The Effect of 2450 mc Radiation on the Development of the Chick Embryo

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INTRODUCTION

THE EFFECT of microwave radiation upon the crystalline lens of the eye has been investigated for several microwave frequencies, particularly 2450 mc. Daily et al. (1) reported the induction of lens opacities in the dog, and the same year Richardson et al. (2) reported similar results in the rabbit. The cataractogenic effect of this frequency has been confirmed for the dog eye by Salisbury et al. (3) and for the rabbit eye by Daily et al. (4), Williams et al. (5), and Carpenter et al. (6, 7).

The normal process of growth in the lens is similar in several respects to the process of embryonic development. The lens grows throughout life by proliferation of new cells and their subsequent differentiation into lens fibers, the two processes taking place concurrently. In the developing embryo, proliferation and differentiation are taking place at the same time and at a relatively rapid rate. It therefore became of interest to test the effect of microwave radiation on the developing embryo.

Materials and Methods

All experiments were carried out on chick embryos which had been incubated at 39°C to approximately the 48-hr stage of development. The microwave source was a Raytheon CMD4 Microtherm, which emits continuous wave radiation at a frequency of 2450 mc. A corner reflector antenna, the Microtherm Director C,

was mounted at the top of an anechoic chamber measuring 12 x 12 x 15 in. and lined by microwave absorbent. The egg was supported on a Lucite table 2 in. beneath the antenna.

Although the radio-frequency energy dissipated in the microwave absorbent during irradiation caused it to become heated, uniform incubator temperature was maintained by means of an exhaust fan mounted at the top of the chamber. Temperature was monitored by a thermistor-thermometer.

Before results could be assayed, it became necessary to know approximately how much radio-frequency energy was being received by the egg. Input power meter settings were therefore equated with power density measurements made calorimetrically in the anechoic chamber. An egg shell of known volume and profile area, filled with physiological saline and placed in the position of an egg during irradiation, served as the calorimeter. A hypodermic needle thermistor probe was inserted through the shell so that its tip approximated the normal position of the embryo. The energy absorbed by the embryo could then be determined for any selected power level. The maximal output of the generator yielded a power density of 400 mw/cm^2. Measurements at 70 and 50% of maximal output were proportionately less, 280 and 200 mw/cm^2, respectively. At these power levels, embryos at the 48-hr stage were irradiated through the intact shell, thus causing minimal interference with their natural internal millieu.

Temperature increases within the egg during irradiation were measured by inserting the thermistor probe through the blunt end of the egg with the tip of the needle placed in the yolk directly below the embryonic disc. After exposure, the thermistor probe was withdrawn, the needle hole sealed with Scotch Tape, and the egg returned to the incubator. These embryos developed in a manner similar to those exposed for identical periods of time but without penetration by a thermistor probe.

Thermal gradients within the egg were also determined. Nonfertile eggs heated in the incubator to 39°C were punctured in the minor axis and each hole sealed with Scotch Tape. The thermistor probe was inserted from below through the Scotch Tape and adjusted so that its tip was approximately 4 mm from the shell of the upper surface of the egg. The temperature at this point was recorded during the minimal lethal exposure period for each of the power densities used. After the power was turned off, temperatures were

recorded after 1, 5, 10, and 20 min as the egg cooled. Similar measurements were made at positions 8, 16, and 28 mm below the shell.

After irradiation, the eggs were allowed to continue development for another 48 hr, at which time the embryos were removed and examined before being preserved and stained. To facilitate correlation of gross and microscopic observations, each embryo was photographed before being embedded in paraffin and sectioned serially at 10 micra.

Results

The total number of eggs incubated was 507, of which 366 were exposed to radiation, 109 served as controls, and 32 were not fertile. At the maximal power of 400 mw/cm^2, embryos which were irradiated for 1 to 4 min showed no appreciable deviation from the control embryos. Irradiation for 4½ to 5 min caused abnormalities and if prolonged for another half minute became lethal. At lower power levels, the difference between exposure periods which were ineffective and those which were lethal was similarly a matter of no more than 2 or 3 min.

