On some stochastic models of the effects of radiation on cell survival

Preliminary draft for discussion only

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1. Introduction.

The present paper is the result of an effort at understanding models for the effect of radiation on cells in *culture*. We concentrate entirely on the effects of *photons* and this only in the range where electron-positron pairs creation or nuclear effects can be neglected. Also, we shall discuss only cell *survival* and will not get into problems of cell transformation.

The reasons for all these restrictions are multiple. The effects on living animals can be very complex. See for instance the work of M. Kripke [12]. Radiation other than photons or electrons can act in a very different manner, producing heavily ionized tracks. It will be seen that photons are complex enough to defy accurate description.

Several models, mostly deterministic in nature have been used in the literature. The best known are probably the Kellerer-Rossi "dual action" theory and Tobias' "repair-misrepair" theory. For references see the book [3] by Chadwick and Leenhouts and the recent symposium proceedings [14]. We shall concentrate on those models that have been fully or partially stochasticized or could be made stochastic at little cost.

Our point of departure is a recent paper [17] by Grace L. Yang and Charles Swenberg. There were previous papers by J. Neyman and P.S. Puri, [13]. As will be shown later the Neyman-Puri model does not fit experimentally observed facts. This is partly because of poor choice of correspondence between the mathematical and the biological definition of cell survival. Another feature is that the model does not provide for the interactions that yield the observed curvature of survival functions in experiments kindly carried out for us by Dr. Tracy C.H. Yang.

The Yang-Swenberg model provides for such interactions at the level of initial action of radiation. Several authors, including C. Tobias in [14] and Goodhead in [10] have disputed the physical possibility of such interactions. They claim that the interaction occurs at the repair level. Because of this, we make a special effort to count how many "hits" are involved at doses commonly used in experiments. This is the subject of Section 4 below. Our conclusion is that interaction is possible but only in a very

weak sense. Still, it can be sufficient to explain certain experimental results.

The paper is divided as follows. Section 2 is a brief description of a "typical" experiment. Section 3 gives a short overview of the space and time scales involved. Section 4 is a speculative essay on the production of ions by photon hits. At the time of this writing it has not been reviewed by a physicist and should be taken with due caution. Section 5 describes some of the repair system. Section 6 gives a classification of stochastic models by category. Section 7 gives our objections to the Neyman-Puri model and describes an experiment of T.C.H. Yang. Section 8 is about the model recently proposed by G.L. Yang and C. Swenberg. Our conclusion is that it probably needs some modifications-except for the case of very soft X-rays.

Section 9 is a very brief comment on the repair-misrepair model of C. Tobias *et al.* together with a remark on saturable repair models.

Our main general conclusion is that, even though several of the models can be made to fit, at least roughly, the observed dose response curves, the general situation is too unclear to permit recommendation of particular models, since, as we shall see, the parameters used to fit the models do not necessarily agree with what we would expect from the physico-biological mechanisms involved.

In the preparation of this draft we have had considerable assistance from Grace Lo Yang, Charles Swenberg, Cornelius Tobias, James Schmidt and many others. We owe very special thanks to Dr. Tracy C.H. Yang who gave us much information and was willing to carry out experiments specially for our benefit.

Many graphs and charts have been taken from available publications. At the time of this writing permission to reproduce them has not been secured.

2. A typical experiment.

We shall take as "typical" the experiments described by T.C.H. Yang and C. Tobias in [18]. Briefly the experiments are conducted as follows.

1) The cells are grown in culture, either in flasks or in Petri dishes, often till confluence

2) The cells are irradiated

3) the cells are dissociated (by trypsin) and put in suspension. For certain cells this may be a medium that permits repair but not growth.

4) Part of the suspension is counted to ascertain the number of cells per ml

5) Part of the suspension is pipeted and distributed over Petri dishes with growth medium

6) The dishes are incubated for a period of time

7) The cells are fixed and colonies are counted.

Note particularly that many variations can occur in these steps. In step (I) one can either synchronize the cells or let them grow as an asynchronous culture. One can irradiate at confluence or before.

In step 2 one needs to select a form of radiation, let us say X-rays. Results may depend on the "hardness" of the rays.

To vary the dose several procedures may be used. A common one is to select a beam intensity and vary the duration of exposure. An alternative is to select a duration of exposure and vary the intensity. In this alternative, results may depend on the procedures used. For instance masks may change the spectral distribution of the energy.

We shall see that such variations are very relevant to the discussion of stochastic models.

In step 3, the holding time in non-growth conditions is very important as cells undergo a repair process during that time.

The experimental variability in step (4) and especially (5) is important in the discussion of the statistical variability of the results.

Step 7 is very important in the definition of "survival" of the cells. Typically only visible colonies, consisting of 50 or more cells, will be counted.

In step 5 it is usual to plate enough cells to yield approximately 100 surviving colonies per dish. This means that the density of cells on the plate increases as the dose increases. One can raise questions about the possibility of interaction between

cells at high densities.

For step 2, one can use a variety of modes of irradiation. We shall discuss only the use of X-rays or γ-rays. They interact with matter in the cell, mostly water, to release electrons, create ion pairs and free radicals.

As we shall see, one may need to take into account in the models whether the cells are covered with a layer of growth medium, or whether they are irradiated from below.

3. Time scales and the size of things.

The time scale for various events are diagrammed in figure 1 taken from Tobias *et al* [15]. Note particularly that the radiation physics and the ensuing chemistry are fast processes taking times of at most 10^{-4} seconds while enzymatic reactions are much slower. Repair takes times of the order of minutes to hours.

For the doses to be discussed here and for the range of photon energies to be considered it is convenient to assume that photons act by discrete hits. More precisely, they act by glancing collisions that lead to molecular excitation and by "knocking out" electrons in closer collisions. It is said that the molecular excitation is of little relevance to the biology of the cell, at least as far as survival goes [3].

In view of this we shall concentrate on the effects represented by photoelectric emission of electrons or by Compton scattering. In the range of energies to be considered, (.3 kev to about 1 Mev) electron-positron pairs or nuclear effects are deemed to be negligible.

It will be taken for granted that the main target is the DNA and that free radicals formed in its very close vicinity can yield to damage in the form of base pair damage, single strand breaks (SSB) or double strand breaks (DSB). Many authors seem to believe that the damage leading to lethal effects on the cell is the formation of unrepaired or unrepairable DSB. Experiments where radiation was confined to the cytoplasm have shown that such irradiation is rarely lethal.

The DNA occurs in a double helix about 2 nanometers (nm) in diameter. Each helix consists of a sugar-phosphate back bone to which are attached bases. These pair to link the two strands of the double helix. The base pairs are situated about .34 nm apart. A turn of the helix takes about 3.4nm or 10 base pairs.

Human DNA is approximately 310^9 base pairs long, but the cells are diploid, (each double helix occurs in two copies) yielding a total of 6.10^9 base pairs. The double helix is wound around protein molecules called histones. The configuration of DNA in the nucleus is uncertain. It is coiled in a complex way, with loops attached to the nuclear membrane. According to [4], page 47, these loops may be more fragile than the rest. During mitosis, the DNA gets condensed into tightly coiled visible chromosomes. Otherwise it is much looser. At time of DNA replication the two strands of the helix separate locally. This may be one of the factors that makes the cells less sensitive to radiation during that phase, but the effect may also be due to the fact that enzymes are more available.

It has been suggested that the radiation target consists of the DNA together with a hydration sheath bringing the effective diameter of the target to 3nm. If one considers that this hydrated DNA does not reintersect itself and if one neglects possible

curvature effects, the target would have a total volume of

$$\frac{\pi}{4}(3)^2(.34)\,6\times 10^9 = 1.44\,10^{10}(\text{nm})^3$$

or equivalently $1.44\,10^{-11}\,(\text{cm})^3$.

The size of the cells themselves vary according to the kind of cell used. In some typical experiments, the cells are flattened out, resting either on the bottom wall of a flask or on a feeder layer. According to numbers communicated to us by Dr. T.C.H. Yang the human cells used in some of his experiments have a nucleus that projects orthogonally on the bottom of the flask on a $144(\mu\,\text{m})^2$ area. The thickness of the nucleus is not uniform. Its resting shape may be likened to that of the yolk of a very fresh egg just released in a frying pan.

Assuming, arbitrarily that the average thickness of the resting nucleus is $5\mu\,\text{m}$, this could give a total volume of $720(\mu\,\text{m})^3 = 72 \times 10^{10}\,(\text{nm})^3$. This seems a bit large compared to the $1.44 \times 10^{10}\,(\text{nm})^3$ computed above for a non overlapping DNA "cylinder". However, that cylinder, if parallel to the bottom of the flask, would project onto an area of $6.12 \times 10^9\,(\text{nm})^2 = 6.12 \times 10^3\,(\mu\,\text{m})^2$ if there was no overlapping of the projections. Thus, thee may be little overlapping of the actual DNA hydrated cylinder in space but there must be significant overlaps in the projections. This may be of importance if the cell is hit by a particle that can make a track perpendicular to the bottom of the flask.

As to the radiation, absorbed doses are usually measured in Grays. One Gray is 100 rads = 10^4 ergs / gram of absorbed energy. Since the density of DNA is close to that of water, one Gray will yield approximately $1.44 \times 10^{-11} \times 10^4 = 1.44 \times 10^{-3}$ ergs in the (hydrated) DNA. For various computations, it is more convenient to use another unit of energy, namely the electron volt (= ev). One electron volt is about $(1.6) \times 10^{-12}$ ergs. Thus, one DNA Gray represents $\dfrac{1.44\times 10^{-7}}{1.6\times 10^{-12}} \sim 9 \times 10^{4\text{ev}} = 90\,\text{kev}$. A similar computation shows that, for a nucleus of $720(\mu\,\text{m})^3$, one nucleus Gray is about 4500 kev.

If one assumes that it takes an average of 34 ev to create one ion pair, the 90 kev would represent approximately 2690 ion pairs. However the energy is not entirely used to form ion pairs. Furthermore, these are short lived. They produce free radicals according to a complex chemistry. It is not clear what the chemistry is, even for interaction of radiation with pure water.

If the radiation is administered in the form of photons, the number of photons needed to deposit such an amount of energy is important in the stochastic modelling for several reasons. One of them is that, if the number of photons involved is large,

one can simplify the models, replacing certain random variables by their expectations. It shall be argued in Section 7 that the Yang-Swenberg model incorporates a simplification of that nature.

Another important reason to consider the number of photons involved is that, if there are many photons, some may hit close to each other and create a local accumulation of ionization with consequent severe damage. This is the scenario in the Kellerer-Rossi dual action theory. It is also involved in the Yang-Swenberg model. If one counts all pairs of photons within distance x of each other and assume independence and uniform distribution, the expected number of pairs would be $\frac{1}{2}\frac{a}{A}N(N-1)$ where A is the total volume of the target and where a is the expected volume of the intersection of a ball of radius x with the target for centers of balls distributed uniformly there.

Note that this "expected number" counts *all* pairs. If for instance 5 hits occur all within distance x of each other, they count for 10 pairs. Note also that one should not take it for granted that photons from a beam perpendicular to the bottom of the flask or Petri dish and distributed Poisson wise in cross section will yield an homogeneous Poisson three dimensional process of hits.

There are other phenomena that may yield high local concentration of ions. For instance, one photon can eject a very energetic electron that proceeds to dissipate its energy locally as a result of multiple primary and secondary collisions. These are typically not Poisson distributed. Photons interact with matter differently according to their energy or wavelength. Since it takes about 34ev to create one ion pair, photons of smaller energy will not create many ion pairs. Photons of higher energy, up to about 30 to 40 kev act mostly through a photoelectric effect: the energy of the photon is entirely transferred to one electron. Thus, this electron carries the same energy as the photon, except for a correction to remove the binding energy of the electron in the atom.

For still higher energies, the Compton effect takes over. At 100kev it is about 10 times as much as the photoelectric effect. At 200 kev the photoelectric effect is negligible. At about 4 Mev formation of electron-positron pairs starts.

We shall stay below that range and, arbitrarily, limit our range of consideration to the interval (.3) to 1400 kev.

In the Compton effects the energy of the ejected electron has the form

$$E\left[1+\frac{506}{(1-\cos\theta)E}\right]^{-1}$$

where E is the photon energy in kev and where θ is the angle between the pre and

post collisions trajectories of the photon. One often sees diagrams where the photon makes a ''track'' in the cell, going along a straight or slightly curved path with ''spurs'' spaced along the path. This is not a good picture of what happens. A photon absorbed by photoelectric effect is just gone. The electron knocked off may make a track in the form of a very tortuous path.

A photon that interacts by Compton effect still retains a large amount of energy. According to the formula given above the maximum energy of the ejected electron is $E\left[1+\frac{506}{2E}\right]^{-1}$. (This happens when the photon is reflected back on its path). The photon retains the rest of the energy. The probability that it will suffer another collision within the same cell is rather small. For instance a photon that still carries 100 kev has a probability $\exp\{-(1.7)\,10^{-4}\}$ of passing through a 10μm layer of water. Thus, if tracks there are they are most likely to consist of just two events: an initial Compton scattering that yield one weakened photon absorbed by photoelectric effect. That could happen for photons in the range where the Compton and photoelectric effects are competitive.

Our next task is to try to find out a little more about the energy dissipation in the nucleus.

Note: The ''gamma ray track'' on Fig 1B is the track of a Compton electron ejected by the gamma ray.

4. Energy absorption in the nucleus.

The purpose of this section is to describe a bit more closely how the energy of the photons gets distributed in the cell nucleus and in the DNA. We consider three main cases. The simplest one refers to very soft X-rays. There the picture is reasonably clear. The second case is that of X-rays with a continuous spectrum located mostly in the photoelectric range. It is argued that they act mostly through the low energy part of the spectrum. The third case is that of photons in the Compton range. There the situation can be very complex. In most of the discussion we argue as if the cells were sitting on top of a feeder layer without any layer of water on top. If there is such a layer the situation is very different. We just say a little about it. For the gamma rays we assume irradiation from below.

It should be emphasized that the arguments given below are those of the author. They may not stand scrutiny if reviewed by a physicist or biophysicist.

a) *Very soft X-rays.* Let us take as an example the case of monochromatic X-ray with an energy per photon of .3 kev. Ignoring excitation processes, they will act through a photoelectric effect each colliding photon knocking off one electron that carries almost (.3) kev.

Such an electron does not travel very far in water. Chadwick and Leenhouts give it a range of at most 7nm. The formation of ions can then be described as follows. Consider the DNA with an hydration sheath as in Section 3. Some photons will impinge on that cylinder and create at least one ion by removing an electron. The electron will be absorbed nearby creating one or more ions. It has the potential of creating as many as 8 or 9 ions pairs and corresponding free radicals within a short distance of the DNA. Some of these pairs will be at distances too large to react chemically with the DNA. However we can assume that hits in the vicinity of the DNA will yield electrons absorbed in our cylinder. Thus the picture that emerges is one composed of simple ions and of related or unrelated small clusters of ions, quickly transformed into reactive chemical species. The chemistry is complex. Some of the common reactions are as follows:

$$H_2O + \text{Ionizing radiation} \rightarrow H_2O^+ + e$$

$$H_2O + e \rightarrow H_2O^-$$

$$H_2O^+ \rightarrow H^+ + OH\bullet$$

$$H_2O^- \rightarrow OH^- + H\bullet$$

$$H\bullet + OH\bullet \rightarrow H_2O$$

$$OH\bullet + H_2 \rightarrow H_2O + H\bullet$$

$$H\bullet + H_2O_2 \rightarrow H_2O + OH\bullet$$

$$H\bullet + O_2 \rightarrow HO_2\bullet$$

$$HO_2\bullet + H\bullet \rightarrow H_2O_2$$

($OH\bullet$ is the hydoxyl radical, with a dot ($\bullet$) representing an unpaired electron)

Since each electron carries about (.3) kev, to deposit 90 kev in the DNA, we shall need approximately 300 photon hits in the required range.

Besides the range of the electrons, one should take into account the fact that the radicals will diffuse around. Chadwick and Leenhouts say that the diffusion path of the radicals is very short. So, it may be legitimate to ignore it. We shall do so.

Even so, the problem of figuring out whether there is a non negligible number of photon pairs hitting close enough to each other to produce a very high local ionization (and consequently more severe damage) is not simple.

According to the formula $\frac{1}{2}\left[\frac{a}{A}\right]N(N-1)$ given in Section 3 one would need to compute the average volume of the intersection with the DNA of a ball of radius x centered at random in the DNA. This assumes that the spatial distribution of hits is a Poisson style process.

