Cytopathological Effects of Radiofrequency Electric Fields on Reproductive Tissue of Adult *Tenebrio molitor* (Coleoptera: Tenebrionidae)[1]

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ABSTRACT

Adult *Tenebrio molitor* L. were exposed to 39-MHz radiofrequency (RF) electric fields and examined to determine cytopathological changes in ovarian and testicular tissues.

Disintegration of trophocytes and primary oocytes followed by vacuole formation in the germarium and germinal vesicle characterized the effects in ovarian tissue. Condensation of the chromatin material in the follicular epithelial cells was also evident. In testicular tissues, RF treatment caused disintegration of the cyst membranes and spermatocytes and clumping of spermatid nuclei. Mature spermatozoa were not observed in the lumina of the vasa efferentia.

Studies of the effects of radiofrequency (RF) energy on insects in the past have been mainly on its mode of action, on mortality to various species, and on morphological and physiological changes resulting from RF treatment.

The effects of other forms of electromagnetic energy on reproductive tissues in insects have been reported by Dederer (1940), who noted the formation of giant cells within "cysts" of growing spermatocytes when pupae of *Samia* (=*Philosamia*) *cynthia* (Drury) were irradiated with X rays at 8000 R. Histological studies of the embryos exposed to higher X-ray doses showed enlargement of nuclei, reduction in cell division, and complete inhibition of the developmental process (Amy 1955). Welshons and Russel (1957) noted the death of both secondary spermatogonia and young spermatocytes in testes of *Drosophila* spp. after X-ray treatment. Riemann (1967) observed necrotic spermatogonial cells and young spermatocytes in pupae of the screw-worm fly, *Cochliomyia hominivorax* (Coquerel), which were irradiated with X rays at 6000 R. Information concerning the effects of RF energy on the reproductive tissues of insects is not available. Our studies were made in an attempt to disclose any cytological changes in the reproductive tissues of *Tenebrio molitor* L. adults which might occur following exposure to RF electric fields.

MATERIALS AND METHODS

T. molitor cultures were maintained according to the method of Murray (1960). Equipment and techniques used to expose test insects to RF energy were the same as those described by Rai et al. (1971), except that the cell dimensions in the polystyrene sample holders were modified to confine the insects in separate cells 15.88 mm long, 5.56 mm wide, and 3.18 mm high. The assembled height of the sample holder (distance between the electrodes) was 18.85 mm.

Three-day postemergence test insects (30 ♂ and 30 ♀) were exposed to continuous-wave RF electric fields of 39 MHz at an electrode voltage of 2.5 kV for 4.0 s and subsequently maintained in petri dishes. At 3 and 6 days posttreatment, the testes and ovaries of RF-treated adults were dissected in Yeager's physiological saline solution and fixed in Bouin's fluid. Untreated adults were identically handled 6 days postemergence. Reproductive tissues from RF-treated and untreated insects were next dehydrated in ethyl alcohol, cleared in benzene, infiltrated with paraffin, embedded in pure paraffin, and sectioned serially at 6 μ. Finally, the sections were affixed to slides with Mayer's albumin, stained with standard alum hematoxylin, counterstained with eosin, and mounted in Permount®.[5] The general procedure adopted for tissue preparation was similar to that of Galigher and Kozloff (1964).

More detailed information on all methods and procedures has been presented by Rai (1970).

RESULTS

Ovarian Tissue.—Each ovary of *T. molitor* consists of 12 telotrophic ovarioles. Detailed histological studies of the ovaries of normal *T. molitor* adults were made by Schlottman and Bonhag (1956).

At 3 days posttreatment (PT), trophocyte disintegration and subsequent vacuole formation were visible (Fig. 1 A). Further cellular disintegration occurred at 6 days PT with an increase in vacuole formation (Fig. 1 E). Further examination revealed that most of the cells in the posterior region of the germarium had disintegrated in the 6-day PT samples.

