5. S. Kit, D. R. Dubbs, L. J. Piekarski, T. C. Hsu, *Exptl. Cell Res.* **31**, 297 (1963).
6. J. W. Littlefield and P. F. Sarkar, *Federation Proc.* **23**, 169 (1964).
7. J. W. Littlefield, unpublished material
8. W. Szybalski, E. H. Szybalska, G. Ragni, *Natl. Cancer Inst. Monograph* **7**, 75 (1962).
9. G. Pontecorvo and E. Käfer, *Advan. Genet.* **9**, 71 (1958).
10. The frequency of $10^{-10}$ was estimated from previous work (*4*) and unpublished studies.
11. Supported by USPHS grant CA-04670-05. This is publication No. 1173 of the Cancer Commission of Harvard University. We acknowledge the assistance of Susan J. Whitmore.

4 May 1964

## Seed Radiosensitivity: A New Constant?

Abstract. *Dormant seeds of different species tolerate varying amounts of ionizing radiation, species having smaller nuclei in the apical meristem generally withstanding greater exposure. Nuclear volume (in $\mu^3$) multiplied by radiation exposure (in roentgens) equals a constant, estimated from 12 species to be $(10.14 \pm 1.17) \times 10^6$. From nuclear volumes alone, predictions of radiation response for two unknown species were made; experimental values in both cases fell below the 95 percent but within the 99 percent confidence intervals of the predictions.*

Sparrow *et al.* (*1*) observed that sensitivities of 16 actively growing plant species to acute x- or $\gamma$-irradiation may vary up to 125-fold as measured by total exposure, but only 4-fold when the criterion is energy absorbed per chromosome at the lethal exposure. The implication is that a similar quantity of energy is absorbed for a similar amount of nuclear damage regardless of total radiation exposure. This striking concept was hinted at in earlier papers (*2*) describing high positive correlations of nuclear volume (or DNA content) with sensitivity of growing plants to chronic irradiation and with frequencies of somatic mutation and chromosome aberration. Similar correlations exist for the sensitivity of dormant (seed) embryos to acute irradiation (*3*). We here derive a constant with which seed radiosensitivity can be predicted from nuclear volume of certain embryonic cells.

Controlling important modifiers such as seed moisture (*4*), we have found that most interspecies differences in radiosensitivity are attributable to nuclear volumes in the apical initial cells of shoot meristems (*5*), although eight additional measurements are required to account for all genetic variability (*6*). For comparisons between species, we calculated the maximum radiation exposure tolerated by seeds before the dry weight of seedlings grown in controlled-environment rooms was reduced by 50 percent ("50 percent exposure"). When growth values were transformed to probits, an almost linear response was obtained with the logarithm of the radiation exposure. Nuclear volumes were determined for dormant seeds stored at 35 to 60 percent relative humidity; volume has been found to remain unchanged within this range (*4*). Embryos were excised from dormant seeds, fixed in chrom-acetic-formalin, infiltrated with tertiary butyl alcohol, and embedded in paraffin. They were then sectioned at 10 $\mu$, stained with warm safranin, and counterstained with fast green in clove oil. Cells in the apical meristem region were examined at $\times$ 930 with an ocular micrometer, and two measurements at right angles were made for each nucleus: the longer axis was designated "*a*" and the other "*b*." After all nuclei of the meristems of several embryos were measured, "*a*" and "*b*" values were averaged and average nuclear volume, *V*, was computed from the ellipsoid formula:

$$V = \frac{4}{3}\pi\left(\frac{a}{2}\right)\left(\frac{b}{2}\right)\left(\frac{a+b}{4}\right)$$

Chromosome numbers were obtained from Darlington and Wylie (*7*) except for *Festuca elatior*, which was determined in our laboratory from root tips.

The nuclear measurements, 50 percent exposures, and calculated values for energy absorption for 12 species from 10 botanical families are listed in Table 1. The second column from the right is comparable to the pertinent numbers of Sparrow *et al.* (*1*), except for our using the 50 percent rather than the lethal exposure. In our computations, however, a spread of more than 11-fold was obtained despite a range of only 10-fold in tolerance as measured in radiation units (kr, third column from the right). When comparisons were made on a per-nucleus rather than a per-chromosome basis (right-hand column), a spread of about 3.3-fold was found.

