RADIATION DAMAGE TO BULL SPERM MOTILITY. I.

X-RAY EFFECTS AND TARGET THEORY

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ABSTRACT Diluted bull semen samples were irradiated with 180 kv X-rays. Dose response curves were measured for the survival fraction of the spermatozoa, and for the average velocity of the surviving cells. The dose response curves did not show a sensitivity threshold. The half-value dose was determined as 11 kr for the survival fraction and 10 kr for the average velocity. Target theory was adapted specially to explain the form of the measured dose response curves. From this target theory it was found that a small sensitive element is present in the sperm cell with a volume of approximately $0.7^5 \times 10^{-15}$ cm^3.

INTRODUCTION

Experiments to determine the effects of X-rays on spermatozoa have been performed since the discovery of this radiation in the late nineteenth century. The early experiments were done mainly to establish the damage to the fertilizing capacity of the sperm. From the results obtained the conviction has carried over that spermatozoa are quite resistant to radiation damage (1). A review of the old literature can be found in reference 2.

Since the middle 1950's a number of papers have appeared in which the radiation damage to the *motility* of sperm was studied (1, 3–10). The evaluation of the motility before and after the irradiation was generally done by visual evaluation, resulting in an estimated percentage of cells in the preparation which showed movement, or in a qualitative criterion such as "good" or "poor". Table I shows a summary of the main results reported in the papers (1, 3–10). It can be seen that a value for the dose at which 50% of the sperm survives insofar that it still shows motility, cannot be estimated to within an order of magnitude.

In recent years methods have been developed (11, 12), however, which make it possible to measure sperm motility quantitatively. It was hoped that application of these methods would enable us to come to an unequivocal value for the radiation sensitivity of sperm motility.

The forward velocity of a given sperm, which can be observed over a period of many seconds, is usually found to be constant over this period (13, 14). Further, the

TABLE I

SUMMARY OF RESULTS REPORTED IN RECENT STUDIES OF RADIATION DAMAGE TO SPERM MOTILITY

Author	Species	Temperature during irradiation	Dose	Shortly after irradiation			Remarks	Reference
				Motility	Respiration	Fructolysis		
		°C	*kr*	%	%	%		
Mann, 1954	Ram		100	good		normal		3
Gerber, 1955	Human		50	−50				4
Chang et al., 1957	Rabbit		6.5	quite good				5
			32.5	poor				
			65	none				
Tyler, 1964	Sea-urchin		30	−50				6
			60	−75				
			90	−90				
Schmermund et al., 1959	Bull	4	10	unchanged				8
			20	unchanged				
Wu and Prince, 1964	Bull		10	unchanged	unchanged			7
			100	unchanged	unchanged			
			320	unchanged	+6			
Mounib and Chang, 1964	Bull		1	unchanged	−30	−30	undiluted	1
			10	−10	−40	−50		
			100	−70	−65	−80		
			100	−15	−35	−40	diluted with egg yolk diluent	
van Herpen, 1965	Bull	2	4	−25			diluted with egg yolk diluent	9
			12	−65				
			300	−90				
Wu and Prince, 1967	Bull		500	little affected				10

frequency and amplitude of the wave in a given sperm flagellum remain constant over a period of around 1 sec (1, 5). However, not all cells in a preparation have the same frequency or amplitude of the flagellar wave. These observations have led to the thought that a "tuning mechanism" is present which actually controls the motion of the flagellum. An organelle acting as such a "control center" would have to be small in dimension in view of the small cross section of a flagellum (0.2 ≈

0.4 μ). By the application of "target theory" (16), it should therefore be possible to make an estimate of the size of this control center, provided it can be established that the radiation damage is affected on the control center and not on other regions, for example on the contractile elements. In preliminary experiments by one of us (GVH), dose response curves were obtained which showed no evidence for a threshold dose for the occurrence of radiation damage (9). This indicates that the conditions for application of target theory are probably fulfilled in the case of sperm motility.

The experiments described in this paper were directed towards establishing precise dose response curves for the damage of bull sperm motility by X-rays. Target theory has been adapted specially for the case of sperm motility to estimate the volume of the element which is primarily sensitive to irradiation.

In the second paper results are reported of experiments aimed at elucidating the function of the sensitive element.

EXPERIMENTAL METHODS

Semen was obtained from Frisian bulls at the Research Institute for Animal Husbandry at Zeist, Holland. Upon ejaculation the semen was cooled slowly to room temperature and diluted in an egg yolk containing medium (EM), described below.

