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MAIN SUBJECT HEADING:

AN	HU	AT	IH	M
ANALYTICS	HUMAN EFFECTS	ANIMAL TOXICITY	WORKPLACE PRACTICES-ENGINEERING CONTROLS	MISCELLANEOUS

SECONDARY SUBJECT HEADINGS: AN HU AT IH M

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Animal Toxicology

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Transportation/Handling/ Storage/Labelling

CELLULAR EFFECTS OF MICROWAVE RADIATION[1]

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BIOLOGICAL STUDIES

Extensive experiments at the New England Institute since 1958 have demonstrated previously unsuspected but highly significant "nonthermal" biological effects of radio frequency fields (1–6). We have shown that such fields:

(1) orient free-swimming protozoans (highly frequency-specific);
(2) inhibit cell division temporarily in cells grown *in vitro*;
(3) induce genetic changes (mutations and chromosome abnormalities in living cells, both plant and animal, including human);
(4) induce crossing over in male germ cells of Drosophila; and
(5) stimulate breaking of dormancy in gladiolus bulbs.

Since heat from such sources is a function of average energy input, we undertook experiments using small, intense pulses of radio frequency energy. Sufficient intervals between pulses made the overall rise in temperature insignificant, substantially ruling out the effect of heat.

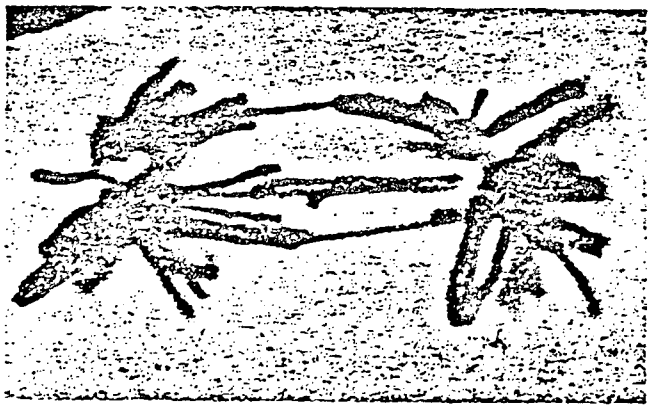

Figure 1. Multiple chromosome bridges at anaphase in a dividing garlic cell treated with radio frequency at 21 MHz.

In experiments with colloidal particles, bacteria, and protozoa (1), remarkable events were seen under the microscope, not related to or accompanied by production of heat. For instance, at certain specific frequencies (5 to 7 MHz) motile protozoa could be constrained to migrate solely parallel to the electrical component of the radio frequency field. At other frequencies (27 to 30 MHz), they were constrained to swim perpendicular to the initial orientation.

Figure 2. Another garlic cell showing anaphase chromosome bridging and chromosome fragments.

Even more intriguing was the effect of radio frequency on intracellular organelles, where similar orientation to specific frequencies occurred (1). It was obvious that, if r. f. could interact on this level, it might play a significant role in mitosis with consequent implications of importance in genetics and in biology and medicine. In experiments using garlic root tips (2, 3, 5), a great variety of chromosome aberrations occurred,[2] including bridging, fragmentation, micronuclei, etc. Figure 1 shows multiple chromosome bridges at anaphase in a dividing garlic cell; Fig. 2 shows another bridge

[1] This work has been supported in part by the Office of Naval Research (Contract No. NR 101-736) and by the John A. Hartford Foundation, Inc.

[2] The conditions of treatment covered a wide range: frequencies from 5 to 40 MHz, pulse duration from 15 to 30 microseconds, repetition rates from 500 to 1000 per second, and voltages from 250 to 6000 peak-to-peak per centimeter. The most effective frequency was 21 MHz.