Another procedure is as follows. Ignore the fact that an diploid DNA is cut in 2×46 segments and consider it as a single double helix filament $2.04\,10^9$nm long. If the corresponding 3nm diameter cylinder does not intersect itself and if it is not curved sufficiently to make the computation of volumes different from what was done in Section 3, one can proceed as if the N hits were distributed independently and uniformly on the segment of length $L = 2.04\,10^9$nm. Along that segment the number of pairs at distance x or less from each other is $\sum_{i<j} I[|X_j - X_i| \leq x]$ where the X_i are independent uniformly distributed. Thus the expected number of such pairs is $N(N-1)\frac{x}{L}$ ignoring end effects.

In order to have this expectation equal to unity one would need to take x equal to $L/N(N-1)$ or $2.52\,10^3$nm for a number N = 900 corresponding to 3 Grays. This is a very large distance along the DNA. Thus, one would not expect much of that to happen.

There are however other possible considerations. One possibility is that, in a diploid cell, two chromosomes of the same pair are very near each other and that a break in one weakens the other or makes it more vulnerable. This is suggested by the fact that haploid yeast cell do not repair double strand breaks as easily as diploid yeast cells. The distances involved also make it difficult to explain the very common

occurrence of sister chromatid exchanges. These occur as the DNA is duplicating and involve substitution of a parent strand by a copy of its mate.

To obtain a larger probability of close pairs one could increase the effective diameter of the DNA "hydration sheath", for instance by taking into account the average range of the electrons. For an average range of 7 nm, this would give an effective diameter of 16 nm. For a range of 11 nm one would get a diameter of 25 nm. In such a case the foregoing computations are not applicable. For a diameter of 16 nm, the "sheath" is a considerable fraction of the volume of the nucleus. For 25 nm it would be the entire nucleus. For 3 Grays the nucleus would receive about 45000 photon hits.

Ignoring the effects near the nuclear membrane (which may be very important!) one would have an expected number of close pairs equal to $\frac{1}{2}(\frac{a}{A})N(N-1)$ where $A = 72 \times 10^{10}(nm)^2$ and where a is the volume $a = \frac{4\pi}{3}x^3$ of a ball of radius x.

To have an expected number of close pairs equal to, say 2, one would have to take $x = 7$ nm. Although this may still be considered large, it is not totally unreasonable. It could, for instance, account for the simultaneous breakage of two sister chromatids.

b) *Continuous spectrum X-rays in the photoelectric range.*

Consider X-rays produced by high energy electrons hitting a metal plate, say copper. The X-rays are produced by bremsstrahlung as well as relaxation emission when an electron knocked out of its atomic shell is replaced by another. However, except for high scallops near the K and L edges for copper, the spectrum of the X-rays is generally describable by a density of the type $\frac{2}{a^2}[a-x]^+$ for $0 \le x \le a$, where a is the maximum possible energy. For instance a potential difference of 200kv will produce photons all in the range $[0,a]$, $a = 200$kev with a density as described. Part of the photons so emitted are in the photoelectric range and the following applies to them.

The probability that a photon of energy E be absorbed (by photoelectric effect) in a layer of water of thickness y is given by an expression of the type

$$p(E) = 1 - \exp\{\frac{Ky}{E^3}\}$$

where K is a certain coefficient.

For a thickness $y = 1\mu m$ and for E expressed in kiloelectron volts Ky is approximately equal to 17.

It follows that the average energy of *absorbed* photons (photoelectric effect only) will be given by the ratio

$$\mathrm{AvE} = \frac{\int_0^a x(a-x)(1-e^{-\frac{Ky}{x^3}})dx}{\int_0^a (a-x)(1-e^{-\frac{Ky}{x^3}})dx}.$$

Note that $1-\exp\{-\frac{Ky}{x^3}\} < \frac{Ky}{x^3}$ and that $\frac{Ky}{x^3}$ can be used as an approximation to $1-\exp\{-\frac{Ky}{x^3}\}$ for $\frac{Ky}{x^3}$ small, that is for large energies.

From this one can argue that for a large, say a = 100 or 200 kev the bulk of the absorbed energy will come from low values of x, small compared to a. Thus the factor $a-x$ plays little role and can be omitted. Also the range of integration can just as well be taken from zero to infinity.

If so we obtain an approximate formula of the type

$$\mathrm{AvE} \sim \frac{\int_0^\infty x\{1-\exp[-Ky/x^3]\}dx}{\int_0^\infty \{1-\exp[-Ky/x^3]\}dx}.$$

It shows that AvE increases about like $(Ky)^{1/3}$ as Ky increases.

The integral is not easy to evaluate analytically. A very rough numerical approximation shows that for Ky = 17 (that is for a thickness of 1μm), AvE is of the order of 2.8 kev. For a 20μm layer it would be of the order of 6.9.

However that is not the end of the story. The electrons ejected by the photoelectric effect carry considerable energy. They can travel some distance in the cells or in water. Here again one may argue as if the probability of passing without interaction through a layer of thickness y is of the form $e^{-\mu y}$ where μ depends on the energy of the electron. The value of μ can presumably be determined from tables of the stopping power of the material and tables of electron fluence.

Another aspect of the situation is the range of the electron. According to a graph in Dertinger that range is approximately $R = (.05)E^{1.7}$ for R in μm and E in kev. Whatever is meant by "range" is not explained in detail there. There are also tables (ICRU 35) of the "mean path lengths". by this is presumably meant the expected length of a path including all collisions of the primary electron until rest. In each collision the outgoing "primary electron" is the one carrying the maximum energy. The

ICRU tables give mean path length that are somewhat higher than the range values of Dertinger. None of these references give tables of the "penetration length" that could be defined as the maximum distance between the origin of the electron path and other points on the path. However the "penetration length" seems to be of the same order of magnitude as the "mean path length". See ICRU 35 section 2.8. Thus we would have penetration ranges R of the following order of magnitude according to electron energies.

E kev	.3	10	20	100	500
R μm	610^{-3}	2.0	7.5	120	2000

Along their path electrons lose energy by collisions, bremsstrahlung, Cerenkov radiation and perhaps other effects. For the energies under consideration here, one can probably neglect the Cerenkov radiation. The bremsstrahlung is present but it does not represent the major source of loss of energy. It would give additional photons absorbed nearby by photoelectric effect or by excitation.

It should be clear from the ranges indicated above that one needs to take into account the geometry of the radiation target and that the main effect on the DNA will not be due to photons that pass through it but to electrons knocked off in the material, perhaps at some substantial distance from the DNA.

We shall assume that the cells are dispersed in a monolayer, attached to a plane surface and that they are flattened in a manner analogous to ordinary eggs juxtaposed contiguously in a frying pan. (Sunny side up type of cooking!) If so, their thickness need not be all that uniform. We shall assume that the nucleus is flattened a little, having an average thickness of 5μm. If there is no other material between the X-ray source and the cells, the computation carried out in this subsection would show that the average energy per absorbed photon is about 4 kev. For a nucleus of $720(\mu m)^3$ diameter and for 3 Grays this works out to approximately 3400 photon hits in the nucleus. The damage to the DNA would be mostly due to electrons liberated within .5μm of it. However this means at least the entire nucleus and possibly part of the cytoplasm.

Note however that this computation is not entirely correct. Even though the average photon hit yields 4 kev, most of the hits will be of much smaller energy and yield electrons of much smaller range.

Some of the ejected electron will end their trajectories outside of the cell. In order to keep the cell live, they have to be in a minimum of moisture. Even if there is only

1 μm of water above the cells, this should be taken into account since it acts as a source of electrons that may penetrate the cells. There are other phenomena that should be taken into account. For instance, some of the energy is dissipated by excitation of the atoms. It may be a sizeable fraction of the photon energy. If so it should be deducted from the ionization computations. It is true that the 34ev average we have used for the formation of ion pairs includes a sizeable amount of energy used to excite the atom. For instance the binding energy of L-electrons in oxygen is about 14ev. The rest of the 34ev namely 20ev is then dissipated by excitation. However we have not taken directly into account near collisions that lead to excitation only.

If one computes as in subsection (a) above, to have two photons acting close enough to lead to high local ionization one would have to take a radius of about 8 nm. This is small compared to the possible range 500nm of the electrons. It does not take into account the very tortuous and fractal like shape of the electron "spur". Nor does it take into account the fact that electrons will tend to be shot off with preferential direction in the continuation of the path of the incident photon.

One could perhaps get a better picture of what can happen by looking at the "efflorescence" created by one single high energy electron as it collides repeatedly shooting off other electrons that repeat the process themselves. One possible picture is an efflorescence similar to twigs of the flower "baby's breath" (*Gypsophila paniculata*) often used to fill out floral arrangements. (Baby's breath tends to have 3-prong forks in its terminal twigs. Here there should be 2-prong ones).

In any event one can probably assume that for 2 to 3 Grays the nucleus is fairly saturated with electrons with a complex non uniform distribution of local densities.

c) *X-rays and gamma rays in the Compton effect range.*

Here we shall consider only a range where the formation of electron-positron pairs is negligible and where the photoelectric effect is small compared to the Compton effect. This means looking at photons with energies E between 100kev and 3Mev, approximately.

If, for instance, one uses 225kvp X-rays with continuous spectrum, one would have to consider both the photoelectric effect and the Compton scattering. The analysis given in subsection (b) above would still imply that the photons that do most of the damage are the ones with fairly small energy. However we shall not discuss that situation further and concentrate on a special case as follows.

Many experiments are carried out with the gamma rays from Cobalt 60. These come in two colors, one situated at 1.17 Mev and the other at 1.33 Mev. To simplify matters and reading of tables we shall consider only the case of 1 Mev photons. Here

the actual geometry of the target seems to be of primary importance.

One standard design would be as follows: the cells are grown in a culture flask of horizontal section 5×5cm. The flask is plastic with a wall of a 1mm thickness. The cells are attached to the bottom wall. They represent a layer of thickness approximately equal to 3.64μm. The radiation is directly vertically from *under* the flask.

Now a 1 Mev photon has a mean free path of approximately 14cm in water, and a slightly larger mean free path in standard plastics. On a Compton scattering, it will lose at most 80% of its energy. Thus the continuing photon will have at least 200kev. Its mean free path is still of the order of 7cm. Thus photons that scatter within the wall of the flask or in the cell layer are quite unlikely to score a second hit as that same wall or layer. (Except for those where the new photon is at right angle with the primary one) The probability that a 1 Mev photon would scatter within the cell layer is negligibly small.

This means that the action of the radiation will be indirect. The action on the cell layer will be mostly due to electrons (and perhaps bremsstrahlung photons) *generated in the plastic wall of the flask.*

The electrons ejected by 1 Mev photons do not all carry the same energy. In fact the energy has a continuous spectrum. Its density has a shape of the following kind

This is a flattened out J-shape with a sharp drop in the J at 800 kev (more precisely 798 kev if the formula of section 3 is correct). The peak at 800 kev corresponds to photons that are reflected. The other maximum, at zero, to photons that are barely deflected.

Even though the peak at 800 kev is about 3 times as high as the trough near 500 kev, we shall argue as if the distribution was uniform from 0 to 800. This does not matter all that much in the very rough computations carried out below. Most of the ejected electrons will spend a good part of their life in the plastic wall. As an indication of their possible range here are some values for the mean total path length in Polyvinyl chloride. (Source: 1CRU 35).

Electron energy in kev	mean total path length mm
10	$3\,10^{-3}$
20	$1\,10^{-2}$
50	$5\,10^{-2}$
100	$1.6\,10^{-1}$
150	$3.2\,10^{-1}$
200	$5.1\,10^{-1}$
500	2.0
800	3.7

This could be fitted by a formula of the type $R = (10^{-1})\,E^{1.48}$, with E in kev and R in μm. As explained previously, this is not the actual penetration depth, but we do not know how to compute the penetration depth and will take the range R described here just for illustration purposes.

For electrons in the range of energy considered here bremsstrahlung is not totally negligible but it is not major compared to collision losses. Thus we shall neglect it. We shall neglect also other phenomena where a photon hit ejects more than one electron. This could happen if, for instance the electron ejected belongs to a layer close to the nucleus and the atom rearranges the other electrons in a hurried manner, ejecting two or more in the process.

If all these other possibilities are neglected, the damage to the DNA will be due to electrons that somehow make their way from the plastic wall to the cell layer and interact there. The probability of interaction within the cell layer will depend on the electron energy and on the angle of its path. If one neglects electrons that travel almost parallel to the cell layer and if one considers the nucleus as spherical the probability of interaction within the nucleus will depend only on the electron energy. This however seems to be an over simplification.

In any event, electrons that arrive to the cell layer with something like 50 kev or more should have a small probability of interaction with a nucleus that is only 10μm in diameter.

For the case of 1 Mev photons considered here one can assume that the distribution of hits within the flask wall is given by a homogeneous Poisson process. Each hit is assigned an energy release E independently of it location. Ignoring the fact that the actual distribution is more complex, we can argue as if E was distributed uniformly between 0 and 800 kev.

Each hit is followed by a collision process scattering a large number of electrons in a geometrical pattern that we likened to a "baby's breath" efflorescence earlier.

Measure "depth" in the plastic from the bottom of the cell layer. Then if the hit is at large depth x and E is small the efflorescence will not even touch the cell layer. If x is large but E is also large, the efflorescence will reach the cell layer. It will contain many electrons, some of them with small enough energy to interact with the cell layer, some of them with enough energy to pass through unscathed and settle in the liquid above the cells.

For a small depth x, the picture is reversed. The electrons with high energy E will just go through while those with small enough energy will make a small efflorescence in the cell layer.

How this happens is of importance for the stochastic models. It should be clear from the above that a photon hit does not result in a single event in the cell or the nucleus. In other words each primary event leads to a complex distribution for the number of events in the cell. The fact that the "primary events" occur in the plastic wall does not change the fact that the distribution of the number of "secondary events" in the cells must be taken into account.

Note that these "secondary events" are not necessarily distributed independently in the cells. An efflorescence with roots at small depth (and small energy) can readily distribute secondary events in close proximity to each other in the cell.

Note also that if the goal is to justify a Kellerer-Rossi approach, or the Chadwick-Leenhouts linear plus quadratic formula, the interaction term that gives the quadratic part of the formula must occur at the photon level. More precisely, two photons hitting at different depths and different locations will "interact" if and only if the part of their respective efflorescences that act in the cells act closely enough there.

Thus to obtain information about the possibility of "interaction" at the cellular level of the impinging photons one needs to know the distribution, spatially and energywise of the electrons that may reach the cell layer. This is not available in the texts I have. The document ICRU 35 does give some number relative to so called "Mass scattering power". However, from the description, these numbers seem to refer to the angle between the initial path of the primary electron and its path after traversing some layer of matter. Since the "primary electron" is defined as the one that carries the maximum energy after collision, these numbers give a distorted view of the angle spanned by our "efflorescence."

One can obtain a better approximation to what actually happens by the following technique. (This is not a realistic description of the physics involved but it seems to match reasonably the pictures found in the literature.) Assume that between collisions

the trajectory of an electron is rectilinear and that it has a random length L such that $Pr[L>x] = \exp\{-\lambda(E)x\}$, as for photons but with a different function λ of the electron energy E. At collision with an orbital electron of an atom two electrons will be ejected at respective angles θ_i, i = 1,2 to the trajectory of the incident electron. Electron #i will carry an energy E_i. The pairs (θ_1,E_1) and (θ_2,E_2) are not independent since they must be compatible with the laws of conservation of energy and momentum. However the pairs are not functionally related since part of the energy can be absorbed by the excitation, recoil or rotation of the atom and since part must be spent overcoming the binding energy of the orbital electron.

If one considers that the orbital electron is fixed with a given binding energy to characterize what happens at the collision it is enough to give what is called a "double differential cross section" $\sigma_E(e_2,\theta_2)$. The differential $\sigma_E(e_2,\theta_2)de_2d\theta_2$ gives the probability that, for an incident electron of energy E, the secondary electron will have an energy in the interval $[e_2, e_2+de_2]$ and an angle to the incident trajectory in $[\theta_2, \theta_2+d\theta_2]$.

Some information is available on such cross sections.

For instance, the document ICRU 36 says that, for large e_2, the marginal density $\int\sigma_E(e_2,\theta_2)d\theta_2$ behaves approximately proportionally to e_2^{-2}. It also reproduces a picture of a cross section $\sigma_E(e_2,\theta)$ for electrons of energy E = 500ev in water but for an angle $\theta = \theta_2 - \theta_1$. It can be seen from the picture that the dependence of σ_E on e_2 can probably be fairly closely represented by a formula of the type $K_1\{1-\exp[-K_2e_2^{-2}]\}$ with coefficients K_1 and K_2 that depend in a complex manner on the angle θ. With information of this type for a suitable range of the initial energy E and with knowledge of the function $\lambda(E)$ one could in principle obtain the distribution of the entire efflorescence created by one photon. We have not done so here because of a lack of solid information on the cross sections σ_E and on the function $\lambda(E)$.

There is also a problem about computing how many photons are needed to give the DNA a 3 Gray dose. One cannot assume that this is achieved by giving the plastic wall a 3 Gray dose, because the energy deposition is not homogeneous in the wall (even though to the primary photon collisions can be taken to form a homogeneous Poisson process).