A section of the vitellarium from an RF-treated adult 3 days PT is depicted in Fig. 1 B, in which it appears that the columnar epithelium had separated from the growing oocyte. Sections of oocytes from RF-treated adults 6 days PT stained considerably darker than comparable tissue from control insects (Fig. 1 C, D). In germinal vesicle tissue from adults 6 days PT, numerous clear areas were observed which were not visible in the same tissue of untreated adults. An additional anomaly was that the columnar epithelium of the follicle transformed into cuboidal

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epithelium in RF-treated adults 6 days PT, whereas the nuclei of the cuboidal epithelium appeared more dense (Fig. 1 F) than did similar nuclei in the epithelium of untreated adults.

Testicular Tissue.—The testes of *T. molitor* are spherical bodies divided into 6 round lobes arranged in a rosette. Spermatogonial cells are massed on the outer aspect of the testicular lobes. Cysts of mature cells lie toward the center of the rosette. The testes of adult *T. molitor* contain predominantly spermatids and spermatozoa. Spermatocytes are packed closely and grouped inside the cysts.

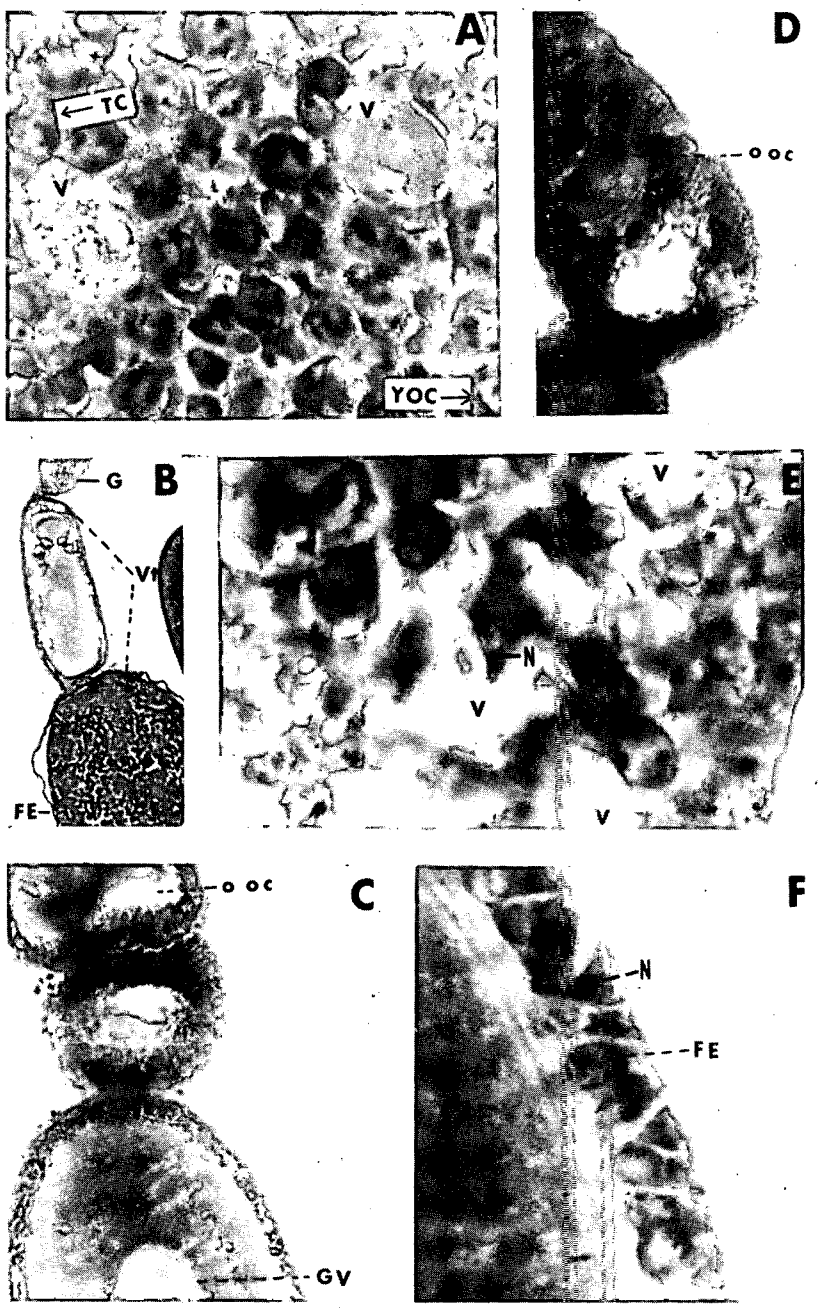

FIG. 1.—Longitudinal sections of various areas of ovaries from RF-treated *T. molitor* females exposed for 4.0 s at 39 MHz and 2.5 kV. A. Germarium 3 days after exposure to RF energy showing trophocytes (TC), young oocytes (YOC), and vacuoles (V) (1000 X). B. Ovariole 3 days following RF treatment showing germarium (G), vitellarium (Vt), and follicular epithelium (FE) (100 X). C. Ovariole 6 days following RF exposure showing oocytes (OOC) and germinal vesicle (GV) (40 X). D. Vitellarium 6 days folowing RF treatment showing distintegrating oocytes (OOC) (40 X). E. Cellular disintegration (V) in the germarium 6 days post RF treatment (1000 X). F. Longitudinal section of a necrotic oocyte 6 days posttreatment showing dense nuclei (N) in the follicular epithelium (FE) (1000 X).

