that a particular gene is not hit is Q_1, given by (7a). The n genes are independent, therefore the probability that none is hit is $S = Q_1^n$, or:

$$S = e^{-nc_1D} \tag{9}$$

Case 2, genes close together compared to d_1. The probability that none of the n genes of the gene complex is hit is $Q_{n,n}$, given by (7) with $k = n$, or:

$$S = e^{-c_{n,n}D} \tag{10}$$

Survival of the diploid cell. We have to make a further distinction between the two extreme cases: a) chromosomes far apart compared to d_2; b) chromosomes close together compared to d_1 (i.e. homologous genes close together compared to d_1). Together with the two extreme cases 1 and 2 of gene proximities in each chromosome we have four different extreme cases, which we shall denote by 1a, 1b, 2a, and 2b. Case 1a, genes and chromosomes far apart. All genes are far apart, therefore hits in different genes are statistically independent. The probability that a certain gene is not hit is Q_1, given by (7a), and the probability that it is hit is $1 - Q_1$. The probability that both homologous genes are hit is $(1 - Q_1)^2$, so the probability that of a pair of homologous genes at least one survives is $1 - (1 - Q_1)^2 = 2Q_1 - Q_1^2$. Since we have n independent gene pairs, the probability of survival of the diploid cell is $S = (2Q_1 - Q_1^2)^n$, or, with help of (7a):

$$S = (2\,e^{-c_1D} - e^{-2c_1D})^n \tag{11}$$

Case 1b, chromosomes close together, genes in a chromosome far apart. The n gene pairs are still statistically independent of each other, but the two homologous genes of a pair are not independent since they

are close together compared to d_1. Hence, the probability that one of the genes is not hit, regardless of what happens to the other homologous gene, is $Q_{2,1}$ given by (7) with n = 2, k = 1, whereas the probability that both genes are hit is $Q_{2,2}$. A simple calculation leads then to the probability that not both genes are hit as $2Q_{2,1} - Q_{2,2}$. The probability of survival of the diploid cell is then $S = (2\,Q_{2,1} - Q_{2,2})^n$, or, using (7):

$$S = (2\,e^{-c_{2,1}D} - e^{-c_{2,2}D})^n \qquad (12)$$

Before treating cases 2 a and 2 b we shall derive a formula for the probability $P_{n,k}$ that in a gene complex of n genes every gene of a subgroup of k genes is hit, and every gene of the remaining n-k genes is not hit. We want to express the $P_{n,k}$ in terms of the $Q_{n,k}$.

We consider a subgroup of p genes. The probability that any q out of these p genes are hit, and no other gene is hit, is $\binom{p}{q} P_{n,q}$. If we let q run from 0 to p we get the probability $Q_{n,n-p}$ that a specific group of n-p genes is not hit:

$$Q_{n,n-p} = \sum_q \binom{p}{q} P_{n,q} \qquad (13)$$

As a rule we shall not explicitly indicate the limits of summation, since they may be taken as $-\infty$ and $+\infty$. The unwanted terms will be automatically zero due to the property of the binomial coefficient $\binom{a}{b}$ that it is only different from zero if $a \geq 0$ and $b \geq 0$ and $a \geq b$. This fact enables us also to interchange the order of two summations without having to worry about the limits of summation.

We operate on both sides of (13) with $\sum_p \binom{k}{p} (-1)^{k+p}$ and obtain

$\sum_p \binom{k}{p} (-1)^{k+p} Q_{n,n-p} = \sum_p \binom{k}{p} (-1)^{k+p} \sum_q \binom{p}{q} P_{n,q}$. On the right hand side of this equation we interchange the order of summation. The sum over p is $\sum_p \binom{k}{p} \binom{p}{q} (-1)^{k+p} = (-1)^{k+q} \binom{k}{q} \sum_p (-1)^{p-q} \binom{k-q}{p-q} = (-1)^{k+q} \binom{k}{q} \delta_{k,q} = \delta_{k,q}$, where $\delta_{a,b}$ is the usual Kronaecker δ-symbol. The summation over q

gives then simply $\sum_q P_{n,q}\ \delta_{k,q} = P_{n,k}$, so that we have:

$$P_{n,k} = \sum_p \binom{k}{p}\ (-1)^{k+p}\ Q_{n,n-p} \qquad (14)$$

Case 2a, chromosomes far apart, genes in a chromosome close together. The probability of survival is obtained by considering all cases in which at most one gene in each homologous gene pair is hit. The probability that m gene pairs are affected in such a way that k genes in one chromosome are hit, m-k in the other, and the remaining n-m gene pairs not at all, is $P_{n,k}\ P_{n,m-k}$. We have made use of the fact that two genes in different chromosomes are statistically independent. For fixed m there are $\binom{n}{m}$ ways of choosing the affected gene pairs, and for any of these choices and fixed k there are $\binom{m}{k}$ ways of distributing the hit genes over the m gene pairs. Thus we get for the probability of survival:

$$S = \sum_{m,k} \binom{n}{m}\ \binom{m}{k}\ P_{n,k}\ P_{n,m-k}.$$

After the substitution of (14), interchanging the order of summations and summing over m and k, we obtain:

$$S = \sum_{p,q} \binom{n}{pq}\ (-1)^{n-p-q}\ Q_{n,n-p}\ Q_{n,n-q}$$

in which $\binom{n}{pq}$ stands for $n!/(p!\ q!\ (n-p-q)!)$. By using (7) we obtain:

$$S = \sum_{p,q} \binom{n}{pq}\ (-1)^{n-p-q}\ e^{-(c_{n,n-p} + c_{n,n-q})\ D} \qquad (15)$$

Case 2b, all genes close together. We pick out k specific gene pairs and consider the probability that in each of these pairs one and only one gene is hit, whereas the remaining n-k gene pairs are unaffected. Since in each gene pair either of the two genes may be the hit one, we have for this probability $2^k\ P_{2n,k}$. We can make $\binom{n}{k}$ different assignments of the affected k gene pairs, and if we let k run from 0 to n we

get for the probability of survival $S = \sum_k \binom{n}{k} 2^k P_{2n,k}$. Using (14), interchanging the order of summation and summing over k leads to :

$$S = \sum_p \binom{n}{p} (-1)^{n-p} 2^p Q_{2n,\,2n-p}.$$

After substitution of (7) we obtain:

$$S = \sum_p \binom{n}{p} (-1)^{n-p} 2^p e^{-c_{2n,\,2n-p} D} \qquad (16)$$

Formulas (11), (12), (15) and (16) are in general for radiation producing tracks with track density n_x. We may consider X-rays, especially hard X-rays as the limiting case $n_x \longrightarrow 0$. Due to the relation (8) the expression for the probability of survival under X-radiation, which we shall denote by S_X, will be essentially of the form (11) in each of the four cases 1a through 2b. In case 1a S_X is, of course, given exactly by (11). In case 1b we have $c_{2,2} = 2\, c_{2,1}$, so that:

$$\text{(case 1b)} \quad S_X = (2\, e^{-(c_{2,1})_X D} - e^{-2(c_{2,1})_X D})^n \qquad (12a)$$

By using relation (8) we can perform the summations in (15) and (16), with the result:

$$\text{(case 2a)} \quad S_X = (2\, e^{-(c_{n,1})_X D} - e^{-2(c_{n,1})_X D})^n \qquad (15a)$$

$$\text{(case 2b)} \quad S_X = (2\, e^{-(c_{2n,1})_X D} - e^{-2(c_{2n,1})_X D})^n \qquad (16a)$$

Equations (12a), (15a) and (16a) can also be obtained at once, by following the reasoning given in case 1a, after observing that in the case of X-radiation all the hits are statistically independent.

