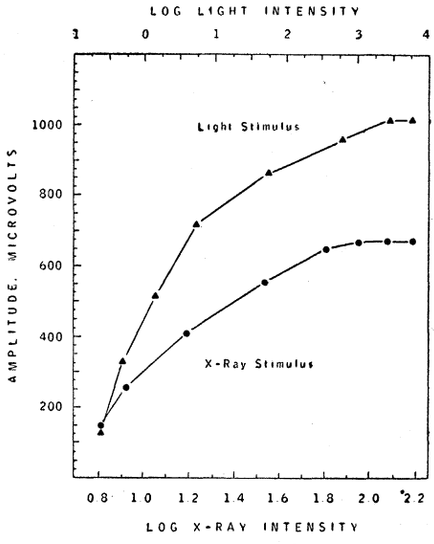

Fig. 4. Mean amplitude of *b*-wave in the on response to x-rays and to light, plotted as a function of the logarithm of light intensity in meter-candles and of x-ray intensity in roentgens per second. Duration of stimulus as in Fig. 3. The values indicate that a maximal response was obtained in both situations. Values are based on experiments involving 35 animals.

indifferent electrode in all cases was placed in contact with the skin over the nose. It was possible with this arrangement to record responses to light and responses to x-rays from the same animal under identical conditions without disturbing the assembly, since stimulating and recording equipment was controlled from a shielded room that was adjacent to the room containing the x-ray tube, the light source, and the animal.

A typical series of "on" responses to light and to x-rays is shown in Fig. 3, ranging from near-threshold responses to maximal responses obtainable for that particular retina. There is a striking similarity between the two sets of responses reproduced in Fig. 3, especially between the responses to light from threshold to 1.5 m-ca and the responses to x-rays from threshold to 66 r/sec. Lest it be concluded, therefore, that the mechanism of action of the two stimuli are the same, certain differences should be pointed out.

The mean maximal amplitude of the *b*-wave from 35 animals in response to x-rays was 665 μv, whereas the mean maximal amplitude obtained from the same animals in response to light was 1010 μv (Fig. 4). Increasing the magnitude of the stimulus in either case did not increase the amplitude beyond these maximal values. It is remarkable that the magnitude of the stimulus necessary to evoke the responses shown in Fig. 2 covered a 12,000-fold range for light, whereas the range for x-rays was only 25-fold. This point is emphasized in Fig. 4 by the different logarithmic scales for light intensity and for x-ray intensity.

The latent period for the response to x-rays in Fig. 2 varied from 135 msec for the maximal response to 190 msec for the minimal response; the latent period for the response to light, by contrast, varied from 60 msec for the maximal response to 150 msec for the minimal response. That Elenius and Sysimetsä did not detect a difference in the latent period of the two responses is probably due to the fact that they considered only a threshold response. At such low levels, the latent period of the two responses does not differ as greatly as at higher amplitudes, although, in the work under discussion, considerable difference was found even at the lower amplitudes. In studies on 35 animals, for example, the mean latent period for 120-μv responses to x-rays was 181 msec, whereas for comparable responses to light it was 140 msec. The mean latent period for maximal responses to x-rays for the same animals was 129 msec, whereas for maximal responses to light the latent period was 71 msec.

The period of latency of the two responses is of considerable significance. Differences in latency suggest different mechanisms in the interaction between the photoreceptors and light, on the one hand, and the photoreceptors and x-rays on the other. Absorption of x-ray photons by the rods is apparently responsible for the electroretinogram. This assumption is based on two lines of evidence. First, no electroretinogram in response to x-rays could be elicited in this laboratory from the horned toad, an animal which lacks rod vision. Second, when the logarithm of the brightness of light necessary to produce a constant response is plotted as a function of time in the dark, the resulting curves for dark adaptation show a break which characteristically occurs during the early stages of adaptation. This break is similar to breaks which have been reported in curves for dark adaptation in human beings and which have been shown to indicate a shift from cone to rod function. Such a break was observed in the response to light but not in the response to x-rays. In view of the results reported here, we propose that, in some way analogous to the manner in which the rods react with visible light, the pigment of the rods, rhodopsin, absorbs x-ray quanta, undergoes chemical change, and leads to excitation which produces the electroretinogram. In this sequence of events the delay, and hence the difference in the response, is to be found in the chemical change involved in the bleaching of rhodopsin. It is suggested that the reaction of radicals produced by x-rays (*4*) is involved in the chemical change (*5*).

