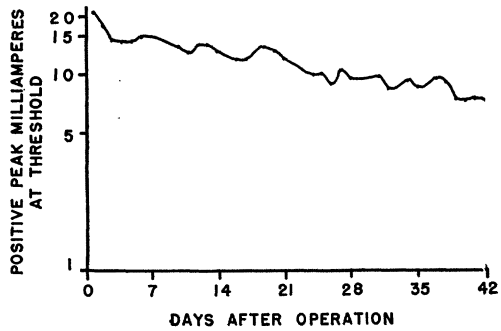

Fig. 2. Variation of threshold with time. The stimulus frequency is 60 pulse-pairs per second; the train duration is 2 sec; and the threshold is the positive peak current necessary to elicit a visible movement. The total quantity of the electricity for the positive pulse summed for the whole train is 18 μcoul. Time is given as days after operation. For the subsequent 9 wk, the threshold remained constant at the value shown at 42 days.

first monkey was observed for 7 wk; at the time of writing, the second monkey had been observed for 17 wk.

Figure 2 shows the slow fall of the threshold current (about 50 percent in the first month) for the pulse-pairs at 60 per second for a typical electrode during a period of 6 wk. Some of this decrease in threshold is probably due to recovery from anesthesia and operative trauma, and some to a slow decrease in distance between the electrode array and the cortex. In the earlier work with rectangular pulses, it was found that a relatively rapid increase in threshold takes place in a few hours (*4*).

Electrocorticograms taken from the electrodes showed no signs of injury. Neurological and behavioral examinations postoperatively showed only some minimal transient signs of injury. Slight flexor weakness in the contralateral hand was seen, but this weakness disappeared gradually during a period of 3 wk. We attribute this to mechanical operative trauma.

Some of the neurohistological studies (frozen sections) have been completed on the first monkey. Under the area of the trephined opening in the skull, there is a moderate proliferation of astrocytes and a slight meningitis. Minute amounts of lipid are present in a few macrophages in the leptomeningeal exudate but not elsewhere. The nerve cells are intact and have approximately the same appearance and population density as on the opposite, nonoperated hemisphere. Whether this degree of change is greater than that due to operative trauma alone remains to be determined.

From these results, it is concluded that this form of electric current does not detectably injure cellular function or structure when it is passed through the cortex near threshold values 4 to 5 hr per day for 5 to 15 wk. Other similarly balanced brief waveforms would probably give similar results. Further details of these observations and their interpretation are in preparation.

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Use of Haploid and Diploid Embryos of *Habrobracon* in the Study of Cell Poisons

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Various genetic and cytological tests have been used in the evaluation of the injurious effects of x-rays on cell nuclei. These have included the study of radiation-induced mutations and chromosome breaks. Additional means of evaluating substances that affect cell nuclei are needed. One such test for chemical agents is reported here and is based upon the fact that the radiosensitivity of cells and organisms may be modified by their degree of ploidy (*1, 2*).

Studies on the parasitic wasp, *Habrobracon juglandis* (Ashmead), have shown that haploid embryos are more resistant to x-rays than diploid embryos when the embryos are treated during the cleavage stages of development (*2*). Similar results have been obtained with the nitrogen mustard, methyl bis(beta-chloroethyl)amine hydrochloride (*3*). Since both x-rays and this nitrogen mustard compound are known to affect cell nuclei and also to be more injurious to diploid than to haploid embryos of *Habrobracon,* various other chemicals were tested (*4*). The fact that some chemicals were found to be more toxic to diploid embryos than to haploid embryos, whereas other chemicals were not, is of interest and points to the possibility that the criterion of greater toxicity to diploid than to haploid embryos might be of some use in the evaluation of cell poisons.

In *Habrobracon,* haploids develop from unfertilized eggs and diploids from fertilized eggs. Haploid cultures are obtained from unmated females, whereas cultures containing both haploid and diploid eggs ("mixed cultures") are obtained from mated females. Usually about 65 percent of the eggs from mated females are fertilized and, accordingly, are diploids. In material used in the present report, all haploids developed into males and all diploids into females. Eggs stored in the uterine sacs of the females remain

Table 1. Hatchability ratios for eggs from mated and unmated females (eggs treated during the cleavage stage).