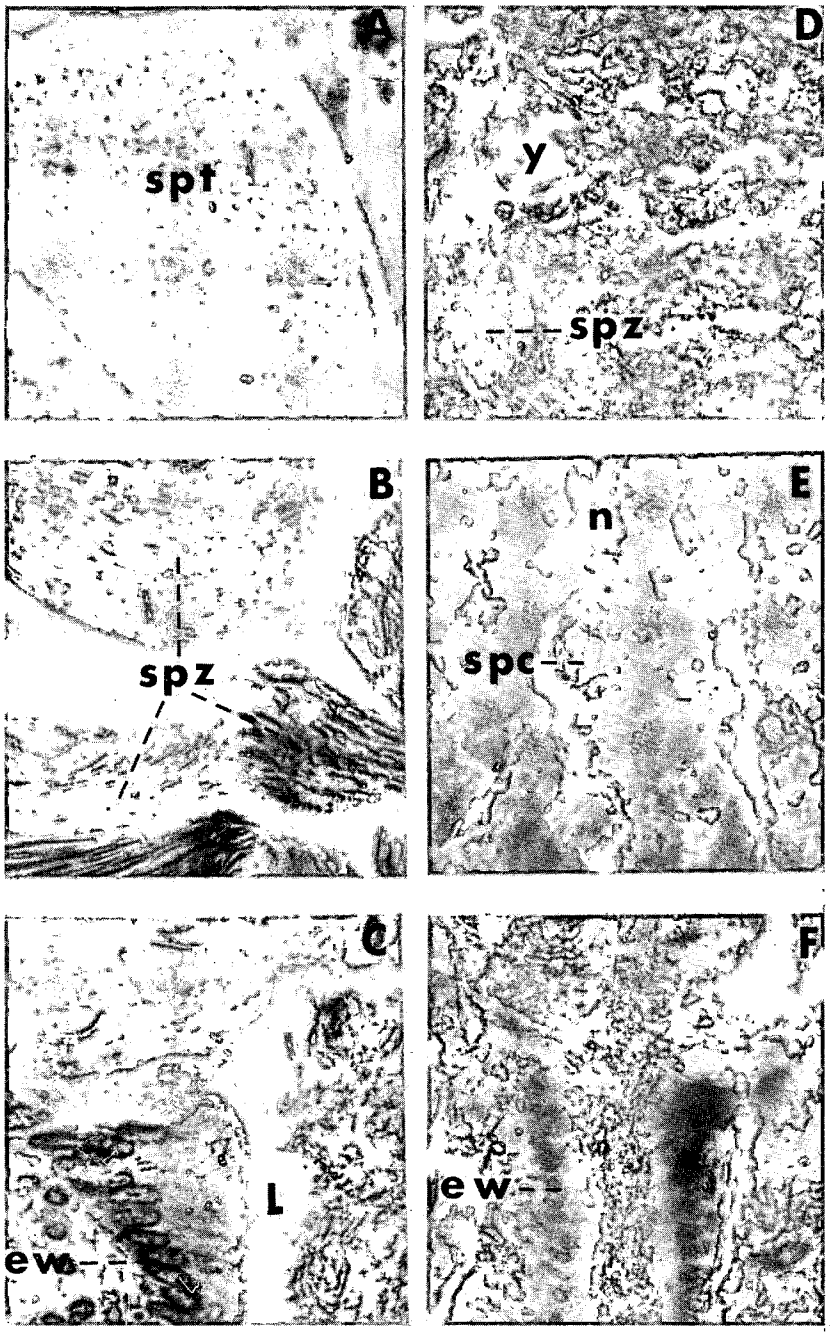

FIG. 2.—Longitudinal sections of testes from untreated (A, B, and C) and RF-treated (D, E, and F) *T. molitor* males. D, E, and F treated at 39 MHz at an electrode voltage of 2.5 kV for 4.0 s. A. Developing spermatids (spt) within a cyst 3 days postemergence (1000 X). B. Bundles of spermatozoa (spz) at the posterior region of the testis 6 days postemergence (1000 X). C. Epithelial wall (ew) of the vas efferens showing lumen (L) 6 days postemergence (1000 X). D. Testis 6 days posttreatment showing disintegrated spermatocytes inside cyst (y) and spermatozoa (spz) (1000 X). E. Spermatocytes (spc) 6 days posttreatment showing darkly stained nuclei (n) (1000 X). F. Vas efferens of 6-day posttreatment testis showing lack of spermatozoa and indistinct cells of epithelial wall (1000 X).

All the usual developmental stages of spermatogenesis were observed in testes from RF-treated adults (Fig. 2). Disintegration of spermatocytes (Fig. 2 D) and dissolution of the cyst membrane (Fig. 2 E) were observed in the testicular tissues removed 3 days PT. The spermatocytes appeared to be normal in size (Fig. 2 E), but disintegrated within the cyst (Fig. 2 D). The cells of the epithelial wall

were indistinct (Fig. 2 F), as compared with those of a normal testis (Fig. 2 C). Spermatozoa were not observed in the lumina of the vasa efferentia of RF-treated testes (Fig. 2 F), whereas numerous mature spermatozoa were present in the lumina of the vasa efferentia of untreated males (Fig. 2 C).

DISCUSSION

The lethal effects of X and gamma radiation on the reproductive tissue of insects are well documented. The effect of RF energy on insect reproductive tissue has not been reported. The results of this study present evidence of damage to the testes and ovaries of *T. molitor* resulting from exposure to RF energy.

Possible nonthermal effects of RF energy on *T. molitor* have been suggested for pupae exposed at microwave frequencies (Carpenter and Livstone 1971). However, the influence of thermal energy arising from dielectric heating is likely to account for tissue damage noted in the studies reported here, because considerable elevation of body temperature results from the type of RF exposures employed (Kadoum et al. 1967).

RF treatments affected principally the trophocytes and oocytes of the ovary. Schlottman and Bonhag (1956) suggested that the nutritive substances are passed from both the follicular epithelium and the apical trophocytes to the developing oocytes. The possibility that nucleic acids or their derivatives contribute to the enlargement of the oocytes was also suggested by these authors. From the nature of damage resulting in developing ovarian tissue following exposure to RF energy, it is suggested that a reduction in the number of eggs developing in RF-treated females could result either from the direct lethal effect of RF energy on the developing oocytes or indirectly by depriving the developing oocytes of nutritive materials as a result of trophocyte degeneration.

