

Effects of Microwave Radiation on the Lens Epithelium in the Rabbit Eye

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• These experiments were conducted to determine the effect of cataractogenic doses of microwave radiation at 2.45 gigahertz (GHz) on the lens epithelium of the rabbit. One hour before animals were killed, tritiated thymidine was injected into the anterior chamber of both eyes at postirradiation intervals varying from six hours to one month. Epithelial peels were made and autoradiographic techniques used to identify cells manufacturing DNA. Comparison of counts from both experimental and control epithelia revealed two patterns, depending on the presence or absence of vesicle strings. Those lenses without vesicle strings showed an initial pronounced suppression of mitotic activity followed by gradual return to normal levels. Those lenses with strings showed a precipitous rise in DNA synthesis on the fourth to fifth day after irradiation. This increased activity may be the result of lens hydration.

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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.¹ These posterior subcapsular cataracts are similar in appearance to those induced by ionizing radiation,²⁻⁴ but 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^{5,6} in which the equatorial opacities appear two days after galactose feeding.

Several investigators have shown that ionizing radiation and galactose feeding affect the lens epithelial cells,

from which the lens fibers differentiate. Ionizing radiation⁷⁻⁹ inhibits mitosis in the lens epithelium and causes a loss of cells in the germinative zone. Von Sallmann,⁹ studying flat mounts of the lens epithelium prepared according to Howard's¹⁰ method, showed that ionizing radiation causes in rabbits an almost complete inhibition of mitosis that lasts about three days, after which there is gradual recovery during the next two weeks followed by a period of greater than normal mitotic activity.

The development of techniques for labeling cells using radioactive compounds such as tritiated thymidine makes identification of cells that are in the process of DNA synthesis as clear cut as identification of those that are undergoing mitosis. Von Sallmann¹¹ and Scullica et al,¹² using intraperitoneally administered injections of tritiated thymidine in rats, found that mitosis in the lens epithelium was blocked immediately after x-irradiation and also that the number of cells incorporating tritiated thymidine decreased, although less promptly. Recovery of DNA synthesis and mitosis began approximately 24 hours later.

Similar studies have been done, with different results, on rats fed galactose. Hanna and O'Brien⁵ presented evidence of a dramatic increase in the number of DNA-synthesizing epithelial cells during the first week of galactose feeding. Grimes and von Sallmann⁶ reported a transient stimulation of cell division in the lens epithelium that reached a peak at four days and subsided by seven days after galactose feeding had begun.

Since ionizing radiation and galactose feeding differ in the effect they produce on the lens epithelium, the experiments reported here were con-

ducted to determine whether or not microwave radiation affects the lens epithelium and, if so, whether the effect is similar to that of ionizing radiation or of galactose feeding.

METHODS

The microwave beam was generated by a magnetron tube driven by a voltage-regulated power supply. The continuous-wave power at 2.45 gigahertz (GHz) was fed through an isolator that protected the magnetron from power reflected back through the system. Two-directional couplers were employed to measure forward-going and backward-reflected powers, both of which were monitored continuously by means of thermistor mounts feeding to power meters. A slotted line was inserted beyond the couplers to tune out reflected power. The power then passed through a short section of coaxial cable to a dipole antenna that was mounted in an anechoic chamber 3×3×2 feet (Fig 1). The anesthetized rabbit was placed in this chamber behind a barrier of microwave-absorbent material with only the head exposed to the microwave field. The right eye of the animal, positioned exactly opposite the dipole antenna crossover and two inches away from the surface of its plastic housing, was exposed to a known cataractogenic dose of radiation. The nonirradiated left eye served as a control.