In the first experiments the dilution rate was 10 times. The cooled, diluted semen was once more diluted five or six times in EM. This sample was split into five parts of 1 cm^3 each, four of which were irradiated (see below) with increasing dosage. The fifth sample served as control.

After the gross effects had been established a modified procedure was used in subsequent experiments. After a first threefold dilution the sample was split into five parts. The irradiations were done on this threefold diluted sperm. For the second dilution in EM the five tubes were diluted differently; the dilutions were adjusted so that a concentration of moving sperm optimal for the motility measurement was obtained. In practice this second dilution varied such that the final dilutions varied from 150-fold for the control sample to 24-fold for the tube with highest dosage.

Medium

The medium (EM) used for diluting the semen was a modified Krebs-Ringer solution containing 140 mM NaCl, 4 mM KCl, 1 mM $MgSO_4$, and 1 mM $CaCl_2$, to which 10% (v/v) of an egg yolk extract had been added to protect the spermatozoa during the cooling to 4°C. The EM was made optically clear and free of unwanted minerals by centrifuging and dialysis and sterilized by filtration. The preparation procedure has been described before in detail (17).

2 mM Na-lactate and 250 $\mu g/cm^3$ fructose were added in EM as substrate. The pH was adjusted to 7.0 and buffered by 2% of 0.1 N phosphate buffer.

Irradiation and Dosimetry

180 kv X-rays were available from a Siemens Tota Stabilivolt therapy tube. The intrinsic filter of the tube consisted of 0.15 mm Cu, to which externally 0.5 mm Al was added, resulting

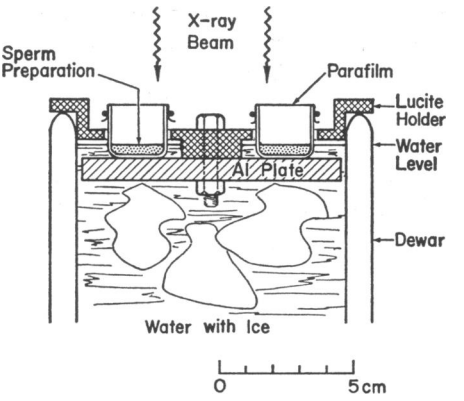

FIGURE 1 Arrangement of sperm samples during irradiation. The anode of the X-ray tube was centered overhead, 30 cm above the top of the lucite holder.

in an HVL of 0.26 mm Cu. The anode of the X-ray tube was placed 30 cm centrally overhead the sperm preparations.

Fig. 1 shows the arrangement used during the irradiation. The 1 cm^3 diluted semen samples formed a layer of approximately 4 mm deep in the glass vessels. The aluminum plate on which the glass vessels rest assures that their temperature is well maintained at 4°C, while the amount of backscattering of X-rays remains acceptable.

Doses were monitored with a thimble ionization chamber and model 37470 dosimeter (Philips Co., Eindhoven, Holland). The radiation intensity over the area of the Dewar flask was found to be uniform to within 2%.

During actual irradiations the ionization chamber was placed beside the Dewar flask containing the sperm preparations at a place where the radiation intensity was three times less than at the top of the Dewar. This ratio proved repeatable within 3%. The radiation dose in the actual experiments was derived from the measured dose at the ionization chamber placed beside the Dewar by multiplying the measured dose by the known ratio of radiation intensity there and at the semen samples. The dose was delivered at a rate of 320 r/min. For the highest dose of 24 kr, the irradiation thus lasted 75 min.

Motility Measurement

The photoelectric method developed by Rikmenspoel and van Herpen (11, 12) was used to measure the motility of the sperm samples. A slide containing a layer of 40 μ thick of diluted sperm is placed under a microscope with dark field illumination. A photomultiplier observes an area of 10 μ diameter in the slide. Whenever a sperm passes over the observed area the photomultiplier receives a light signal which is recorded. From the form of this record the velocity of the sperm in question can be determined. Over a period of several minutes a number of sperm passages can be observed from which the average velocity of the sperm in the sample is derived. Provided the number of passages is sufficient, the form of the velocity distribution is obtained as well. The rate of passages of sperm gives, after appropriate calibration, the concentration of moving sperm in the preparation. Extensive discussion of the method can be found in references 11 and 12.