VI. PROPERTIES OF THE THEORETICAL SURVIVAL CURVES, (I)

The curve representing the probability of survival of the cell as a function of the radiation dose is called the survival curve.

The haploid survival curves, given by eqs. (9) and (10), are simple decreasing exponentials in the dose. If ln S is plotted as a function of D, a straight line through the origin is obtained with slope $-nc_1$ and $-c_{n,n}$ respectively.

The diploid survival curves are not easy to handle. The simplest one is (11), which depends only on two parameters. For each value of n the survival S can be plotted as a function of $c_1 D$, so that the shape of the function can be studied in detail. It turns out that the shape is of the so-called "sigmoid-type", i.e. a curve with approximately the shape of a reversed S. The curve is monotonic decreasing, since it is a product of monotonic decreasing factors:

$$\frac{d}{dD}\left(2\,e^{-c_1 D} - e^{-2c_1 D}\right) = -2\,c_1\left(e^{-c_1 D} - e^{-2c_1 D}\right) \leqslant 0.$$

The equal sign in this inequality holds if $D = 0$, so that $\frac{dS}{dD} = 0$ at $D = 0$, or in words: the initial slope of the survival curve (11) is zero.

The next simplest case is the survival curve given by (12). Even to prove that the curve is monotonic decreasing requires a property of the c's which we have not proved yet. It is from (6) obvious that $c_{n,k}$ increases with k, but it is not so obvious that $(1/k)\,c_{n,k}$ decreases with k. This property, which depends upon the fact that $(1/x)(1-e^{-x})$ is a decreasing function of x (Chapter VII, Corollary 3), will become clear in Chapter VIII. Assuming the truth of this statement for the moment, we have, in particular, $c_{2,1} < c_{2,2} < 2\,c_{2,1}$. With help of this we get by differentiation: $-\frac{d}{dD}\left(2\,e^{-c_{2,1}D} - e^{-c_{2,2}D}\right) = 2\,c_{2,1}\,e^{-c_{2,1}D} - c_{2,2}\,e^{-c_{2,2}D} > 2\,c_{2,1}\left(e^{-c_{2,1}D} - e^{-c_{2,2}D}\right) \geqslant 0$, so that, indeed, eq. (12) represents a monotonic decreasing curve. The initial slope is not zero, as in case 1a, but equal to $-(2\,c_{2,1} - c_{2,2})$.

The expressions for S in the cases 2a and 2b, given by equations (15) and (16) are quite difficult to handle. It is not even obvious mathematically that the expressions are non-negative, since in the sums positive as well as negative terms occur. We shall return to the treatment of these curves in Chapter VIII.

In order to study the dependence of the diploid survival curves on the type of radiation, i.e. on n_x, we propose the following procedure: On the theoretical side we introduce a new variable x, proportional to the dose D, in such a way that all the haploid curves are given by the equations $S = e^{-x}$. The coefficient of proportionality between x and D is different in the various cases: in case 1, $x = nc_1D$ and in case 2, $x = c_{n,n}D$, as is clear from (9) and (10). It should be kept in mind that c_1 and $c_{n,n}$ depend on n_x. The object is to compare the diploid curves for particle radiation with the diploid curves for X-rays, after they are written in terms of the new variable x. We shall call the process outlined above <u>normalization</u>, and shall denote the probability of survival, represented by the normalized expression, by $S^{(n)}$. Normalization of (11), (12), (15) and (16) gives the following equations:

$$\text{(case 1a)} \quad S^{(n)} \quad (2\exp\left[-\frac{x}{n}\right] - \exp\left[-2\frac{x}{n}\right])^n \tag{11b}$$

$$\text{(case 1b)} \quad S^{(n)} \quad (2\exp\left[-\frac{c_{2,1}}{c_1}\frac{x}{n}\right] - \exp\left[-\frac{c_{2,2}}{c_1}\frac{x}{n}\right])^n \tag{12b}$$

$$\text{(case 2a)} \quad S^{(n)} = \sum_{p,q}\binom{n}{p}\binom{n}{q}(-1)^{n-p-q}\exp\left[-(c_{n,n-p}+c_{n,n-q})\frac{x}{c_{n,n}}\right] \tag{15b}$$

$$\text{(case 2b)} \quad S^{(n)} = \sum_{p}\binom{n}{p}(-1)^{n-p}\,2^p\exp\left[-c_{2n,2n-p}\frac{x}{c_{n,n}}\right] \tag{16b}$$

In particular for X-rays we have, in view of (8) and (6b):

$$\text{(case 1b)} \quad S_X^{(n)} = (2e^{-a_2\frac{x}{n}} - e^{-a_2\frac{2x}{n}})^n \tag{12c}$$

$$\text{(case 2a)} \quad S_X^{(n)} = \sum_{p,q}\binom{n}{p}\binom{n}{q}(-1)^{n-p-q}\,e^{-(\frac{n-p}{n}+\frac{n-q}{n})x} \tag{15c}$$

$$\text{(case 2b)} \quad S_X^{(n)} = \sum_p \binom{n}{p} (-1)^{n-p} \exp\left[-a_{2n} \frac{2n-p}{n} x\right] \qquad (16c)$$

while in case 1a $S_X^{(n)}$ is still given by (11b). Eqs. (15c) and (16c) are left in the form of a sum in order to facilitate comparison with (15b) and (16b).

It is seen that in case 1a the normalized curves for X-rays and for particle radiation coincide, since they are both given by (11b). In cases 1b, 2a and 2b the X-ray curves are different from the particle radiation curves. Our aim is to prove that in all these cases the particles radiation curve lies entirely below the X-ray curve.

When the theoretical and experimental curves are compared, the experimental diploid curves for different types of radiation are also plotted on such a dose scale that their corresponding haploid curves are all given by e^{-x}. In other words, we also normalize the experimental curves.