The results of all experiments are summarized in Table I. Of the 109 control embryos, 106 continued their development normally, as did also 142 embryos which received ineffective doses of radiation. Of the 103 embryos which died, some had continued to develop for only a few hours after irradiation and others had continued for a day.

At 48 hr of development, the stage at which embryos were irradiated, the following features are evident: Torsion has occurred to a level slightly posterior to the heart so that the anterior portion of the embryo lies on its left side. Lateral undercutting of the body folds is proceeding so that the gut is being floored in and the embryo is being isolated from the yolk. The brain has just begun to differentiate from three into five vesicles. The primary optic vesicle has invaginated to form a cup and the lens is in vesicle form. Two visceral arches are present, with the beginning of a third. Although blood circulation has been established, the heart has not yet begun to be partitioned into right and left sides. It is essentially a tubular organ located at the level of the rhombencephalon. Lying outside the body cavity and bulging to the right of the embryo, it has begun to undergo the flexions and torsions which will bring its regions into their adult relationship. The region posterior to the heart is relatively undifferentiated. (See Figs. 1 and 2.)

TABLE I

Irradiation of Chick Embryos at 48 Hours of Incubation

Power (mw/cm²)	Time (Min.)	Number of Cases	Results			
				Abnormalities		
			Normal	General	Restricted	Dead
Controls		109	106			3
400	1	10	10			
400	2	8	8			
400	3	10	10			
400	4	22	20		2	
400	4½	46	9	26	4	7
400	5	32	2	17	4	9
400	5½	10				10
400	6	10				10
280	4	9	9			
280	5	15	15			
280	6	15	15			
280	6½	10	10			
280	7	17	4	8	2	3
280	7½	18		8	1	9
280	8	32		17	8	7
280	8½	10		5		5
280	9	20				20
200	8	10	10			
200	10	10	10			
200	12	9	9			
200	13	14	1	10	1	2
200	14	14		4	4	6
200	15	15				15
Totals		475	248	95	26	106

At 96 hr of incubation, the time at which all embryos were preserved for study, the normal chick embryo exhibits the following characteristics: It is C-shaped and lying on its left side; torsion, therefore, is complete. The body folds have isolated the embryo from the yolk except in the region of the yolk stalk. The brain has become compartmented into five secondary vesicles, and its walls have thickened. Hypophysis and epiphysis are well defined. The two layers of the optic cup are beginning to differentiate into pigment and

neural layers. Lens fibers have elongated so that they nearly occlude the primary lens cavity. Four visceral arches are present. The heart has come to occupy a position more caudal with reference to other structures and lies at the level of the wing buds. The partitioning of the heart into four chambers has already begun, the interatrial and interventricular septa being present but not complete. The myocardium has thickened, particularly in the ventricular region. Lateral undercutting of the body folds has occurred and the heart is thus enclosed within the body. The anterior and posterior appendage buds appear as paddle-shaped outgrowths of the body wall directed ventrad and slightly caudad. The allantois is a well formed sac evaginated from the hind gut. (See Figs. 3 and 4.)

In the 121 embryos in which irradiation had interfered with development, the anomalies fell into two categories:

1. General inhibitory effects: In these embryos, gross abnormalities were observed which were not restricted to any particular region of the embryo. The most obvious effect was the smaller body size (Figs. 5 and 6), which resembled a 72-hr embryo rather than a 96-hr one. Torsion had progressed posteriorly only as far as the wing bud. The brain possessed only the same degree of differentiation present when the embryo was irradiated. The eyes were abnormally small and frequently were atypical in shape (Figs. 5, 6, and 7). Wing buds frequently were absent; when present, they were of subnormal size. The development of hind limb buds had been suppressed in almost every embryo. Only three visceral arches, instead of four, were present. The heart showed no evidence of partitioning into four chambers and its myocardium was extremely thin (Fig. 8). Posterior to the level of the heart, the degree of development equalled that of 48-hr embryos. Neither allantois nor tail bud had developed.