All exposures were carried out at 280 milliwatts (mW)/sq cm for seven minutes' duration. The absorbed power density of 280 mW/sq cm was calculated from calorimetric measurements made in a phantom eye. This method of determining absorbed power has been described in detail by Carpenter and Van Ummersen.¹³ Since that time it has been possible to measure the power incident at the position of the eye in the anechoic chamber using a probe that measures all components of the microwave field. The incident power under these conditions is 585 mW/sq cm. It should be made clear, however, that these two power figures cannot be equated exactly because the former was an attempt to determine the power absorbed by the eye, while the latter was a measure of power perhaps incident on the eye. Another important point

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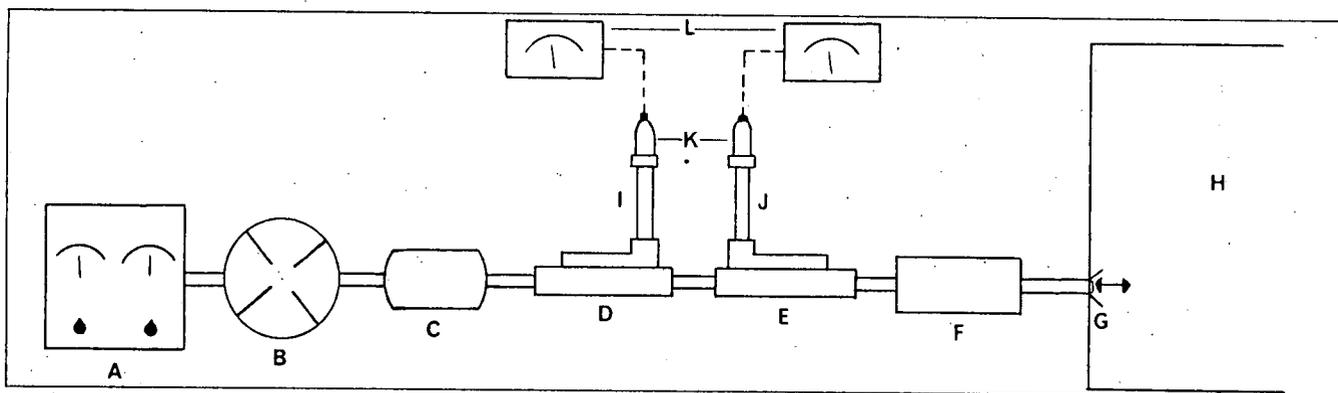


Fig 1.—Schematic representation of system used for irradiating rabbits. A indicates regulated power supply; B, magnetron; C, isolator; D and E, directional couplers; F, slotted line; G, dipole antenna; H, anechoic chamber; I and J, fixed attenuators; K, thermistor mounts; and L, power meters.



Fig 2.—Representative field of epithelium from germinative zone of right lens of animal killed 24 hours after irradiation (hematoxylin-eosin, original magnification $\times 250$).

is that the exact power used was not critical to the study under discussion so long as the dose produced a cataract.

The right eyes of 82 New Zealand white rabbits between four and five months in age were thus exposed to microwave radiation, followed by killing at postirradiation intervals varying from six hours to one month. With the exception of those animals killed 18 hours after irradiation, all animals were killed at mid-afternoon between 3 and 4 o'clock. The 18-hour animals were killed between 10 and 11 in the morning. One hour before death, the animals were anesthetized by pentobarbitol sodium administered intravenously and both eyes were treated topically with tetracaine. Physiologic saline (.05 ml) containing four microcuries of tritiated thymidine was injected into the anterior chamber of each

eye through a 26 gauge, 0.96 cm needle. To avoid aqueous loss, the needle was inserted almost parallel to the corneal surface and just in front of the limbus. After injection, the needle was crimped close to the cornea and severed, leaving about 0.32 cm of it in the cornea. This piece was removed just before the animal was killed. Following death, the eye was enucleated and the anterior hemisphere fixed in Carnoy fixative for 24 hours and then transferred to 70% alcohol. The lens was removed, with the 12-o'clock position marked on both the anterior and posterior parts of the lens surfaces with small dots of india ink applied to the lens equator through the capsule.