The curves (12b) and (12c) will be compared in this chapter, although we shall need some results from the next chapters. It is clear, from the asymptotic behavior of (12b) and (12c), that a necessary condition for $S^{(n)}$ to lie below $S_X^{(n)}$ is $a_2 < c_{2,1}/c_1$, or

$$a_2 c_1 / c_{2,1} \leq 1 \qquad (17)$$

From Chapter IV, relation (A1), it follows that $a_{2n} < a_n$, so that if $k > 0$ and $f(s) \geq 0$ we have $k\, a_{2n} f(s) \leq k\, a_n f(s)$. Using the result of Chapter VII, Corollary 3, we have

$$\frac{1}{a_{2n}} \left(1 - e^{-k a_{2n} f(s)}\right) \geq \frac{1}{a_n} \left(1 - e^{-k a_n f(s)}\right)$$

and after integration over s and bringing all factors to one side, we obtain:

$$\frac{a_{2n}}{a_n} \quad \frac{\int_0^\infty \left(1 - e^{-k a_n f(s)}\right) s\, ds}{\int_0^\infty \left(1 - e^{-k a_{2n} f(s)}\right) s\, ds} \leq 1$$

Comparing this with (6) we see that we have proved for arbitrary n, k:

$$\frac{a_{2n}}{a_n} \frac{c_{n,k}}{c_{2n,k}} \leq 1 \qquad (18)$$

from which (17) follows by taking n = 1, k = 1.

We shall show now that (17) is also a sufficient condition that $S^{(n)}$ lies below $S_X^{(n)}$ in case 1b. Putting $b = a_2 c_1/c_{2,1}$ we have by (17) $b \leq 1$. Putting $y = (c_{2,1}/c_1)(x/n)$ we have $a_2 x/n = by$, so that by (12c)

$$S_X^{(n)} = (2 e^{-by} - e^{-2by})^n.$$

Equation (12b) in terms of y is:

$$S^{(n)} = (2 e^{-y} - e^{-(c_{2,2}/c_{2,1})\, y})^n$$

In the last equation $c_{2,2}/c_{2,1} \leq 2$ (see Chapter VIII), so that $2 e^{-y} - e^{-(c_{2,2}/c_{2,1})\, y} \leq 2 e^{-y} - e^{-2y} = 1 - (1-e^{-y})^2 \leq 1 - (1-e^{-by})^2 = 2 e^{-by} - e^{-2by}$, which proves $S^{(n)} \leq S_X^{(n)}$ for all x.

Before we are able to treat the diploid curves in cases 2a and 2b we have to develop some more powerful mathematical tools. This will be the purpose of the next chapter.

VII. MATHEMATICAL INTERLUDE: COMPLETELY MONOTONIC FUNCTIONS

In this chapter we shall develop the properties of completely monotonic functions only so far as we need for our applications. We shall give a list of definitions and theorems first, and give the proofs of the theorems afterwards. Some of the first theorems in our list are mentioned by WIDDER (1941) without proof.

Definition 1. A function f(x) is called completely monotonic in an interval (ab) if f(x) has derivatives of all orders, and $(-1)^n \frac{d^n f}{dx^n} \geqslant 0$ $(n = 0, 1, \ldots)$ in the interval (ab).

Definition 2. A sequence a_k is called completely monotonic if $(-1)^n \Delta^n a_k \geqslant 0$, where the n^{th} difference operator Δ^n is defined by the recursion relation $\Delta^n a_k = \Delta^{n-1} a_{k+1} - \Delta^{n-1} a_k$.

Definition 3. A function f(x) is called absolutely monotonic in an interval (ab) if f(x) has derivatives of all orders and $\frac{d^n f}{dx^n} \geqslant 0$ $(n = 0, 1, \ldots)$ in the interval (ab).

Notation. In the following, completely monotonic will be denoted by CM, absolutely monotonic by AM. The intervals in which the functions are CM or AM will not always be mentioned in the following theorems.

Theorem 1. If f(x) is CM in (ab), then f(b-x) is AM, $0 < x < b-a$.

Theorem 2. If f(x) is AM and $\phi(y)$ is AM, then $\phi(f(x))$ is AM.

Theorem 3. If f(x) is CM and $\phi(y)$ is AM, then $\phi(f(x))$ is CM.

Theorem 4. The sum of two CM functions is CM.

Theorem 5. The product of two CM functions is CM.

Theorem 6. If f(x, y) is a CM function of x for every y in interval (ab), and $g(y) \geqslant 0$ a continuous function of y, then $\int_a^b f(x, y)\, g(y)\, dy$ is a CM function of x.

Definition 4. The n^{th} difference function $dif^n f(x)$ of a function f(x) is defined by the recursion relation $dif^n f(x) = dif^{n-1} f(x) - dif^{n-1} f(x+1)$, $n = 1, 2, \ldots$, and $dif^0 f(x) = f(x)$. (It is customary to define the n^{th}

difference as $\Delta^n f(x) = \Delta^{n-1} f(x+1) - \Delta^{n-1} f(x)$, but for our purposes the function $\mathrm{dif}^n f(x)$ is more convenient. By comparison we have $\mathrm{dif}^n = (-1)^n \Delta^n$.)

Theorem 7. If f(x) is CM then all difference functions $\mathrm{dif}^n f(x)$, n = 0, 1, 2, ..., are CM.

Corollary 1. If f(x) is CM then $\mathrm{dif}^n f(x) \geq 0$.

Theorem 8. The n^{th} difference function of f(x) can be written as the following sum:

$$\mathrm{dif}^n f(x) = \sum_{k=0}^{n} \binom{n}{k} (-1)^k f(x+k).$$

Corollary 2. If f(x) is CM then

$$\sum_{k=0}^{n} \binom{n}{k} (-1)^k f(x+k) \geq 0.$$

Theorem 9. The n^{th} difference function of a product of two functions f(x) and g(x) can be written as the following sum:

$$\mathrm{dif}^n (f(x)g(x)) = \sum_{k=0}^{n} \binom{n}{k} \mathrm{dif}^k f(x) \, \mathrm{dif}^{n-k} g(x+k).$$

Theorem 10. If g(x) is CM and $f(x) = x\, 2^{-x} g(x)$, then $\mathrm{dif}^n f(x) \geq 0$ if $x \geq n$.

Theorem 11. If of a function f(x) the derivative $\frac{df}{dx}$ is CM, then $e^{-f(x)}$ is a CM function of x.

Theorem 12. If of two functions f(x) and g(x) the derivatives $\frac{df}{dx}$ and $\frac{dg}{dx}$ are CM, and $e^{-f(x)} - e^{-g(x)} = h(x)\,(g(x) - f(x))$, then h(x) is CM.

Theorem 13. If of a function f(x) the derivative $\frac{df}{dx}$ is CM, and f(0) = 0, then f(x) = x g(x), in which g(x) is CM.

Examples of AM functions are x^m ($x \geq 0$, m = 0, 1, 2, ...), a^x ($a \geq 1$) and $(1/x)(e^x - 1)$.