2. Posterior inhibitory effects: These embryos characteristically exhibited deficiencies only in the region of the body posterior to the wing bud, a region which at 48 hr of incubation has undergone little differentiation. Microwave radiation seemed to have inhibited the differentiation which would have taken place in the ensuing 48 hr. These embryos either had an anterior portion normal to the 96-hr stage but with the posterior part of the body lacking (Figs. 9 and 10) or there were anterior defects similar to those already described above and the posterior body region consisted of what appeared to be an undifferentiated thin-walled balloonlike vesicle (Figs. 6 and 11).

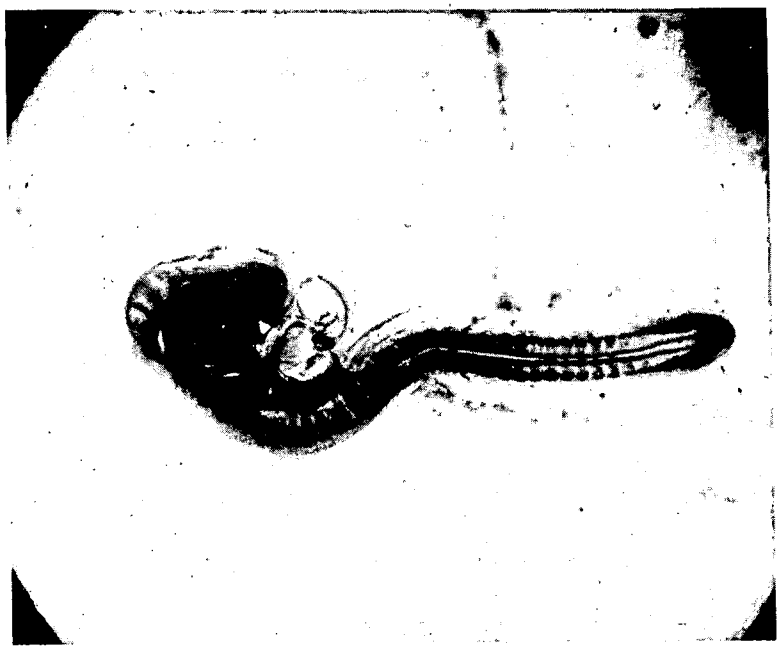

FIG. 1. Normal 48-hr chick embryo, whole mount. 12X.

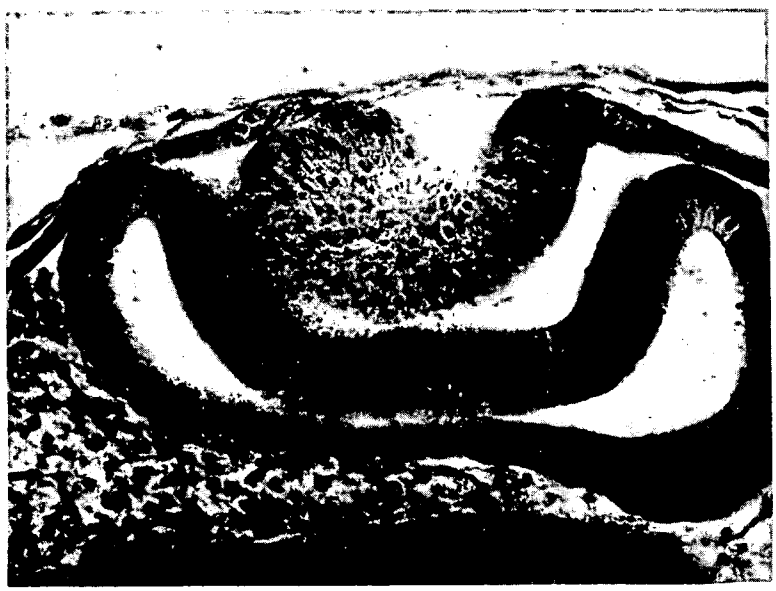

FIG. 2. Transverse section through the optic cup and lens vesicle of a normal 48-hr embryo. 200X.

