dryness at reduced pressure and dissolved in water. Glutamic acid, glutamine, aspartic acid, and γ-ABA were determined by two-dimensional chromatography according to Gaitonde (*6*). In some cases γ-ABA was determined also by the enzymatic method of Scott and Jakoby (*7*).

Gamma-aminobutyric and other amino acids were released from the surface of the individual cerebral cortex at a constant rate for 3 to 4 hours, once the resting state of the animal had become stabilized—as judged by a constant pattern of the ECG. The amounts obtained simultaneously from the left and right hemispheres were approximately the same, unless asymmetrical lesions had been made in the brain stem. There was, however, considerable variability from animal to animal.

In neuraxially intact or "encephale isolé" preparations with relatively few "sleep" spindles appearing in the ECG, the rate of γ-ABA release was about 0.7 μg hour^{-1} cm^{-2}, as shown in Table 1. In the animals with midbrain transections and constant sleep patterns in the ECG the rate of γ-ABA release was about three times as great. In two animals, midcollicular hemisections were made so that the rates of release of amino acids from an activated and a sleeping hemisphere could be determined simultaneously in the same animal, thus the possible effect of general factors such as changes in blood pressure were controlled. The rate of γ-ABA released from the "sleeping" hemisphere was higher than that from the intact side. The rate of glutamic acid release was higher in the intact "waking" hemisphere, with no difference apparent for glutamine and aspartic acid.

In two animals, (not included in Table 2) with unilateral mesial coagulation of the brain stem at the mesodiencephalic junction, stimulating electrodes were placed in the midbrain reticular formation on the opposite side. The ECG on the side of the lesion showed constant "sleep" spindles and did not react to electrical stimulation of the reticular formation below. The same stimulation (5-second trains of 100 per second, 1-msec pulses of 5-volt peak intensity administered periodically at 2- to 5-minute intervals) maintained a constant desynchronized arousal pattern in the ECG on the side of the intact brain stem. In these animals no γ-ABA could be detected in the perfusate from this activated side, while γ-ABA was released at rates of about 1 to 2 μg hour^{-1} cm^{-2} from the side of the lesion.

The relatively constant rate of release of glutamine and aspartic acid in different preparations (Table 1) makes it unlikely that the differences in γ-ABA release might be due to circulatory changes or factors other than the effects of the brain stem lesions upon the afferent supply to the cerebral cortex. It seems that the major portion of the ascending systems of neurones which liberate γ-ABA must lie in the mesial portions of the brain stem in close association with those systems regulating the spontaneous electrical activity of the cortex.

Sensory cortical evoked potentials and responses to direct electrical stimulation are rapidly depressed and changed in form by the topical application of γ-ABA (*8*), while spontaneous slow waves and rhythmic spindle waves are enhanced. Topical γ-ABA produces a marked increase in the slow-wave component of an experimental spike and wave complex which can be produced by rhythmic electrical stimulation of the mesial thalamus; this wave is associated with inhibition of cortical neuronal activity (*9*). Iontophoretically applied γ-ABA through multibarrelled microelectrodes has also been shown to cause rapid arrest of spontaneous firing in individual cortical cells (*10*).

Our studies show that free γ-ABA is released from the cerebral cortex in amounts (probably in much larger amounts than could be detected by the methods used in our experiments) which bear a definite relation to its functional activity. They suggest the possibility that γ-ABA or some related substance may be of physiological importance as a mediator of inhibitory controls of brain activity.

That a significantly greater amount of glutamic acid was found in the activated (aroused) cortex with intact brain stem is consistent with the known excitatory action of this substance (*4*); this may mean that factors in addition to acetylcholine may have to be sought for a full explanation of the excitatory aspects of the control exerted upon the activity of the cortex by the brain stem in different states of vigilance.