Examples of CM functions are x^{-m} $(x > 0,\ m > 0)$; a^{-x} $(a > 1)$, in particular e^{-x} and 2^{-x}; $(1/x)(1 - e^{-x})$. That $(1/x)(1 - e^{-x})$ is CM follows from the fact that $f(x) = 1 - e^{-x}$ satisfies the hypotheses of Theorem 13. As a consequence we have the following corollary:

Corollary 3. $\frac{1}{x}(1 - e^{-x})$ is a monotonic decreasing function of x.

PROOFS OF THE THEOREMS

Proof of Theorem 1. If we put $z = b - x$, then $\frac{d^n}{dx^n} f(b-x) = (-1)^n \frac{d^n}{dz^n} f(z) \geq 0$ since f(z) is CM.

Proof of Theorem 2. The n^{th} derivative of $\phi(f(x))$ is a sum of products of derivatives of ϕ and derivatives of f, all of which are non-negative. It follows that $\frac{d^n}{dx^n} \phi(f(x)) \geq 0$.

Proof of Theorem 3.* If we put $z = b - x$, and $f(x) = f(b-z) = g(z)$, then g(z) is AM by theorem 1, and therefore $\phi(g(z))$ is AM by Theorem 2. It follows that $(-1)^n \frac{d^n}{dx^n} \phi(f(x)) = \frac{d^n}{dz^n} \phi(g(z)) \geq 0$.

Proof of Theorem 4. This theorem follows immediately from the fact that the n^{th} derivative is a linear operator.

Proof of Theorem 5. The proof goes in the same way as the proof of Theorem 3, after observing that the product of two AM functions is obviously AM.

Proof of Theorem 6. The integral may be considered as the limit of a sum, in which case the theorem follows from Theorem 4, or one can differentiate under the integral sign and keep in mind that $(-1)^n \frac{\partial^n}{\partial x^n} f(x, y) \geq 0$.

Proof of Theorem 7. For the first difference function dif f(x) we have $(-1)^n \frac{d^n}{dx^n} \operatorname{dif} f(x) = (-1)^n \frac{d^n}{dx^n} (f(x) - f(x+1)) = \int_x^{x+1} (-1)^{n+1} \frac{d^{n+1}}{dy^{n+1}} f(y)\, dy \geq 0$, since the integrand is ≥ 0.

This proves that the first difference function of a CM function is CM, and for the difference functions of higher order it follows by induction.

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* This proof was suggested to the author by Dr. R. M. Robinson, Department of Mathematics, University of California, Berkeley.

Proof of Theorem 8 (Compare: JORDAN (1947), page 8). Proof by induction. The formula is obviously true for n = 0. Assume the formula to be true for n-1, then we have $\mathrm{dif}^n f(x) = \mathrm{dif}^{n-1} f(x) - \mathrm{dif}^{n-1} f(x+1) =$

$$\sum_k \binom{n-1}{k} (-1)^k f(x+k) - \sum_k \binom{n-1}{k} (-1)^k f(x+1+k) =$$

$$\sum_k \binom{n-1}{k} (-1)^k f(x+k) + \sum_k \binom{n-1}{k-1} (-1)^k f(x+k) =$$

$$\sum_k \binom{n}{k} (-1)^k f(x+k), \text{ since } \binom{n-1}{k} + \binom{n-1}{k-1} = \binom{n}{k}.$$

Proof of Theorem 9 (Compare JORDAN, page 96). Proof by induction. The theorem is true for n = 0. Assume that the theorem is true for n-1, then we have $\mathrm{dif}^n (f(x)g(x)) = \mathrm{dif}^{n-1}(f(x)g(x)) - \mathrm{dif}^{n-1}(f(x+1)\,g(x+1)) =$

$$\sum_k \binom{n-1}{k} \left\{\mathrm{dif}^k f(x)\, \mathrm{dif}^{n-1-k} g(x+k) - \mathrm{dif}^k f(x+1)\, \mathrm{dif}^{n-1-k} g(x+1+k)\right\}.$$

After subtracting and adding $\mathrm{dif}^k f(x)\, \mathrm{dif}^{n-1-k} g(x+1+k)$ the expression in curly brackets may be written as

$$\mathrm{dif}^k f(x)\, \mathrm{dif}^{n-k} g(x+k) + \mathrm{dif}^{k+1} f(x)\, \mathrm{dif}^{n-1-k} g(x+1+k)$$

and we get $\mathrm{dif}^n(f(x)g(x)) =$

$$\sum_k \binom{n-1}{k} \mathrm{dif}^k f(x)\, \mathrm{dif}^{n-k} g(x+k) + \sum_k \binom{n-1}{k} \mathrm{dif}^{k+1} f(x) \mathrm{dif}^{n-1-k} g(x+1+k) =$$

$$\sum_k \binom{n-1}{k} \mathrm{dif}^k f(x)\, \mathrm{dif}^{n-k} g(x+k) + \sum_k \binom{n-1}{k-1} \mathrm{dif}^k f(x)\, \mathrm{dif}^{n-k} g(x+k),$$

from which Theorem 9 follows, in view of $\binom{n-1}{k} + \binom{n-1}{k-1} = \binom{n}{k}$.

Proof of Theorem 10. We make use of the following lemma: if $h(x) = x\, 2^{-x}$ then $\mathrm{dif}^n h(x) = (x-n)\, 2^{-(x+n)}$. This lemma follows immediately from application of Theorem 9 (we have $\mathrm{dif}\, x = -1$, $\mathrm{dif}^2 x = \mathrm{dif}^3 x = \ldots = 0$ and $\mathrm{dif}^n 2^{-x} = 2^{-(x+n)}$). After applying Theorem 9 again we have:

$$\mathrm{dif}^n f(x) = \sum_k \binom{n}{k} \mathrm{dif}^k h(x)\, \mathrm{dif}^{n-k} g(x+k).$$

Since g(x) is CM, $\mathrm{dif}^{n-k} g(x+k) \geq 0$ for all k. According to the lemma $\mathrm{dif}^k h(x) = (x-k)\, 2^{-(x+k)}$ which is ≥ 0 if $k \leq x$. Since k runs from 0 to n all terms in the sum are ≥ 0 if $x \geq n$.

Proof of Theorem 11. Consider the function $F(x) = C - f(x)$, where C is an arbitrary real constant. C can be chosen so large that $F(x) \geq 0$ in some interval (ab). Since $\frac{df}{dx}$ is CM we have in (ab): $(-1)^n \frac{d^n}{dx^n} F(x) \geq 0$ so that F(x) is CM in (ab). Since e^{+y} is an AM function of y, we have by Theorem 3 that $e^{F(x)} = e^{C} e^{-f(x)}$ is a CM function of x in (ab). Since C may be chosen arbitrarily large, and therefore $F(x) \geq 0$ in any arbitrary interval, we conclude that $e^{-f(x)}$ is CM in any interval in which $\frac{df}{dx}$ is CM.