This being as it may, and pending further evaluation, one can still guess that the effect of the radiation on the cells on the DNA is *not* linear in the photon dose.

In summary, even though the above description is very oversimplified, one should expect that the shape of dose response curves will depend on the particular features of the experiments under study. Soft X-rays may be efficient killers because they yield localized spurs of high ion density. One would not expect them to yield pronounced

"shoulders" because of the small probability of interaction. Yet it is an experimental fact that the dose response curves exhibit shoulders.

For harder X-rays or gamma rays it seems necessary to take into account not only the cell monolayer but the wall it rests on or the layer of culture fluid above it, depending on how the radiation is administered. For instance for gamma rays directed from under the flask, the thickness of the flask wall and its composition should be important. If our interpretation of the situation is at all correct, extremely thin flasks should yield little opportunity for interaction while flasks of ordinary thickness (about 1mm) allow a significant interaction between the electron efflorescences produced by the incident photons.

5. The repair system and some other complications.

Many experiments have shown that cells are capable of repairing a large amount of radiation induced damage. According to Cole *et al* in [4] a 2.5 Gray dose of gamma rays applied to a mammalian cell induces approximately 1000 single strand breaks (SSB) and 20 double strand breaks. Elkind says 50 DSB and Bryant says 40. If the cell is allowed to rest in non-growth conditions for a few hours most of the SSB will be rejoined, leaving an average of 7.5 SSB unrejoined and 2 unrejoined DSB. The repair of SSB is reasonably fast with a half-time of approximately 6 to 15 minutes. The repair of DSB can take a considerably longer time. At the 2.5 Gray dose approximately 50% of the cells are "killed." The actual cause of death is not well understood. The considerable amount of repair carried out by the cell is probably the result of many different mechanisms some of which must be rather complex.

Even though a cell can often rejoin DSB, this does not mean that the repair restores the cell to its initial state. The text of Chadwick and Leenhouts contains pages of diagrams that display a few of the things that can go wrong. However misrepair is not always lethal. Apparently mammalian cells contains a great abundance of DNA that is not crucial to the function of the cell. A misrepair at such locations will not lead to transformation or to cell death unless it prevents the DNA from separating and replicating.

There is quite a bit of evidence that some breaks are easier to repair than some others and that some are, to all intents and purposes, impossible to repair.

One of the experiments that shows such differences was carried out not by irradiating cells but by treating them with restriction enzymes. P.E. Bryant in [2] reports that the restriction endonuclease called Pvu II generates "blunt end" DSB that are difficult to repair. The restriction endonuclease Bam H1 on the contrary generates frazzled DSB's that get repaired much more easily. In fact most of them get repaired, even though the distance between the cuts on the two strands of DNA is only about 4 base pairs.

Besides repair performed in some way, the cell carries out other processes. Bryant estimates that one Gray produces some 40 DSB directly and that about as many 40 DSB are produced "via endonucleolytic cleavage of the DNA during the first few hours after irradiation." These cuts may be due to base lesions that are cleaved out in an attempt at repair. Note that Bryant's estimates are considerably larger than Cole's.

How the repair system works does not seem to be well understood. Several questions remain largely unanswered: (1) Are the repair enzymes always in place patrolling the DNA as a matter of routine or are they floating around and called in by the unwinding of the DNA? (2) Are the enzymes used in a catalytic process that

regenerates them or are they destroyed in the repair process? (3) Are some of the enzymes induced by the DNA damage?

The answer to these questions seems to be a definite "may be". For instance Chadwick and Leenhouts ([3] pages 179-180) report that a small dose of radiation applied to *Saintpaulia* epidermis cells makes them more resistant to a subsequent large dose. (Saintpaulia = African violet). They mention some other organisms where a similar phenomenon has been observed.

The destruction of enzymes is the basis for a theory of Goodhead [10]. This particular author claims that the shape of observed dose response curves is best explained by an assumption that the repair process is saturable. This means that the quantities of the necessary enzymes are limited. They are used up in the repair process so that too large a dose leaves the cell unable to repair the total damage. It is indeed a fact that almost any sort of dose response curve can be simulated by such a saturable process, see [10]. However the evidence of saturation is not compelling. Goodhead cites some evidence on irradiated nerve cells, but it refers only to rather large doses and can perhaps be explained otherwise.

The experiments yielding evidence of a repair mechanism are at least of two different kinds. One typical kind is to irradiate cells and keep them in suspension for several hours in a medium that does not promote cell division. Then the cells are plated in growth medium. Presumably in non growth conditions the cells can repair some of the damage. On the contrary at division time the damage either prevents completion of the division or else it gets fixed in the progeny and eventually leads to cessation of the division process or to transformation. Thus cells allowed repair time will survive better than cells that are plated immediately.

Another kind of experiment involves irradiating cells and counting the number of DSB immediately after irradiation or after various liquid holding recovery periods.

It seems to be an accepted fact that after plating in growth medium very little repair will take place (see [17]). However this is not a hard and solid rule. The "human T.1" cells used by T.C.H. Yang do not seem to follow the rule. They keep dividing all the time unless acutely starved. To hold them in liquid suspension does not seem to lead to better repair or survival. (The "human T.1" line is supposed to be a non malignant cell issued from a human kidney. Even though they do not display the usual characters of malignant cells they have become aneuploid in the culture process)

One of the most detailed description of the effect of repair in number of DSB is given in a paper by M Frankenberg-Schwager, D. Frankenberg *et al* [8]. The authors worked with yeast cells and irradiation doses of up to 2400 Grays. Note that yeast

cells are much more radiation resistant than mammalian cells, presumably because their DNA content is small.

The following figures 2, 3 and 4 are reproduced from the Frankenberg-Schwager paper. Note that the number of DSB is drastically reduced by liquid holding for 24 to 48 hours. In fact at 2400 Grays it gets reduced from approximately 150 to 25. However the damage does not get repaired totally, even for holding times of 72 hours. Note also, from figure 3, that even though the cells seem to be unable to repair all the damage, their repair system is not "saturated" since damage due to an additional dose of radiation gets repaired almost as much as the initial damage.

Finally note the shape of the dose response curves. An evaluation immediately after irradiation yield a number of DSB that is essentially a linear function of the dose. Evaluation 24 hours or more after irradiation gives definitely curved dose responses.

This has been cited (see [5] ,[15]) as evidence that the effect of initial radiation damage is in fact a linear function of the dose (That is, at dose D the expected number U of initial lesions is aD.) but that a non-linear process is at work during the repair period: the expected number of remaining uncommitted lesions at time t satisfies an equation of the type

$$\frac{dU}{dt} = -\lambda U - \kappa U^2.$$

Presumably the term in U^2 represents an interaction term between lesions situated either on the same DNA strand, two different coiled strands, different chromatids or different chromosomes.

The many chromosomes aberrations visible at mitosis in irradiated cells confirm that interaction does take place. A quadratic term could also be due to other sources. For instance one may conceive of repair enzymes being induced proportionally to the number of lesions present. However if the enzymes are not consumed in the repair process this would lead to an acceleration in time of the rate of repair. There is no mention of such a change of rate having been observed except for the organisms described in Chadwick-Leenhouts pages 179-180. In fact the Frankenberg-Schwager curves show a decrease of repair rate in time.

It is likely that the situation is more complex than what can be described by the simple linear-quadratic kinetics just mentioned. Indeed the Frankenberg-Schwager experiments suggest that the radiation induces many different kinds of damage some of it easily repairable and some of it essentially impossible to repair. A variation in the amount of severe damage according to dose would lead to the same kind of curvature in the dose response graphs. This is essentially what is claimed in the Kellerer-Rossi dual action theory or in the book by Chadwick and Leenhouts.

Tobias *et al* in [15] cite other evidence for the existence of a non linear repair term. One of them is the shape of the dose response curves for various strains of *Saccharomyces cerevisiae* (brewer's yeast). Using a tetraploid strain one can replace up to four copies of a wild type gene that codes for repair of DSB by a mutant called "rad 52" that is repair deficient. It can be seen from fig 5 that the dose response curves vary in a systematic manner as the number of deficient genes is increased. Tobias *et al* interpret this as an increase in the ratio $\frac{\lambda}{\kappa}$ of the coefficients of the linear and quadratic terms in the kinetic equation $U' = -\lambda U - \kappa U^2$. Here again one could try to interpret the data in a different manner using different degrees of severity of the radiation induced damage. A cell with four wild genes could repair all the damage except the most severe. A cell with 4 deficient genes would repair only the most minor damage. Even though such an explanation may seem artificial, it is not ruled out by the existing evidence.

There are other complications in the interpretation of experimental data. One of them has to do with the fact that the sensitivity of cells to radiation varies during the cell cycle. According to a graph reproduced in Chadwick and Leenhouts ([3], page 41), cells in the mitotic phase are about 20 times more sensitive than cells in the DNA synthesis phase. One possible explanation is that during synthesis the two strands of the DNA helix are at some distance from one another while in the mitotic phase they are condensed in chromosome form thus allowing a single bad hit to cause more severe damage. However that explanation does not take into account the fact that Okada, quoted in [7], has found that the initial number of DSB and SSB immediately after radiation is independent of the phase in the cell cycle.

Most fully stochastic models are meant to describe cell population irradiated in a synchronous state. For instance they could all be in synthesis phase, or they could all be in the resting G_1 phase. However many experiments are conducted with ordinary asynchronous cell cultures. To see how this can affect the shape of dose response curves, suppose that one distinguishes m different phases in the cell cycle, with cells in phase j responding according to a survival probability $S_j(D)$ for dose D. If the proportion of cells in the j^{th} phase of one asynchronous culture is p_j, the combined survival probability at dose D will be $T(D) = \Sigma p_j S_j(D)$. Now the survival curves are usually plotted with D on the abscissa and log percent survivors on the ordinate. Thus what will be plotted is $\log T(D)$ versus D. If for instance $S_j(D) = \exp\{-\lambda_j D\}$ so that $\log S_j(D)$ is linear in D the curve $\log T(D)$ will be convex. More generally if $S_j(D)$ is written in the form $S_j(D) = \exp L_j(D)$ then the second derivative of $\log T(D)$ has the form

$$\Sigma \pi_j L_j'' + \Sigma \pi_j (L_j')^2 - [\Sigma \pi_j L_j']^2$$

with coefficients $\pi_j \geq 0$ such that $\Sigma \pi_j = 1$ and $\pi_j = C p_j \exp\{L_j(D)\}$. Thus the effect of the mixture is to add to an average of the second derivative a term that has the structure of a variance for the first derivatives.

Since most observed dose response curves are concave for small D, the variance term is usually not able to overwhelm the term $\Sigma \pi_j L''_j$.

(This type of computation is also relevant for some other purposes: If a large human population is exposed to low doses of radiation, the aggregated dose response curve may be convex while individual responses are concave).

The variation during the all cycle suggests that other phenomenon should be investigated. For instance, from the mechanisms involved, one would expect that although the cell is least sensitive during the synthesis phase, the frequency of induced sister chromatid exchanges should be highest at that stage.

Although the theory for synchronized cells is simpler than a theory for asynchronous cultures, both cases must be considered since the literature contains hints that the synchronization process may modify the enzymatic repair system.

Several experiments have been conducted to find out if for a given total dose D the rate at which the radiation is applied modifies the response. Indeed such effects have been observed but typically only at very low or very high dose rates. For very low rates a possible explanation is that the repair mechanism has enough time to act during the period of irradiation. For instance, if a dose of 2 or 3 Grays is given uniformly over a 60 minute period, one would expect that the SSB repair mechanism, with a half-time of perhaps 10 minutes, would have ample time to act enough to yield observable results. However, here again, the culture medium and the cell line will make differences. On the contrary the same dose of 2 or 3 Grays applied in just one minute does not allow significant repair during irradiation.

Keeping this information in mind, it is now time to look at some of the stochastic models, even though we have said nothing about sister chromatid exchanges and about the fact that DNA can conduct electrons for a long distance, passing them from one base to the next.

6. Some general stochastic models.

Stochastic models, or deterministic models that could readily be stochasticized have been used for a long time to describe the effects of radiation on cells. The simplest idea is that the cell contains one or more separate vital domains such that if the radiation hits at least one of them the cell will no longer proliferate. Assuming that the photons arrive in a Poisson manner each vital domain will receive an amount of radiation proportional to the dose D and the probability S of survival will take the form $S = e^{-\lambda D}$ or equivalently $\log S = -\lambda D$.

It turns out that the observed $\log S$ dose response curves do not have such a simple linear shape. Near the origin they are concave displaying what is called a "shoulder". This is true at least for the low LET radiation represented by our photons. The case for the high LET radiation is entirely different. It does not lead to shoulders. For the low LET several "models" have attempted to mimick the observed curves. Here are a few:

a) *Multiple hit models*

In the simplest ones the cell contains one vital domain. It can withstand k hits but not $k+1$. Under the same Poisson assumptions the dose response curve is

$$\log S = -\lambda D + \log [\, 1 + \frac{\lambda D}{1} + \cdots + \frac{(\lambda D)^k}{k!} \,].$$

The most common value to be considered is $k = 1$. If there are, say, m separate vital domains with different sizes but all corresponding to the same $k = 1$ one would have

$$\log S = -(\sum_{i=1}^{m} \lambda_j) D + \sum_j \log (1 + \lambda_j D).$$

Such a curve would still have a zero first derivative at $D = 0$ while many observed dose response curves do not. This can be cured by adding to the above a term $-\mu D$ corresponding to other vital domains that are killed by just one hit.

There is something a bit unaesthetic about allowing the domain to sustain k hits but not $k+1$. If one assumes that a vital domain that gets k hits has a probability s^k of still functioning, then Poisson takes over again and the curve become linear in D.

Although it is clear that the formulas above or similar ones can be used to fit many things such models have passed from favor, mostly because they do not really "model" or "explain" much.

b) *The Kellerer-Rossi dual action theory*

The description is hard to understand for non-physicists. It is not entirely stochastic. What it seems to be is a continuous variant of the multiple hit models just described. The radiation impinges on the cells in a Poisson manner homogeneously on the average. However due to random fluctuations the actual deposition of energy is not uniform. Those areas that receive higher concentration of energy are likely to get extensively damaged, thus leading to higher death probabilities.

A similar argument is carried out by Chadwick and Leenhouts: a particle that passes close to the DNA may cause a SSB. It is less likely to cause a DSB. Some other DSB (and SSB) are caused when two different particles pass near the DNA in close proximity to one another. The computation involves then computing the average number of such effects, leading to a formula of the type $\alpha D + \beta D^2$. The survival probability S is then computed as if these effects had a Poisson distribution. That is $\log S = -\alpha D - \beta D^2$.

Even though the Chadwick-Leenhouts text is very impressive one cannot say that it is entirely convincing. An actual description of what happens would take us back to the speculations of Section 4 above. Except for the case of very soft X-rays these speculations do not readily support the idea of "Poisson Statistics".

c) *The Neyman-Puri model.* This is an attempt to describe what happens during exposure and after by a Markov process system. It is roughly as follows: Primary particles impinge Poissonwise on the cell nucleus. Each produces a burst of secondary particles. These now behave independently of one another creating lesions. The lesions can be of three types, "uncommitted", "transforming", "lethal". Each lesion independently of the rest performs a Markov process in which it can change from uncommitted to transforming or lethal or can be repaired. Similarly a transforming lesion can become repaired or lethal. The lethal ones stay that way.

It is clear that such a model does not actually provide for any interactions between incident particles or their progency. Since Neyman and Puri nevertheless obtain a shoulder effect we shall return to a closer examination of the situation in Section 7 below.

d) *The G.L. Yang-C.E. Swenberg model.*

This starts very much like the Neyman-Puri model with primary particles causing burst of secondary ones that now behave independently of each other. However there are some major changes. The radiation is administered in time and the probability $\pi_1(t)$ that a secondary particle will create a lesion is an increasing function of t. One

can make that function depend in addition on the rate at which the radiation is administered. For radiation given uniformly over an interval of time, the time t is an index of the amount of radiation received up to that time. Thus one could also make the function π_1 depend not on time but on the previously accumulated dose and on the dose rate. In any event this provides a semi-stochastic interaction possibility intermediate between a deterministic Chadwick-Leenhouts type interaction and a completely stochastic description.

Other features involve the time from end of irradiation to plating time, under the assumption that in a liquid holding phase in non-growth medium the cells will repair but not divide. After plating in growth medium they will divide (perhaps) but not repair. This description is applicable to yeast cells, but not to the human T1 cells. They will divide in the holding phase and continue repair after plating. Finally cell "survival" is equated to the absence of lesions except those that are correctly repaired or have become transforming lesions. In the Neyman-Puri model survival was equated to the absence of "lethal" lesions. We shall see that this is not a tenable proposition.

e) *Repair-misrepair models.*

These, proposed by C. Tobias and colleagues make no assumptions about the mechanism of production of lesions except that their average number is proportional to the applied dose D.