The data from reference (*1*) were used to determine energy absorbed per nucleus at the lethal exposure, and a 28-fold range was found; however, the range of the nine polyploid species was only 8-fold and that of the seven diploid species was less than 5-fold. The apparent lack of concordance between the two series of experiments may be ascribed to the facts that, in the experiments of Sparrow *et al.*, actively growing meristems were irradiated to the lethal point and most species—9 out of 16—were polyploid while, in our experiments, dormant meristems were irradi-

Table 1. Test of the hypothesis that in dormant seeds of quite different sensitivity, as measured by total radiation exposure, similar or identical sensitivity exists as measured by energy absorbed per chromosome or per nucleus. (SE, standard error.)

| Plant group and chromosome number | Average nuclear volume ($\mu^3 \pm$ SE) | Energy per chromosome per roentgen (ev)* | Energy per nucleus per roentgen (ev) | 50% exposure (kr $\pm$ SE)† | Energy per chromosome at 50% exposure (Mev) | Energy per nucleus at 50% exposure (Mev) |
|---|---|---|---|---|---|---|
| 1. *Cucumis sativus* (14) | 117 $\pm$ 2.2 | 502.9 | 7,041 | 46.3 $\pm$ 0.21 | 23.28 | 326.0 |
| 2. *Trifolium incarnatum* (14) | 126 $\pm$ 1.4 | 541.6 | 7,583 | 135.0 $\pm$ 3.74 | 73.12 | 1023.7 |
| 3. *Brassica napus* (38)‡ | 125 $\pm$ 0.2 | 198.0 | 7,522 | 142.2 $\pm$ 8.11 | 28.16 | 1069.6 |
| 4. *Linum usitatissimum* (30) | 164 $\pm$ 3.8 | 329.2 | 9,870 | 71.3 $\pm$ 5.91 | 23.47 | 703.7 |
| 5. *Lycopersicon esculentum* (24) | 193 $\pm$ 5.5 | 483.8 | 11,615 | 47.5 $\pm$ 2.01 | 22.98 | 551.7 |
| 6. *Lactuca sativa* (18) | 193 $\pm$ 0.3 | 645.1 | 11,615 | 47.3 $\pm$ 3.66 | 30.51 | 549.4 |
| 7. *Arachis hypogaea* (40)‡ | 249 $\pm$ 2.9 | 224.5 | 14,985 | 29.3 $\pm$ 0.82 | 6.58 | 439.1 |
| 8. *Festuca elatior* (42)‡ | 435 $\pm$ 7.1 | 623.5 | 26,178 | 14.0 $\pm$ 0.69 | 8.73 | 366.5 |
| 9. *Hordeum vulgare* (14) | 467 $\pm$ 2.1 | 2,007.6 | 28,104 | 25.9 $\pm$ 1.99 | 52.00 | 727.9 |
| 10. *Allium cepa* (16) | 901 $\pm$ 21.0 | 3,388.7 | 54,222 | 13.0 $\pm$ 0.38 | 44.05 | 704.9 |
| 11. *Gossypium arboreum* (26) | 435 $\pm$ 3.6 | 1,006.8 | 26,178 | 16.8 $\pm$ 0.27 | 16.91 | 439.8 |
| 12. *Daucus carota* (18) | 114 $\pm$ 1.8 | 379.7 | 6,830 | 61.8 $\pm$ 2.32 | 23.47 | 422.1 |
| | | | | (Averages) | (29.44) | (610.4) |

* Based on 1.77 ionizations per cubic micron of tissue per roentgen and 34 ev per ion pair; for the computations it is assumed that nuclei are composed entirely of chromosomes. † Maximum exposure to seeds causing 50 percent reduction in seedling dry weight. ‡ Polyploids.

ated to a sublethal endpoint and most species—9 out of 12—were diploid.

The search for a unifying concept of seed radiosensitivity can be carried one step further. The values in the last column of Table 1 may be estimates of a single number, representing the maximum energy (in Mev) which can be absorbed by a dormant nucleus in the apical meristem before growth of the ensuing seedling will be reduced by 50 percent; the average value is 610.4 ± 70.4 Mev. The only variables making up this "constant" value ($k$) are the nuclear volume and the 50 percent exposure; thus either could be used to estimate the other. Since the experimenter is usually interested in predicting radiation tolerance, it would be relatively simple to section a few embryos and measure apical nuclei. It follows that

$$\frac{k}{\text{nuclear volume}} = \frac{}{\text{the 50 percent exposure in roentgens}} \quad (1)$$

for dormant embryos in their most resistant state, and

$$k = \frac{610.4 \pm 70.4 \text{ Mev/nucleus}}{(1.77)\ (34)\ \text{ev}/\mu^3/\text{roentgen}} = (10.14 \pm 1.17) \times 10^6 \quad (2)$$

therefore

$$\frac{(10.14 \pm 1.17) \times 10^6}{\text{average nuclear volume } (\mu^3)} = \frac{}{\text{the 50 percent exposure in roentgens}} \quad (3)$$

for dormant embryos in their most resistant state.

This method was tested on the last two species of Table 1 prior to the performing of dose-response experiments. For the first 10 species, average energy per nucleus at the 50 percent exposure was 646.3 ± 80.1 Mev, hence $k$ was calculated to be $(10.74 \pm 1.33) \times 10^6$. The 50 percent exposure for *Gossypium arboreum*, with an average nuclear volume of 435 $\mu^3$, was thus predicted to be 24.7 kr with a 95 percent confidence interval of 17.5 to 32.1 kr and a 99 percent confidence interval of 14.8 to 34.6 kr. The experimental value (Table 1) was 16.8 ± 0.27 kr.