The normal movement of the sperm flagellum is a helical wave, accompanied by a rotation of the cell about its longitudinal axis. Normal cells swim along essentially straight paths, thus

ensuring random sampling in our photoelectric measurements (14, 15). It is known, however, that the most frequently occurring abnormal type of movement exhibits planar flagellar waves and no rotation (14, 15). In this abnormal movement the sperm always swim in closed circular paths. This means that with the photoelectric measuring method random sampling of these abnormal cells is not obtained. The average velocity and the concentration of these abnormal cells cannot be measured photoelectrically.

In analyzing the records of the experiments the signals from the abnormal cells were disregarded. To have some information about these cells visual estimates were made in a number of experiments of the percentage of abnormal cells in the preparations.

The measurements of motility were made at 37°C. The slides were allowed 3 min after being placed in the thermostated microscope stage to warm to this temperature. Photoelectric signals were recorded for 5 min immediately following. In each motility measurement the data on three slides prepared from the same sample tube were averaged.

RESULTS

Dose Response Curves

The split samples of a number of ejaculates were subjected to X-ray doses of either 0, 2, 4, 8, and 12 kr; or 0, 3, 6, 9, and 12 kr; or 0, 8, 16, and 24 kr. The average velocity and the concentration of the normally moving cells after irradiation were measured.

Fig. 2 shows the results obtained for the average velocity as a function of dosage. The average velocity of the surviving cells has decreased to half the control value at a dose of 10 kr.

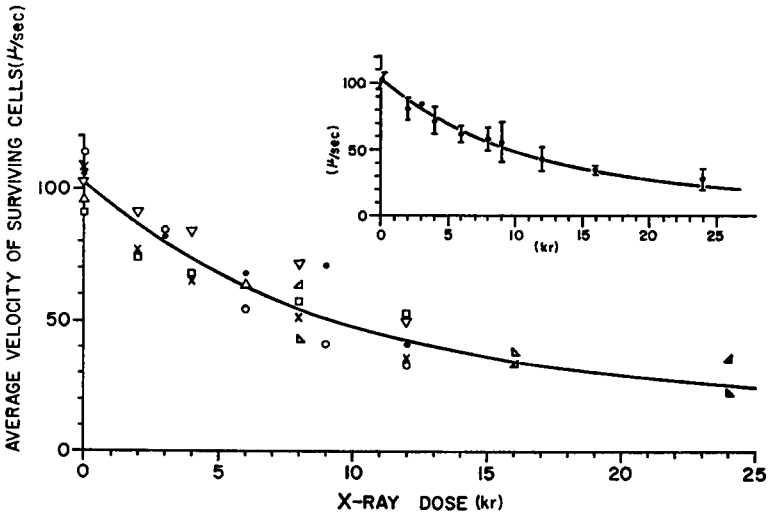

FIGURE 2 Average velocity of the sperm as a function of X-ray dose. Each symbol represents one ejaculate, at various radiation doses. The insert shows the average and standard deviation of the data at the various doses.

The data for the concentration of normally moving sperm yield (after taking into account the varying dilution rates for the different samples) the fraction of cells that still show movement after irradiation. Fig. 3 shows this "survival fraction" as a function of dose. At a dose of 11 kr half of the cells have ceased movement.

It can be seen in Figs. 2 and 3 that both dose response curves are monotonously decreasing lines. No evidence for a sensitivity threshold is present. Semi-logarithmic

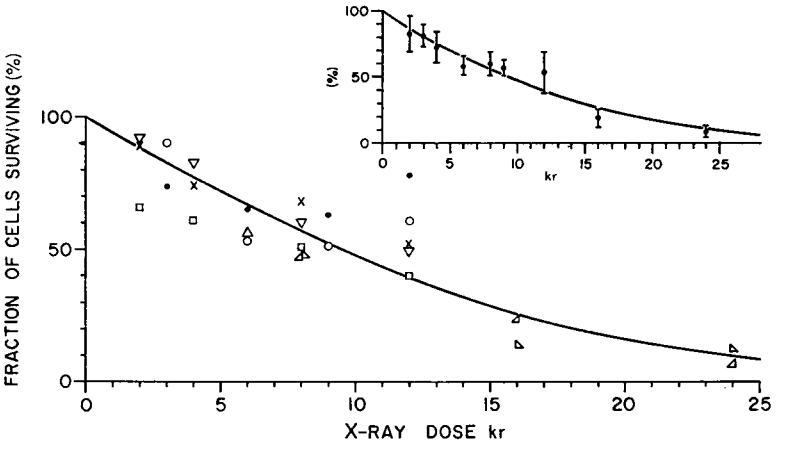

FIGURE 3 Fraction of surviving sperm as a function of X-ray dose. One ejaculate is represented by the same symbol in Figs. 2 and 3. The insert shows average and standard deviation of the data pooled at each dose.