One piece of evidence cited for this is the fact that SSB and DSB counted immediately after radiation have an average proportional to D. Also Goodhead, [10], says that the incident particles cannot possibly be close enough to interact in their creation of lesions.

Instead the model proposes that the interaction takes place between *lesions* at the time of *repair*.

Tobias' model was semi-deterministic. One writes differential equations that given the average flow of lesions in their various states. Then one uses Poisson Statistics. A fully stochastic description by the corresponding Markov model has been worked out by N. Albright. There is also a further modification called the LPL model in which interactions are allowed during the repair and before it.

This, of course, leads to a merger of the "dual action" with the "repair-misrepair" models.

f) *The saturable repair models.*

There is still a further class of models. So far they are still in deterministic or semi-deterministic form but they could be stochasticized. The model proposed by

Goodhead in [10] assumes that repair does take place but that it can be saturated. He shows that one can get in this manner a large variety of dose-response curves.

One of the main shortcomings of such models is that, except for special cells at very high doses of radiation, there is very little evidence that the repair mechanism can be saturated. See for instance the discussion in Frankenberg-Schwagger. [8].

In some other models, the repair system is not ''saturated'' but its enzymes get inactivated by the radiation.

Since all the models described above (except (c)) readily yield dose-response curves with shoulders, selection among them must be based on other criteria. We shall review the situation again after a discussion of models (c) and (d).

7. The Neyman-Puri model.

The model, described in [13], has the following features.

1) primary particles impinge on the cell according to a Poisson process with intensity λ

2) Each primary generates a cluster of secondary particles. The number ν in a cluster is a random variable with generating function g.

3) The secondary particles move independently of each other. Each has a probability π_1 of creating a potentially repairable lesion and a probability π_2 of creating a lethal lesion.

4) If at any time t a cell that is still alive contains repairable lesions these can change status independently of each other according to the following Markov process rates

i) the damage may be repaired at rate α

ii) it may be converted to cancerous damage at rate β

iii) it may become lethal at rate γ.

Furthermore there is a possibility of death unrelated to radiation, at total rate δ. The Markov rates $\lambda, \alpha, \beta, \gamma$ and δ may be functions of time.

Actually Neyman and Puri state the assumption called (4) above in a different manner. For instance they say that conditionally given that at t the cell has k unrepaired lesions the probability of a single repair in (t, t+h) is $\alpha kh + o(h)$ and so forth. The fact that these infinitesimal probabilities are linear in k is equivalent to the fact that lesions behave independently of each other.

It should be obvious that such a model does not provide for any interaction between incident primary particles, secondary particles, or lesions of various types. *Each secondary particle generates a Markov process independent of the processes generated by the other particles.*

Neyman and Puri go in on to describe the processes in terms of a three dimensional Markov process $t \to \{X(t), Y(t), Z(t)\}$ where X(t) is the number of unrepaired hits at time t, where Y(t) is the number of cancerous moves in (0,t) and Z(t) is the number of cell killing events in (0,t). They obtain for the process a joint generating function $G(s_1,s_2,s_3;t) = Es_1^{X(t)} s_2^{Y(t)} s_3^{Z(t)}$ whose logarithm has the form

$$\log G(s_1, s_2, s_3;t) = -(1 - s_3)\int_0^t \delta(\tau)d\tau$$

$$-A\int_0^t \lambda(\tau)\{1 - g[\,\phi(s_1,s_2,s_3;\tau,t)\,]\}d\tau$$

where

$$\phi(s_1,s_2,s_3;\tau,t) = (1-\pi_1-\pi_2)+\pi_2 s_3+\pi_1\psi(s_1,s_2,s_3;\tau,t)$$

with

$$\psi(s_1,s_2,s_3;\tau,t) = s_1 \exp\{-\int_\tau^t(\alpha+\beta+\gamma)du\}$$

$$+\int_\tau^t(\alpha+\beta s_2+\gamma s_3)\exp\{-\int_v^t(\alpha+\beta+\gamma)du\}dv.$$

The only place where the dose appears in $\log G$ is in the integral term, coefficient of A. As must be expected from the fact that secondary particles generate independent processes, the dose enters in $\log G$ in a purely linear manner.

In spite of this Neyman and Puri do go on to obtain equations that show that the dose response curve can exhibit a "shoulder". The phenomenon occurs as a result of two assumptions. One of them, call it (A), is that a cell is "killed" only by "lethal lesions". It can survive any number of unrepaired "repairable" hits (or cancerous mutations). This occurs at the point where they set the probability S(t) that the cell is alive at t as G(1,1,0;t). Another, call it (B), is that the radiation is applied at a certain rate ρ during a period of time T and *that the total dose is varied by keeping* ρ *constant and varying* T.

The combined effect of the assumptions (A) and (B) just described is to lead to shoulders in $\log S(t)$ as function of the total dose D because the second integral in $\log G$ can then be written as an integral from zero to D/ρ (for $t \geq \frac{D}{\rho}$). (See [13] equations (19) and (20).)

However the combined effect of (A) and (B) also leads to conclusions that are not tenable. To see this keep λ constant in $[0,T]$ and zero afterwards. Keep all the other rates constant with $\beta = 0$. Consider two pairs (T_i, t_i), $i = 1,2$. Suppose that in (T_1,t_1) the times T_1 and t_1 are equal but that in (T_2,t_2) one has $T_2 < T_1$ but t_2 very much larger than $T_1 = t_1$. For the second pair the total dose D_2 is smaller than the dose D_1 of the first pair but the probability of survival can be smaller because in the period of time from T_2 to t_2 unrepaired lesions can turn into lethal ones.

This phenomenon disappears if assumption (A) is changed to define the survival probability S(t) as G(0,1,0;t) so that the cell is considered "'dead" at time t if it has either lethal lesions or unrepaired ones.

Unfortunately, as shown by Yang and Swenberg in [17], with S(t) = G(0,1,0;t) the shoulder phenomenon also disappears.

Assumption (B) appears natural in that many dose response experiments are indeed carried out by fixing a beam intensity and varying the exposure time. However one can vary the dose in different manner. In an experiment kindly carried out for us by Dr. Tracy C.H. Yang the time of exposure was kept constant, The dose was varied by varying the distance to the gamma ray source. In such a case the second integral in log G becomes

$$A\lambda\int_0^T [\,1-g(\phi)\,] = D\frac{1}{T}\int_0^T [\,1-g(\phi)\,]\,d\tau$$

the dose enters only in a linear manner. Thus log S is a linear function of D. However the observed results of the experiment do have a visible "shoulder". See fig 6

For these reasons, it appears necessary to modify the Neyman-Puri model. We shall consider a modification due to G.L. Yang and C.E. Swenberg.

8. The Yang-Swenberg model.

The model is described in a paper [17] that is not yet published. At first glance it looks a bit like the Neyman-Puri model. However there are three major differences.

1) As in the Neyman Puri model, the primary particles generate secondary ones that act ''independently''. However the probabilities π_1 and π_2 of producing ''potentially lethal'' lesions and ''irrepairable'' lesions respectively have been made to depend on the time since the start of irradiation. This is to take into account the fact that a particle impinging on an already damaged area has a higher probability of creating even more severe damage. Actually the case discussed in detail in [17] makes only π_1 depend on time. It is understood that π_1 might also depend on other factors, such as the rate of which the dose is administered.

2) Cell survival is defined as absence of potentially lethal or irrepairable lesions. In the formulation described in Section 7 above

$$S(t) = G(0,1,0;t).$$

3) The lesions in the cell proceed to follow a Markov process exactly as in the Neyman-Puri model except that provision is made for a period of time where the cells are in non-growth medium. During that time the Markov processes proceed as described. At the time of plating on growth medium the repair activity ceases.

These modifications entail a behavior that is very different from the behavior in the Neyman-Puri model as follows.

Assumption 3 above is just there to reflect how the experiments are conducted. Just as in the case of the Frankenberg-Schwager yeast, holding the cells in non-growth (but not starving) conditions will allow repair to proceed before the damage gets ''fixed'' during mitosis. By ''fixed'' is meant that the damage becomes permanent.

The assumption that repair ceases at the time of plating is subject to discussion. It seems to be a fairly common assumption, justified by the fact that deleterious damage will either prevent mitosis or get transmitted in an irrepairable form to the progeny. However for Human T.1 cells the cell cycle takes about 22 to 24 hours. They will be cultured for 10 days or so to allow them to form colonies. If a cell is in the G_1 phase when plated, it may take almost 20 hours before finding itself in a position to divide. Thus, during that stage, it may have an opportunity to repair quite a bit of damage.

In fact one can gather that this must happen. In the experiment on human T.1 cells carried out by Dr. Tracy C.H. Yang the cells were plated ''immediately'' after irradiation. Here ''immediately'' means the time to get from the irradiation chamber to the cell culture laboratory, a matter of perhaps 3 to 5 minutes. A cell hit by some 2.5 or 3 Grays would have about 1000 SSB and 50 DSB and yet a survival probability around

.50. It could repair some, but not all, SSB during the transit time, but certainly would have little chance to repair the DSB since the half time for that is measured in hours. In one experiment the radiation was applied for 1 minute, leaving little chance of repair during that time. It is often assumed that a cell will not survive if it is left with 2 or more DSB. If so the Human T.1 cells must have performed repair while plated in growth medium.

The cell cultures used by Dr. Yang were asynchronous with perhaps 10% in or very near the mitotic phase. Those may not have had much chance to repair, but, apparently, many of the cells did. Thus, although assumption (3) above can be realistic for some cells, it cannot be generally applicable. It is however a good working assumption for certain cell lines, such as yeast.

The assumption (2) in which survival is defined as absence of unrepaired potentially lethal or lethal lesions is commonly made in the literature. See for instance the repair-misrepair model of C. Tobias. As already mentioned in Section 7 the alternative definition of Neyman and Puri is not in accordance with observation.

The Yang-Swenberg assumption that more serious damage is done by particles that arrive later in the irradiation period than by the early ones needs further discussion. It is easy to show that making the probability π_1 of creating a potentially lethal lesion depend only on calendar time since the start of irradiation does not fit with certain observations: If one increases the dose D without changing the length T of the irradiation period the model does not yield a shoulder. This is as it should be since the secondary particles act independently. However, as we have seen, Dr. Tracy Yang experiments, carried out for fixed T did yield shoulders.

To understand the situation better let us return to the intent behind the assumption in question. It is intended to provide for the fact that a particle that hits an already disturbed area is more likely to yield severe damage than a particle hitting an undamaged area. This suggests that π_1 increases not with time, but with the accumulation of damage. In fact one could conceive of a very crude and unrealistic scenario as follows. A first hit by a particle is not very damaging. It disturbs a certain volume, say v. The total disturbed volume grows as more radiation is applied. A second particle arriving in the cell can either hit outside the disturbed part, this adding to the disturbed volume but not to severe damage, or it can hit a previously disturbed volume with a significant probability of creating a potentially lethal lesion. This is a bit like the Kellerer-Rossi dual action theory, or the Chadwick-Leenhouts explanation of their $\alpha D + \beta D^2$.

If one puts the above into a differential equation, ignoring overlap phenomena and boundary effects, one obtains that π_1 would have the form

$$\pi_1(D,t) = \zeta + \pi_0\{1 - \exp[-qD\frac{t}{T}]\}$$

where D is the total dose applied uniformly over an interval of length T. The π_0 and ζ are constants, ζ representing the probability that a first hit leads to a potentially lethal lesion by itself. The term q is a constant. This is to be compared with the formula $\pi_1(t) = \zeta + \pi_0\{1 - \exp[-at]\}$ of Yang-Swenberg (equation 22). This latter formula is not derived from biophysical arguments in [17]. It is presented as reasonable and leading to mathematical tractability. The $\pi_1(D,t)$ formula given above amounts to take the Yang-Swenberg equation with their coefficient a proportional to dose rate. The argument leading to it is very crude indeed. One can ask whether the formula itself or moderate modifications of it are at all defensible. We shall attempt to argue the case better.

Let us consider a situation where the cells are irradiated rapidly, say in a minute or less, so that enzymatic reactions have little chance of taking place during that time. Consider a small segment of DNA, of, say, 10 or 20 base pairs. With a hydration sheath, it forms a cylinder of about 3nm diameter and 3.4 to 6.8nm length. A photon hitting that cylinder, or perhaps the close vicinity of the cylinder, will knock off a photoelectron that makes a track or better an "efflorescence" that will deposit a certain random number of ions in the cylinder. Suppose for instance that very soft X-rays are used, as in Section 4, part a. Then the efflorescence will have a length of about 7nm. It will contain approximately 10 ion pairs. So there may be anywhere from zero to 20 ions in the cylinder. That is enough to create a lot of damage. Another photon hitting in the same vicinity will add to the damage. One could argue that this second photon is likely to create not only more damage, but more severe damage. Indeed suppose that the electrons from the first photon have created disturbances in one strand of the DNA helix. This will weaken the double helix structure and make it more likely that the next photon will create DSB's. One can also argue that, since the time of irradiation is short all that needs to be taken into account is the total number of ions in the cylinder.

Thus one would be led to evaluate two quantities: 1) the distribution of the number of places in the DNA where a cylinder of length 20 bases contains at least k ions and 2) the probability that in a cylinder that contains k ions there will be SSB's or DSB's. (Some of these perhaps created later by enzymatic cleavage).

The evaluation of the distribution of number of places that contain k ions is a complex non linear problem. The Kellerer-Rossi or Chadwick-Leenhouts formulas are perhaps to be considered guesses at approximate solutions of the problem, together with further guesses at the effect of the ions.

What Yang and Swenberg suggest is to look at the problem in a different way. Suppose that at time t after the start of irradiation one knew the exact position of all the free radicals that are attached to the DNA or close to it. If one assumes (contrary to evidence, but for simplicity's sake!) that all radicals are the same chemical species, the position of the radicals can be specified by a certain measure $M(t) = \sum_j \delta_{X_j(t)}$ where $X_j(t)$ is the position of the j^{th} radical at time t. In a short time, from t to $t + \varepsilon$, the chances are that at most one photon will impinge in the vicinity of the support of $M(t)$. The added radicals are placed independently of the previous ones, leading to an updated measure $M(t + \varepsilon)$. Presumably $M(t)$ has already created a certain distribution of lesions. The increment $M(t + \varepsilon) - M(t)$ creates further lesions in a manner that depends on it and on the previous measure $M(t)$.

If one considers the entire nucleus as a ball and if one knows the configuration of the efflorescence or shower created by one photoelectron, one can in principle write an evolution equation for the measure $M(t)$. To do this one could, for instance, take Laplace transforms of the type

$$\phi(s, t) = E \exp \{ \int s(u) M(t, du) \}$$

for a variable s that ranges through a suitable space of continuous functions on the nucleus.

One should take into account in the evolution equation of the fact that some ions or free radicals are "captured" and thereby rendered inoffensive while some other attach to the DNA and may eventually lead to trouble. In any event the task of writing an evolution equation for the measure $M(t)$ does not look impossible. It may even be possible to incorporate a modification taking into account different chemical species and their own interactions.

To go from the measure $M(t)$ to the actual damage to the DNA and to the additional damage created by a new hit seems to be more complex. This is, at least in part, due to the fact that we do not know the mechanisms by which the radicals cause serious damage and we do not know what is "serious damage".

Assuming that it is a matter of chemistry involving the positions of ions and free radicals, the above analysis suggests a simplifying assumption. It is obviously not correct, but may be close enough to warrant study. It is the assumption that the damage created by a new photon or photoelectron arriving between t and $t + \varepsilon$ depends only on the measure $M(t)$ and not on the distribution and form of the serious lesions already created up to time t.

More precisely, the simplifying assumption is that given $M(\tau)$ for $\tau \leq t$ the probability that a new incident photon creates a serious lesion depends only on $M(t)$ and of

the disposition of the ionization this new photon creates. Such an assumption would be reasonable if the form and position of lesions is a deterministic function of the position of free radicals, described by M(t), or if it is a purely random one independent of variables other than M(t).

Under such an independence assumption one can split the problem into two parts. First find and solve an evolution equation for M(t). Then compute the damage production as M(t) evolves and new photons arrive.

The equations proposed by Yang and Swenberg could be obtained by a procedure of this nature: If the conditional probability that a new photon arriving in $(t, t+\varepsilon]$ given the entire past of the process creates a lesion depends on M(t) but not on the number and place of previously inflicted lesions then the evolution equation for the total number of lesions can be reduced to the Yang-Swenberg form.

In the Yang-Swenberg model, as in the Neyman Puri model, each incident photon is assumed to generate a random number of secondary particles that proceed to create lesions independently of one another. This description probably needs some revision. A single hit by a (.3) kev photon will create many ions and many radical in close proximity to each other. They may act together to create, perhaps, just one lesion, but perhaps a more severe lesion than would be expected from the electron shower of a 1 Mev photon since this latter is more spread out spatially.