The data from *G. arboreum* were then added to the preceding 10 species and the average energy per nucleus at the 50 percent exposure became 627.5 ± 74.8 Mev, and $k$ was thus $(10.43 \pm 1.24) \times 10^6$. *Daucus carota* (average nuclear volume 114 $\mu^3$) was predicted to have a 50 percent exposure of 94.2 kr, with a 95 percent confidence interval of 65.2 to 119.4 kr and a 99 percent confidence interval of 57.0 to 125.9 kr. The experimental value (Table 1) was 61.8 ± 2.32 kr. Thus in both tests the observed values fell below the 95 percent but within the 99 percent confidence interval (*8*).

Since this report was first submitted, we have been permitted access to relevant unpublished data from two Spanish authors (*9*). Their study provides nuclear volume and $LD_{50}$ (lethal dose to 50 percent of the population) values for 20 species. Pertinent technical features are: all species were from the family *Cruciferae* and 16 of the 20 species were diploid; dormant seeds were equilibrated at 70 percent relative humidity then x-irradiated at 1200 r/min; exposures reducing survival by 50 percent were determined after 2 months of growth; and nuclear volumes of apical meristems were measured in sprouted seedlings. Average nuclear volumes ranged from 25 to 270 $\mu^3$; chromosome numbers, from 10 to 64; and $LD_{50}$'s, from 15 to 240 kr. Ranges of energy per chromosome and per nucleus at the $LD_{50}$ were inconclusive, being 6.6-fold in the former case and 5.4-fold in the latter. Means and standard errors were $(24.97 \pm 2.99) \times 10^6$ ev per chromosome and $(553.4 \pm 65.5) \times 10^6$ ev per nucleus, values quite in agreement with ours of Table 1. From their data one obtains a $k$ value of $(9.20 \pm 1.09) \times 10^6$, which compares favorably with our value of $(10.14 \pm 1.17) \times 10^6$.

The theoretical significance of such a constant is obscure, but the practical importance is clear. Heretofore a person embarking on a radiation study with dormant seeds of an untested species could not predict whether his material would be devastated by 1 kr or be unaffected by 100 kr. Now a few microscopic measurements and some easy arithmetic will reveal the approximate amount of radiation he can expect the seeds to tolerate before a significant reduction in growth will occur.

THOMAS S. OSBORNE
ALLYN O. LUNDEN
*Agricultural Research Laboratory,**
*Oak Ridge, Tennessee*

**References and Notes**

1. A. H. Sparrow, L. A. Schairer, R. C. Sparrow, *Science* **141**, 163 (1963).
2. A. H. Sparrow and J. P. Miksche, *ibid.* **134**, 282 (1961); A. H. Sparrow, R. S. Cuany, J. P. Miksche, L. A. Schairer, *Radiation Botany* **1**, 10 (1961).
3. H. J. M. Bowen, *Radiation Botany* **1**, 223 (1962).
4. T. S. Osborne, A. O. Lunden, M. J. Constantin, *ibid.* **3**, 19 (1963).
5. *Univ. of Tennessee Agricultural Research Laboratory Semi-Annual Progress Report Jan. 1–June 30, 1963, ORO-614* (1964).
6. A. O. Lunden, *Radiation Botany*, in press.
7. C. D. Darlington and A. P. Wylie, *Chromosome Atlas of Flowering Plants* (Allen, London, 1955).
8. It is possible that the numbers in the last column of Table 1 are estimates, not of a single value, but of a distribution having a rather small variance. It may be that further work will reveal different $k$ values for different classes of seeds—for example, different ploidy levels.
9. C. Gómez-Campo and L. Delgado, *Radiation Botany*, in press
10. We thank C. Gómez-Campo and L. Delgado for permission to use unpublished data, D. A. Gardiner and R. J. Monroe for statistical advice, and A. H. Sparrow for helpful discussions.

* Operated by the University of Tennessee Agricultural Experiment Station for the U.S. Atomic Energy Commission.

18 May 1964

## Protein Synthesis During Development: Control through Messenger RNA

Abstract. *Utilization of long-lived messenger RNA appears to be the exception rather than the rule in cells which are differentiating and synthesizing large amounts of specialized product at the same time. The fact that polyribosomes synthesize protein after RNA synthesis is turned off by actinomycin D is used to demonstrate messenger RNA of long half-life. The data suggest that most tissues examined have short-lived messenger RNA's, but the ocular lens can synthesize protein after an incubation of 24 hours in 40 μg of actinomycin D per milliliter. A common basis for the presence of long-lived messenger RNA in the cells of the lens, the feather, and in reticulocytes is discussed.*

A mark of the differentiated cell is its capacity to synthesize structural or enzymatic cell specific proteins. Some cells, such as skin, liver, muscle, connective tissue, reticulocyte, pancreas, and thyroid, produce large amounts of one or a few kinds of protein. We have asked whether all or only some differentiating cells synthesize their specialized product on messenger RNA which has a long half-life. It has already been shown that hemoglobin (*1*) and feather