An encircling cut was made with a sharp razor blade just posterior to the lens equator, and, with a soft brush, the epithelium and anterior capsule were carefully peeled from the cortex. This epithelial "peel" was positioned capsule side up on a piece of cellophane tape, and several radial cuts were made around the periphery so that it could be brushed flat. The peel was then transferred directly to a clean glass slide and spread epithelial side up. This flat spread of the lens epithelium was dipped in bulk photographic emulsion according to Jofte's¹⁴ method. After a month in darkness in an oxygen-free atmosphere having a relative humidity of less than 15%, the emulsion was developed, the epithelium stained with Ehrlich hematoxylin, and the preparation mounted in synthetic mounting medium. Each pair of preparations, representing an irradiated eye and a nonirradiated control eye from the same animal, was then studied microscopically, and all mitotic figures and tritium-labelled cells in the entire epithelium were counted, with a minimum of 15 silver grains constituting the criterion for a labelled cell. Mitotic figures and labelled cells were tallied separately.

In addition, both eyes of 27 nonirradiated control animals with normal lenses were injected with tritiated thymidine,

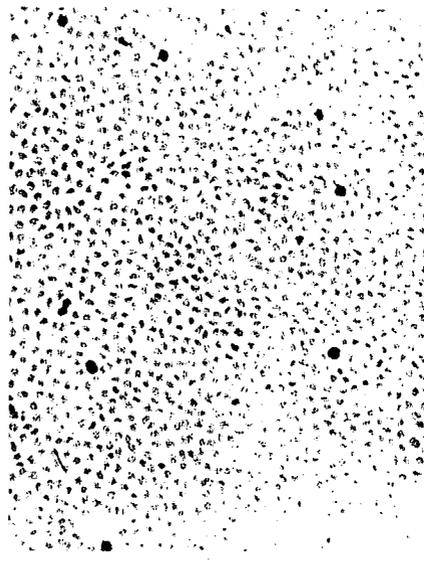


Fig 3.—Similar field from corresponding nonirradiated left lens epithelium from same animal as Fig 2 (hematoxylin-eosin, original magnification $\times 250$).

and flat spreads of the lens epithelium and capsule were made according to this procedure.

In both the control and the experimental groups, peels in which epithelium was missing in the germinative zone owing to errors of technique or that were unusable for other reasons, such as bubbles in the emulsion or folds in the spread, were discarded. In the control group, all counts in which the difference between right and left eyes varied by more than two standard deviations from mean were discarded. In all, 95 pairs of acceptable peels were studied, of which 74 were from experimental animals and 21 were from controls.

RESULTS

Control Group

None of the eyes in the 21 animals in this group were irradiated and

none had any visible lenticular changes when examined by slit lamp and by ophthalmoscope. Counts of the control group showed that there was no constant proportion of labelled cells to mitotic figures even within a pair. For example, rabbit 6-36 had 49 labelled cells and 10 mitotic figures OD, while there were 2 labelled cells and 59 mitotic figures OS; rabbit 6-40 showed 234 labelled cells and 155 mitotic figures OD, while 288 labelled cells and 82 mitotic figures were seen OS. For this reason we have used "total counts" that include both labelled cells and mitotic figures.

Further analysis of the control counts showed that although the activity in the epithelium of the right and left lenses of any pair was about the same (of 21 cases, the greatest difference between right and left counts was 8.5%), the difference from one pair to another pair was considerable and made averaging all right epithelial and all left epithelial counts meaningless. For example, rabbit 5-31 had a total OD count of 400 and an OS count of 411, a difference of 2.7%; rabbit 6-36 had a total OD count of only 59 and an OS count of 61, a difference of 3.2%. We therefore chose to use the percent difference in the total counts of right and left epithelia (OS was considered as the standard). These could then be averaged, and the calculated deviations were reasonable (See Table 1).

Experimental Group

The findings in 74 experimental animals grouped according to post-irradiation interval are shown in Table 1, which presents a comparison of the differences in the number of lens epithelial cells undergoing either mitosis or DNA synthesis in the microwave irradiated eyes and in the contralateral control eyes.