This concludes our theoretical argument for the Yang-Swenberg approach. One can however point out certain aspects that seem to need further study.

One of them is that the discussions of the repair system suggest that it is not enough to have only one kind of "potentially lethal" lesions. For instance DSB must be classified according to whether they are blunt or frazzled. There is also the matter of distinguishing them from chromosome breaks or breaks affecting two sister chromatid at conjugated spots.

Another feature that needs clarification is as follows. Yang and Swenberg fitted their model to several sets of experimental data. The curves fit all right, but the values of the fitted parameters are not always compatible with what is known of the mechanisms involved. For instance the coefficient b in the fit the Tradescantia data is given as 151/min. This is rather fast for enzymatic action while the .077/hour of the fit for the yeast experiment seems slow.

We attempted to fit the same model to the experimental data on Human T.1 cells of Dr. Tracy C.H. Yang. The fit was not particularly good. However this can be attributed to the fact that we did not take into account the asynchronous nature of the cell cultures. As we have argued before in Section 5, this must be taken into account.

9. The repair-misrepair model, (RMR).

This model was proposed by C. Tobias and his colleagues. It is described in [15] and reviewed in [14]. The model is based on two main assumptions:

1) Radiation produces in the cells certain lesions called "uncommitted" because their fate will be decided later. The lesions are treated as if they were all alike. Their expected number is proportional to the radiation dose.

2) There are two kinds of repair mechanisms. In one of them the rate of repair at time t is proportional to the number U(t) of uncommitted lesions present at that time. In the other kind, the repair rate is proportional to $[\,U(t)\,]^2$.

Both linear and quadratic repair can lead to correct repair, to transformation or to lethal misrepair.

In the papers cited, the authors derive differential equations for the "expected number" of various forms of lesions. For instance U will satisfy an equation of the type $\frac{dU}{dt} = -\lambda U - \kappa U^2$. The equations are solved and then recourse is made to "Poisson Statistics". This means that for instance at time t the actual number of uncommitted lesion is taken to be a Poisson variable with expectation equal to the solution U(t) of the above equation. The same type of procedure is implicit in the Chadwick Leenhouts argumentation, the Kellerer-Rossi theory and the LPL and a model of S. Curtis that merges the Kellerer-Rossi approach with the RMR.

That kind of argumentation could be defended if the numbers such as U(t) were large. However this will not be the case from some point on if the cell is to survive. Indeed the assumption is made that "survival" means absence of uncommitted and misrepaired lethal lesions.

It is easy to translate the assumptions made above to describe evolution in the form of a Markov process. The procedure is as follows. Let N(t) be the actual number of uncommitted lesions at time t. Then the probability that one such lesions will change status in the interval $(t,t+\varepsilon]$ is $[\,\lambda N(t)+\kappa N^2(t)\,]\varepsilon+o(\varepsilon)$. The probability that more than one change of status takes place is $o(\varepsilon)$. If a change takes place it can be either to correctly repaired status, to transformed (misrepaired but non lethal) or to lethal. The probabilities of these various possible changes can be made different for the linear and the quadratic form of repair. For instance a simple model is obtained if linear repair is always correct and quadratic repair is always misrepair.

The Markov process obtained in this manner have been studied by N. Albright in [1]. Later Albright modified the model to take into account the track structure of the radiation damage, using different interaction coefficients κ for the within track and between track interactions.

One of the main difficulties with the Markov process RMR is its mathematical intractability. Albright was able to obtain explicit solutions, but they are complex and difficult to study except numerically.

It was shown by I. Janssen, [11], that although the deterministic + Poisson statistics approach and the Markov process approach lead to dose response curves of the same general shape, interpretation of coefficients in fitted curves has to be done carefully. Coefficients derived from the deterministic-Poisson statistics approach do not yield Markov process curves that fit.

This was not surprising. However another surprise was in store: when the Markov Process curves were fitted to actual data, they gave an expected number of initial uncommitted lesions of the order of two. This is not in keeping with the estimates of Cole, of Elkind and of Bryant reported in Section 5.

Strong arguments have been advanced in favor of the RMR model. From the purely biological side one knows that lesions can interact since one observes chromosome aberrations of many different types. Tobias *et al* also argue that the RMR model is best able to explain the observations on cell lines that have lost part or most of their repair ability. We already mentioned the case of brewer's yeast with 0 to 4 defective repair genes. There are other examples given in [14] and [15].

A point in favor of the RMR model or of saturable repair models is given by Goodhead in the introduction of [10]: "Because of the low probability of multiple tracks coinciding in a given small volume, these multiple track effects must involve long distances in the macromolecular scale".

This can be taken to mean that the interaction claimed to be present in the Kellerer-Rossi dual action theory would be either a long distance one or would be too weak to be of significance.

However the case for the RMR model is not a conclusive one. For instance Yang and Swenberg, quoting Elkind, say that after plating in growth medium there is very little repair. Yet cells plated immediately after X-ray exposure (250 kvp. X-rays) show the typical shoulder that RMR would attribute to repair. The situation is uncertain however. Elkind in [7] seems to say that repair takes place under growth conditions, at least for that fraction of repair that is fast repair.

Tobias *et al* point out that A-T cells (cells from Atasia telangectasia patients) have lost their repair ability and, as expected in RMR, yield dose response curves without shoulders. However Elkind says that the cells must be able to carry out a very considerable amount of repair. Also experiments where cells were put in hypertonic solution for some time after irradiation show that this exposure does not affect further the behavior of A-T cells but prevents normal cells from repairing properly. However,

according to van der Schans [16], reported by Elkind in [7] the A-T cells repair DNA breaks at a normal rate and to a normal extent.

It is difficult to interpret the effect of oxygen through the RMR model, because it starts functioning only after the lesions are created. Indeed it has been said that cells irradiated in hyperbaric oxygen are much more radiation sensitive than hypoxic cells but that the effect is very small if the oxygen is applied a few milliseconds after irradiation.

One could modify the RMR model in many ways. One procedure would be to allow different kinds of initially uncommitted lesions. An attempt of this nature has been described by S.B. Curtis in [5]. Curtis' initial model, called the LPL (lethal-potentially lethal) model allows the radiation to create two kinds of lesions, lethal ones that cannot be repaired and potentially lethal ones that can be repaired. This allows a bit of flexibility in the initial slope of the dose response curve. Curtis, following Tobias, allows the radiation to be spread out in time and takes that into account in his differential equations. Otherwise the model is a small modification of the RMR model. It becomes more distinct from RMR by introduction of two kinds of potentially lethal lesions to discuss the effect of hypertonic medium culture. There Curtis distinguishes a class of lesions that can be repaired rapidly from a class that admit only slow repair.

This certainly makes the model much more flexible and thereby able to fit a larger variety of observations.

Curtis treats the model by deterministic kinetics followed by Poisson statistics. One could use the Albright procedure to make it more fully stochastic. (Note that if this is done the distribution of remaining lesions at any time t is not going to be a Poisson distribution!)

The first part of Curtis' technical report [5] assumes that lesion production is proportional to dose, as in Tobias's RMR. However in a second part Curtis allows "prelesions" to combine to form an irrepairable lesion if "two or more prelesions occur within a critical region of average extension X_0 along the track". This brings him back to the Kellerer-Rossi dual action theory. Curtis seems to need this to explain the oxygen effect.

It should be obvious that a model that combines the dual action theory with the RMR model has a lot of flexibility. After all each of the two did reasonably well by itself. Their combination can only do better as far as fitting experimental observations.

It should also be clear that one could combine the dual-action-RMR merger with the saturable repair model of Goodhead. To make the whole affair fully stochastic is not difficult, in principle, however the resulting equations will be rather complex and

difficult to solve. The solutions will be difficult to interpret. But perhaps the main question is what would actually be gained? The models do not give a particularly good insight in what goes on in the cells. Thus, at this point it is probably good to reflect back on other avenues.

10. Concluding remarks.

Stochastic models of natural phenomena are, by their very nature, simplified descriptions of the mechanisms involved. They do serve a purpose only if they catch some of the essential features and lead to a better understanding of the situation. In the present case it seems that much more work needs to be done before one will feel at ease with applications of the models. The work will have to be mathematical, but also experimental.

The fact that several disparate models lead to dose response curves that fit reasonably well the experimental ones does not by itself show that any of the models actually represents what is happening. According to the literature, the Kellerer-Rossi dual action theory, the Yang-Swenberg model, the repair-misrepair models and the saturated repair models are all able to provide dose response curves that fit fairly well at least a good part of the experimental evidence. Since they rely on quite different mechanisms to explain observed interactions one is left with the uneasy feeling that neither of them has yet caught the essentials of the process.

In the present paper we dealt only with the possible effects of photons in a range from .3 kev to 1 Mev. We understand the pressures that have led many authors to elaborate theories encompassing all forms of ionizing radiation, be they X-rays, neutrons or fast heavy ions. However these various forms of radiation act in disparate ways. It does not seem sensible to expect to find in this manner a clear and simple picture of the phenomena involved. As we have explained here, photons are already a very complex affair.

The speculations of Section 4 suggest that even the very start of the Neyman-Puri and Yang-Swenberg models may be in need of modification. Both assume that a primary particle generates a random number ν of secondaries with $Es^{\nu} = g(s)$ and that these secondaries act independently of each other. The picture suggested by Section 4 is different. For very soft X-rays a primary photon leads to a very localized (7nm length) cluster of around 5 to 10 ion pairs. These are unlikely to act "independently". They may act in concert and perhaps yield something that could be called "one lesion". This means that the choice $g(s) \equiv s$ made by Neyman and Puri for X-rays can perhaps be sustained if "secondaries" is replaced by "lesions". The picture changes drastically as soon as one looks at somewhat more energetic photons. If a photoelectric electron acquires 4 kev, it will disperse its energy along a 500 nm "track" or, better, "efflorescence". Many of the small branches of the efflorescence will create ion clusters similar to those discussed just above. It seems highly unlikely that the actions of these clusters can be described by considering them stochastically independent.

One of the complications suggested by our Section 4 is that the shape of dose response curves ought to vary according to small modifications of the experimental protocol such as thickness of container walls or thickness of the culture medium situated above the cells. We do not know of any report of experiments carried out for the specific purpose of checking such variability. They seem feasible. If the shape of the response curves turns out to be independent of such protocol variations it could mean that the dual action theory of Kellerer and Rossi and similar models do not capture the essence of the interactions.

The Neyman-Puri and the Yang-Swenberg models use two basic kinds of lesions: lethal ones and potentially lethal ones. (They also use transforming or cancerous lesions that we have not discussed at all here). This does not seem to be enough to describe what happens. We already know that there are SSB's, DSB's and that the DSB's can be blunt or frazzled. We know from Bryant's experiments that blunt DSB's are less easily repairable than the frazzled ones. Such a variability in the nature of initial lesions can be embedded in most models. For instance it can readily be embedded in the Yang-Swenberg analysis. The trouble is that the resulting flexibility will make the models fit almost anything one wishes without assuring us that indeed we have caught any essential part of the mechanisms involved. It seems that special experiments will have to be devised to sort out the possibilities. It should be apparent that much further progress could depend on a better understanding of what the lesions are and how they are created. However some information can be obtained even from rather crude experiments. Note for instance the explanations by Curtis [5] of the effect of holding cells in hypertonic solutions.

To end on a different matter, let us note the following. We had started the present investigation on the mistaken assumption that effects of photons would admit a reasonably simple analysis. This is clearly not the case. For instance typical 220 kvp X-rays have a part with a continuous spectrum, leading to a complex structure for the effects of the absorbed energy. Monochromatic gamma rays may look simpler but they scatter electrons by Compton effect leading again to a broad continuous spectrum for the ejected electrons.

This suggests that experiments designed to figure out what happens in the cells might be better carried out directly with electrons and that the electron beams used should have as narrow an energy spectrum as possible. One may even be tempted to select the value of the energy for special effects since several molecules react differently to different energies.

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Legends

Fig 1A - From [15].

Fig 1B - From [18]. Note the large swath of destruction caused by high let radiation, suggesting that a single particle may be lethal.

Fig 2 - From [8]. Effect of holding yeast cells in medium that does not allow much replication.

Fig 3 - From [8]. Note that the amount of repair performed at the second dose is very substantial, showing that a good part of the repair system is not saturated.

Fig 4 - From [8]. The amount of curvature increases with repair time.

Fig 5 - From [15].

Fig 6 - From [15]. Survival probability varies according to when the radiation is administered during the mitotic cycle.

Fig 7 - From [17]. Effects of dose rate.

Fig 8 - From Martin Burger "On the spatial correlation of ionization events in water" Schematic picture of a small electron shower.

Fig 9 - From [17]. Fit of the Yang-Swenberg model to data from [8]. (Solid lines. The dotted line is included to show the effect of varying one of the parameters).

Fig 10. Plot from an experiment carried out by Dr. Tracy C.H. Yang varying doses but keeping total time of irradiation constant. Note the pronounced shoulder. It is even stronger than it seems since the cells were not synchronized.

Note also the increase in survival when passing from a 1 min irradiation to a 30 or 60 minute irradiation ltime. The small difference between the 30 and 60 minutes is probably not compatible with an explanation based on a repair mechanism acting at constant rate in time.

Fig 11. Same data as Fig 10, plotted on a D versus $-\frac{\log S(D)}{D}$ coordinate system. For a linear relation between D and log S(D) the lines are horizontal ones. For a linear + quadratic they would be straight lines with slope equal to the coefficient of the quadratic term.

Fig 12. The 1-minute data of plots 10 and 11 with a Yang-Swenberg formula fit with $g(s) = s$.

Fig 13. Data from an experiment carried out by Dr. Tracy C.H. Yang on melanoma cells. The dose was varied keeping total irradiation time constant. Note the pronounced shoulder.

Fig 14. Equations from the Yang-Swenberg model when each primary particle gives only one secondary that can produce lesions.

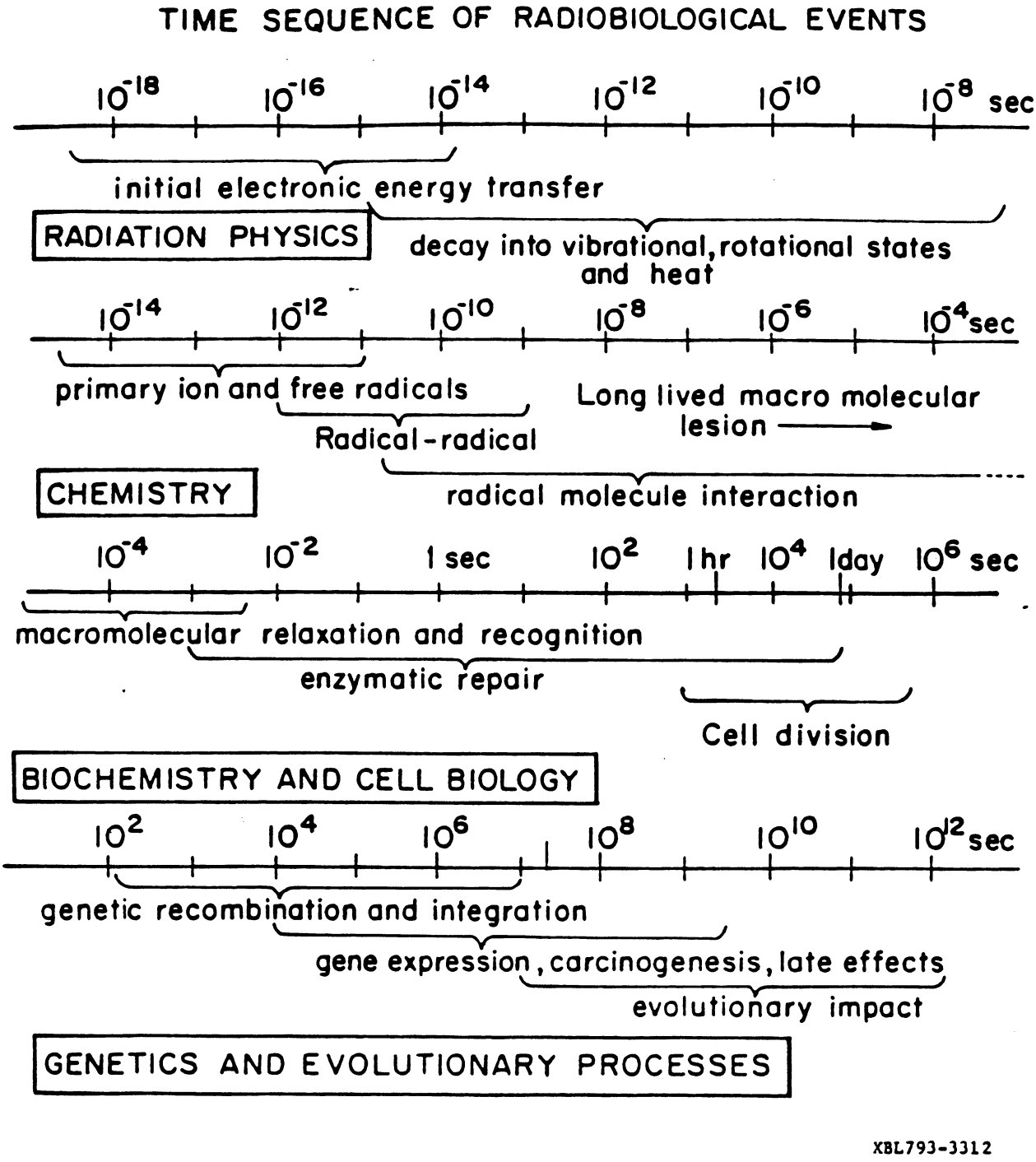


Figure 1A

Time sequence of the radiobiological events found with cell irradiation, from the initial electronic energy transfer through late genetic effects. The physics events include time for heat transport.

