day, radiostimulation was discontinued, but the tone remained connected for 1 week. The next two recorded lever pressings started the tone and produced some head-turning and behavioral inhibition in Ali, but extinction appeared quickly, and successive tones were not effective. The number of lever pressings by Elsa diminished to 9 the first day and to 1 to 8 per day during the following 6 days. No evidence of increased self-stimulation appeared in these studies. Immediately after these experiments the radiostimulator was attached to Sarah for three consecutive days, and the tone was changed to 500 cy/sec. For each monkey, including Sarah, 0 to 6 daily lever pressings were recorded with no significant increase in the number for any animal during the 3 days.

Curiosity probably was not the cause of the increase in lever pressing because fewer were recorded on the first than on the fourth experimental day. Correlation with radiostimulation seems more probable because lever pressings during the third and fourth days were more than twice as many as during any of the seven extinction days when only the tone could be activated. The lever was permanently attached to the cage and competed for the monkeys' attention with padlocks and food on the floor, swings, and other parts of the living quarters. This competition may explain the low number of lever pressings, and it makes more significant the increased pressing resulting when Ali was radiostimulated. Observation of the colony and analysis of films showed that several times Ali's threatening attitude was followed by Elsa's lever pressing (*6*).

The studies continued with the radiostimulator again strapped on Ali, this time connected to a contact in the posteroventral nucleus of the thalamus, and with the tone set at 900 cy/sec. Previous radiostimulations of this area had increased Ali's aggressiveness. When the lever was attached to the cage, it was triggered only seven times during three consecutive days. Then the lever was removed and was actuated by a timer once every minute for half an hour. After the fourth trial, signs of conditioning were evident. At the onset of the tone, Ali showed increased aggressiveness, and the other three monkeys grimaced and climbed to the cage ceiling. On several occasions this escape reaction to the tone started before Ali initiated any threat. Later the stimulation was discontinued, and during 30 trials the tone continued to sound once every minute and induced a reaction 25 times in Elsa, 11 times in Ali, and 7 times in both Sarah and Lou. This experiment was duplicated on three different days with results showing similar characteristics and indicates that conditioning may be established through association of the tone with aggressive behavior evoked in Ali. In another series of investigations, there was no individual or social conditioning when motor areas were stimulated in Ali and in Sarah by radio-timed control.

Performance of instrumental responses may be induced by cerebral stimulation and may be conditioned to auditory or visual cues (*7*). The fact that "spontaneous-like" behavior evoked by brain excitation may also be conditioned to an indifferent stimulus is a relatively new finding. These results have been confirmed in further experiments (*8*). Behavioral conditioning has also been established on a time basis by programmed stimulations of the superior vestibular nucleus of the thalamus without giving the monkey any cue other than fixed interval of 1 minute between stimulations (*9*).

Social conditioning may help in the analysis of cerebral stimulation because each member of the colony is an interpreter of the reactions of the stimulated animal. Heterostimulation presents obvious questions about hierarchical control, reciprocal punishment, instrumental self-defense, and other problems related to human behavior (*10*).

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6. Two months later Elsa and Ali were part of a new colony with three other monkeys. The radiostimulator was then strapped to Ali and connected to his caudate nucleus. Heterostimulation of Ali by Elsa was recorded 22 times in 1 day, and during the bar pressing Elsa's attention was usually directed toward Ali, in a way similar to that shown in Fig. 2. Reproducibility of the phenomenon of heterostimulation was thus demonstrated.
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Relationship between Nuclear Volumes, Chromosome Numbers, and Relative Radiosensitivities

Abstract. *An inverse relationship between a volume estimated to be associated with interphase chromosomes and acute lethal exposure to x- or gamma radiation has been found in 16 plant species. The apparent differences in radiosensitivities found would seem spurious, since the estimated average energy absorbed in the nucleus per chromosome (3.6×10^6 ev) approaches a constant (variation less than fourfold) in spite of wide ranges of lethal exposures (0.6 to 75 kr), of nuclear volumes (43 to 1758 μ^3), and of somatic chromosome numbers (6 to 136). The regression line obtained can be used to predict the radiosensitivities of other plant species if their nuclear volumes and chromosome numbers are known.*

The radiosensitivity of a species, as indicated by degree of growth inhibition, is correlated with the average volume of interphase nuclei and with chromosome number (*1*, *2*). If these variables are controlled one at a time, an increase in nuclear size with chromosome number constant increases sensitivity and an increase in chromosome number with nuclear volume constant decreases sensitivity. Apparently the number and size of targets in the nucleus (excluding the nucleoli) are the major factors determining radiosensitiv-