Postirradiation Interval, 6 to 24 Hours

The eyes in the 16 animals in this group showed no lens changes on either ophthalmoscopic or slit-lamp examination, but study of the epithelial spreads revealed substantial inhibition of both mitotic activity and DNA synthesis in the irradiated eyes. For

Post-irradiation Interval	No. of Cases	Degree of Opacification			Average % Difference*	Result†
		Minimal	Moderate	Maximal		
Controls	21	5.6 ± 3	...
Reaction of Lenses Without Vesicle Strings						
6 hr	5	91.4 ± 8	—
12 hr	4	87.5 ± 7	—
18 hr	3	82.9 ± 7	—
24 hr	3	81.6 ± 9	—
48 hr	5	3	2	...	79.4 ± 6	—
3 to 4 days	12	6	6	...	63.2 ± 15	—
5 days	7	...	7	...	52.7 ± 20	—
7 days	5	1	4	...	47.2 ± 12	—
14 days	2	1	...	1	14.0 ± 8	—
	1	1	0	...
	1	1	20.7	+
28 days	3	2	1	...	28.9 ± 7	+
	2	...	1	1	23.0 ± 4	—
Reaction of Lenses With Vesicle Strings						
3 days	2	...	2	...	820.0 ± 102	+
4 days	2	...	2	...	3.2 ± .6	—
5 days	1	...	1	...	4,060	+
	1	...	1	...	3,620	+
	1	...	1	...	1,020	+
	1	...	1	...	1,090	+
	1	...	1	...	315	+
7 days	2	...	1	1	36.0 ± 13	+
	1	...	1	...	9	—
14 days	3	...	2	1	51.8 ± 10	+
	1	...	1	...	3.6	—
	1	...	1	...	70	—
28 days	2	...	2	...	42.8 ± 8	+
	2	...	2	...	15.5 ± 4	—

* Average percent difference between right and left eyes in total number of mitotic and DNA-synthesizing cells.

† Plus (+) indicates total for right eye was greater than total for left eye; minus (—) indicates total for right eye was less than total for left eye.

example, in one animal the lens epithelium of the irradiated eye had only 17 mitotic or labelled nuclei compared to 650 in the nonirradiated eye (Fig 2 and 3). The irradiated eyes averaged $87.3\% \pm 5\%$ fewer mitotic figures or labelled cells than the nonirradiated ones.

Postirradiation Interval, 48 Hours

The irradiated eyes of the four animals in this group showed moderate lens changes. There were small, circumscribed, crescent-shaped opacities, postequatorial granules, and posterior cortical banding.¹⁵ This banding appeared in the posterior subcapsular cortex as concentric bands of greater optical density separated by clear zones. Sometimes there

were one or two such bands parallel to the posterior capsular band. In other cases, three bands were evident, with the outer one not separated from the posterior capsular band. As in the 6- to 24-hour group, the lens epithelium showed severe inhibition of both mitosis and DNA synthesis in the irradiated eyes.

Postirradiation Interval, Three to Four Days

By three to four days, the irradiated lenses in this group of 16 animals exhibited an extensive range of response. Minimal changes included posterior cortical banding and small aggregates of granules, vesicles, or fibrillar processes in the vicinity of or along the posterior suture; these changes could be observed only by slit



Fig 4.—In vivo photograph of moderate opacity. Note vesicular patches at nasal and temporal ends of posterior suture and clouding in posterior part of cortex (original magnification $\times 10$).



Fig 6.—In vivo photograph showing extensive or maximal response that involves major portion of posterior part of cortex (original magnification $\times 10$).