Proof of Theorem 12. The function h(x) may be written as

$$h(x) = \frac{e^{-f(x)} - e^{-g(x)}}{g(x) - f(x)} = e^{-g} \frac{1 - e^{-(f-g)}}{f - g} = e^{-g} \int_0^1 e^{-(f-g)\,y}\, dy =$$

$$\int_0^1 e^{-y\,f(x) - (1-y)\,g(x)}\, dy.$$

In the latter integral $\frac{\partial}{\partial x}(y\,f(x) + (1-y)\,g(x))$ is CM for $0 \leq y \leq 1$ since $\frac{df}{dx}$ and $\frac{dg}{dx}$ are CM. By Theorem 11 the integrand is a CM function of x, and by Theorem 6 this holds then for the integral too.

Proof of Theorem 13. If $\frac{df}{dx} = h(x)$, h(x) CM, then $f(x) = \int_a^x h(y)\, dy$, where a is some constant. From f(0) = 0 it follows that a = 0, so $f(x) = \int_0^x h(y)\, dy$. We have for the function g(x): $g(x) = \frac{1}{x} f(x) = \frac{1}{x} \int_0^x h(y)\, dy = \int_0^1 h(xz)\, dz$, and since h(xz) is a CM function of x, it follows from Theorem 6 that g(x) is CM.

VIII. PROPERTIES OF THE THEORETICAL SURVIVAL CURVES. (II)

In this chapter we shall discuss the properties of the diploid curves in cases 2a and 2b, using the properties of completely monotonic functions developed in the previous chapter. We shall show that in both these cases:

1) $S(D) \geq 0$ for all $D \geq 0$;

2) $-\frac{dS}{dD} \geq 0$ for all $D \geq 0$, therefore S is monotonic decreasing;

3) $S_X^{(n)}(x) - S^{(n)}(x) \geq 0$ for all $x \geq 0$, i.e. the normalized particle radiation curve lies entirely below the normalized X-ray curve.

The key to the proofs is the study of the dependence of the $c_{n,k}$ on k, $k = 1, 2, \ldots\ldots$, $c_{n,k}$ given by (6). Equation (6) defines a continuous function of k, for $k \geq 0$, which we shall denote by $c_n(k)$. The $c_{n,k}$ are the values of $c_n(k)$ in the integers $k = 1, 2, \ldots\ldots$ The function $c_n(k)$ has the following properties: $c_n(0) = 0$ (since if $k = 0$ the integrand in (6) is 0), and

$$\frac{dc_n(k)}{dk} = \frac{1}{n_x} \int_0^\infty e^{-k\, a_n\, f(s)}\; a_n\, f(s)\, 2\pi s\, ds$$

is a CM function of k since $e^{-ka_nf(s)}$ is a CM function of k for all s, and because of Theorem 6. By Theorem 11 we have then:

$$e^{-A\, c_n(k)} \text{ is a CM function of } k \qquad (19)$$

in which A is an arbitrary constant ≥ 0. By Theorem 13 we have:

$$c_n(k) = k\, g(k), \qquad g(k) \text{ being CM } *) \qquad (20)$$

A second observation is that the sums in (15) and (16) can be related to the sum in Theorem 8. Finally we make use of Corollary 2 to prove the desired inequalities.

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* There will be a different function g(k) for each value of n.

1) Proof that $S \geq 0$ for all $D \geq 0$.

Case 2a. S is given by (15). We shall suppress the first index on the c's and write c_k instead of $c_{n,k}$. We introduce a new summation variable $k = n-p-q$, so that $n-q = p + k$, and $\binom{n}{pq} = \binom{n}{pk} = \binom{n}{p}\binom{n-p}{k}$. If we keep in mind the remark about the limits of summation, made in Chapter V, we can write (15) as

$$S = \sum_p \binom{n}{p} e^{-c_{n-p} D} \sum_k \binom{n-p}{k} (-1)^k e^{-c_{p+k} D} \qquad (15d)$$

By (19) we have that $e^{-c(p+k) D}$ is CM, and since $c_{p+k} = c(p+k)$ for $k = 0, 1, \ldots\ldots$, we have by Corollary 2:

$$\sum_k \binom{n-p}{k} (-1)^k e^{-c_{p+k} D} \geq 0.$$

Since also $\binom{n}{p} e^{-c_{n-p} D} \geq 0$ for all p, we have proved $S \geq 0$.

Case 2b. S is given by (16). We shall suppress the first index on the c's and write c_k instead of $c_{2n,k}$. After introducing the summation variable $k=n-p$, we can write (16) in the form

$$S = 2^{2n} \sum_k \binom{n}{k} (-1)^k 2^{-(n+k)} e^{-c_{n+k} D} \qquad (16d)$$

In (16d) $2^{-(n+k)}$ is CM and $e^{-c_{n+k} D}$ is CM by (19), so that $2^{-(n+k)} e^{-c_{n+k} D}$ is CM by Theorem 5. Hence $S \geq 0$ by Corollary 2.

2) Proof that $-\frac{dS}{dD} \geq 0$ for all $D \geq 0$.

In the notation we shall again suppress the first indices on the c's.

Case 2a. By differentiating S given by (15) we obtain:

$$-\frac{dS}{dD} = \sum_{p,q} \binom{n}{pq} (-1)^{n-p-q} (c_{n-p} + c_{n-q}) e^{-(c_{n-p}+c_{n-q}) D}$$

$$= 2 \sum_{p,q} \binom{n}{pq} (-1)^{n-p-q} c_{n-p} e^{-(c_{n-p}+c_{n-q}) D}$$

and after introducing $k = n-p-q$:

$$-\frac{dS}{dD} = 2 \sum_p \binom{n}{p} c_{n-p} e^{-c_{n-p}D} \sum_k \binom{n-p}{k} (-1)^k e^{-c_{p+k}D}.$$

In the last equation the factors $\binom{n}{p} c_{n-p} e^{-c_{n-p}D}$ are all ≥ 0, and the sum over k has already been shown to be non-negative in the proof of $S \geq 0$ in case 2a. This completes the proof that $-\frac{dS}{dD} \geq 0$ for all $D \geq 0$.

Case 2b. By differentiating (16d) we get:

$$-\frac{dS}{dD} = 2^{2n} \sum_k \binom{n}{k} (-1)^k c_{n+k} 2^{-(n+k)} e^{-c_{n+k}D},$$

and since by (20) $c_{n+k} = (n+k, g(n+k))$, in which g is CM, we have:

$$-\frac{dS}{dD} = 2^{2n} \sum_k \binom{n}{k} (-1)^k (n+k) 2^{-(n+k)} g(n+k) e^{-c_{n+k}D}.$$

If we put $f(k) = k 2^{-k} g(k) e^{-c_k D}$, then $-\frac{dS}{dD} = 2^{2n} \operatorname{dif}^n f(n)$, according to Theorem 8. Since $g(k) e^{-c_k D}$ is CM by (19) and Theorem 5 we have $\operatorname{dif}^n f(n) \geq 0$ by Theorem 10, which proves $-\frac{dS}{dD} \geq 0$ for all $D \geq 0$.