FIG. 3. Control 96-hr embryo, whole mount. 8X.

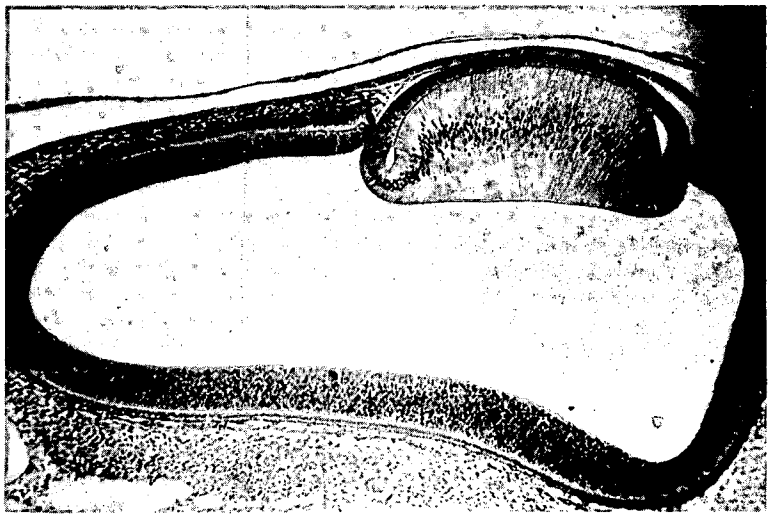

FIG. 4. Transverse section through the optic cup and lens of a normal 96-hr embryo. 60X.

FIG. 5. 96-hr embryo which had been irradiated 7 min at 280 mw/cm², whole mount. 12X.

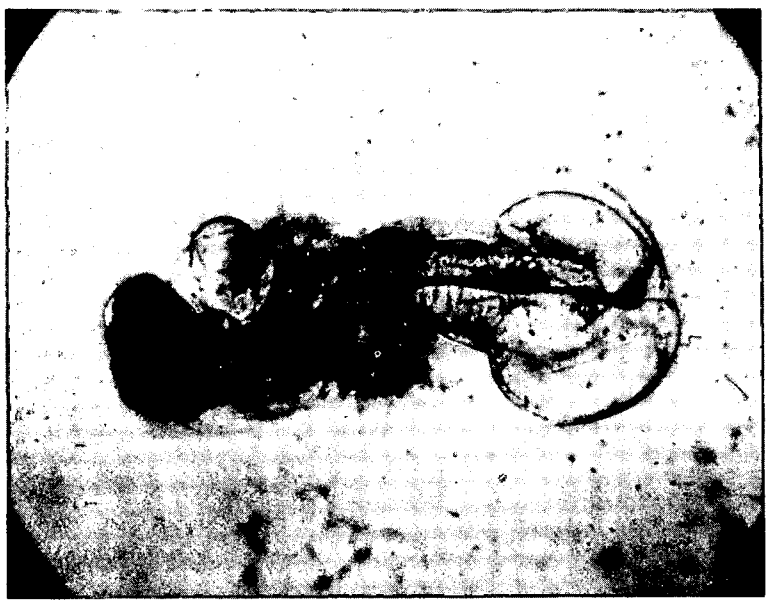

FIG. 6. 96-hr embryo which had been irradiated 8 min at 280 mw/cm², whole mount. 12X.

FIG. 7. Transverse section through the optic cup and lens vesicle of an embryo which had been irradiated 5 min at 400 mw/cm². 200X.