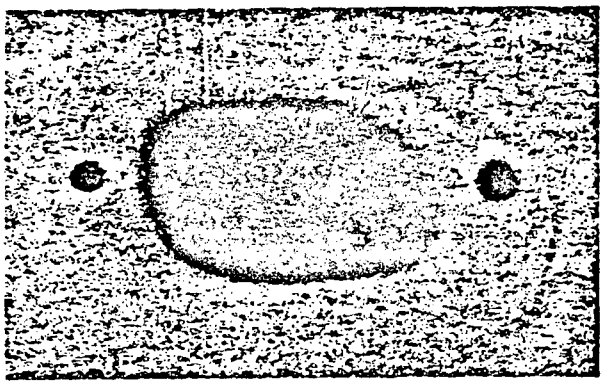

Figure 3. Garlic cell with 2 micronuclei, one on each side of the nucleus.

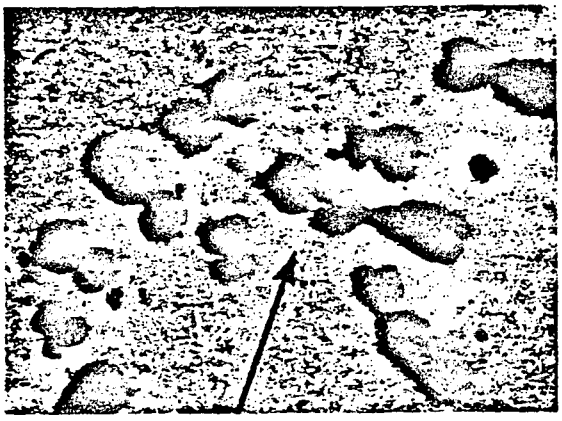

Figure 4. Cultured human lymphocyte showing a chromatid break following radio frequency treatment at 21 MHz.

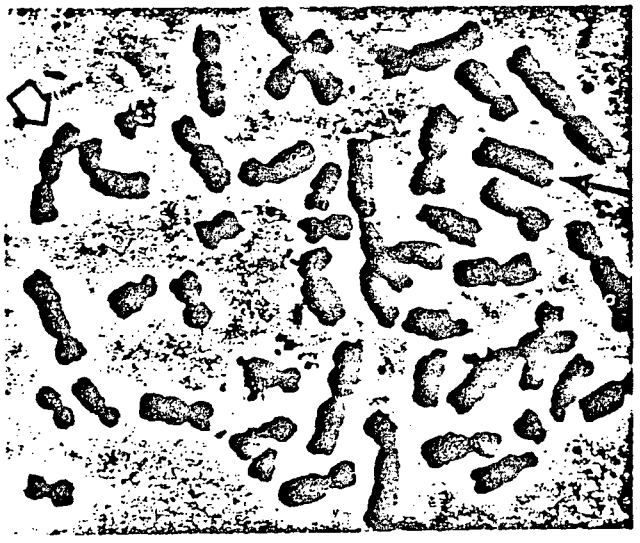

Figure 5. Another human lymphocyte after radio frequency treatment showing both a dicentric chromosome (hollow arrow) and an acentric fragment (solid arrow).

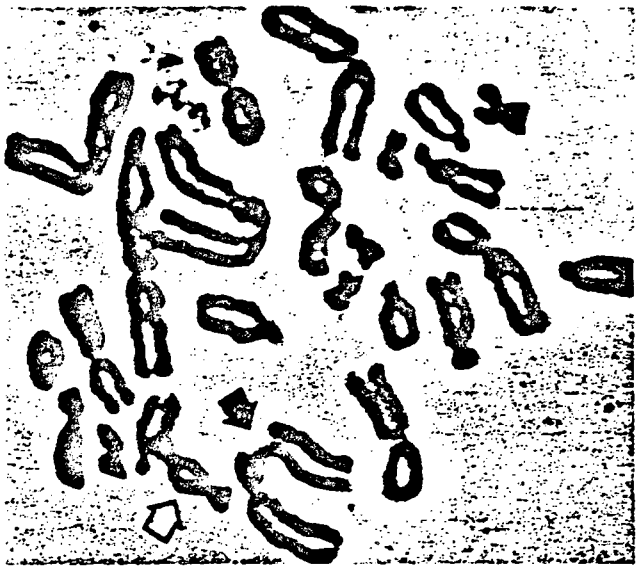

Figure 6. A Chinese hamster cell treated in tissue culture with radio frequency showing a dicentric chromosome (hollow arrow) and a chromatid break (solid arrow) lying adjacent to each other.