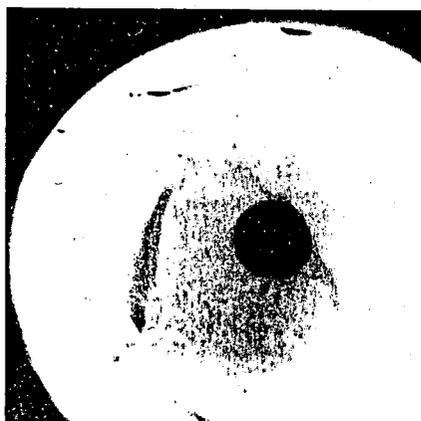


Fig 5.—In vivo photograph showing moderate opacity with supertemporal vesicle string. Note large vesicles (original magnification $\times 10$).

lamp. Circumscribed crescent-shaped opacities, usually at the ends of the posterior suture (Fig 4), and strings of large vesicles just posterior to the equator in the temporal quadrants (Fig 5) were categorized as moderate changes. A maximal response was considered to be a diffuse opacity involving either the entire posterior part of the cortex or at least a large part of it (Fig 6).

The data for experimental animals developing lenticular vesicle strings were treated separately because of the obvious difference in epithelial response compared to those lenses that did not develop vesicle strings. In the animals of this group with 5- and 14-day postirradiation intervals, the variability of the percent differences of the counts was so great that averaging them as a group was meaningless. Therefore, they were recorded individually.

Without Vesicle Strings.—The changes in the irradiated lenses varied from minimal to maximal. In each of these 12 cases, the total of labelled nuclei and mitoses counted in the irradiated lens was appreciably lower than in the control. The average percentage difference between OD and OS was $63.2\% \pm 15\%$.

With Vesicle Strings.—In four animals, strings of vesicles had developed just posterior to the lens equator. In two of these, the total count was much higher in the irradiated lens. Average percentage difference was $820\% \pm 102\%$. In the other two, the total count was slightly less in the irradiated lens (the average difference was $3.2\% \pm 0.6\%$). In three of these four cases, regardless of the total count, most of the labelled cells were in the area of epithelium lying directly in front of the equatorial vesicle strings. In the fourth case, there were too few cells labelled to effect any concentration.

Postirradiation Interval, Five Days

In this group of 12 animals, all of the irradiated lenses developed moderate opacities.

Without Vesicle Strings.—In each of these seven cases, the total count of labelled cells and mitotic figures in the irradiated lens was lower than that of its control. The average difference was 52.7% .

With Vesicle Strings.—In these lenses from five animals, the count was considerably higher in the irradiated eye than in the control eye.

Fig 7.—Representative field of epithelium peripheral to germinative zone of right lens in animal killed five days after irradiation. Field is adjacent to vesicle string shown in Fig 5 and shows increase of cells and mitotic figures and irregular arrangement of cells (hematoxylin-eosin, original magnification $\times 250$).

The percentage differences varied so much from animal to animal that they have been reported individually (Table 1). Again, as in the three- to four-day group, the labelled nuclei and mitotic figures were concentrated in the epithelium of that region lying immediately anterior to equator adjacent to the vesicle string. In this same region, instead of being organized into radially arranged rows, the cells exhibited a random or irregular arrangement (Fig 7), a condition not apparent at three to four days post-irradiation. The mitotic activity seems to have been so accelerated that the cells were unable to enlarge or to align themselves normally.

In the 26 experiments in which the postirradiation interval was from one to four weeks, radiation-induced lens changes varied from minimal to maximal.

Postirradiation Interval, One Week

Without Vesicle Strings.—In these five animals, the total counts were lower in the irradiated lenses. The average percentage difference was

47.2% ± 12%.

With Vesicle Strings.—Two of these three animals had a somewhat higher count in the irradiated lens. The average percentage difference of lenses in these two animals was 36% ± 13%, while the remaining animal had a count 9% lower than the control lens. The wave of DNA synthesis and mitosis in the region of vesicle strings had subsided, and there was a lower concentration of labelled cells in that area. The cells approached normal size and were less randomly arranged.

Postirradiation Interval, Two Weeks

Without Vesicle Strings.—In two of these four animals, the number of labelled cells was lower in the irradiated lenses; the average percentage difference was 14% ± 8%. In one, the total count was essentially equal (398 OD and 399 OS) and in the other animal was 20.7% higher in the irradiated lens.