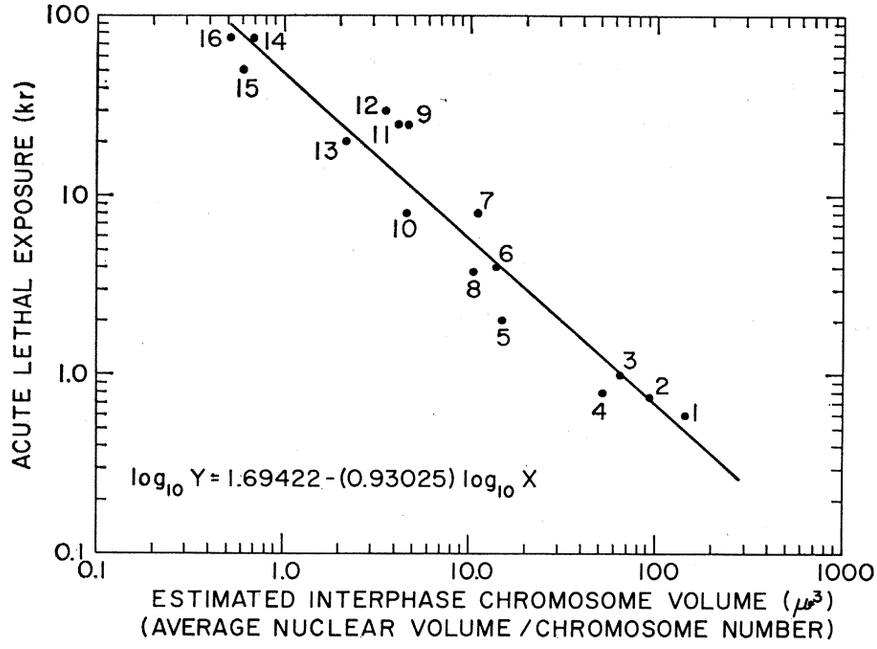

Fig. 1. Relationship between interphase chromosome volume and acute lethal exposure (kr) for 16 plant species (see Table 1 for names of species).

ity. A single index of radiosensitivity might, therefore, be obtained by dividing average nuclear volume by the number of chromosomes characteristic of the species. The present report provides confirming data, using mortality as an end point. Within an organism, if chromosome number is constant, chromosome size or volume will be the major factor determining the relative sensitivity of cells or tissues.

The value obtained by dividing the interphase nuclear volume by the chromosome number will be referred to here as average interphase chromosome volume. Since no allowance has been made for nucleoli or interchromosomal space and since in many species there is considerable variation in chromosome size as seen at metaphase or anaphase (and presumably also in interphase), the average interphase chromosome volume is thus more of a concept than an actual biological entity. It is, however, a highly useful statistic for calculations of energy absorption by the genetic targets. Possibly more refined methods eventually will allow us to define more accurately the actual volume of these sensitive targets. A start in this direction has been made by correlating mean DNA content per nucleus with nuclear volume and mean DNA content per chromosome with radiosensitivity (*3*), but the variations in DNA values and chromosomal or nuclear size during the nuclear cycle present some difficulties which have not yet been resolved.