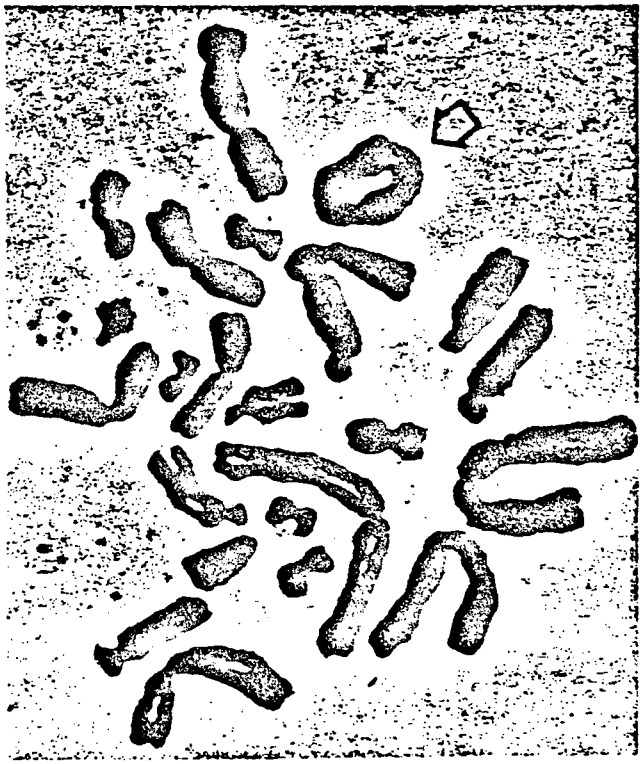

Figure 7. A Chinese hamster cell in tissue culture following treatment with radio frequency. The cell contains 23 chromosomes instead of the normal number of 22, and one is a ring chromosome.

TABLE 1

Results of treatment of cultured human peripheral lymphocytes with pulsed radio frequency at 21 megacycles

Type of culture	Number of cells scored	Aberrations*						Mean abn./cell	χ^2	*P* value
		a	c	d	e	f	p			
Control	600		6			6	4	0.016		
Fixed immediately	500	4	2			6	12	0.036	5.2	0.02
24 hours recovery	2000	11	24	8	9	19	60	0.056	16	<0.01
36 hours recovery	850	4	10	3	8	8	41	0.077	24	<0.01

* a = chromosome break.
c = chromatid break.
d = dicentric chromosome.
e = endoreduplication figure.
f = acentric fragment.
p = polyploid number chromosomes.

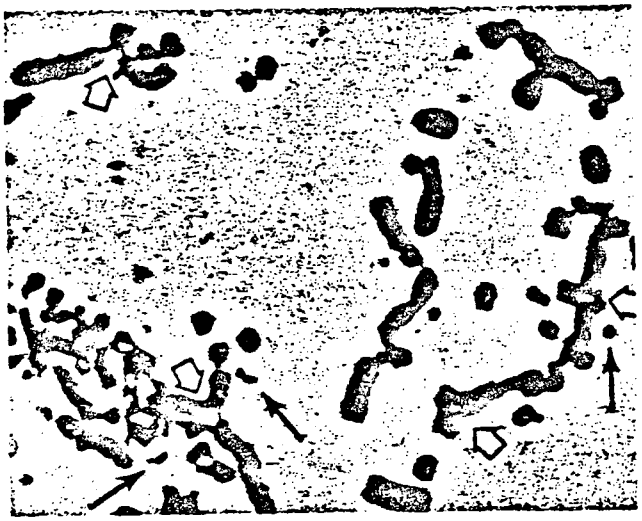

Figure 8. Parts of two Chinese hamster cells showing numerous fragments (solid arrows) and translocations (hollow arrows).

and fragments; and Fig. 3 shows a cell with two micronuclei, one on each side of the nucleus.