It is easily established that $\operatorname{dif}^n f(n) = 0$ if and only if $g(k) e^{-c_k D}$ is a constant in k. This can happen only at $D = 0$, and even then g(k) is only constant in the case of X-rays. If we have particle radiation g(k) is strictly decreasing, so that $-\frac{dS}{dD} > 0$ for all $D \geq 0$. In particular we have the result: the initial slope of the particle radiation survival curve in case 2b is negative.

3) Proof that $S_X^{(n)} - S^{(n)} \geq 0$ for all $x \geq 0$.

Case 2a. $S_X^{(n)}$ is given by (15c), $S^{(n)}$ by (15b). We shall suppress the first index n on all the c's and write c_k instead of $c_{n,k}$. We have:

$$S_X^{(n)} - S^{(n)} = \sum_{p,q} \binom{n}{p,q} (-1)^{n-p-q} (e^{-(n-p+n-q)x/n} - e^{-(c_{n-p}+c_{n-q})x/c_n})$$

After introducing $k = n-p-q$ we get:

$$S_X^{(n)} - S^{(n)} = \sum_{p,k} \binom{n}{pk} (-1)^k \left(e^{-(n-p+p+k)x/n} - e^{-(c_{n-p}+c_{p+k})x/c_n}\right).$$

Since $\frac{d}{dk}\,(n-p+p+k)x/n\ (= x/n)$ and $\frac{d}{dk}\,(c(n-p)+c(p+k))x/c_n \left(= \frac{x}{c_n}\frac{dc(p+k)}{dk}\right)$ are both CM functions of k, we have, by Theorem 12:

$$e^{-(n-p+p+k)x/n} - e^{-(c_{n-p}+c_{p+k})x/c_n} = h(k)\left(\frac{c_{n-p}}{c_n} + \frac{c_{p+k}}{c_n} - \frac{n-p}{n} - \frac{p+k}{n}\right)x,$$

in which h(k) is CM. By making this substitution we get

$$S_X^{(n)} - S^{(n)} = x \sum_{p,k} (-1)^k h(k) \left(\frac{c_{n-p}}{c_n} - \frac{n-p}{n}\right) + x \sum_{p,k} (-1)^k h(k) \left(\frac{c_{p+k}}{c_n} - \frac{p+k}{n}\right)$$
$$x\,(S_1 + S_2),$$

where S_1 stands for the first sum and S_2 for the second one. We shall prove $S_1 \geqslant 0$ and $S_2 \geqslant 0$ separately.

The only property of the c_k of which we shall make use is

$$\frac{c(k)}{c_n} - \frac{k}{n} \geqslant 0 \quad \text{if} \quad 0 \leqslant k \leqslant n \tag{21}$$

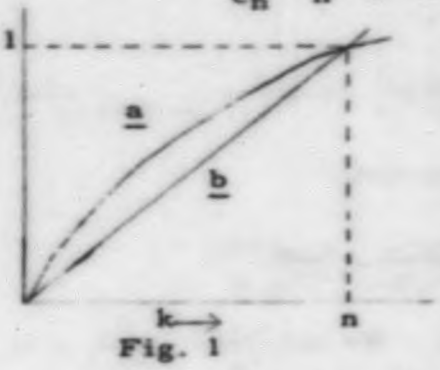

Fig. 1

To see this we recall that $\frac{dc(k)}{dk}$ is CM, so that $\frac{d^2c(k)}{dk^2} \leqslant 0$ and therefore c(k) is concave. The curves $c(k)/c_n$ and k/n have been plotted qualitatively in Fig. 1. Curve a represents $c(k)/c_n$ and curve b represents k/n. The two curves intersect in $k = 0$ and in $k = n$. It is seen that curve a lies above curve b in the interval (On), which establishes (21).

We can write S_1 as

$$S_1 = \sum_p \binom{n}{p} \left(\frac{c_{n-p}}{c_n} - \frac{n-p}{n}\right) \sum_k \binom{n-p}{k} (-1)^k h(k).$$

For $0 \leq p \leq n$ we have $\binom{n}{p}\left(\frac{c_{n-p}}{c_n} - \frac{n-p}{n}\right) \geq 0$ by (21), and

$\sum_k \binom{n-p}{k} (-1)^k h(k) \geq 0$ by Corollary 2, so $S_1 \geq 0$.

In S_2 we introduce a new summation variable $q = p+k$, and after interchanging the order of summation we obtain:

$$S_2 = \sum_q \left(\frac{c_q}{c_n} - \frac{q}{n}\right) \sum_k \binom{p}{k} (-1)^k h(k) \binom{n-k}{q-k}.$$

We have $0 \leq q \leq n$, since otherwise $\binom{n-k}{q-k}$ would be zero, so that by (21) $c_q/c_n - q/n > 0$. The sequence $b_k = \binom{n-k}{q-k}$ is CM in k: $\mathrm{dif}^0 b_k = b_k = \binom{n-k}{q-k} \geq 0$, $\mathrm{dif}^1 b_k = b_k - b_{k+1} = \binom{n-k}{q-k} - \binom{n-k-1}{q-k-1} = \binom{n-k-1}{q-k} \geq 0$, and by repeating this process we get $\mathrm{dif}^m b_k = \binom{n-k-m}{q-k} \geq 0$, $m = 0, 1, \ldots\ldots$. Since h(k) is CM, the sequence $h(k)\binom{n-k}{q-k}$ is CM and thus $\sum_k \binom{p}{k} (-1)^k h(k) \binom{n-k}{q-k} > 0$ by Corollary 2. This proves that $S_2 \geq 0$, and since $S_x^{(n)} - S^{(n)} = x(S_1 + S_2)$, we have proved $S_x^{(n)} - S^{(n)} \geq 0$ for all $x \geq 0$.