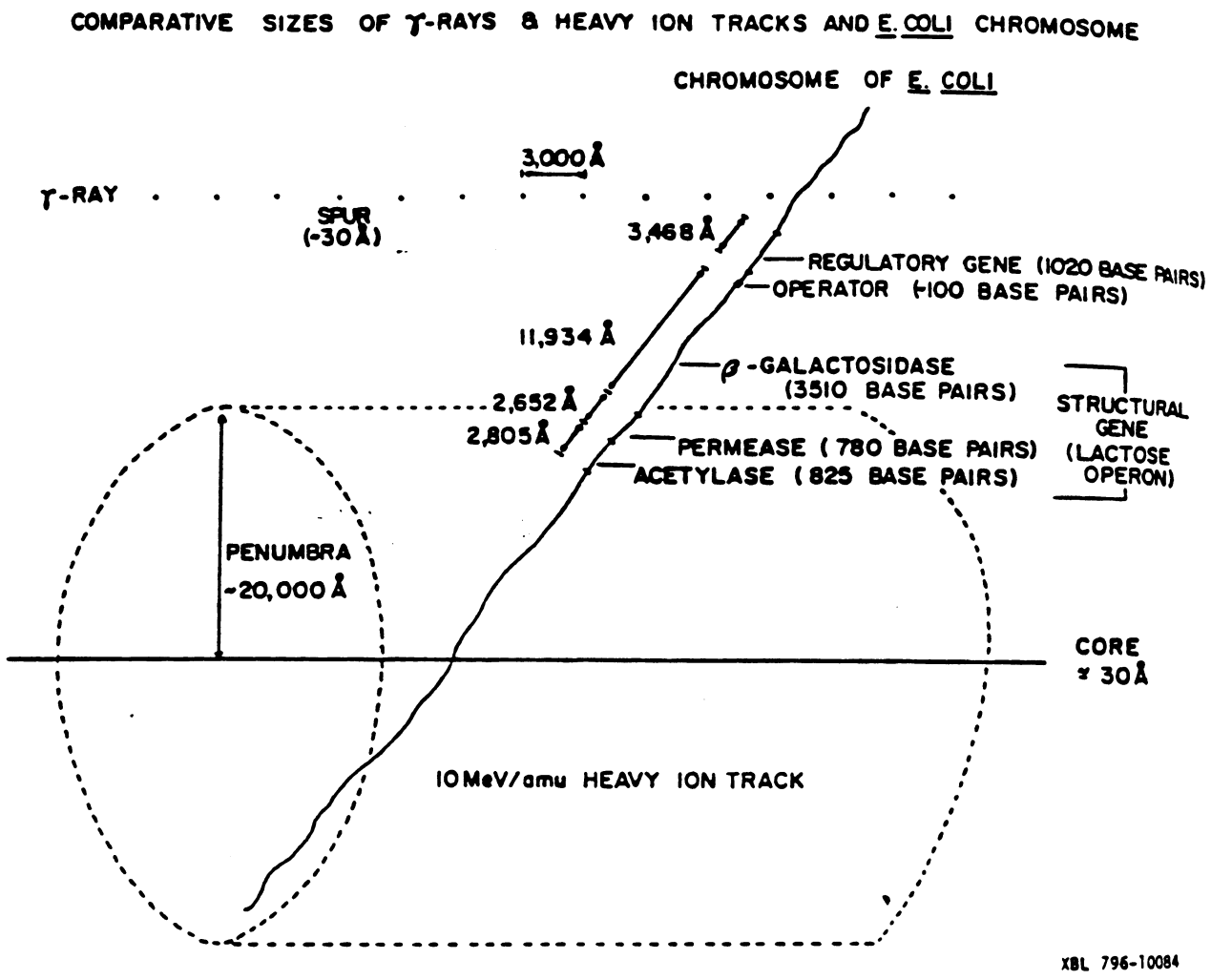


FIGURE 18

(XBL 796-10084)

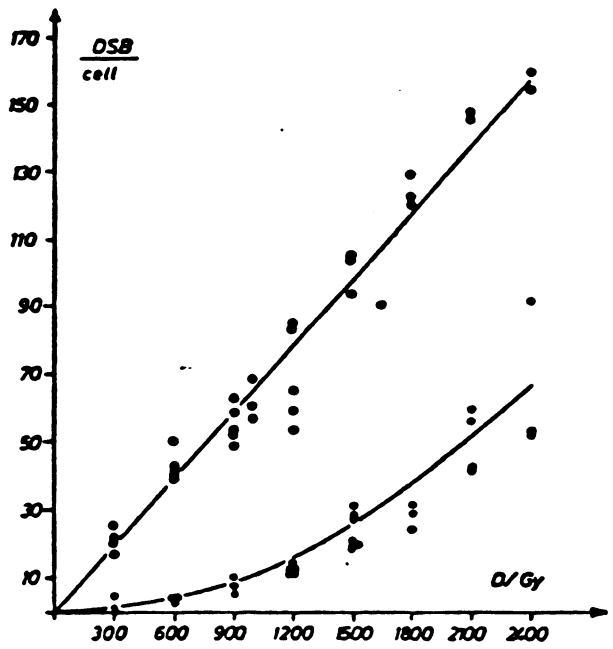


FIG. . Effect of 24 hr liquid holding on the number of dsb/cell. The number of dsb/cell is plotted as a function of dose. Cells were analyzed for DNA double-strand breakage immediately after irradiation (O) and after a subsequent liquid holding treatment of 24 hr (●).

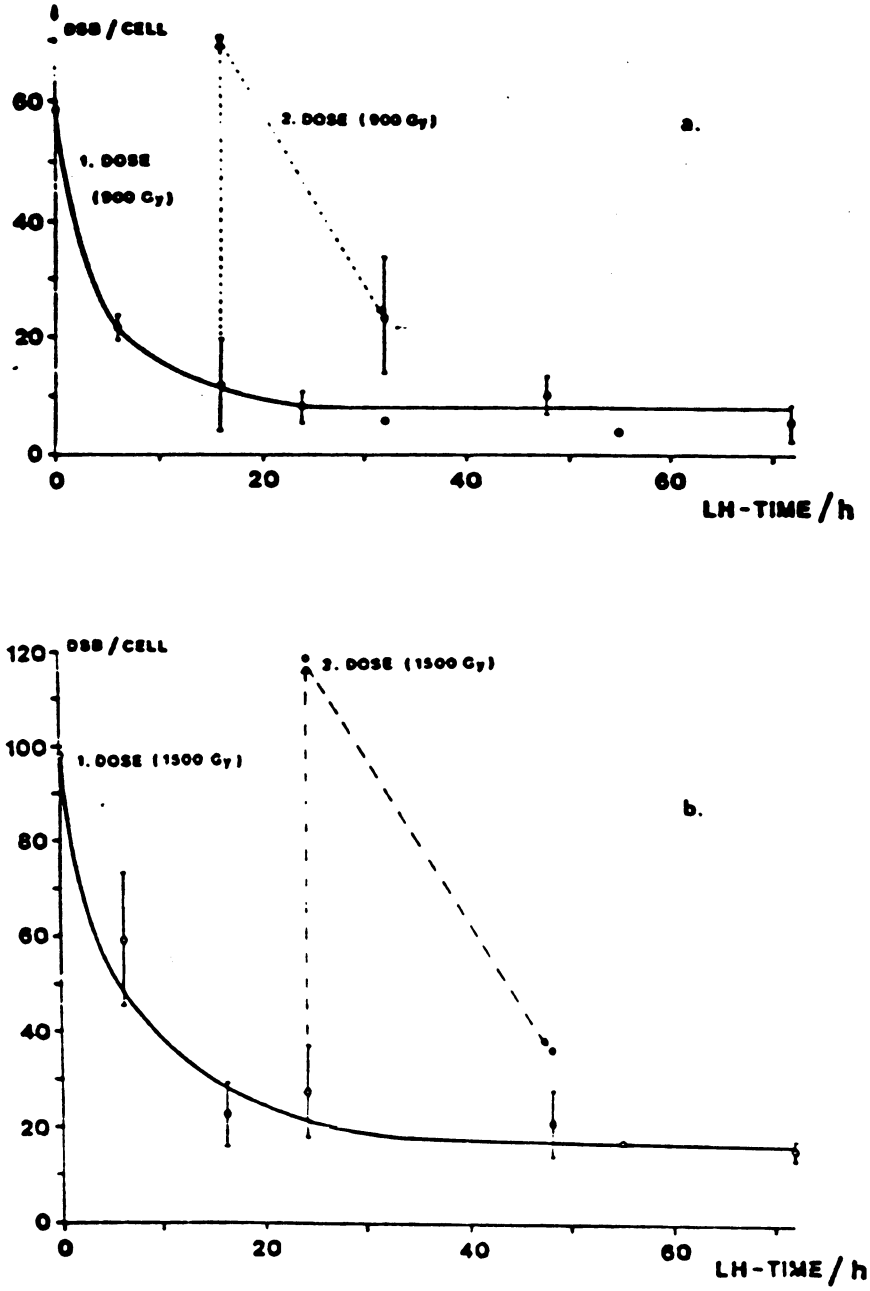


FIG. Split-dose experiments with a liquid holding interval. The number of dsb/cell is plotted as a function of time of liquid holding treatment. After having received a first dose of 900 (a), 1500 (b), and 2500 Gy (c) cells were allowed a period in liquid holding medium to perform maximum repair of dsb, the time in each case depending on the first dose. Then a second dose was given followed by another liquid holding period in fresh medium sufficiently long to allow maximum repair of dsb induced by the second dose.

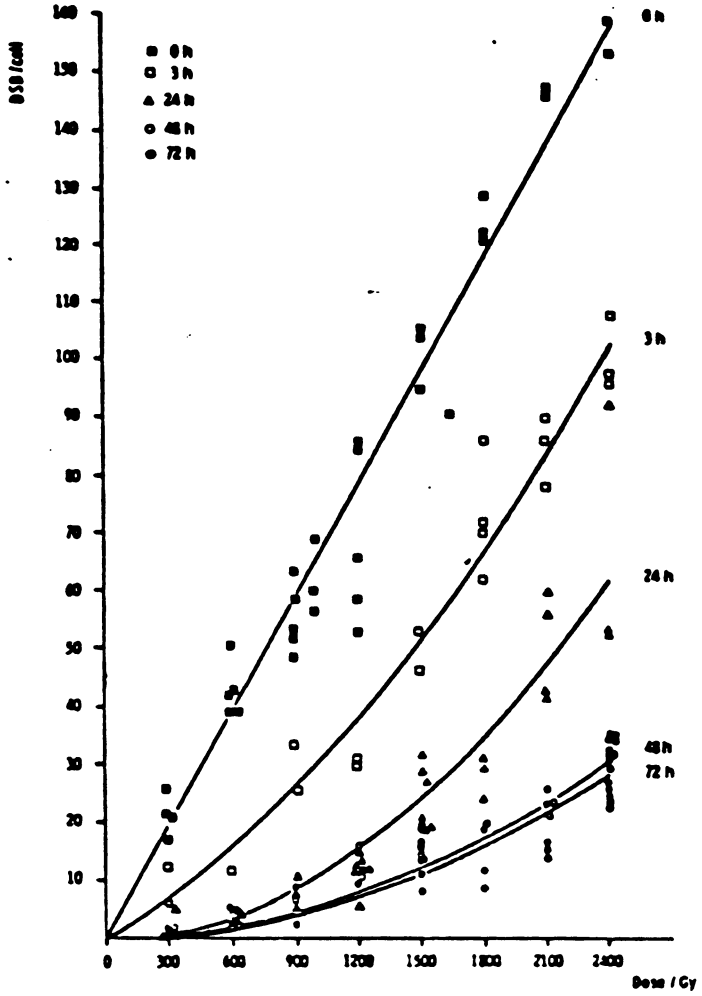
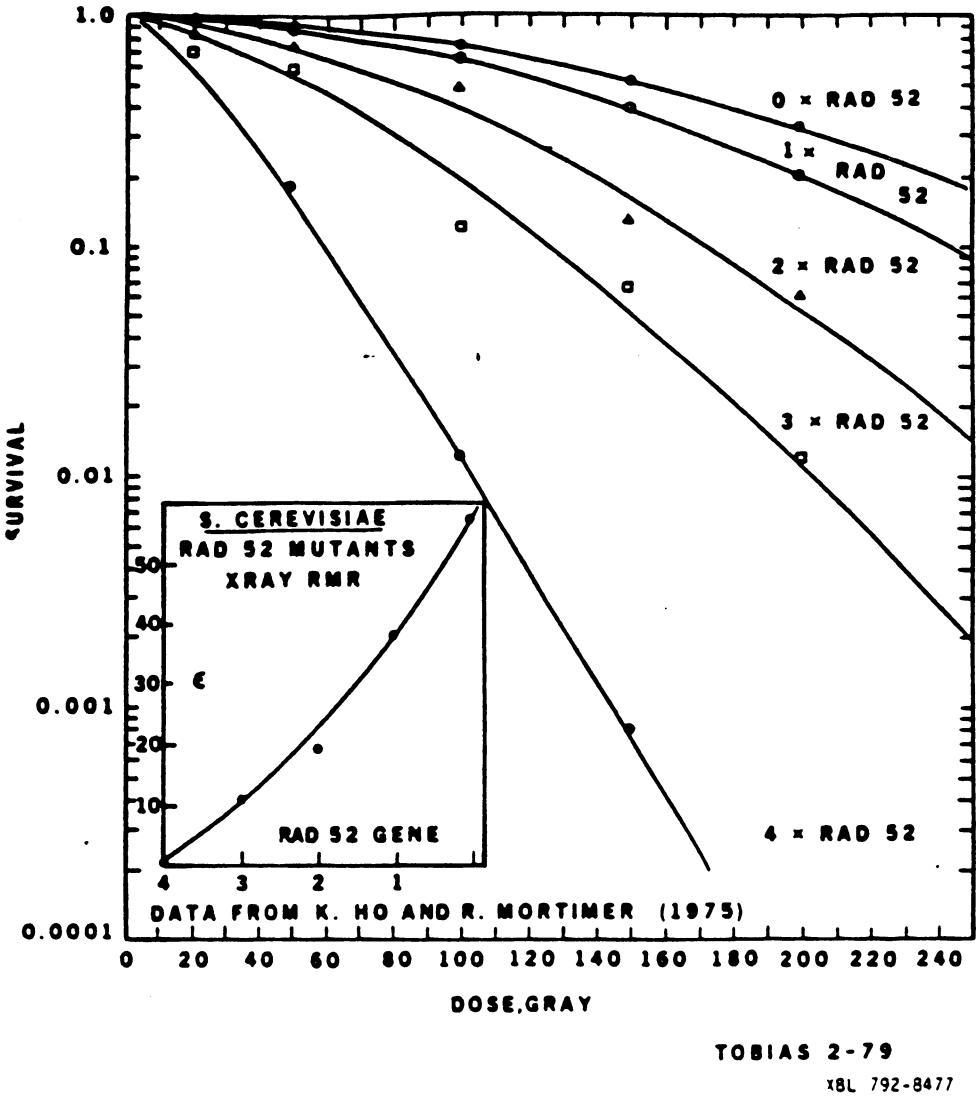