Human lymphocytes separated from peripheral circulation were placed in culture and stimulated to transform and undergo mitosis by means of phytohemagglutinin. After two days in culture, the cells were subjected to pulsed radio frequency at 21 MHz.[3] Following the experimental treatment, the cultures were either fixed immediately or allowed to recover for periods of 24 to 36 hours before fixation. Standard air-dry films were stained with aceto-orcein and scored for types of mitotic or chromosome aberrations.

Results are summarized in Table 1. The incidence of chromosome abnormalities in the experimental groups was significantly greater than in the controls. Chromosome damage ranged from single chromatid breaks through dicentric chromosomes to occasional severe erosion of all chromosomes within a given cell. Varying degrees of pycnosis of nondividing nuclei were evident. Figure 4 shows a chromatid break, and Fig. 5 shows both a dicentric chromosome and an acentric fragment in the same cell.

Cultured lung cells from Chinese hamster, when treated with radio frequency in a similar fashion to the human lymphocytes, produced significant num-

[3] Five hundred volts peak-to-peak per centimeter, distance of 2 cm between electrodes, 10 microseconds per pulse, 100 pulses per second for 30 minutes. Temperature was controlled at 27 °C.

TABLE 2

Crossing over induced in male germ cells of Drosophila melanogaster *by r. f. treatment*

Expt. No.	Experiment agent	No. tested	Cross over events	Percent of cross over
1	r. f., 20 MHz	3116	6	0.19
2	r. f., 20 MHz	1110	2	0.18
3	r. f., 20 MHz	1045	2	0.19
4	Heat, 4 hrs.	1851	0	0
5	Heat, 1 hr.	2210	0	0
6	Control	4358	0	0
7	Cold	20,107	0	0
8	r. f., 27 MHz	7829	2	0.0255
9	r. f., 30 MHz	2758	2	0.072

