RADIOBIOLOGY

Effects of X-rays on Electrical Activity in the Eye of the Cockroach *Blaberus giganteus*

Significant changes in electroretinograms (ERG) and in spontaneous electrical activity recorded from the compound eyes of cockroaches have been found here during investigations of the effects of radiation on insects. These electrical discharges, although similar to the activity recorded from the optic nerve of *Limulus*[1], do not exhibit as marked a change in frequency with light stimulation. In this communication, we present the results of preliminary studies of the effects of X-rays on both the ERG and the spontaneous electrical discharges in the eye of the cockroach *B. giganteus*.

Whole adult insects, wrapped in polythene, were fastened to a wax platform. Fine platinum wire electrodes were inserted into the eye and into the head capsule by means of a semi-micromanipulator. Most consistent results were obtained when the reference electrode was located immediately posterior to the margin of the eye. Electrical activity, displayed on a dual-beam 'Tecktronix' oscilloscope, was recorded with a 'Brush' pen recorder. The insects were irradiated with a 150 kV X-ray machine (inherent filtration 3 mm beryllium; target-specimen distance 20 cm) at a dose rate of 2,000 r./min. The source of light was fluorescent room-lights giving an illumination of approximately 4 ft.-candles. All experimental animals were light-adapted.

Fig. 1, tracing *A* shows the typical spontaneous electrical activity prior to irradiation. With irradiation, this activity gradually decreased and, at a total dose of approximately 120 kr, all activity had virtually disappeared (Fig. 1, *B*). Partial recovery from this effect occurred during a one-hour rest period without X-rays (Fig. 1, *C*). Subsequent irradiation-and-rest cycles showed that suppression and recovery would occur several times, although less radiation and shorter rest periods were required for succeeding cycles of irradiation and rest. The amplitude and frequency of the electrical activity never returned to the pre-irradiation values.

B. giganteus reacted to changes in light intensity with the typical 'on-off' response (Fig. 2, *A*) of some insects as described by Roeder[2]. Exposure to X-rays decreased the amplitude of the 'on' response to light, but no change in amplitude was observed in the 'off' response. The reduction in the 'on' response was small, reaching a maximum of 15 per cent only after an exposure of 60 kr. Recovery from this effect was rapid, occurring in approximately 30 sec after the cessation of exposure to X-rays. After short rest periods of about 5 min following irradiation, this decrease in amplitude could be produced with very small doses (about 300 r.). No attempt was made to measure latent periods in these preliminary tests. It was interesting that an 'on-off' response was also found with X-rays (Fig. 2, *B*).

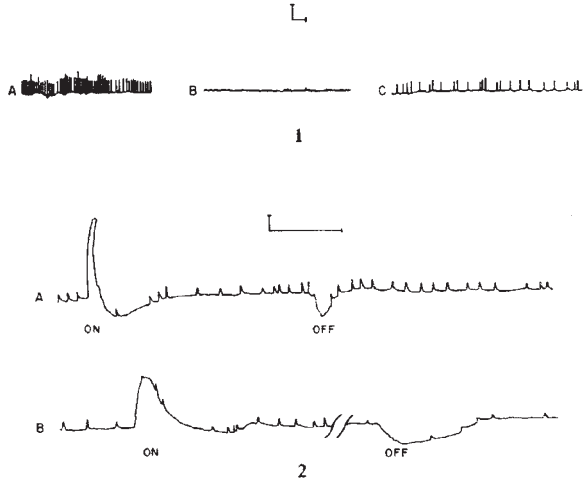

Fig. 1. Spontaneous electrical activity in the eye of *B. giganteus*. (*A*) Normal activity prior to X-rays, (*B*) after 120 kr; (*C*) recovery after a rest period of 45 min. Calibration values 275 μV and 1 sec

Fig. 2. 'On-off' response to (*A*) light and (*B*) X-rays. (Note that in tracing *B* a time lapse of 15 min occurred between the 'on' and 'off' responses.) Calibration values 200 μV and 1 sec

This work has demonstrated a marked effect of large doses of radiation in reducing the amplitude and frequency of the electrical activity recorded from the eye of *B. giganteus*. Also, an effect of X-rays was found in the 'on' response to light. Recovery from the effect occurred in both responses, but at very different rates. Further work will involve the study of recovery from radiation damage in these two systems.

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BIOLOGY

Species Identification of Animal Cell Strains by Immunofluorescence

Animal cells cultivated *in vitro* can be readily identified with respect to their species of origin by several immunologic procedures, including: hæmagglutination[1], and mixed cell agglutination[2], both involving detection of species antigens common to erythrocytes and cultured cells, and an immunofluorescence method[3], which provides direct visualization of species-specific antigen–antibody reactions on cell surfaces. By means of these procedures, a number of established cell lines have been shown to be of a species other than that originally claimed[4,5], a finding obviously due to heterologous cell contamination and overgrowth or other laboratory errors.

This communication describes the use of the immunofluorescence procedure to detect individual cells of different species within mixed cell populations (as occurs in contamination, especially in early phases), as well as its use in species identification of a number of established cell lines and sub-lines from various laboratories.

Antisera against verified prototype strains (human: Detroit-6, HeLa; monkey: *LLC-MK*$_2$; mouse: *L-M*, *S*-180; Chinese hamster) and/or primary kidney cultures of various animal species (human, monkey, mouse, rat, rabbit) were prepared by immunizing guinea pigs or rabbits with washed cells scraped from monolayers cultured in Eagle's medium supplemented with 5 or 10 per cent human or calf serum as previously described[3]. Antisera were heated at 56° C for 30 min and the globulins precipitated in 18 per cent sodium sulphate (1 vol. serum/2 vol. 27 per cent sodium sulphate) at 37° C for 16 h. Following dialysis, the resulting globulin solutions, adjusted to *p*H 9, were conjugated with fluorescein isothiocyanate (FITC) by adding them directly to a dry mixture of 1 part FITC (0·025 mg. FITC/mg protein) and 9 parts cellulose powder ('Celite') and shaken for 5 min[6]. The 'Celite' was removed by centrifugation and the unbound dye by 'Sephadex' gel filtration.

Cells to be tested were obtained by trypsinizing monolayer cultures to yield $3–4 \times 10^6$ cells per ml. After 3 washes in buffered saline (*p*H 7·5), 0·1 ml. of cell suspension was mixed with 0·1 ml. labelled antibody, agitated for 30 min, again washed 3 times, and a drop of the final sediment sealed under a coverslip and observed by fluorescence microscopy[3]. Specific staining reactions