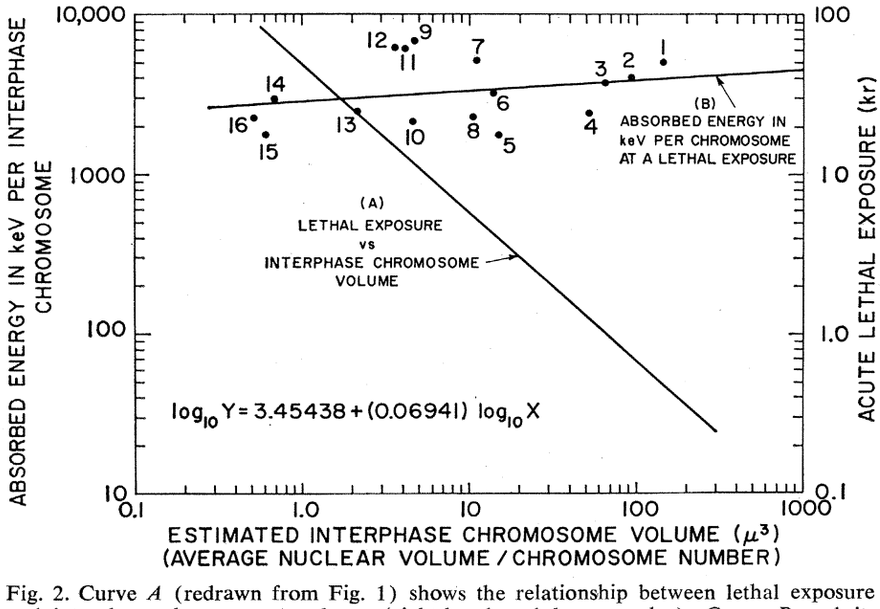

Fig. 2. Curve *A* (redrawn from Fig. 1) shows the relationship between lethal exposure and interphase chromosome volume (right-hand and lower scales). Curve *B* and its regression equation show that the absorbed energy per chromosome at a lethal exposure approaches a constant of approximately 3624 kev regardless of chromosome size (left-hand and lower scales).

Vegetative shoot meristems of the 16 species listed in Table 1 were fixed in Craf III and, after being embedded and sectioned at 10 μ, they were stained with safranin and fast green. Interphase nuclear volumes were calculated from the mean diameter obtained by measuring 2 diameters at right angles to each other in each of 20 nuclei of the inner tunica or corpus cells of the meristems. The chromosome numbers used are from Darlington and Wylie (*4*) or were determined in our laboratory.

The 16 species of herbaceous plants were chosen because they represent a wide range of both nuclear volume and chromosome number (Table 1) and include both diploids and polyploids. The latter include three high polyploids (Nos. 14 to 16, Table 1). The number of plants per exposure varied from 8 to 15 and the lethal dose was the lowest dose at which all plants in the exposed group died at a time when the controls still had their normal size and vigor. Tolerance data were obtained by acute exposure of the plants to six to eight different acute dosages from 250 kv (peak) x-rays or Co^{60} gamma rays. The lethal exposures for the species studied varied from a low of 0.6 kr for the most sensitive to a high of at least 75 kr for the most resistant—a difference of approximately 130-fold.

The data obtained as described above are presented in Table 1 and Fig. 1. The slope of the regression line of Fig. 1 (also curve *A* in Fig. 2) does not deviate significantly from -1. From this relationship, one can conclude that the product of the two variables, interphase nuclear volume divided by chromosome number and acute lethal exposure, is nearly a constant. If exposure is expressed as energy absorbed per unit volume of tissue, the product of the

Table 1. Summary of nuclear, chromosomal, and mortality data for 16 species of plants, and calculated amount of energy absorbed per interphase chromosome at the lethal exposure. SE, standard error.

Species and somatic chromosome number ($2n$)	Nuclear volume ($\mu^3 \pm$ SE)	Average volume per chromosome * ($\mu^3 \pm$ SE)	Energy absorbed per chromosome per roentgen (ev † $\pm$ SE)	Lethal exposure (kr)	Energy absorbed per chromosome at lethal exposure (kev)
1. *Trillium grandiflorum* (10)	1452 ± 81.42	145.19 ± 8.1420	8348 ± 468.17	0.60	5008
2. *Podophyllum peltatum* (12)	1118 ± 56.96	93.16 ± 4.7463	5357 ± 272.91	0.75	4017
3. *Hyacinthus* c.v. Innocence ‡ (27)	1758 ± 98.19	65.12 ± 3.6367	3744 ± 209.11	1.00	3744
4. *Lilium longiflorum* (24)	1252 ± 42.18	52.18 ± 1.7575	3000 ± 101.06	0.80	2400
5. *Chlorophytum elatum* ‡ (28)	422 ± 27.58	15.08 ± 0.9850	867 ± 56.64	2.00	1733
6. *Zea mays* (20)	279 ± 11.81	13.95 ± 0.5907	802 ± 33.97	4.00	3209
7. *Aphanostephus skirrobasis* (6)	67 ± 5.40	11.19 ± 0.8996	643 ± 51.73	8.00	5147
8. *Crepis capillaris* (6)	64 ± 4.95	10.59 ± 0.8243	609 ± 47.40	3.75	2284
9. *Sedum ternatum* (U643) ‡ (32)	149 ± 6.22	4.64 ± 0.1944	267 ± 11.18	25.00	6671
10. *Lycopersicum esculentum* (24)	110 ± 5.43	4.60 ± 0.2261	265 ± 13.00	8.00	2116
11. *Gladiolus* c.v. Friendship ‡ (60)	252 ± 10.44	4.19 ± 0.1741	241 ± 10.01	25.00	6027
12. *Mentha spicata* ‡ (30)	107 ± 6.02	3.57 ± 0.2006	205 ± 11.53	30.00	6152
13. *Sedum oryzifolium* ‡ (20)	43 ± 2.63	2.16 ± 0.1317	124 ± 7.57	20.00	2486
14. *Sedum tricarpum* ‡ (128)	89 ± 4.92	0.69 ± 0.0384	40 ± 2.21	75.00	2985
15. *Sedum alfredi* var. *nagasakianum* ‡ (128)	78 ± 1.87	0.61 ± 0.0146	35 ± 0.84	50.00	1755
16. *Sedum rupifragum* ‡ (136)	71 ± 3.36	0.52 ± 0.0247	30 ± 1.42	75.00	2246