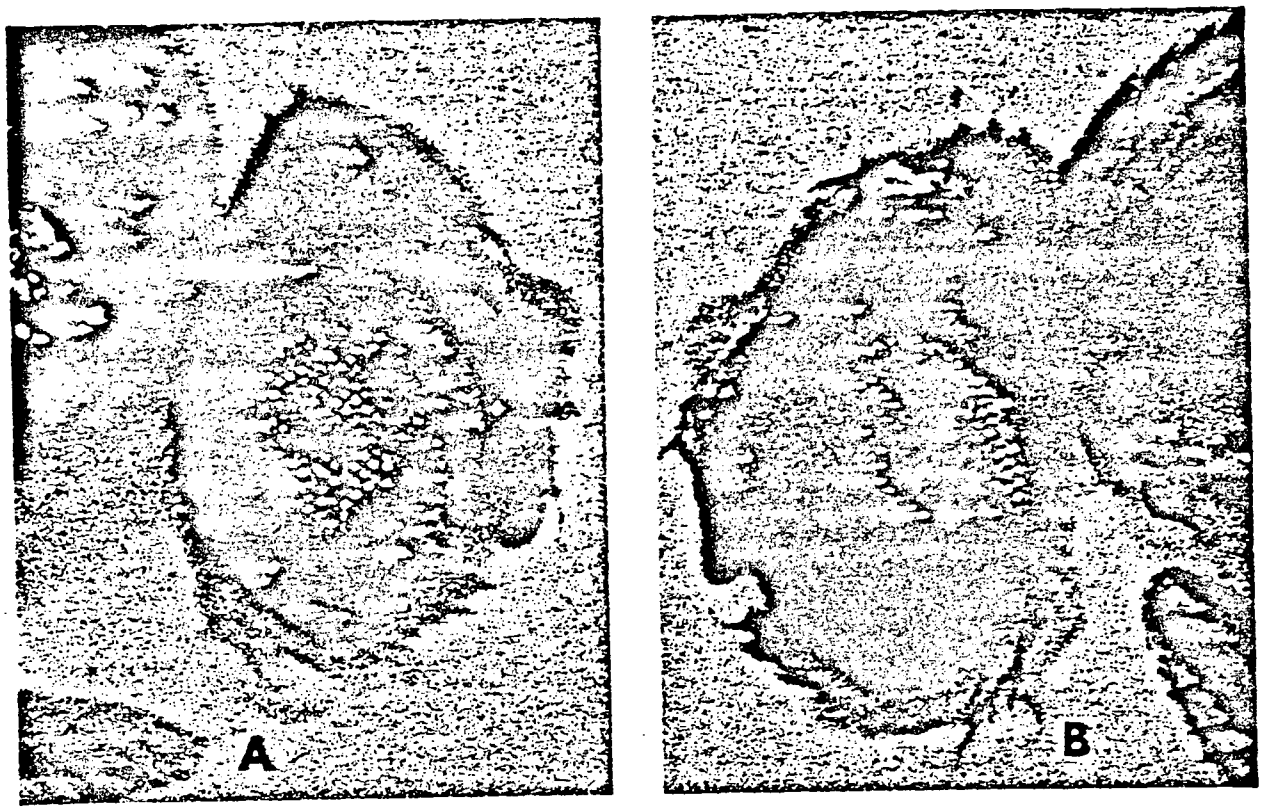

Figure 9. *Drosophila melanogaster* mutant eye (spotted) induced by radio frequency treatment, A; normal or wild-type eye, B.

bers of chromosome aberrations. Figure 6 shows a chromatid break and a dicentric chromosome in one cell. Figure 7 shows a ring chromosome in a cell with 23 chromosomes rather than the normal number of 22. Figure 8 shows a plethora of chromosome fragments and translocations.

Pulsed radio waves[4] applied to the mature germ cells of male Drosophila (5) resulted in the production of numerous visible mutations; some were sex-linked (white eye, singed bristles, yellow body, etc.), others were autosomal (blister wing, spotted eye); some were dominant, and some recessive; and many were sex-linked recessive lethals. Figure 9 shows the mutant spotted eye (A) compared to a normal eye (B). A mutant wing (blister wing, A) and a normal wing (B) are shown in Fig. 10. A sex-linked recessive visible mutant, singed bristle, is illustrated in A of Fig. 11, and the normal or wild-type bristles are shown in B.

[4] The length of treatment varied from 5 minutes to 1 hour; the pulse width, from 30 to 50 microseconds; the most effective frequency was 21 MHz; the repetition rate was 500 to 100 per second; and the voltage, from 500 to 1000 peak-to-peak per centimeter. The flies were placed in a plastic compartment 1 cm^2 and were treated in air.

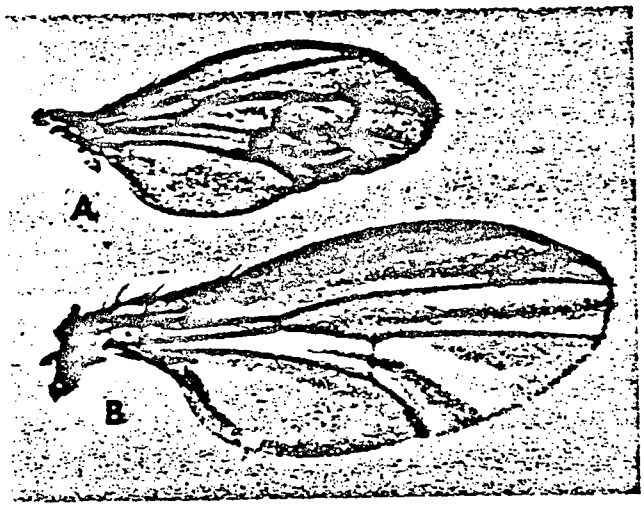

Figure 10. Drosophila mutant (blister wing) resulting from radio frequency treatment, A; normal or wild-type wing, B.