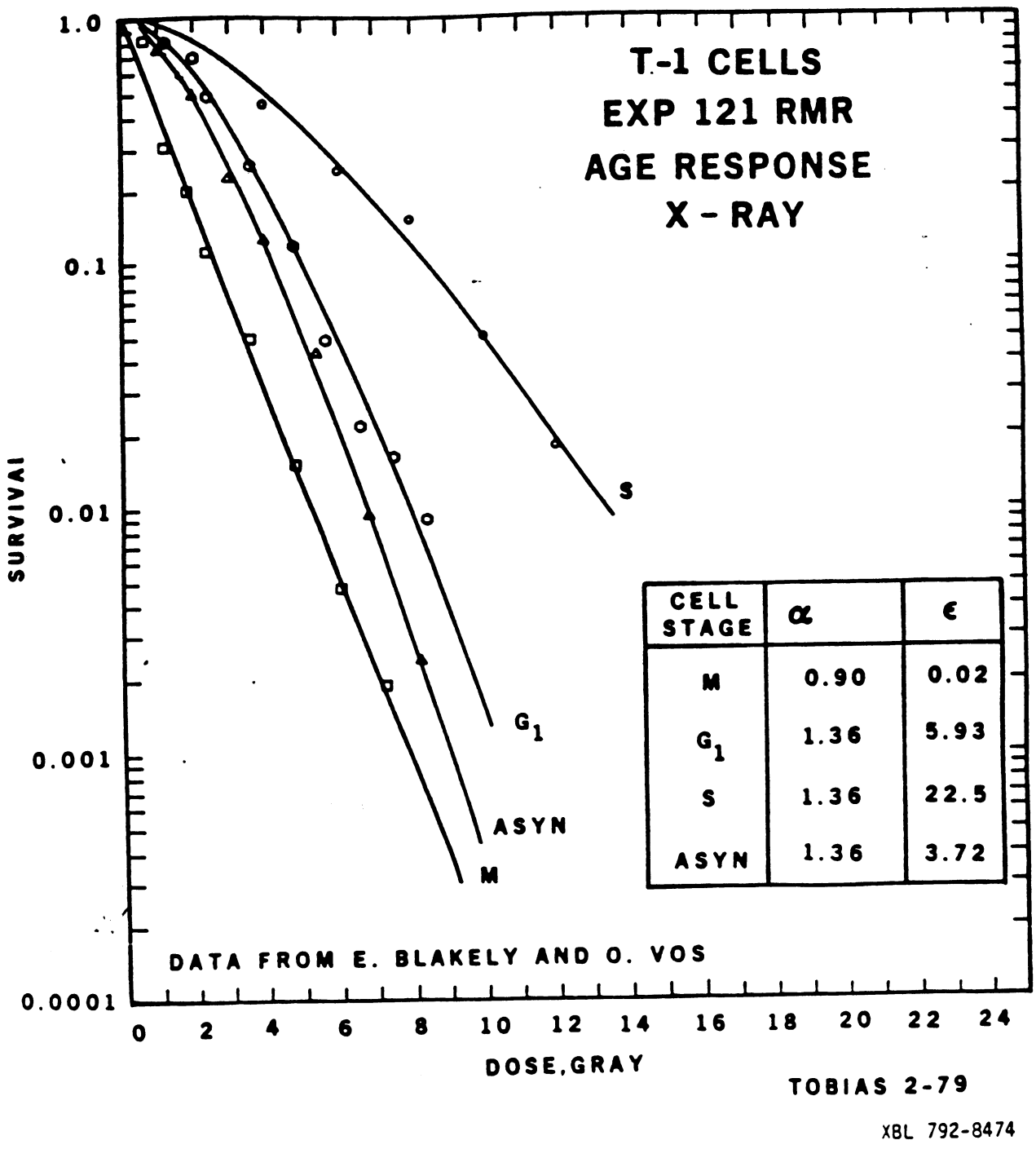
FIG. . . Repair of dsb under nongrowth conditions. The number of dsb/cell is plotted as a function of ...e. Irradiated cells were liquid held for 0 (■), 3 (□), 24 (▲), 48 (○), and 72 hr (●) before analysis ... double-strand breakage.

... dsb repair in irradiated cells also demonstrate that after a 24-hr liquid holding ...atment not all the reparable dsb are repaired and that cells need longer treatment ... repair these dsb. When irradiated cells are plated after 24 hr treatment on ...trient agar as is the case in survival assays, these remaining reparable dsb may be ...paired. That this is so has been demonstrated in our preliminary experiments ...sults not shown) and in the work of Resnick (*32*). Our results do, however, ...ow a clear correlation between an increase in colony-forming ability and a ...ncomitant decrease of the number of dsb per cell due to liquid holding treatment ... irradiated cells; this indicates a *possible* causal relationship between dsb and ...ll killing. Other evidence in favor of a causal relationship between dsb and yeast



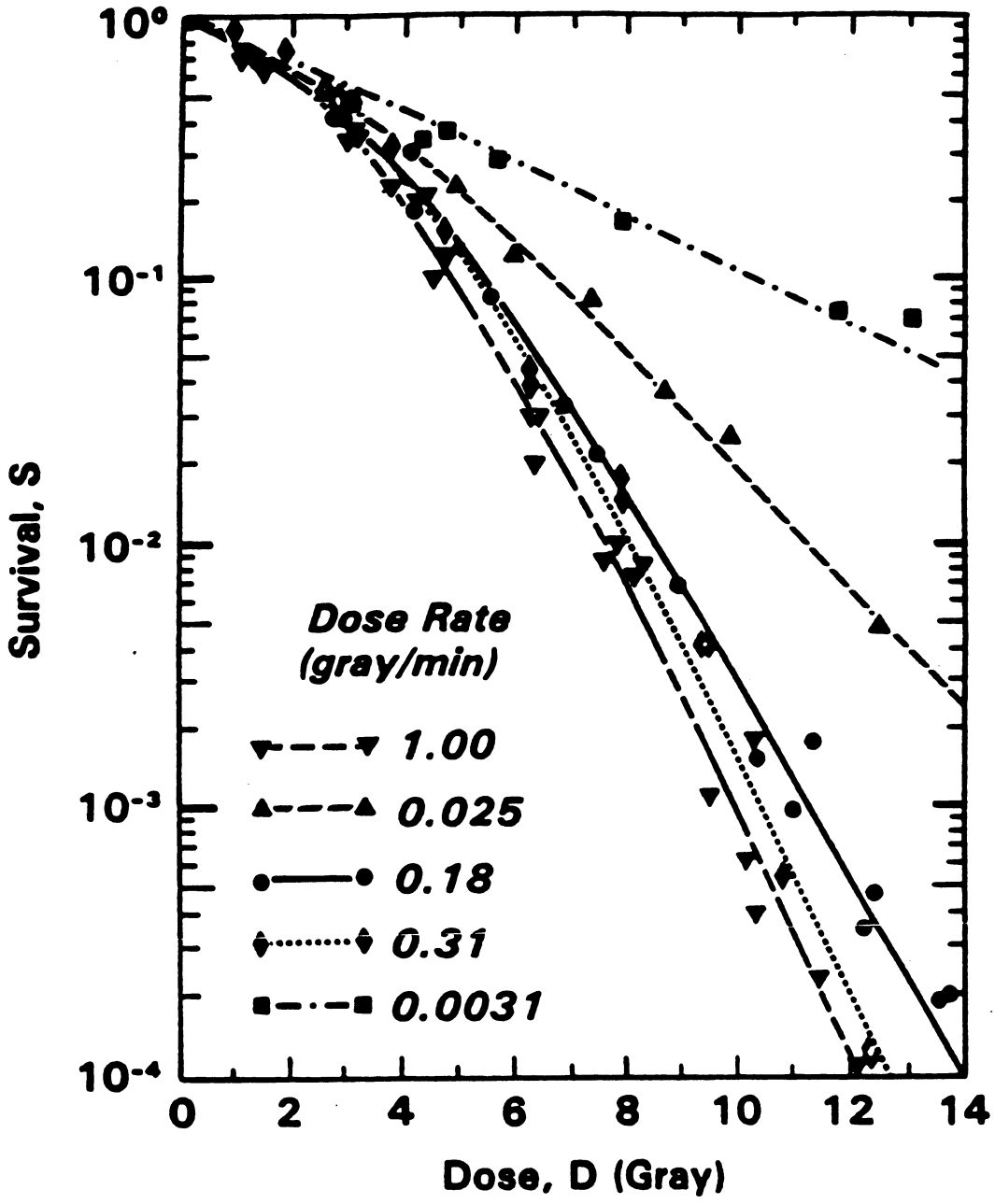
Figure

Experimental survival curves for tetraploid yeast cells (from the work of Ho and Mortimer 1973), fitted by the RMR model. Rad 52 is a repairless gene. The survival curves vary with gene dose in a manner generally in agreement with the RMR model. With α and k fixed, the values for ε are given in the insert. If we assume that ε measures the repair rate and that ε is proportional to the repair enzymes, then it appears that the amount of repair enzyme available increases approximately proportionally to the gene dose of the wild type gene (+).



Figure

T-1 cell survival curves obtained with 220 kV x rays for cells synchronized by mitotic shake-off. The data are from Vos et al. (1966) and Blakely et al.


10⁰
10⁻¹
10⁻²
10⁻³
10⁻⁴
Survival, S
0
2
4
6
8
10
12
14
Dose, D (Gray)
Dose Rate
(gray/min)
1.00
0.025
0.18
0.31
0.0031