* (Nuclear volume/$2n$). † Based on an average value of 32.5 ev per ion pair and 1.77 ionizations per cubic micron of wet tissue per roentgen [Table 2 in Lea (*10*)] and assuming all chromosomes within a genome to be the same size, which is seldom the case. ‡ Polyploids.

two variables is the total energy absorbed per chromosome at specified doses and volumes. Energy absorbed could be expressed as ergs, ion pairs, or electron volts (ev). We have arbitrarily chosen electron volts or kiloelectron volts (kev) (see Table 1 and footnote) and these values are plotted in Fig. 2.

The relationships shown in Figs. 1 and 2 indicate that mortality occurs in all individuals of each species when the absorbed energy per chromosome reaches a critical level within fairly narrow limits. While in the experiments reported here the average value is slightly above 3.6×10^6 ev, the exact value varies from about 1.7×10^6 to 6.7×10^6 ev. Some of this variation may result from different frequencies of the telo-, acro-, and metacentric chromosomes in the species used or from differences in relative volumes occupied by nucleoli in the different species. Thus, the critical absorbed energy per chromosome at the lethal dose would approach a constant as long as the physical variables employed in the experiment are essentially uniform. When one uses a dose response curve based on energy absorption per chromosome, instead of the more usual energy absorption per unit volume of air or tissue, it may be concluded that the tolerance of the different genetic systems found in the 16 species studied is essentially uniform, that is, varies by a factor of less than 4 (Table 1, right-hand column). It seems reasonable to assume that the same correlation will hold far beyond the range of the particular species studied here. In fact, we would speculate that eventually the same or a similar relationship will obtain for a large part of both the plant and animal kingdoms. Proof that the correlation is generally valid will require a considerable effort by radiobiologists, but the importance of such a unifying concept would seem to justify such an effort.

The volumes were calculated from measurements made on nuclei after they had been through the histological procedure outlined above. For purposes of comparisons between species this procedure is reasonably valid, but it is doubtful that the volumes calculated from the processed slides would be the same as those of living nuclei, since the procedures used are known to shrink nuclei to a considerable extent. This fact leads to some difficulties when energy absorption calculations are made with the values from histologically processed slides. Eventually a correction factor will have to be established to enable one to calculate absolute values for energy absorption in irradiated living nuclei for specified radiobiological end points.

In previous publications it has been shown that the degree of cytogenetic damage (*3*) or of growth inhibition produced (*5*) can be correlated with nuclear volume changes associated with changes in stage of the nuclear cycle (*3*) or with seasonal changes [dormant versus active buds (*5*)]. These results and the data presented in this report would suggest a general relationship between absorbed dose, radiobiological effect, and size [or DNA content (*3*)] and number of genetic targets. The relationship is such that when the average energy absorbed per chromosome reaches a critical level, the accrued effects are lethal to the individual. In our series of experiments the critical level varies from about 1.7 to 6.7×10^6 ev and averages 3.6×10^6 ev for the 16 species even though these species exhibit very much greater variation in both chromosome size and number. In any similar series of experiments the exact value would be expected to vary with the dose rate and with the linear energy transfer of the radiation used.

From known patterns of energy transfer by gamma radiation one can calculate that a minimum of several hundred tracks must cross a cell nucleus of a higher plant before the energy absorbed approaches the critical value given above (*6*). It is known that a single deletion per nucleus can cause cell death and it has been calculated that approximately 100 ionizations are required within 0.1 μ of track length to break a chromatid of *Tradescantia* (*7*). Since the 100 ionizations thought to be required to break a chromosome would involve only about 3250 ev, the total energy absorbed per chromosome (3.6×10^6 ev) is clearly sufficient to produce many breaks per chromosome and an even larger number per nucleus.