Furthermore, radio frequency energy induces "crossing over" in the germ cells of male Drosophila (4, 5), a phenomenon which naturally occurs only in the female fly. Table 2 summarizes results of these experiments. These genetic effects resemble those resulting from application of ionizing radiations, which have tremendously greater energy levels. The rate of crossing over induced by radio

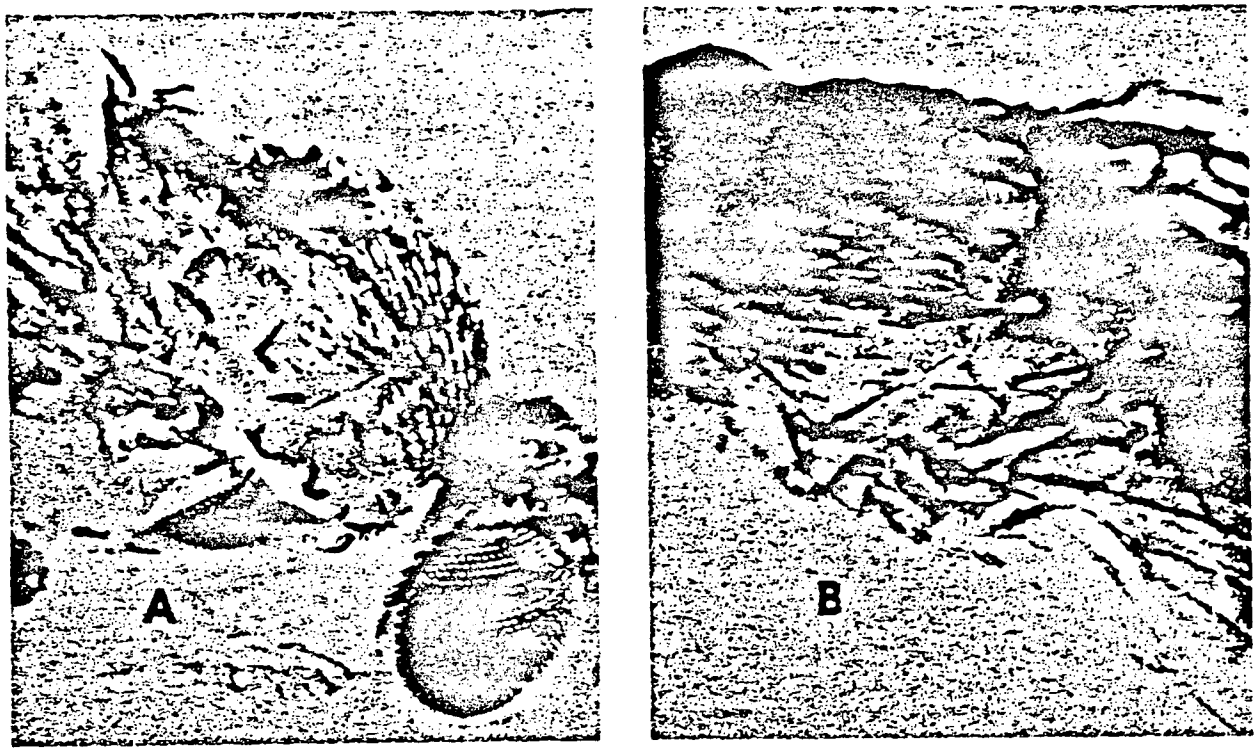

Figure 11. Drosophila sex-linked recessive visible mutant (singed bristles) resulting from radio frequency treatment, A; normal or wild-type bristles, B.

frequency treatment[5] in these experiments is equivalent to that produced by about 250 r of x-rays.

Treatment of gladiolus bulbs (6) with radio frequency of 21 MHz stimulated breaking the dormancy period and produced larger and more vigorous plants which bore more flowers than the other groups.[6] Normal emergence for these bulbs is about 40 to 50 percent; the value of 96 percent emergence for the bulbs treated with 21 MHz is much higher than the next value of 80 percent. The average height of the 21-MHz group was approximately twice that of any other group.

NONBIOLOGICAL STUDIES

While we were actively pursuing these biological studies, we also undertook to secure basic physical data on the marked effect of radio frequency on the zeta potential of polystyrene colloids, demonstrating for the first time the fact that pulsed radio frequency waves could alter the charge on inert materials (7–9).