Case 2b. $S_x^{(n)}$ is given by (16c), $S^{(n)}$ by (16b). By putting $p = n-k$ we obtain:

$$S_x^{(n)} - S^{(n)} = 2^{2n} \sum_k \binom{n}{k} (-1)^k 2^{-(n+k)} \left(\exp\left[-\frac{n+k}{n} \frac{a_{2n}}{a_n} x\right] - \exp\left[-c_{2n,n+k} \frac{x}{c_{n,n}}\right]\right).$$

Using Theorem 12 we can write for the form in brackets

$$h(n+k)\left(c_{2n}(n+k) \frac{x}{c_{n,n}} - \frac{n+k}{n} \frac{a_{2n}}{a_n} x\right) = h(n+k)\, x \frac{c_{2n,n}}{c_{n,n}} \left(\frac{c_{2n}(n+k)}{c_{2n,n}} - \frac{a_{2n}}{a_n} \frac{c_{n,n}}{c_{2n,n}} \frac{n+k}{n}\right),$$

in which h is a CM function of k. We put $\frac{a_{2n}}{a_n} \frac{c_{n,n}}{c_{2n,n}} = b$, so that we have $b \leq 1$ by (18). By applying (20) to $c_{2n}(n+k)$, by keeping in mind that $\frac{c_{2n}(n+k)}{c_{2n}(n)}$ and $\frac{n+k}{n}$ are equal at $k = 0$, and by absorbing all

constants of k in the function g(k), we can write:

$2^{2n} \times \frac{c_{2n,n}}{c_{n,n}} \left(\frac{c_{2n}(n+k)}{c_{2n,n}} - b\,\frac{n+k}{n}\right) = (n+k)\,(g(n+k) - b\,g(n))$, in which g(k) is CM. In this way we obtain:

$$S_x^{(n)} - S^{(n)} = \sum_k \binom{n}{k} (-1)^k (n+k)\, 2^{-(n+k)}\, h(n+k)\,(g(n+k) - b\,g(n)).$$

If we put $f(k) = k\, 2^{-k}\, h(k)$ and $G(k) = g(k) - b\,g(n)$ then

$S_x^{(n)} - S^{(n)} = \mathrm{dif}^n (f(n)G(n)) = \sum_k \binom{n}{k} \mathrm{dif}^k G(n)\, \mathrm{dif}^{n-k} f(n+k)$ by Theorems 8 and 9. By Theorem 10 $\mathrm{dif}^{n-k} f(n+k) \geq 0$, and for $k \neq 0$ $\mathrm{dif}^k G(n) = \mathrm{dif}^k g(n)$ since G(k) and g(k) differ by a constant. Since g(k) is CM we have $\mathrm{dif}^k g(n) \geq 0$, so $\mathrm{dif}^k G(n) \geq 0$ for $k = 1, 2, \ldots$. For $k = 0$ we have $\mathrm{dif}^0 G(n) = G(n) = (1 - b)\, g(n) \geq 0$ since $1 - b \geq 0$. This completes the proof that $S_x^{(n)} - S^{(n)} \geq 0$ for all $x \geq 0$.

IX. COMPARISON WITH EXPERIMENT. CONCLUSIONS

Some of the experimental results, obtained by Tobias and co-workers, have been plotted in Fig. 2, at the end of this chapter. The X-ray data of the haploid and diploid cells have been marked by circles, the α-particle data by crosses. The α-particles were from Polonium and were slowed down to a rate of energy loss of approximately 2 BeV/(g/cm^2).

It is seen in Fig. 2 that ln S for the haploid, both for X-ray and α-particles, is represented well by a straight line for a dose not too large. At larger doses considerable deviations occur, which cannot be explained by the present theory. It will be necessary in the future to develop a better theory, based on a more complicated model, which will be able to explain properties of the yeast cell which are not now understood. In the meantime we shall ignore the so-called "tail" of the haploid survival curve and make use only of the first part of the curve.

In Fig. 2 the haploid curves for the X-rays and the α-particles have been plotted on different dose scales, in such a way that these curves coincide. Thus the data of the survival of the diploid cell under X-rays and α-particle radiation appear in their normalized form (see Chapter VI). It is seen in the figure that the α-particle data lie considerably below the X-ray data. It has been shown in Chapters VI and VIII that the normalized α-particle curve lies entirely below the normalized X-ray curve in the cases 1b, 2a and 2b, whereas these curves coincide in case 1a. Thus the comparison between theory and experiment enables us to reject the possibility of occurence of case 1a (all genes far apart), so that we may conclude:

either: two homologous chromosomes are close together (1b or 2b).

or: two homologous chromosomes are far apart, but the genes in a chromosome are close together (2a).

The investigation of the X-ray data are the most interesting, since the theoretical expressions for S are the simplest in the case of X-rays, so that we can make a quantitative comparison with the experimental points. In all the cases the normalized X-ray curve is represented by

$$S_x^{(n)} = (2\, e^{-cx/n} - e^{-2cx/n})^n \qquad (22)$$

with $c = a_2$ in case 1b
$c = 1$ in case 2a
$c = a_{2n}/a_n$ in case 2b

which can be obtained from the eqs. (12c), (15c) and (16c) in Chapter VI, or by normalizing (12a), (15a) and (16a) in Chapter V.

From (22) it follows that for large dose $\ln S_x^{(n)}$ is asymptotically represented by

$$\ln S_x^{(n)} \longrightarrow n \ln 2 - c\, x$$

In principle one could use this formula for determining n and c from the asymptotic behavior of the normalized diploid X-ray curve. However, this method seems rather inaccurate.

Equation (22) is easiest to handle in case (2a), since then $c = 1$ and $S_x^{(n)}$ depends only on one parameter, namely n. In case (1b) and (2b) $S_x^{(n)}$ depends on the two parameters n and c. We could determine n and c by adjusting these parameters in such a way that they give the best fit to the experimental points. However, it turns out that the experimental points are not very well represented by any equation of the form (22). It seems better to wait with an accurate determination of n and c until more reliable data are available. In the meantime a rough estimate of n will be made as follows. In the first place it is possible, on theoretical grounds, to set an upper and lower bound on c. Indeed, from relation (A_1) in Chapter IV it follows that $a_2 < 1$ and $a_{2n}/a_n < 1$, and from (A_2) it follows that $a_2 > 1/2$ and $a_{2n}/a_n > 1/2$. Therefore we have

in all three cases $1/2 < c \leq 1$. By plotting the right hand side of (22) for various values of n, both with c=1 and with $c = 1/2$, and by comparison with the experimental points, we can set a rough lower and upper bound on n. In Fig. 2 curves are drawn for $c = 1$, $n = 10$ and $n = 30$; $c = 1/2$, $n = 2$ and $n = 10$. Since the experimental points are within bounds set by $c = 1/2$, $n = 2$ and $c = 1$, $n = 30$, we can conclude that n is somewhere between 2 and 30.

By looking at Fig. 2 there is a slight indication that the asymptotic slope of the X-ray diploid curve is less than the haploid curve, i.e. $c < 1$. If this is true it means that we can eliminate case 2a (in which $c = 1$), and the conclusion would be that two homologous chromosomes are close together. It is hoped that in the future X-ray measurements at high radiation dose may prove or disprove this suggestion. Some additional support for this suggestion is obtained from initial slope of the diploid α-particle curve. In Chapter VI and VIII it was shown that in case 2a the initial slope of the survival curve is zero, whereas it is negative in cases 1b or 2b (chromosomes close together). By looking at the diploid α-particle data there is a slight indication that the initial slope is indeed negative. However, more data at low dose are necessary to establish this fact more certainly.