H. H. JASPER
R. T. KHAN
K. A. C. ELLIOTT
Montreal Neurological Institute
and *Department of Biochemistry,*
McGill University, Montreal, Canada

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Relative Radiosensitivities of Woody and Herbaceous Spermatophytes

Abstract. *The sensitivities of several woody and herbaceous species to single acute exposures to cobalt-60 gamma rays have been determined. Within each group the sensitivity of each species is largely determined by its average interphase chromosome volume (interphase nuclear volume divided by chromosome number) of shoot apical meristem cells. On the basis of the calculated amounts of energy absorbed (in kiloelectron volts) per interphase chromosome at an exposure necessary to produce a given biological effect, woody species were approximately twice as sensitive as herbaceous species.*

In herbaceous plants there is a high correlation between exposures of x- or γ-rays required to produce a lethal effect and the average interphase chromosome volume of the shoot apical meristem cells (*1, 2*). The correlation is much better than the previously reported correlation between radiosensitivity and nuclear volume (*3*).

Using the same techniques, we at-

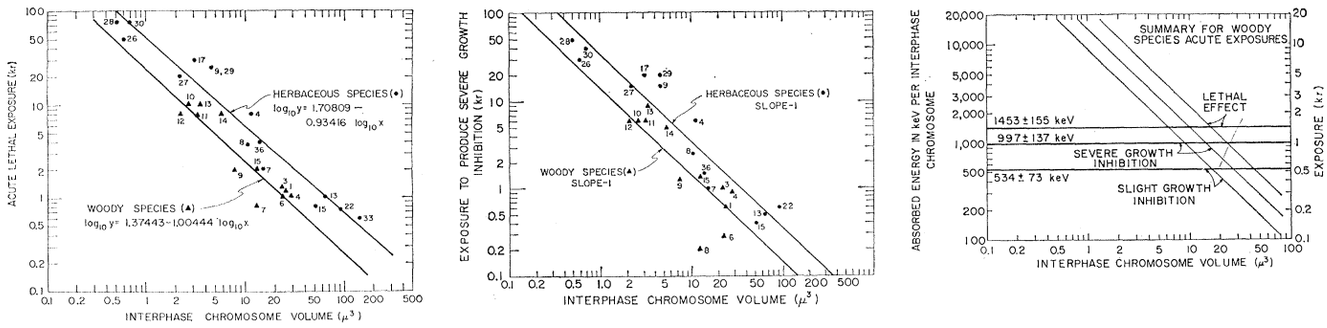

Fig. 1 (left). Relation between interphase chromosome volume (μ^3) and acute lethal exposure (kr) for 15 herbaceous and 12 woody plant species (Table 1 gives names of woody species). Herbaceous species are: 4, *Aphanostephus skirrobasis*; 7, *Chlorophytum elatum*; 8, *Crepis capillaris*; 9, *Gladiolus* c.v. Friendship; 13, *Hyacinthus* c.v. Innocence; 15, *Lilium longiflorum*; 17, *Mentha spicata*; 22, *Podophyllum peltatum*; 26, *Sedum alfredi* var. *nagasakianum*; 27, *Sedum oryzifolium*; 28, *Sedum rupifragum*; 29, *Sedum ternatum*; 30, *Sedum tricarpum*; 33, *Trillium grandiflorum*; 36, *Zea mays*. Fig. 2 (middle). Relation between interphase chromosome volume (μ^3) and acute exposures (kr) required to produce severe growth inhibition for 14 herbaceous and 12 woody plant species. (Table 1 gives names of woody species. Herbaceous species are listed in Fig. 1; No. 33 not included.) Fig. 3 (right). Summary, for woody species, of the relations between interphase chromosome volume (μ^3) and acute exposures (kr) required to produce lethality and growth inhibition (right hand and lower scales) and energy absorption (kev) per chromosome for the same three end points (left hand and lower scales). (Table 1 and Figs. 1 and 2 give details for sensitivity lines; drawn with a slope of −1). Average energy absorption per chromosome and the standard deviation are given for each end point. These lines are drawn with a slope of 0.

tempted to determine if the same relation would hold for woody plants. The 12 species studied included gymnosperms and dicotyledonous angiosperms, which were obtained from commercial nurseries or were available on the Brookhaven National Laboratory site; diploid and polyploid species were included (Table 1). Tolerance was determined by irradiating young plants with suitable exposures between 50 and 18,000 r of γ-rays from a Co^{60} source. In all cases a 16-hour exposure period was used, hence dose-rate varied from approximately 3 r per hour to 1100 r per hour. The number of plants per exposure varied from 25 to 30, and the lethal dose was the lowest exposure at which all the irradiated plants died while the controls were still growing normally. Some of the plants were irradiated in the dormant stage, and some in early stages of active growth. In either case, measurements of the nuclear volume were made from control material fixed on the same date that the plants were irradiated. After irradiation, the plants were moved to outdoor plots and scored for survival 10 to 14 months later. The lethal exposures for