A monodisperse system of polystyrene was prepared by emulsion polymerization. It was dialized for 5 days against running distilled water. The electronegative zeta potential on these particles was on the order of 40 to 45 millivolts negative. These charges are due to the carboxyl and sulphate groups on the surface. These groups are covalently bonded to carbon atoms of the polymeric chain. There is about one charge to every 500 square angstroms of surface. A cuvette was filled with about 15 milliliters of polystyrene colloid. Two platinum electrodes were on either side of the cuvette. As a function of particle size, there was an interaction with an r. f. field. This is at a specific frequency for a particle size. Negative charges appeared to be "stripped" from the surface. There was about a 25 percent reduction of electronegative charge on the polystyrene. Different-sized monodisperses responded to different frequencies. The threshold voltages were of the order of 100 volts peak-to-peak per centimeter, with a maximum effect being found at 1000 volts peak-

[5] The most effective frequency was 20 MHz. Other parameters were the same as above for mutations.

[6] The frequencies ranged from 11 to 41 MHz, at 1-MHz intervals. The chamber in which the cormels were treated measured 6×5×3.5 cm, which accommodated 25 cormels at a time. Other parameters were: 10 minutes' treatment, pulse width 30 microseconds, repetition rate of 1000 pulses per second, peak-to-peak voltage ranged from 12,500 to 15,000.

to-peak per centimeter. This effect reversed itself spontaneously with time. The response, however, was very slow, requiring 4 hours to regain its initial zeta potential. The rate of return was linear.

Other colloids also were examined, ranging from starch granules, where the charge is not ionogenic but adsorbed to spores of various fungi. All manifested the same phenomena. There was no evidence of an increase of ionic groups in the solution in which the colloids were suspended, and certainly the impressed r. f. field was trivial in comparison with circa 3 electron volts needed to break a covalent bond. As yet, no effective explanation of this phenomenon is available.

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EFFECTS OF MICROWAVE RADIATION ON LENS EPITHELIAL CELLS
(Summary)

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It is well established that exposure of the eye to microwave radiation can cause within a few days the formation of opaque areas, or cataracts, in its normally transparent lens. These posterior subcapsular cataracts are similar in appearance to those induced by ionizing radiation except that, in the latter case, weeks or months are required for the cataract to develop. With respect to its latent period the microwave cataract is similar to the galactose induced cataract, in which equatorial opacities appear after two days of galactose feeding.

Several investigators have shown that ionizing radiation and galactose feeding affect the lens epithelial cells, from which the lens fibers differentiate by a process of elongation. For this reason the present experiments were conducted with microwave radiation to determine whether this form of radiation has an effect upon the lens epithelium and, if so, whether the effect is similar to that of ionizing radiation or galactose feeding.

The right eyes of adult New Zealand white rabbits were exposed to cataractogenic doses of CW radiation at 2.45 GHz; the nonirradiated left eye served as the control. At post-irradiation intervals varying from six hours to one month the animals were sacrificed. One hour before sacrifice, tritiated thymidine (a radioactive form of the thymine incorporated by cells synthesizing DNA in preparation for mitosis and cell division) was injected into the anterior chambers of both eyes. This autoradiographic technique was utilized so that every epithelial cell which was preparing to undergo mitosis could be identified as well as those in active mitosis. By counting and comparing the number of such cells in both the irradiated and the control lenses the effect of the radiation could be determined.

Characteristically the irradiated lenses showed an initial pronounced suppression of both DNA synthesis and cell division. This gradually diminished during the ensuing two weeks, by which time these activities had recovered and by one month post-irradiation they were proceeding at a slightly accelerated rate. This sequence of events parallels closely those observed in the lens epithelium after exposure of the eye to ionizing radiation.

In twenty of the irradiated eyes, however, there was superimposed upon the usual course of recovery a precipitous rise in DNA synthesis occurring on the fourth to fifth day after irradiation. In all of these irradiated lenses equatorial vesicle strings had begun to develop in the superior temporal quadrant on the third day post-irradiation. The counts made on these epithelia revealed that the increased activity was localized in the quadrant of the epithelium lying directly in front of the vesicle strings. This sharp rise in DNA synthesis is similar to that which is observed in galactose fed rats where hydration of the lens occurs in the form of equatorial vesicles which seem to stimulate the overlying epithelium to proliferate at a greatly accelerated rate. The same stimulus may be responsible for the greatly increased rate of DNA synthesis in the microwave irradiated lenses in which equatorial vesicles are formed.