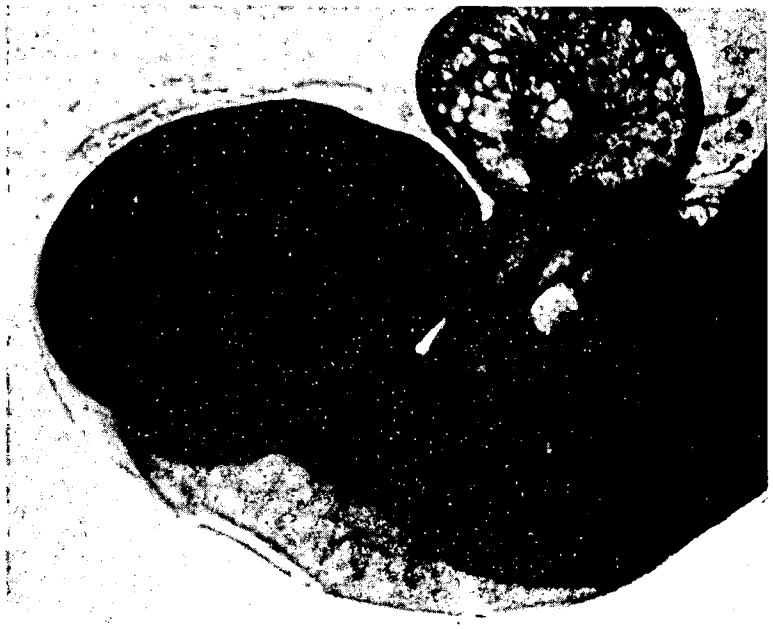

FIG. 8. Anterior region of a 96-hr embryo which had been irradiated 5 min at 400 mw/cm², whole mount. 12X.

FIG. 9. 96-hr embryo which had been irradiated 14 min at 200 mw/cm^2, whole mount. 12X.

FIG. 10. 96-hr embryo which had been irradiated 13 min at 200 mw/cm^2, whole mount. 12X.

FIG. 11. Posterior region of a 96-hr embryo which had been irradiated 5 min at 400 mw/cm², whole mount. 12X.

Microscopic examination of serial sections of the former group showed that the anterior region developed normally but that posterior to the wing bud some atrophy as well as inhibition of growth had occurred. The latter group of embryos exhibited brain, eye, and heart abnormalities similar in nature to those already described but posteriorly the effects were far more extensive. In many embryos, the neural tube posterior to the wing bud had developed as a multiple structure (compare Figs. 12 and 13 with Figs. 14 and 15) or had failed to close. The wing bud, if present, was directed laterad instead of ventro-caudad and there were no posterior limb buds. The

FIG. 12. Transverse section through the neural tube of an embryo which had been irradiated 5 min at 400 mw/cm². 200X.

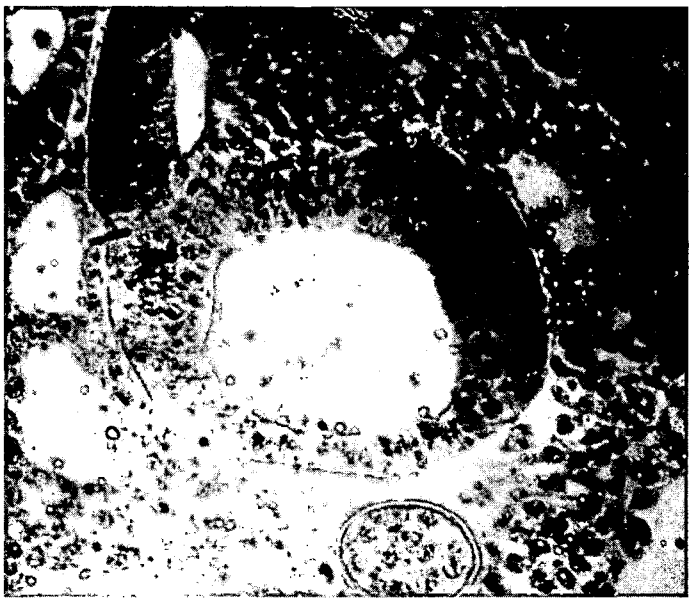

FIG. 13. Transverse section through the neural tube of an embryo which had been irradiated 8 min at 280 mw/cm². 200X.

FIG. 14. Transverse section through the neural tube of a normal 48-hr embryo. 200X.