Table 1. Summary of nuclear, chromosomal, and mortality data for 12 woody plants (indicated by plant number, species, and chromosome number, $2n$) and calculated amount of energy absorbed (E abs) per interphase chromosome (chr) at the acute lethal exposure and at acute exposures required to produce growth inhibition. (SE, standard error)

Plant number, species, and somatic chromosome number ($2n$)	Nuclear* volume ($\mu^3 \pm$ SE)	Chromosome† volume ($\mu^3 \pm$ SE)	Lethality		Severe inhibition		Slight inhibition	
			Exposure (kr)	E abs‡ per chr§ (kev)	Exposure (kr)	E abs‡ per chr§ (kev)	Exposure (kr)	E abs‡ per chr§ (kev)
4 *Picea glauca* (24)	681 ± 40.78 D	28.4 ± 1.70	1.02	1665	0.900	1469	0.30	490
1 *Abies balsamea* (24)	580 ± 25.49 D	24.2 ± 1.06	1.15	1597	0.600	833		
6 *Pinus strobus* (24)	558 ± 31.67 D	23.3 ± 1.32	1.00	1337	0.415	555	.15	201
3 *Larix leptolepis* (24)	543 ± 29.33 D	22.6 ± 1.22	1.25	1626	1.000	1301	.70	910
15 *Sambucus canadensis* (36)	462 ± 32.04 A	12.8 ± 0.89	2.00	1477	1.350	997	.90	665
8 *Taxus media* HV. Hatfield (24)	303 ± 9.22 D	12.6 ± 0.38	0.80	580	0.200	145		
9 *Thuja occidentalis* (22)	168 ± 6.27 D	7.6 ± 0.29	1.50	658	1.250	548	.60	263
14 *Quercus rubra* (24)	133 ± 8.30 A	5.5 ± 0.35	8.00	2541	5.000	1588	2.00	635
13 *Fraxinus americana* (46)	157 ± 6.84 A	3.4 ± 0.15	10.00	1965	9.000	1769	3.50	688
11 *Acer saccharum* (26)	83 ± 4.71 A	3.2 ± 0.18	8.00	1466	6.000	1100	3.00	550
10 *Acer rubrum* ‖ (91)	241 ± 11.23 A	2.7 ± 0.12	10.00	1521	6.000	913	4.50	684
12 *Betula lutea* ‖ (84)	183 ± 11.76 A	2.2 ± 0.14	8.00	1000	6.000	750	2.00	250
			Mean	1453 ± 155		997 ± 137		534 ± 73

* Measurements made from shoot meristems at time of irradiation; D, dormant, A, actively dividing. † 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 (*4*, Table 2) times exposure. § On the assumption that all chromosomes within a karyotype are the same size. ‖ Polyploids.

the 12 species varied from 0.80 to 10 kr.

Figure 1 shows the lethality data for the herbaceous species (*1*) and the new data for the woody species, and Fig. 2 shows the exposures necessary to produce severe growth inhibition, each plotted against the interphase chromosome volumes for the various herbaceous and woody species. In both cases woody species are much more radiosensitive than herbaceous species of comparable interphase chromosome valume. A statistical test indicated that the differences were significant at the 5 percent level.

In Fig. 1 the actual slopes of the regression lines are used but none of the slopes discussed here deviated significantly from −1; all other lines were drawn with a slope of −1 for easier comparison. Since the slopes are essentially −1, the product of the two variables should approach a constant as shown for 16 acutely irradiated herbaceous species (*1*). When exposure is expressed as energy absorbed per unit volume of tissue, the product of the two variables is the total energy absorbed per interphase chromosome at specified exposures and volumes (Table 1). The energy-absorption calculations are based on an average value of 32.5 ev per ion pair and 1.77 ionizations per cubic micron of wet tissue per roentgen. (*4*, Table 2). From a graph of the energy absorbed per chromosome at a lethal exposure against the interphase chromosome volume a regression line was obtained with a slope approaching zero (−0.00453); thus the same principle holds for the sensitivity of woody plants as previously reported for herbaceous species (*1*), that is, the energy absorption per chromosome approaches a constant. A summary of the sensitivities (−1 slope) and the energy absorption (0 slope) for woody species is given in Fig. 3 along with means and standard deviations.