We return now to the question mentioned in the beginning of Chapter II, namely whether or not the radiation affects individual genes, or chromosomes as a whole. We are still not able to make a suggestion one way or the other, due to the fact that the value of n is still rather uncertain. As soon as n is determined more accurately we may be able to make a choice in favor of one or the other possibility of genetic damage. If n turns out to be large compared to unity, the damage is probably done to individual genes, whereas if n turns out to be comparable to unity (< 10 say) it is likely that the damage is done to chromosomes as a whole.

APPENDIX. CALCULATION OF p(r).

(For a more detailed treatment of this problem the reader is referred to a forthcoming paper by the author in the June 1952 issue of the Bulletin of Mathematical Biophysics).

Consider a sphere of radius r_o in an infinite scattering and absorbing medium. A point particle, originally at a distance r_1 from the center of the sphere, moves in this medium with a velocity v which is constant in magnitude. The particle has a mean free path ℓ of being isotropically scattered, and a probability per unit time a of being absorbed by the medium. As soon as the particle passes through the surface of the sphere it is "caught" forever. We describe this by calling the sphere "black". The problem can be made spherically symmetric by distributing the particle homogeneously over the surface of a sphere of radius r_1, concentric with the black sphere. Furthermore the problem can be made time-independent by considering the spherical surface of radius r_1 as a particle source with a strength of one particle per unit time, and asking for the number of particles absorbed by the black sphere per unit time. *)

If $\ell \ll r_o$ the problem reduces to a differential equation (diffusion equation), which, together with the boundary conditions, leads immediately to a solution of the form of eq. (1). However, it is not known whether the condition $\ell \ll r_o$ is satisfied in reality, so that it is advisable to treat the problem in a better approximation and investigate the influence of ℓ.

The position $\vec{r}$ of each particle is measured from the center of the black sphere. The direction of the particle velocity $\vec{v}$ is given by the angle ϑ between $\vec{v}$ and $\vec{r}$. Instead of ϑ we shall use $\mu = \cos \vartheta$. We now introduce a particle density function $\psi(r,\mu)$, such that $4\pi r^2 \psi(r,\mu)\, dr\, d\mu$ is the number of particles between r and r + dr with velocity directions

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* The reduction to a time-independent problem was suggested by Dr. Robert Serber.

between μ and $\mu+d\mu$. Ψ satisfies the Boltzmann transport equation:

$$v\,\mu\,\frac{\partial \Psi}{\partial r} + v\,\frac{1-\mu^2}{r}\,\frac{\partial \Psi}{\partial \mu} + \frac{v}{\ell}\,\Psi + a\Psi - 1/2\,\frac{v}{\ell}\,\Psi_o = \frac{1}{8\pi r_1^2}\,\delta(r - r_1) \qquad (23)$$

in which $\Psi_o(r) = \int_{-1}^{1} \Psi(r,\mu)\,d\mu$ is the particle density at r, and δ is the Dirac δ-function. In eq. (23) the first two terms represent the loss of particles from interval dr $d\mu$ as a result of their motion, the third term is the loss from collisions in $drd\mu$ to other intervals, the fourth term is the loss through absorption in the medium, the fifth is the gain from collisions in other intervals to $drd\mu$, and the right hand side represents the spherical source at r_1. There are two boundary conditions. At $r = r_o$ the requirement is that there are no particles coming out of the black sphere, and at $r \to \infty$ the particle density should approach zero:

$$\Psi(r_o,\mu) = 0 \quad (\mu > 0)$$
$$\lim_{r \to \infty} \Psi(r,\mu) = 0 \qquad (24)$$

Eq. (23) with boundary condition (24) cannot be solved exactly, but MARSHAK (1947) has proposed a convenient approximate method, called the "Spherical Harmonic Method", which we shall use in our problem. The method consists of expanding $\Psi(r,\mu)$ in a series of Legendre polynomials $P_\ell(\mu)$: $\Psi(r,\mu) = \sum_\ell (\ell+1/2)\,\Psi_\ell(r)\,P_\ell(\mu)$, and breaking the series off after a finite number of terms. We shall only keep the first two terms:

$$\Psi(r,\mu) = 1/2\,\Psi_o(r) + 3/2\,\Psi_1(r)\,\mu$$

which is called by Marshak the "P_1 - approximation". In this approximation we have to replace the first of the boundary conditions (24) by an approximate boundary condition, for which we choose, with Marshak, the requirement that the total current leaving the black sphere be 0. Thus we replace (24) by:

$$\int_0^1 \Psi(r_o,\mu)\,d\mu = 0$$
$$\lim_{r \to \infty} \Psi(r,\mu) = 0 \qquad (25)$$

It is reasonable to assume that the absorption probability per scattering collision is small, i.e. $a\ell/v \ll 1$. If we neglect $a\ell/v$ compared to 1, and put $d = \sqrt{\ell v/(3a)}$, the P_1 - approximation leads to the solution

$$p(r_1) = (1 + \frac{2\ell}{3r_o})^{-1} \frac{r_o}{r_1} e^{-(r_1 - r_o)/d} \qquad (26)$$

It can be shown that (26) is a good approximation if $\ell < r_o$. It is seen from (26) that the influence of ℓ is manifested in the form of a factor which is independent of r_1, and therefore immaterial for the purpose of this thesis.

Equation (1) is obtained from (26) by omitting the factor containing ℓ, and by neglecting r_o compared to r_1.

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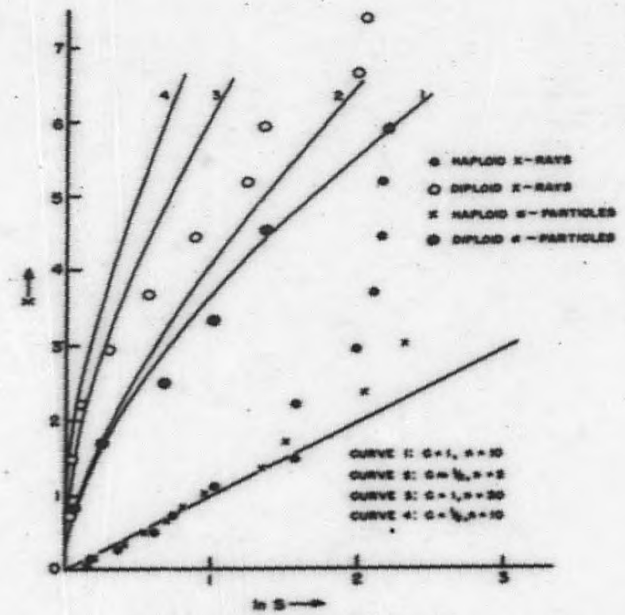

FIG. 2: ln S AS A FUNCTION OF X

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