There are several possible explanations for this discrepancy, such as the fact that energy distribution may not be uniform, and that many breaks may rejoin or yield viable aberrations. However, if chromosome breakage or resultant deletions are the major cause of growth inhibition or death, a very small fraction of total energy absorbed per nucleus would actually be involved in the production of the lethal lesion(s). The correlation described indicates that death depends upon a critical amount of energy being absorbed on the average by each chromosome. However, the fact that the structural organization and thus the actual nuclear volume occupied by the elements of an interphase chromosome are not well known raises important theoretical questions about just where and how critical amounts of energy are absorbed by the nuclear material. Since the amount of DNA per chromosome is not constant in different species but the energy absorbed per chromosome is almost constant, the ultimate biological damage is not due to the production of a constant proportion of damaged DNA molecules but, presumably, to the production of a relatively constant number of some kind or kinds of deleterious event(s). We cannot, at this time, conclude whether these events are gross chromosomal damage (breakage and aberration), more subtle molecular disturbances, or a combination of both.

It has been pointed out that an adequate knowledge of the role of nuclear variables in determining radiosensitivity should allow one to make predictions of expected radiation responses of species or biological material for which no radiobiological data were available (*2*). Such predictions have been made (*8*) and have been in part quite successful. The graphs presented in Figs. 1 and 2 can be used to estimate the expected lethal dose for any plant species for which interphase chromosome volume (interphase nuclear volume divided by somatic chromosome number) is known. The ability to make such predictions should be of considerable theoretical and practical value in radiobiology, radioecology and, if the method can be successfully extended to animal cells, possibly also in radiotherapy (*9*).

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Amino Acid Composition of Hemerythrin in Relation to Subunit Structure

Abstract. *Determination of the amino acid composition of coelomic hemerythrin from* Golfingia gouldii *shows 3 arginine residues and 10 to 11 lysine residues per protein subunit of 13,500 molecular weight. On this basis, the 28 to 30 major peptide spots revealed by electrophoresis and chromatography of tryptic hydrolysates would indicate two kinds of subunit. However, similar evidence from chymotryptic hydrolyses is not unequivocal, since the number of peptide spots is also compatible with an assumption of only one kind of chain. In addition to indicating the possible existence of two types of subunit, the peptide maps of enzymic digests of hemerythrin from individual animals shows at least one and perhaps more differences in peptide composition.*

Hemerythrin, the oxygen-carrying pigment of sipunculid worms, is constituted of eight subunits held together by noncovalent bonds (*1, 2*). The native protein of 107,000 molecular weight can be dissociated into its subunits, *merohemerythrins* (*3*), by a number of chemical or environmental modifications including succinylation, treatment with sulfhydryl-blocking reagents, addition of urea and increase or decrease in *p*H. Thus, because it is made up of separable polypeptide chains, hemerythrin shows macromolecular features analogous to those of hemoglobin.

In comparisons of hemerythrin to hemoglobin, the question immediately arises whether all eight merohemerythrins are basically identical with some occasional changes in constitution resulting from genetic modification (*4*) or whether there are more far-reaching differences between the chains.

A tentative answer to this question of the basic identity or nonidentity of the chains is available from assays of amino acid content of the protein combined with information on the number of peptides occurring in tryptic and chymotryptic digests.

Hemerythrin was isolated from *Golfingia gouldii* (also known as *Phascolosoma gouldii*) and crystallized (*5*). The protein was obtained from the pooled blood of 100 worms. Total amino acids in native protein as well as in hemerythrin from which iron had been removed by treatment with acidified acetone were determined by standard hydrolytic and analytical methods and with the use of a Beckman-Spinco amino acid analyzer (model 120 B). The results are summarized in Table 1.

Samples of pooled hemerythrin and of crystallized protein from individual worms were hydrolyzed by trypsin. Hemerythrin, in 0.02*M* ammonium carbonate of *p*H 8.3, was denatured by heating. Acid-treated trypsin (*6*) was added in small increments over a period of about 4 hours until its total amount was 5 percent with respect to the weight of hemerythrin. The precipitated proteins generally went into solution at the end of 1 hour. Larger proportions of enzyme were also used without significant changes in the results. Controls with trypsin alone showed no peptides. All hydrolysates were lyophilized and then dissolved in a small amount of water. An aliquot of this solution was subjected to electrophoresis on Whatman 3MM paper in pyridine-acetate buffer of *p*H 6.4. This was followed by chromatography with a mixture of butanol, acetic acid, and water (200:35:75 by volume) (*7*). The separated peptides were revealed with a ninhydrin reagent containing collidine (*7*) and with specific reagents for histidine, tyrosine, and tryptophan.

The amino acid analyses show three arginyl residues and 10.5 lysyl residues in each merohemerythrin unit of 13,500 molecular weight. If these units are all