The effect of RF treatment on male reproductive tissues was most obvious in the developing spermatocytes. Disintegration of spermatocytes was evident in tissue from RF-treated testes. The effects of X and gamma rays on the reproductive tissues of insects have been reported by several workers. The effect of X rays on the development of spermatozoa varied from necrosis and death of spermatogonial cells and spermatocytes (Alexander and Stone 1955, Welshons and Russel 1957, Riemann 1967) to the formation of giant cells (Dederer 1940). Irradiation with gamma rays resulted in the inhibition of germ cell production (Ives et al. 1950) and the death of spermatocytes (Riemann 1967).

The nature of damage to the male reproductive tissues following RF treatment indicates that such treatment could reduce the production of spermatozoa by damaging the spermatocytes. From the observations made on the cytological changes resulting from RF treatment of male reproductive tissues, it is suggested that (1) some of the spermatocytes degenerate following RF treatment and lose their reproductive capacity, and (2) the loss of virility in RF-treated males can be attributed to a decrease in the production of spermatozoa resulting from spermatocyte degeneration.

REFERENCES CITED

Alexander, M. S., and W. S. Stone. 1955. Radiation damage in the developing germ cells of *Drosophila virilis*. Proc. Natl. Acad. Sci. U.S.A. 41: 1046–57.

Amy, R. L. 1955. A comparative study of the effects of beta rays, gamma rays, and X rays on development in *Habrobracon*. Radiat. Res. 3: 166–81.

Carpenter, R. L., and E. M. Livstone. 1971. Evidence for nonthermal effects of microwave radiation: abnormal development of irradiated insect pupae. IEEE Trans. Microwave Theory and Techniques. MTT-19: 173–8.

Dederer, P. H. 1940. The production of giant spermatocytes in *Philosamia cynthia* by means of X rays. J. Morphol. 67: 159–74.

Galigher, A. E., and N. Kozloff. 1964. Essentials of practical microtechnique. Lea and Febiger, Philadelphia. 484 pp.

Ives, P. T., R. S. Heilman, and H. H. Plough. 1950. The LD_{50} for gamma radiation in *Drosophila melanogaster*. Genetics 40: 577.

Kadoum, A. M., S. O. Nelson, and L. E. Stetson. 1967. Mortality and internal heating in radiofrequency-treated larvae of *Tenebrio molitor*. Ann. Entomol. Soc. Am. 60: 855–9.

Murray, D. R. P. 1960. The stimulus to feeding in larvae of *Tenebrio molitor* L. J. Insect Physiol. 4: 80–1.

Rai, P. S. 1970. An investigation of the morphological and reproductive changes induced by exposure of the yellow mealworm, *Tenebrio molitor* L., to radiofrequency energy. Diss. Abstr. 71–9580.

Rai, P. S., H. J. Ball, S. O. Nelson, and L. E. Stetson. 1971. Morphological changes in adult *Tenebrio molitor* (Coleoptera: Tenebrionidae) resulting from radiofrequency or heat treatment of larvae or pupae. Ann. Entomol. Soc. Am. 64: 1116–21.

Riemann, J. G. 1967. A cytological study of radiation effects in testes of the screw-worm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae). Ibid., 60: 308–20.

Schlottman, L. L., and P. F. Bonhag. 1956. Histology of the ovary of the adult mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Univ. Calif. Publ. Entomol. 11: 351–94.

Welshons, W. J., and W. L. Russel. 1957. The effect of X-rays on the *Drosophila* testis and a method for obtaining spermatogonial mutation rates. Proc. Natl. Acad. Sci. U.S.A. 43: 608–13.

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