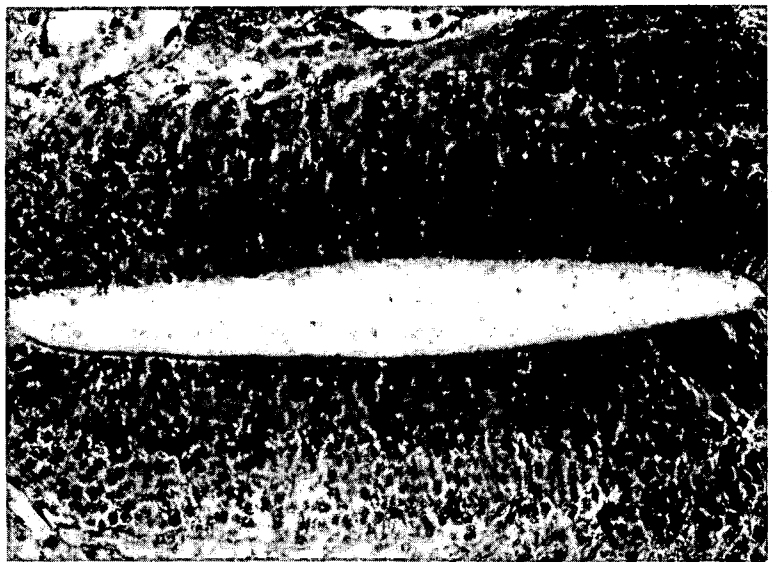

FIG. 15. Transverse section through the neural tube of a 96-hr control embryo. 200X.

balloonlike posterior vesicle in histologic section seemed to be of double nature, one part on each side of the mid-line of the embryo. It appeared to comprise a large coelomic space which had arisen chiefly through failure of the lateral body folds to develop sufficiently to separate an embryonic from an extra-embryonic coelom.

A number of temperature measurements were made to determine the rise in temperature at the level of the embryo during irradiation. The period of time required for the egg to cool to incubator temperature was also recorded. The data are presented in Fig. 16. Regardless of the applied power or duration of exposure, a lethal dose coincided with a temperature of approximately 59°C in the yolk just below the embryo.

The thermal gradient across the egg was also determined. When placed in an incubator, an egg heats uniformly from all points on its surface inward to the center, but when exposed to the radio-frequency energy from an antenna, the media nearest to the antenna heat most rapidly, while the media farthest from the antenna heat most slowly. This is inferred from the fact that 1 min after termination of irradiation, a gradient existed across the egg. Twenty minutes later the entire egg was close to a uniform temperature (Table II).

Discussion

The experiments here reported demonstrate that the normal course of development in the early chick embryo is adversely affected

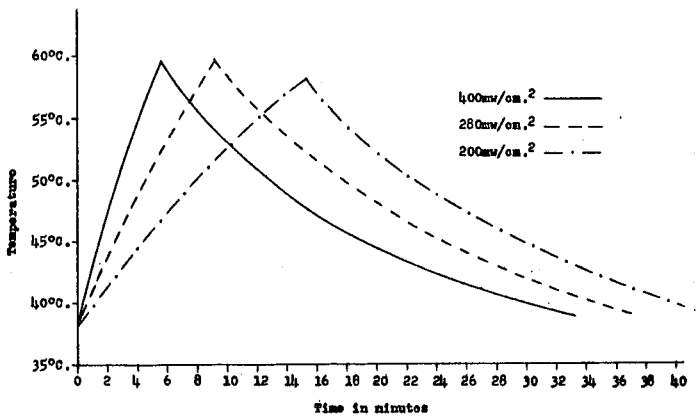

FIG. 16. Temperatures of the yolk below the embryo.