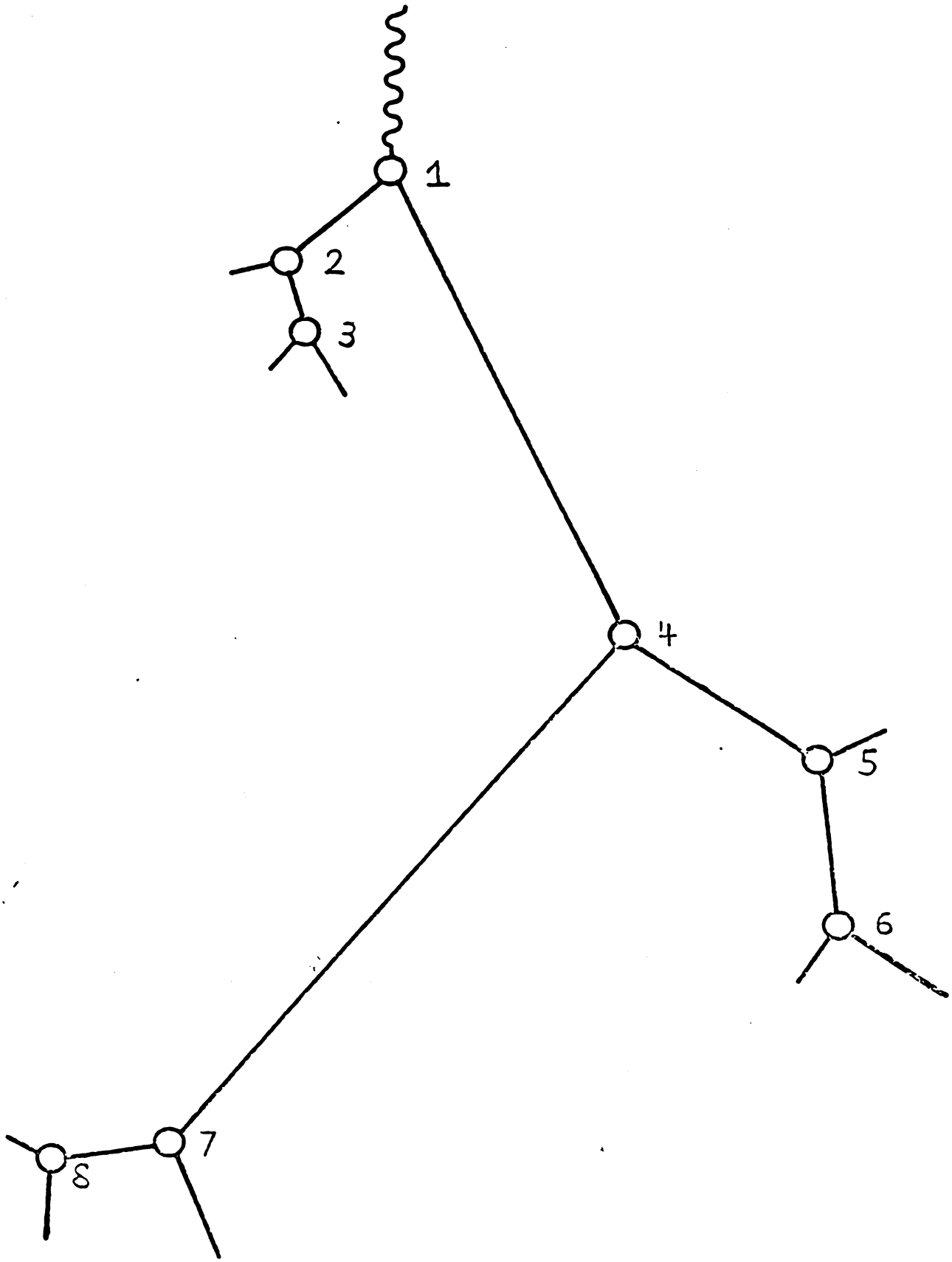
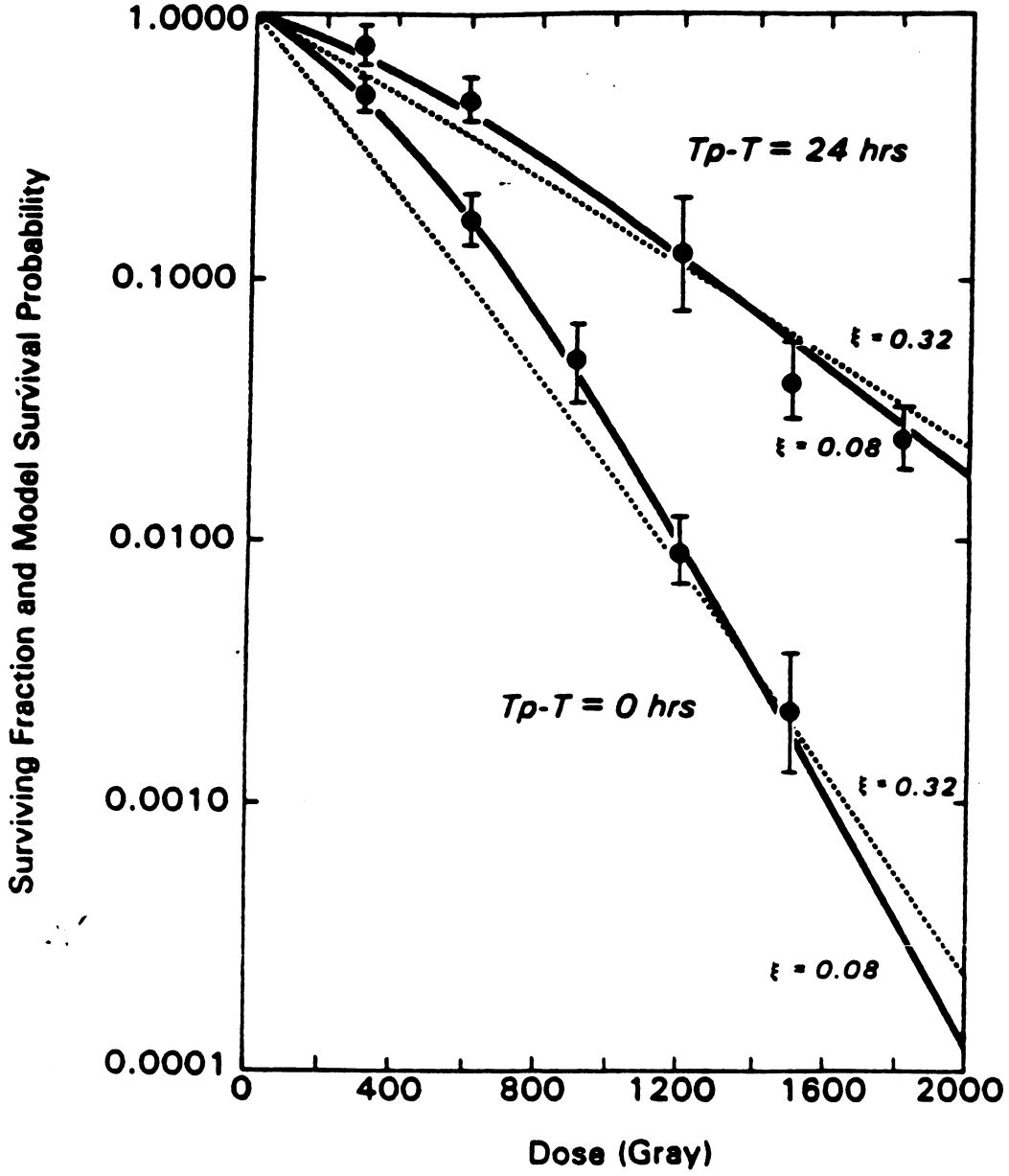
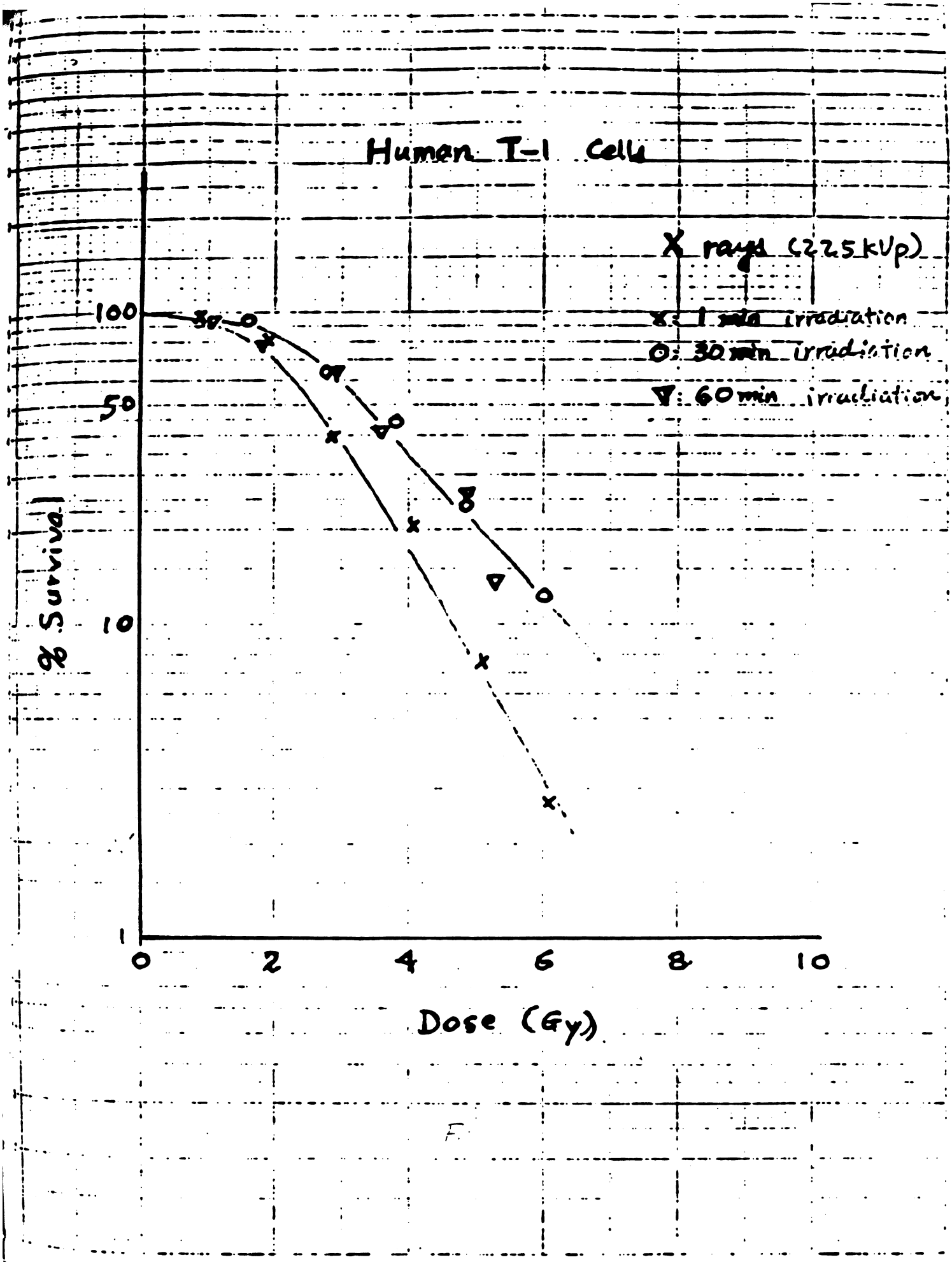
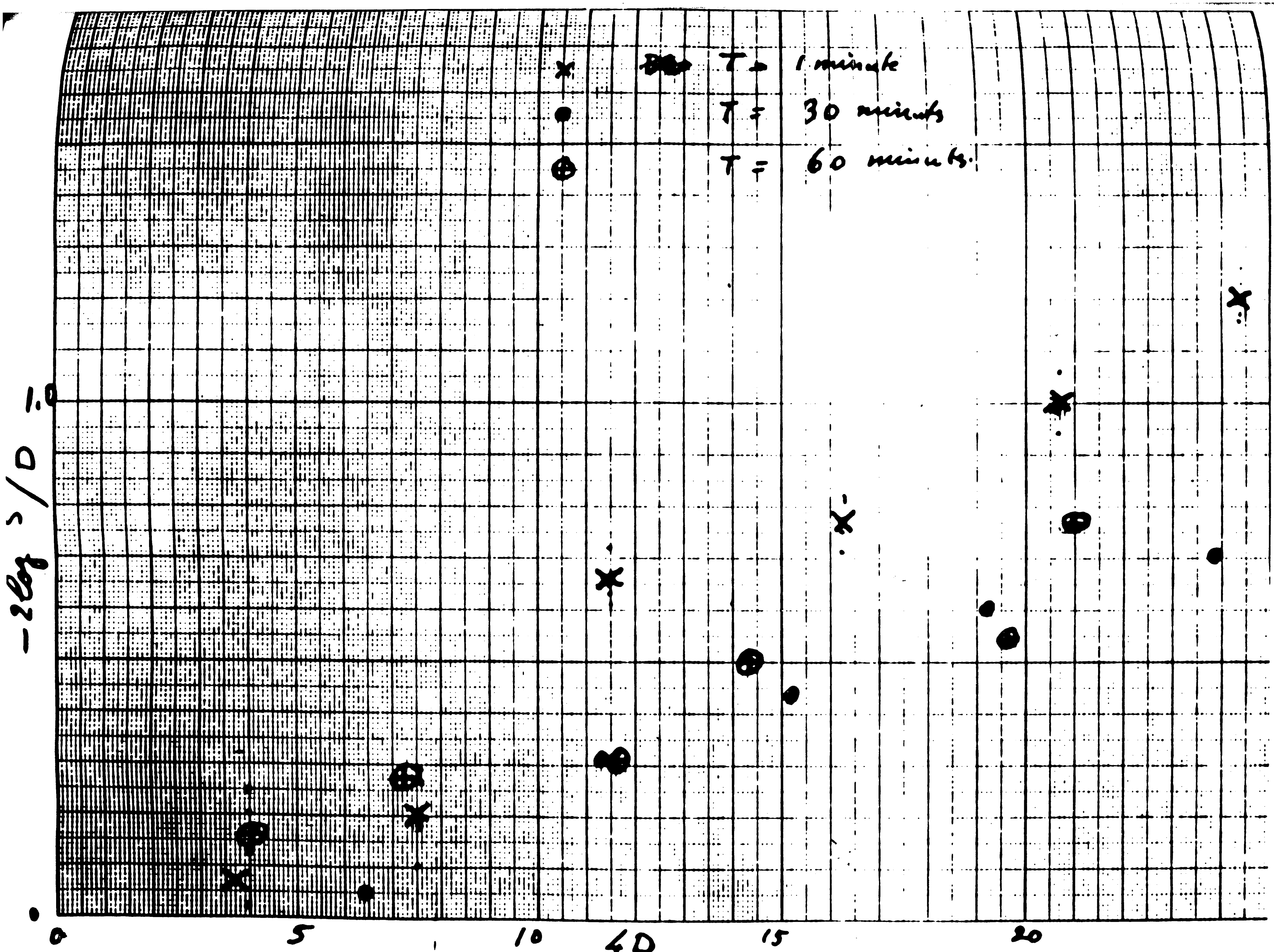
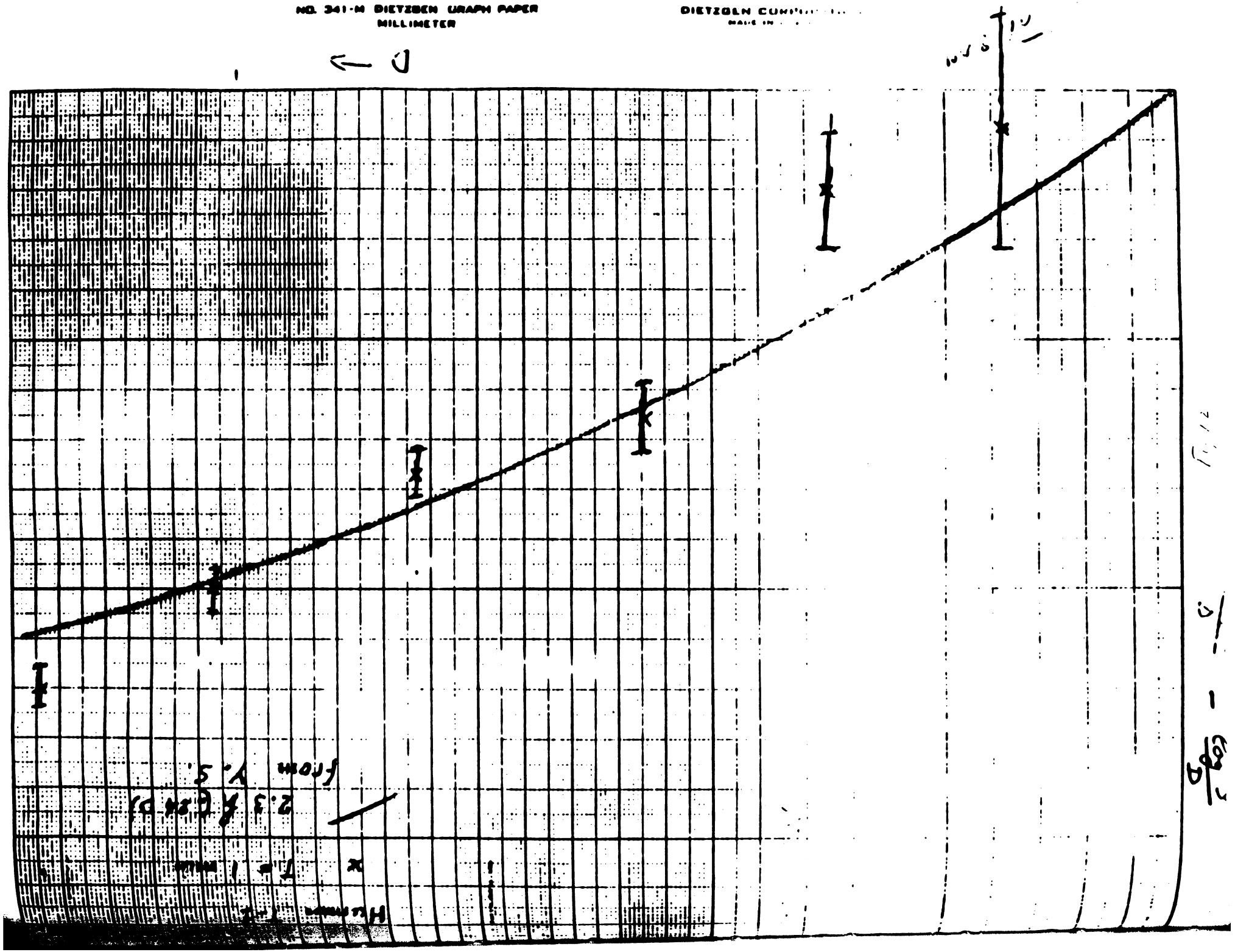


Fig. 1


1.0000
0.1000
0.0100
0.0010
0.0001
Surviving Fraction and Model Survival Probability
0
400
800
1200
1600
2000
Dose (Gray)
Tp-T = 24 hrs
ξ = 0.32
ξ = 0.08
Tp-T = 0 hrs
ξ = 0.32
ξ = 0.08


Human T-1 Cells
X rays (225kVp)
×: 1 min irradiation
○: 30 min irradiation
▽: 60 min irradiation
% Survival
100
50
10
1
0
2
4
6
8
10
Dose (Gy)


× T = 1 minute
● T = 30 minuts
⊕ T = 60 minuts.
−2log ⁰/D
1.0
0
5
10
15
20
4D — Ang


NO. 341-M DIETZGEN GRAPH PAPER
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DIETZGEN CORP.
MADE IN U.S.A.

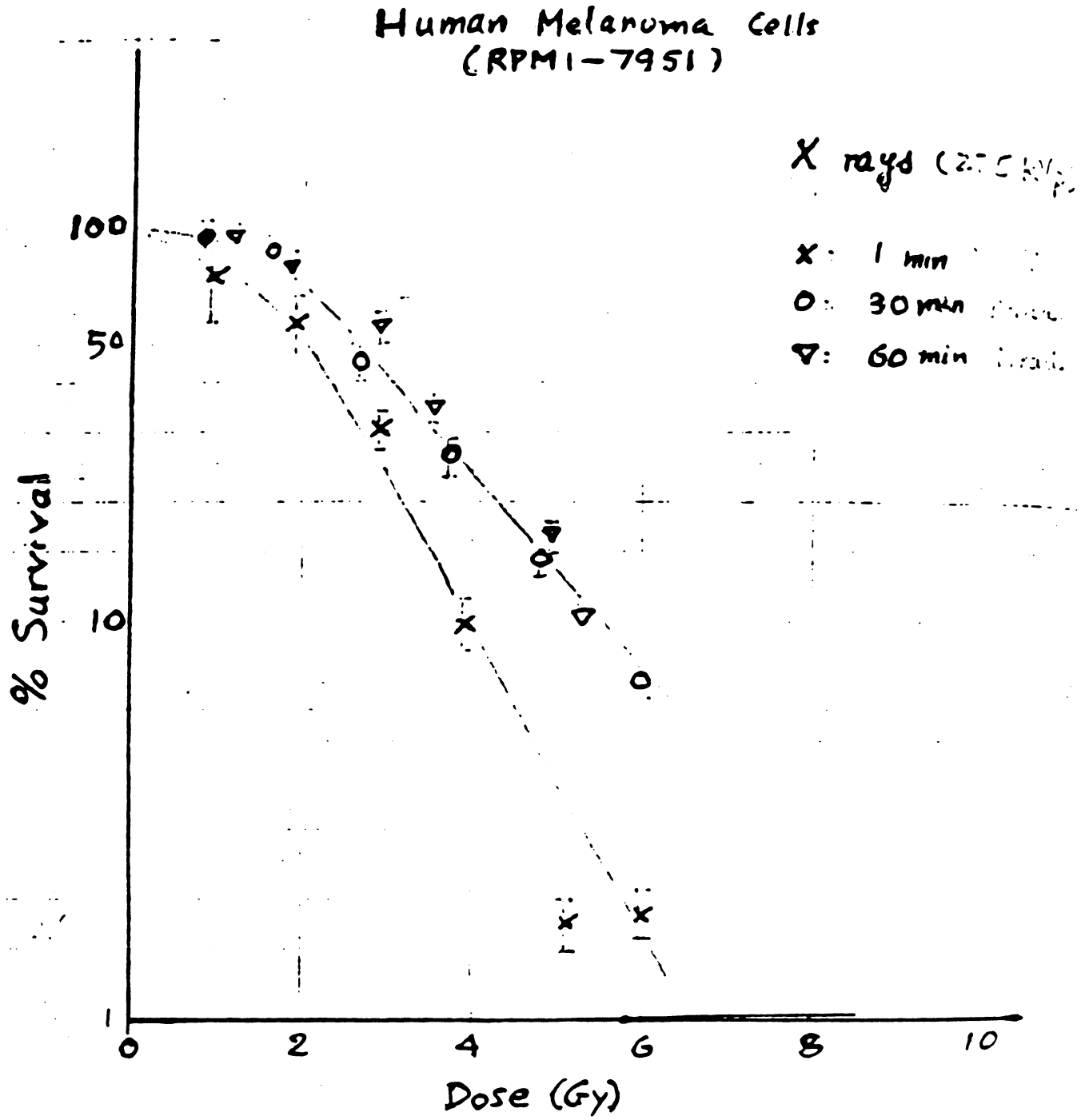
Human Melanoma Cells
(RPMI-7951)
X rays
x: 1 min
o: 30 min
∇: 60 min
100
50
10
1
% Survival
0
2
4
6
8
10
Dose (Gy)

Figure 14

C^{-1} = number of particles per Gray hitting nucleus.

S = survival probability

$$-\frac{C \log S}{D}$$

$$= \pi_2 + \zeta\left[\frac{\gamma}{b} + \left(1 - \frac{\gamma}{b}\right) e^{-b\Delta}\right]$$

$$+ \pi_0\left[\frac{\gamma}{b} + \left(1 - \frac{\gamma}{b}\right) e^{-b\Delta}\right] h(qD)$$

$$-\zeta\left(1 - \frac{\gamma}{b}\right) e^{-b\Delta} h(bT)$$

$$+ \pi_0\left(1 - \frac{\gamma}{b}\right) e^{-b\Delta} \frac{[bT\,h(qD)K - qD\,h(bT)]}{qD - bT}$$

T = time of irradiation

D = dose in Grays

$$\pi_1(v) = \zeta + \pi_0\{1 - \exp[-qD\frac{v}{T}]\}$$

$$h(x) = \frac{e^{-x} - 1 + x}{x}$$

$$b = \alpha + \beta + \gamma$$

Δ = time to plating.

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