With Vesicle Strings.—The tally in this group of five animals showed three with counts higher in the irradiated lens, one with an essentially equal count (difference was 3.6%) and another with an extremely low count in the irradiated lens (difference was 70.6%). Because of the variability of the percent differences, these were reported individually (see Table 1).

By two weeks postirradiation, the cells in all lenses with vesicles were arranged normally in meridional rows and only a slight concentration of labelled cells was observed in the vesicle string area.

Postirradiation Intervals, Four Weeks

Without Vesicle Strings.—Of the five animals in this group, three had counts higher in the irradiated lens (average percentage difference was 28.9% ± 7%) while the other two animals had counts lower in the irradiated lens (average percentage difference was 22.9% ± 4%).

With Vesicle Strings.—Two of these four animals had higher counts in the irradiated lens (average percentage difference was 42.8% ± 8%) and the other two had counts slightly lower in

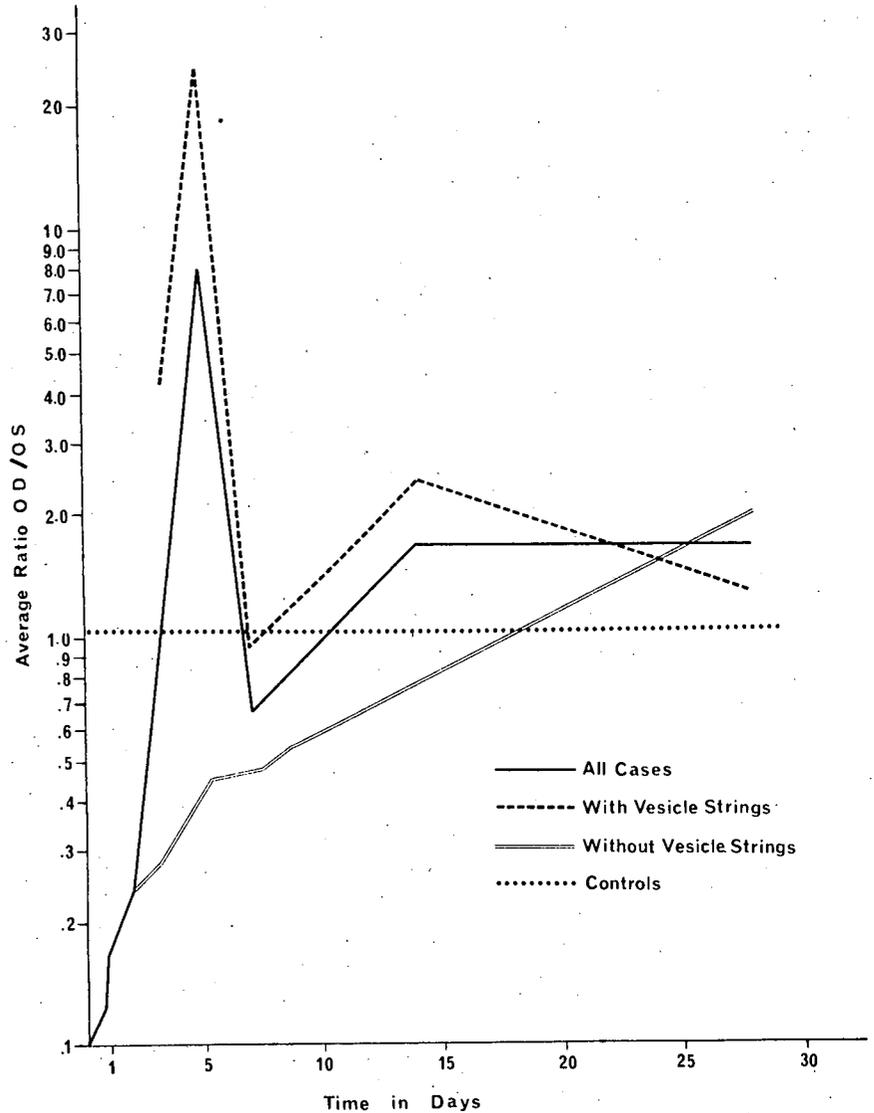


Fig 8.—Average ratios of right eye total counts to left eye total counts at postirradiation intervals varying from six hours to one month.

the irradiated lenses (average percentage difference was 15.5% ± 4%). Four weeks after irradiation, regardless of whether or not vesicle strings were present, no concentration of labelled cells in any one area was evident.