TABLE II

Thermal Gradients Within the Yolk

Distance from shell surface	Temperature during cooling period at:				
	0 min	1 min	5 min	10 min	20 min
A. Previous irradiation at 400 mw/cm² for 5½ min					
4 mm	58.7	56.6	53.3	46.0	40.6
8 mm		56.3	51.6	45.9	40.6
16 mm		55.4	47.7	43.7	39.9
28 mm		43.9	39.5	38.0	36.8
B. Previous irradiation at 280 mw/cm² for 9 min					
4 mm	59.3	57.8	53.1	45.5	40.7
8 mm		57.4	53.0	45.5	40.7
16 mm		56.7	52.6	44.9	40.7
28 mm		45.2	42.1	40.1	37.4
C. Previous irradiation at 200 mw/cm² for 15 min					
4 mm	57.8	56.2	51.5	44.0	39.6
8 mm		55.8	51.2	44.0	39.6
16 mm		54.0	50.8	43.8	39.5
28 mm		45.0	42.3	39.7	37.1

by relatively brief exposure of the embryo to microwave radiation at a frequency of 2450 mc. The only other experiments of this nature which have been reported have been those of Osborne (8, 9), who found that at no stage of development was the chick embryo affected by long exposures to 200 mc radiation. It should be noted, however, that at 200 mc the wavelength is approximately 5 ft. It has been pointed out by Vogelman (10) that if an object in the path of microwave radiation is to absorb any of this energy, its dimension should be at least one-tenth the wavelength. Inasmuch as the greatest dimension of an egg is considerably less than the requisite 6 in., it is quite probable that in Osborne's experiments the radio-frequency energy passed through the egg with none, or very little, of it being absorbed. It may be significant that Osborne found the temperature rise within the egg to be never more than 1°C.

At the 2450 mc frequency, approximately two-thirds of the radiation which enters the egg is absorbed within a depth of 1 cm. This distance coincides quite closely with the position of the embryonic area in the egg. At this frequency, there is also a significant increase

in temperature which is related to the power density and the duration of exposure. All embryos in which effects were produced reached a temperature of approximately 55°C. The question arises as to whether the abnormalities are induced wholly as a thermal effect or whether they are due wholly or in part to some other factor.

Pincus and Fischer (11) showed that cultures of chick osteoblasts were killed in 3.5 to 6 min at temperatures exceeding 50°C, but it must be noted that these were isolated cells in tissue culture. On the other hand, the experiments of Szarski (12, 13) demonstrated that so long as the blood circulation was intact, all tissues of the embryo remained viable. After heating eggs in a high temperature incubator (45.5°C) for an interval of 1 to 16 hr, Szarski opened the eggs and cultured pieces from various organs of the embryo on nutrient media. All tissues removed from embryos in which the circulatory system was still functioning retained full viability and continued to develop when cultured. Heart and intestinal tissues remained viable 1 to 2 hr after heart stoppage. The cerebrum was slightly more sensitive, remaining viable for only 1 hr after cardiac failure. Not until 13 hr of high temperature incubation, or some 11 hr after cessation of blood circulation, did the extra-embryonic membranes begin to lose their viability. These results led Szarski to conclude that the loss of viability of the tissues was not caused by the heat per se, but resulted from the accumulation of products of metabolism and lack of oxygen following circulatory failure. Organs with a higher metabolic rate, therefore, succumbed sooner.

Except in a few instances, the present experiments corroborate Szarski's conclusion with regard to the dependence of tissue viability on intact blood circulation. In most cases, the tissues continued to grow by prolifération but usually did not continue to differentiate. In some embryos, however, the posterior region began to atrophy even though the heart continued to beat.

Irradiation of the rabbit eye, either with X-ray or with microwave, results in the formation of characteristic lens opacities. In like manner, irradiation of the chick embryo by X-ray or by microwave radiation causes the development of characteristic abnormalities. Developmental abnormalities in embryos exposed to X-ray as reported by both Schneller (14) and Reyss-Brion (15) were almost identical with those which are caused by microwave radiation. Both types of radiation act to retard or inhibit development. In both instances

similar defects occur in the anterior region of the embryo. Differentiation of the brain is arrested, the optic cup invaginates only slightly and no lens fibers develop. Development of the allantois is inhibited by both types of radiation. Reyss-Brion (15) also described posterior neural effects identical to those described in this paper, i.e., open neural folds, or two or three rudimentary neural tubes grouped together. There is no mention in the literature of anything similar to the balloonlike tails described here.