The energies per chromosome required to produce each of the three end points used in the irradiation of the woody plant species were calculated and compared with those of the herbaceous species (*1, 2*). The average amount of energy absorbed per chromosome required to produce lethality in woody plants (1453 kev) is 39 percent of that required for the herbaceous species (3711 kev). Comparable ratios hold for the average amount of energy required to produce severe growth inhibition (997 kev for woody species, 2331 kev for herbaceous species) and slight growth inhibition (534 kev for woody species, 1070 kev for herbaceous species). On this basis the woody species are much more sensitive than the herbaceous species. The cause of this difference is not yet understood.

RHODA C. SPARROW
ARNOLD H. SPARROW
Biology Department,
Brookhaven National Laboratory,
Upton, Long Island, New York

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Serum Protein Electrophoresis in Acrylamide Gel: Patterns from Normal Human Subjects

Abstract. *Technical improvements in the method for vertical acrylamide gel electrophoresis (Raymond) have resulted in improved resolution and reproducibility. In a survey of approximately 200 normal human serums, ten lines were found to be common in all specimens. Interposed with these constant lines and in addition to known variations in the pattern reflecting haptoglobin and transferrin types, at least one line followed that of albumin and three followed that of transferrin; the presence or absence of these varied from individual to individual. Based on these variations, the electrophoretic patterns of normal humans may be divided into at least 12 discrete groups.*

Serum protein electrophoresis performed in a gel medium, either starch (*1*) or polyacrylamide (*2*), permits the demonstration of approximately 20 specific protein bands, in contrast to the five or six observed on other supporting media. The question arises whether the distribution of serum proteins studied by high-resolution techniques would give rise to patterns as variable as, for example, finger prints, or whether the electropherogram consists of certain constant features with discrete variations superimposed thereon. The fact that transferrin and haptoglobin, two of the chief features of the protein electrophoresis performed on gels, occur only in several discrete genetically controlled variants suggests that many, perhaps all, of the serum proteins are so controlled. We have made a survey of approximately 200 normal serums employing several modifications to the method of Raymond (*3*), and have found additional evidence for the concept that the serum protein electrophoretic diagram consists primarily of several definable proteins.

In these studies a 5 percent acrylamide gel was used. The gels were prepared before each run from the following refrigerated stock solutions: (*A*) Acrylamide, 20 percent: acrylamide 380 g, *N-N'*methylenebisacrylamide 20 g, dissolved in water to 2 : 1; (*B*) DMAPN-buffer: dimethylaminopropionitrile (DMAPN) 8 ml, buffer (20X, described below) 100 ml, and water to 250 ml; (*C*) ammonium persulfate: 0.2 percent in water, prepared fresh each week. To prepare the gel, 40 ml of acrylamide stock (reagent *A*), 20 ml of DMAPN-buffer (reagent *B*), 80 ml of persulfate (reagent *C*), and 20 ml of water were mixed and poured into the cell just before the run. The buffer contained tris(hydroxymethyl)aminomethane, 431 g; disodium ethylenediaminetetraacetate, 37 g; and boric acid, 220 g in 2 liters (*p*H 8.3–8.4), and was diluted 20 times before use in the electrode vessels. The concentration of boric acid in this buffer is higher than that used by others (*4*) and is an important factor in the improved resolution. All reagents were filtered before use and discarded after each use. The buffer was not recirculated. Voltage was applied for 20 minutes before addition of the sample (*5*). The serums used were obtained from normal, healthy blood-bank donors and were stored not longer than 24 hours at 4°C. All specimens were clear and free of hemolysis. Fifteen microliters of serum were applied to each slot. An eight-place slot former was used.

Water at 0°C (for cooling) was circulated through the cell throughout the run. A potential difference of 200 volts (10 v/cm) was applied which resulted in a current of approximately 25 ma at the end of the run (5 hours). The gels were stained for 1 hour in 1