Treatment of Data

The lens epithelial cells that were synthesizing DNA or were undergoing mitosis were counted in both eyes of the control and the experimental animals. These data were treated in two ways in order to correlate them.

1. The average ratio of right eye counts to left eye counts was calculated with the left eye considered as one. In the control group this average ratio was 1.01:1, with a range of 0.9 to 1.1. The ratios obtained similarly for the irradiated animals were compared graphically with those of the controls in Fig 8 where it can be seen that the average ratios of right eye counts to left eye counts varied according to the postirradiation intervals and also according to whether or not vesicle strings had developed in the lens.

2. Considering that the variation

for any pair was positive when the total count for the right eye was higher than that for the left and negative when the converse was obtained, the probable distribution of positive and negative pairs was calculated for each experimental group. If the microwave radiation had no effect, then an equal distribution should be expected. The results are presented in Table 2.

COMMENT

The earliest effect of microwave radiation on the lens epithelium was inhibition of mitosis and DNA synthesis during the 6 to 24 hour interval after irradiation. This result is similar to that following ionizing radiation. Forty-eight hours after irradiation, the lens epithelium showed some recovery, with DNA synthesis and mitotic activity averaging about 25% of the control. At this time the first moderate changes in the lens were observed in the form of granular aggregates in the posterior part of the cortex near the equator; they could be seen with an ophthalmoscope through a widely dilated pupil. This type of opacity was quite different from the vesicular equatorial strings that occurred in the temporal quadrants in some cases and that might be seen after three days. The equatorial granules seemed to represent an early stage of damage in this area; if followed up for a while, they could be seen either to develop into the vesicular type of opacity or to disappear.

At three and four days following irradiation, the lens changes were much more extensive, with vesicular-type equatorial changes appearing for the first time. As is evident in Figure 8, mitosis and DNA synthesis rose sharply, increasing even more on the fifth day postirradiation. This activity was apparently correlated with the presence of equatorial vesicles (Fig 8), since there was a steep rise in the ratio of total counts of OD to OS in eyes with vesicular strings and a gradual rise in eyes without this type of change. The labelled cells accounted for most of the rise in the eyes with vesicular strings, and, interestingly enough, they were concentrated in the germinal zone of the epithelium in that quadrant of the

Post-Irradiation Interval	No. of Cases	OD Higher	OD Lower	Probability, %
Without Vesicle Strings				
Controls	21	11	10	49
6 to 48 hr	20	...	20	0.00009
3 to 4 days	12	...	12	0.024
5 days	7	...	7	0.78
7 days	5	...	5	3.13
14 days	4	2	2	50
28 days	5	3	2	31
With Vesicle Strings				
3 to 4 days	4	2	2	50
5 days	5	5	...	3.13
7 days	3	2	1	17
14 days	5	3	2	31
28 days	4	2	2	50

lens in which the vesicular string developed.

One week after irradiation, the ratios of total counts in lenses with vesicular strings rapidly decreased to the level of the control series. Since there were only three such animals, this precipitous drop may have been atypical. The ratios in lenses without vesicular strings were still depressed, but were slowly increasing and approaching normal (Fig 8).

At two weeks, the ratio of OD to OS counts in eyes with equatorial vesicles was greater than that of the control series. There were five animals in this group, so that the average ratio may be more truly representative of the extent of DNA synthesis and mitotic activity in the irradiated epithelium. There was also an increase in both types of activity in the eyes without equatorial vesicles that approached conditions in the control series. By one month postirradiation, the activity in all of the irradiated lenses exceeded that of the controls by approximately 50%.