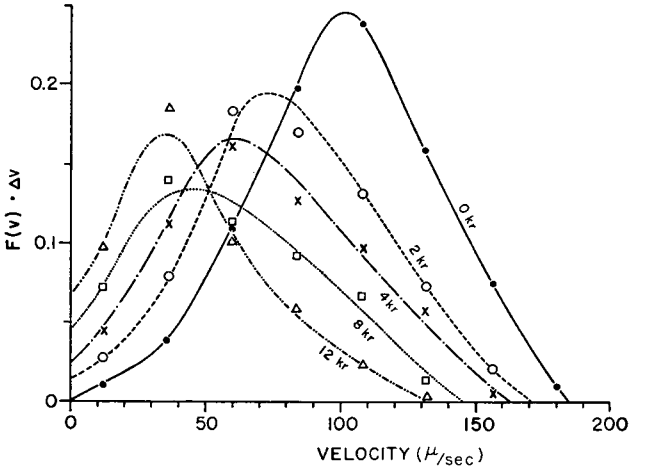

FIGURE 4 Velocity distribution of the sperm, after irradiation at 0, 2, 4, 8, and 12 kr, respectively. The points are obtained by pooling all available data at the different doses. The vertical scale is such that at 0 kr, $\int F(v)\,dv = 1$. At the other doses $\int F(v)\,dv$ corresponds to the average survival fraction shown in the insert of Fig. 3.

plotting of the data of Figs. 2 and 3 is not presented since neither of the two curves corresponds to a simple exponential.

Velocity Distributions

The statistics of the motility data of one experiment (on one ejaculate) were not good enough to make presentation of velocity distributions meaningful. Therefore, the data of all experiments done with a dosage series of 0, 2, 4, 8, and 12 kr were pooled. Fig. 4 shows these pooled velocity distributions. The curves of these data in Fig. 4 have been normalized so that the area under each curve is proportional to the average survival fraction shown in Fig. 3.

Abnormal Cells

In the control preparations for the two experiments done with a dosage series of 0, 8, 16, and 24 kr it was observed that a relatively large fraction (20 and 38%, respectively) of the cells showed abnormal movement. For these preparations visual estimates were made of the percentage normally and abnormally moving cells after irradiation. These figures were then converted to estimated "survival fractions" after irradiation for both the normal and the abnormal cells.

Fig. 5 shows the results of these two experiments. Each point in Fig. 5 is the average of visual estimates on three different slides by both of us.

It can be seen from Figure 5 that the 50% survival dose from estimates on the normal cells is not drastically different from the one obtained with the photoelectric measurements. This points to the validity of our estimated data.

The dose response curve for the abnormal cells, as derived from Fig. 5 is approximately the same as that found for the normally moving cells. We have interpreted this to mean that the damaging effect of the irradiation is not the conversion of

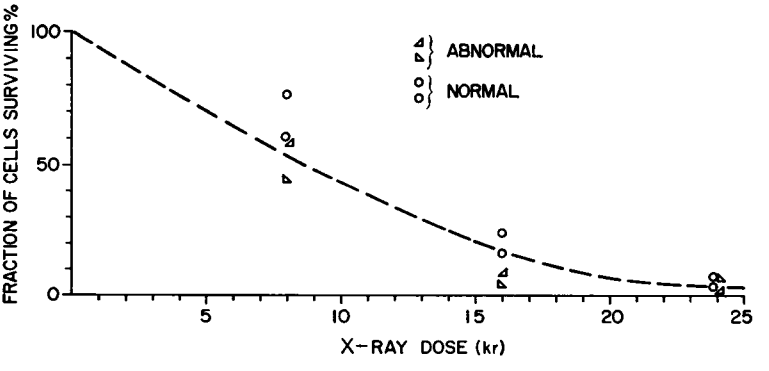

FIGURE 5 Survival fraction of normal and abnormal sperm, as obtained from visual estimation of the percentage of moving sperm.

normal into abnormal cells. The normal, helical type of tail movement apparently persists, even though decreased, after irradiation damage.