Agent	Concn. (%)	Haploid + diploid (from mated females)		Haploid (from unmated females)	
		No. of eggs	Hatchability	No. of eggs	Hatchability
X-rays, 220 r (*2*)		331	0.18 ± 0.02	319	0.51 ± 0.03
Controls		1071	.92 ± .01	573	.93 ± .01
Methyl bis(Beta-chloroethyl)amine HCl* (*3*)	0.0010	878	.22 ± .01	837	.58 ± .02
Ethyl bis(beta-chloroethyl)amine HCl†	.0025	445	.19 ± .02	463	.74 ± .02
2,2′-Dichloro-diethylamine HCl‡	.0200	508	.16 ± .02	417	.53 ± .02
2,2′,2″-Trichloro-triethylamine HCl‡	.0025	269	.21 ± .03	240	.60 ± .03
Controls		543	.95 ± .01	432	.96 ± .01
Sodium azide	.0200	250	.46 ± .04	221	.39 ± .03
Potassium cyanide	.0025	108	.66 ± .05	133	.64 ± .04

* Obtained from Merck and Co., Inc. † Obtained from the Army Chemical Center, Md.
‡ Obtained from the University of Chicago Toxicity Laboratory.

in first meiotic metaphase, and further development does not take place until the eggs are laid (*5*). It is therefore easy to obtain timed embryos of known stages.

Eggs in the cleavage stage of embryonic development were placed in bags made of lens paper, immersed in test solutions of different nitrogen mustards for ½ hr, and then washed. They were then placed in mineral oil and allowed to continue development. The nitrogen mustard solutions were made up immediately before use and were kept at *p*H 7 by means of the Teorell buffer. The number of eggs that hatched as larvae was recorded. This incidence of hatchability (No. larvae/No. eggs) was used to compare the sensitivity of haploid and diploid embryos. Cultures (haploid) arising from unmated females were compared with mixed (haploid-diploid) cultures from mated females. These cultures were immersed in the same solution at the same time and were treated in the same way. In every case the haploid and the mixed cultures were paired. The hatchability values reported in Table 1 are averages from a number of such paired experiments.

Comparison of these cultures (Table 1) shows that for the nitrogen mustard compounds tested the hatchability of purely haploid cultures was greater than that for the haploid-diploid cultures. The same results were obtained for x-rays. The difference in hatchability between these two types of cultures is large and of the same magnitude for each of the nitrogen mustards that were tested, despite the fact that these mustards differ in their toxicity and probably in their mutagenic ability. Comparison of hatchability between haploid and mixed cultures treated with sodium azide or potassium cyanide shows no significant difference (Table 1).

Examination of whole mounts of embryos treated during the cleavage stage shows that embryos treated with x-rays or with the methyl bis(beta-chloroethyl)-amine hydrochloride are arrested in development at the end of cleavage and have enlarged nuclei (*2, 3*). The effects of the other compounds have not been studied cytologically.

Differential effects of chemical agents on individuals differing in their degree of ploidy have been reported for amphibians treated with chloretone (*6*) or with hexenolactone (*7*). It seems worthy of note that a closely related compound, beta-propiolactone, induces mutations in *Neurospora* and causes chromosomal aberrations in *Vicia faba* (*8*). The present work indicates that comparable studies on diploid and polyploid plants and particularly on haploid and diploid yeasts might be pertinent to the analysis of the action of chemicals on cells.

Such comparisons of the toxic effects of chemicals on groups of individuals that differ in their degree of ploidy seem to indicate the following possibilities: (i) a simple and convenient method of screening nuclear and nonnuclear poisons might be obtained; (ii) a method of distinguishing mutagens from nonmutagens or perhaps of separating mutagenic substances that cause gross chromosomal aberrations from mutagens that do not cause chromosome breaks might be obtained; and (iii) further data might be secured on the mechanism of radiation damage by determining which of the chemicals are radiomimetic in this respect.

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