These observations are supported by calculations of the probability of obtaining the results reported here. If microwave radiation had no effect on the activity of the lens epithelium, then the probability of obtaining a distribution in which the right eyes would have a count higher or lower than the left would be 50% (Table 2). This was the case in the nonirradiated control series, in which the calculation from the data was 49%. But

when the distributions were calculated from the data obtained from the experimental groups, the results were so far from 50% that the disparity must be attributed to the effect of the radiation. The probability of the distribution obtained in the 6 to 48 hour group was .00009%, indicating that the radiation must have caused the inhibition of mitotic activity.

Beginning with the three to four day period, it was necessary for proper consideration of the experimental data to divide the experimental animals into two groups: those without vesicle strings and those with vesicle strings. In those lenses without vesicle strings at postirradiation intervals of three to seven days, the pair distribution is significantly different from that of the controls. As the postirradiation interval lengthens, the pair distribution approaches that of the controls. As repair is completed and conditions in the lens epithelium return to normal, one would expect the average ratios (Fig 8) and the calculated probabilities (Table 2) to drop to that of the control series, as they did. The fact that repair had been largely completed is further suggested by the observation that the opacities at one month were well stabilized and showed no further progress.

Characteristically, the irradiated lenses showed an initial pronounced suppression of both DNA synthesis and mitosis. 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 closely parallels those observed in the lens epithelium after exposure of the eye to ionizing radiation.^{11,12}

In 21 of the irradiated eyes, however, there was superimposed on the usual course of recovery a precipitous rise in DNA synthesis occurring on the fourth to fifth day after irradiation. In these 21 irradiated lenses, equatorial vesicle strings had begun to develop in the supertemporal quadrant on the third day postirradiation. The accompanying sharp rise in DNA synthesis is similar to that observed in galactose-fed rats, where hydration of the lens occurs in the form of equatorial vesicles.

In the microwave-irradiated lenses in which equatorial vesicles were formed, the increase in DNA synthesis and mitosis was localized in the quadrant where the vesicles were found. It may be conjectured that these large vesicles represented hydration of the lens and acted as a stimulus to cause the overlying epithelial cells to proliferate at an accel-

erated rate.

Kinoshita and Merola¹⁶ have shown that after two days of galactose feeding, there is an increase of 50% in the water content of the lens. Grimes and von Sallmann⁶ reported that as early as 12 hours after galactose feeding begins, a severe swelling of the lens is evident. This reaches a maximum at four days and does not increase further up to 14 days (the termination of their experiment). They further report that the hydration of the lens is paralleled in the epithelium by a wave of increased mitotic activity that is rapid in onset, reaches a peak at four days, and subsides within seven days. They suggest that the sudden volume increase in the lens with the resulting expansion of the surface area to be covered by the epithelium stimulates cell division.

Ultrastructural studies of galactose cataracts made by Kuwabara et al¹⁷ strongly support the concept that osmotic disturbances play an important role in causing this type of cataract. Three days after beginning galactose feeding, opacities occurred in the equatorial region of the lens. Electron micrographs of lenses at this stage showed cellular edema and intracellu-

lar vacuoles in the epithelial cells and extracellular cysts in the nuclear bow and posterior cortical regions of the lens.

It may be, as Grimes and von Sallmann⁶ suggest in the case of galactose cataracts, that the lens swelling and epithelial proliferation are coincidental effects of the metabolic changes occurring in the presence of galactose, for they have reported that in alloxan-fed rats, lenses show a similar though less severe wave of mitotic activity that is not related to any detectable enlargement of the lens.⁶

It is apparent that microwave radiation affects the lens epithelium in the rabbit eye. In those irradiated lenses uncomplicated by the development of equatorial vesicles, the epithelium follows a course similar to that observed after exposure to ionizing radiation. In those lenses that develop equatorial vesicles, the effect on the lens epithelium closely parallels that observed after galactose feeding in rats. This local increased activity of the lens epithelium may be the result of hydration.

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