TARGET THEORY

The dose response curve for the average velocity of the cells surviving irradiation (Fig. 2) shows that a cell is not rendered motionless by being "hit" once by the radiation. If that were the case the average velocity of the surviving cells would be unchanged after irradiation.

We have to assume that a cell which had before the irradiation a velocity v_o, will have after the irradiation a velocity v with

$$v = v_o - f(\text{dose}) \tag{1}$$

where f is as yet an unknown function of the dose.

The basic thought of target theory is that radiation damage is caused by discrete packages of energy which are deposited along the path of incident radiation. Poisson distribution effects then give rise to the various shapes of dose response curves (16).

For the purpose of target theory equation 1 has to be replaced by

$$v = v_o - f(n, \beta) \tag{2}$$

where n is the number of hits received, and β a sensitivity parameter. A cell is rendered motionless if n is so big that $f(n, \beta) \geqq v_o$.

The number of sperm having initially a velocity v_o is $F_o(v_o)$, where F_o is the velocity distribution function. Assuming the "hits" to have a Poisson distribution, the fraction of these cells which shows movement after receiving a dose D is

$$\sum_{n=0}^{n_{max}} \frac{D^n}{n!} e^{-D} \cdot F(v_0). \tag{3}$$

The summation over n in equation 3 has to be cut off at n_{max}, such that $f(n_{max} + 1, \beta) \geqq v_o$. The total number N of sperm showing movement after receiving a dose D is given by

$$N = \int_{v_0} \sum_{n=0}^{n_{max}} \frac{D^n}{n!} e^{-D} \cdot F_0(v_0)\, dv_0. \tag{4}$$

If $F_o(v_o)$ is normalized as $\int F_o(v_o)\, dv_o = 1$, N in equation 4 represents the total fraction of surviving cells.

It should be realized that the form of the function $f(n, \beta)$ is present in the value for n_{max} in equation 4. The relation between N and D is therefore dependent on $f(n, \beta)$ even though this function does not appear explicitly in the equation.

The average velocity of the surviving cells, $\langle v \rangle$ can be written as

$$\langle v \rangle = \frac{1}{N} \int_{v_0} \sum_{n=0}^{n_{max}} \frac{D^n}{n!} e^{-D} [v_0 - f(n, \beta)] \cdot F_0(v_0) \, dv_0. \quad (5)$$

In order to compute dose response curves for N and $\langle v \rangle$ from equations 4 and 5, a form of the function $f(n, \beta)$ has to be assumed. Three different forms of $f(n, \beta)$ have been used, first such that $v = v_0 - n \cdot \beta$, which represents a straight loss of velocity for every hit, secondly $v = v_0 (1 - n \cdot \beta)$, which corresponds to a fractional loss of velocity per hit, and thirdly $v = v_0 - \sqrt{n} \cdot \beta$, corresponding to a process in which a following hit causes less damage than the previous one.

A program was written for the CDC 3300 computer to calculate dose-response curves from equations 4 and 5 as outlined above. For each of the three forms of $f(n, \beta)$ the dose response curves were computed for a number of values of β, cover-