No measurable heat is produced by X-ray and yet the abnormalities which are produced by microwave are identical to those induced by X-ray. This similarity of results suggests the possibility of a nonthermal influence exerted by microwave radiation which could be acting either concomitantly or synergistically with the obvious thermal effect of the radiation.

At 48 hr of development, the brain, the heart, the optic cup, and the lens are undergoing differentiation as well as growth by proliferation. It appears that irradiation at this stage has the effect of arresting further differentiation without at the same time, in most instances, affecting proliferation. The optic cup and lens vesicle were exceptions in a small number of embryos in which they neither proliferated nor differentiated any further.

There are present at the 48-hr stage the seventeenth to nineteenth somites, which contribute the mesoderm of the wing bud. In some embryos the wing bud developed but was atypically small. Structures such as the posterior limb buds, tail, and allantois, which normally develop in the region posterior to the wing bud, exist at the 48-hr stage only as potential areas. Likewise, the posteriormost region of the embryo consists of merely three germ layers, although neural folds are present and there are differentiated extra-embryonic blood vessels. The effect of microwave radiation on this region is to prevent differentiation of new structures from occurring and they therefore do not develop at all. Obviously a structure must become differentiated before it can grow. In the case of neural folds and extra-embryonic blood vessels, some differentiation has already occurred. Further differentiation is inhibited but, as in the case of the brain, heart, etc., these structures continue to grow by proliferation.

In summary, microwave radiation appears to inhibit cellular differentiation in the developing chick embryo. In structures which have already begun to differentiate, cellular proliferation continues,

but no further degree of differentiation occurs. Structures which have not yet begun to differentiate fail to do so subsequent to irradiation, and their development is therefore suppressed.

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plus intéressante à remarquer que l'apolyse est normalement déclenchée par l'ecdysone.

D'autre part, les relations étroites du réticulum agranulaire avec les mitochondries (figure, e) tendent à montrer, comme c'est aussi le cas dans les glandes ecdysiales de lépidoptères[4,5], qu'un couplage fonctionnel est réalisé entre ces 2 organites essentiels de la stéroïdogenèse. La localisation du réticulum agranulaire à la périphérie des cellules suggère qu'il joue aussi un rôle dans le transport et la libération des produits élaborés.

En définitive, la démonstration morphologique du développement cyclique du réticulum agranulaire qui est la manifestation la plus marquante du processus de mue tend à confirmer le rôle de ces glandes dans la synthèse et la libération d'ecdysone sous une forme probablement couplée à des protéines.

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Effects of 9.4 GHz microwave exposure on meiosis in mice[1]

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Summary. Exposure to 9.4 GHz pulsed microwaves at low power densities for 1 h/day during 2 weeks induces in adult male Balb/c mice disturbances in meiosis, consisting in an increase of translocations and the appearance of cells with several chromosome pair remaining univalents at MI.

The rapidly expanding industrial, scientific, medical and home uses of numerous devices producing nonionizing radiation (NIR) lead to a steady increase of the amount of NIR in man's environment and cause concern because of potential health hazards[2]. Biological effects of microwave exposure were the subject of numerous investigations[3]. It was shown that exposure to 5-40 MHz waves induces chromosomal aberrations and mitotic abnormalities in somatic cells[4-7]. As far as we are aware, effects of microwave exposure on meiosis have not been investigated. The aim of the present work is to verify whether repeated microwave exposures from a typical radar source will interfere with meiosis, because of grave implications of such phenomena.

Results of analysis of methaphase I

Group	Total number of metaphases analyzed	Number of translocations (metaphases with quadri- or hexavalents)	Number of metaphases with univalents	Number of metaphases with number of bivalents N < 20	N = 20	N > 20
Controls	175	3	2	53	116	6
0.1 mW/cm²	92	6*	14*	26	61	5
0.5 mW/cm²	104	3	10*	37	60	7
1.0 mW/cm²	120	10*	9*	51	63	6
10.0 mW/cm²	112	9*	34*	36	72	4

* Significant in comparison to controls at 0.001 level (chi² test).