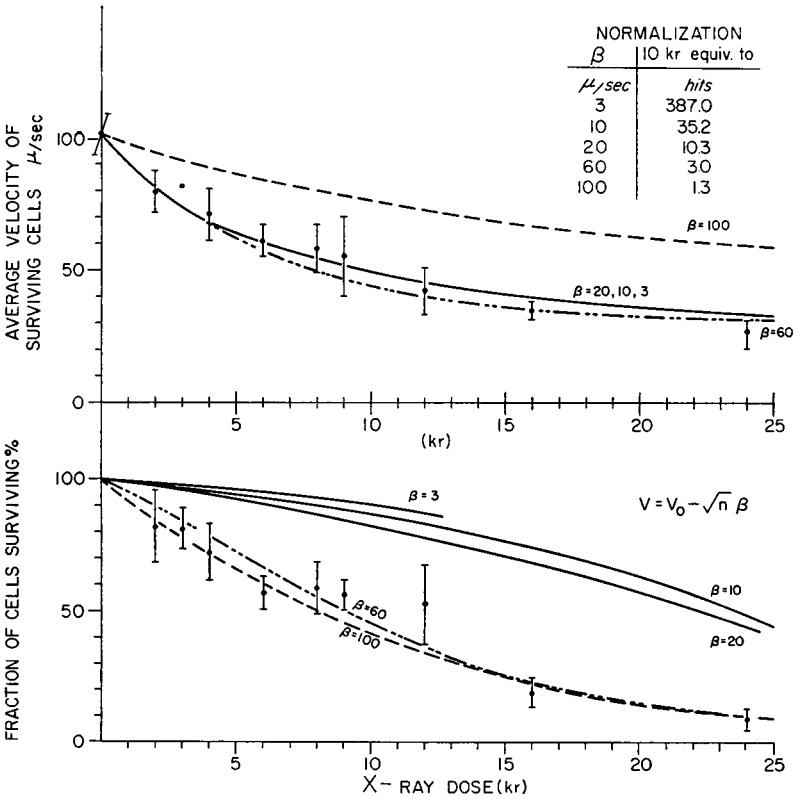

FIGURE 6 Computed dose response curves for a velocity loss due to radiation, which is proportional to the square root of the number of hits: $v = v_0 - \sqrt{n} \cdot \beta$ (see text). For each set of 2 curves the horizontal scale is adjusted as shown in the table, giving the best possible fit.

ing a wide range of assumed "sensitivity". At each value of β the two computed dose response curves for $\langle v \rangle$ and N were normalized such that at least one of the set of two curves showed the best fit to the corresponding experimental data.

It was found that for the two forms of $f(n, \beta)$ mentioned first it was not possible for any value of β to construct dose response curves which agree with *both* the data for N and for $\langle v \rangle$.

Fig. 6 shows the computed dose response curves for the third form of $f(n, \beta)$, such that $v = v_o - \sqrt{n} \cdot \beta$. It can be seen in Fig. 6 that for small values of β (3, 10, and 20 μ/sec, respectively) the dose response curves for $\langle v \rangle$ are not much different and they can be reasonably fitted to the experimental data. The computed curves for N then do not cover the data at all. For $\beta = 100$ μ/sec, the curve for N is fitted to the data, but the one for $\langle v \rangle$ is obviously too high. At $\beta = 60$ μ/sec both curves fit the data.

At the value of $\beta = 60$ μ/sec, the normalization of the curves in Fig. 6 immediately yields a volume for the sensitive target. 1 r produces 1.2×10^{12} ion pairs/cm^3, which are deposited in clusters of an average of three ion pairs (17). Each of these clusters is taken to be able to produce one "hit". The "target" which is on the average hit three times at a dose of 10 kr thus has a

$$\text{volume} \approx 0.75 \times 10^{-15} \text{ cm}^3. \tag{6}$$

If this target volume 0.75×10^{-15} cm^3 were spherical it would have a diameter of $\approx$1100 A.

DISCUSSION

The data presented in Figs. 2 and 3 show no large spread in radiation sensitivity between the different ejaculates. We cannot offer an explanation why the radiation damage has not been observed in bull sperm in references 3, 4, 5, and 10, even at much larger doses.

Our present experiments point to a small structure within the spermatozoa which is most sensitive to X-ray irradiation. During the irradiation our preparations were in liquid water. The medium in which the sperm were suspended contained about 5 mg dissolved protein per cm^3. It can therefore be assumed that sufficient "scavengers" were around to limit the diffusion distance of free radicals formed to a few hundred A before decay (17). This means that the actual structure representing the target should have a volume not far from 0.75×10^{-15} cm^3, as found by means of the target theory. Of the structures which can be easily identified in a sperm flagellum, the mitochondrial sheath has a volume of 6×10^{-12} cm^3 (18). The nine coarse longitudinal fibers which are contractile elements (15, 19) in the flagellum have a total volume of 4.5×10^{-13} cm^3. The double central fiber of which the function is unknown as a volume of 2.5×10^{-14} cm^3. All of these structures are more than an order of magnitude larger than the target volume found.

To obtain more insight into the nature of the target which is sensitive to radiation one would want to have indications for its shape (20) (its length to width ratio), and its function. The following article (21) will describe experiments aimed at this purpose. In these experiments irradiations were done with fast protons in order to obtain a cross-section of the sensitive target. The motility measurements after irradiation were complemented with measurements of respiration and respiratory inhibitions, to ascertain whether damage had occurred in the respiratory enzymes and in the contractile system.

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