C. S. BACHOFER
S. ESPERANCE WITTRY*
Department of Biology, University of Notre Dame, Notre Dame, Indiana

References and Notes

1. G. L. Clark, *Applied X-Rays* (McGraw-Hill, New York, 1955), p. 17.
2. F. Himstedt and W. A. Nagel, *Ber. naturforsch. Ges. Freiburg Breisgau* **11**, 139 (1901); *Festschrift Freiburg Universität* (1902), p. 257.
3. V. Elenius and E. Sysimetsä, *Acta Radiol.* **48**, 465 (1957).
4. A. O. Allen, *J. Phys. & Colloid Chem.* **52**, 479 (1948).
5. This research was supported by contract No. AT(11-1)-205 between the U.S. Atomic Energy Commission and the University of Notre Dame.
* Present address: College of St. Catherine, St. Paul, Minn.

29 August 1960

Micromanipulation in Control and Handling of Zygiella x-notata as an Experimental Animal

Abstract. The spider *Zygiella x-notata* may be brought under direct control analagous to that of the common laboratory animals for an important group of experimental investigations on the nerve, muscle, or secretion of digestive glands. Without anesthetics, chilling, or damage the animal can be fixed for prolonged periods and microinstruments (which include feeding pipettes), positioned by a standard micromanipulator.

The spider *Zygiella x-notata* has been used as a sensitive biological test animal for a number of psychopharmacologic and hallucinogenic drugs (*1*). Characteristic disturbances in web-building activities and patterns reflected the effects of the drugs on the central nervous system of the animal. Important applications of the test to body fluids of man have been made in the search for hallucinogenic substances in schizophrenia by Witt and Weber (*2*).

Administration of psychotrophic substances has been either by direct injection (with a high mortality due to irremediable chitinous damage), or by the ingenious indirect technique of Wolff and Hempel (*3*); the latter consisted in the injection of the drug dissolved in sugar solution into the abdomen of a desiccated fly which was weighed and thrown on the web, which was then vibrated by a tuning fork. When the spider had accepted this artificial prey the latter was again weighed to determine the amount of the drug taken.

The present investigations were designed to explore the possibility of direct dosage without injury in this small

(5 to 6 mm) animal and the placing of microinstruments before and after the administration of various substances, including those of the psychopharmacologic and hallucinogenic group (Fig. 1). A method was devised to maintain the animal in a position suitable for operations for prolonged periods without anesthetics, chilling, or damage.

Before fixation of the animal a small drop (approximately 1 to 2 mm^3) of collodion-amyl acetate was placed on a microscope cover slip; this was allowed to thicken for 20 to 30 seconds. The cover slip was inverted onto the dorsal surface of the abdomen of the spider and held down for a few seconds. The cover slip was then turned over to bring the ventral surface of the animal under the microscope to allow access for the micromanipulator instruments, which could be positioned quickly and accurately by the standard micromanipulator (Leitz) equipped with three instrument carriers; a stereo-binocular microscope facilitated observation. The animal could be maintained in position in this way for several days without any ill effects. Outbursts of activity took place, but the spider also remained immobile for long periods. Micro-saltbridges made from glass capillaries, the terminal points being filled with agar (*4*) or 25 μ microelectrodes (*5*), may be applied for nerve (or muscle) studies; capillary tubing with a flexible taper of 2 to 3 mm and a diameter of 0.1 to 0.2 mm (with a tip diameter of 20 to 30 μ) permitted movement without breakage. Though insertion of micro-saltbridges (as distinct from injection) at the side of the thorax resulted in about a 50-percent mortality (8 in 15 animals), the remainder survived for approximately 24 hours, long enough for most investigations into electrical changes.

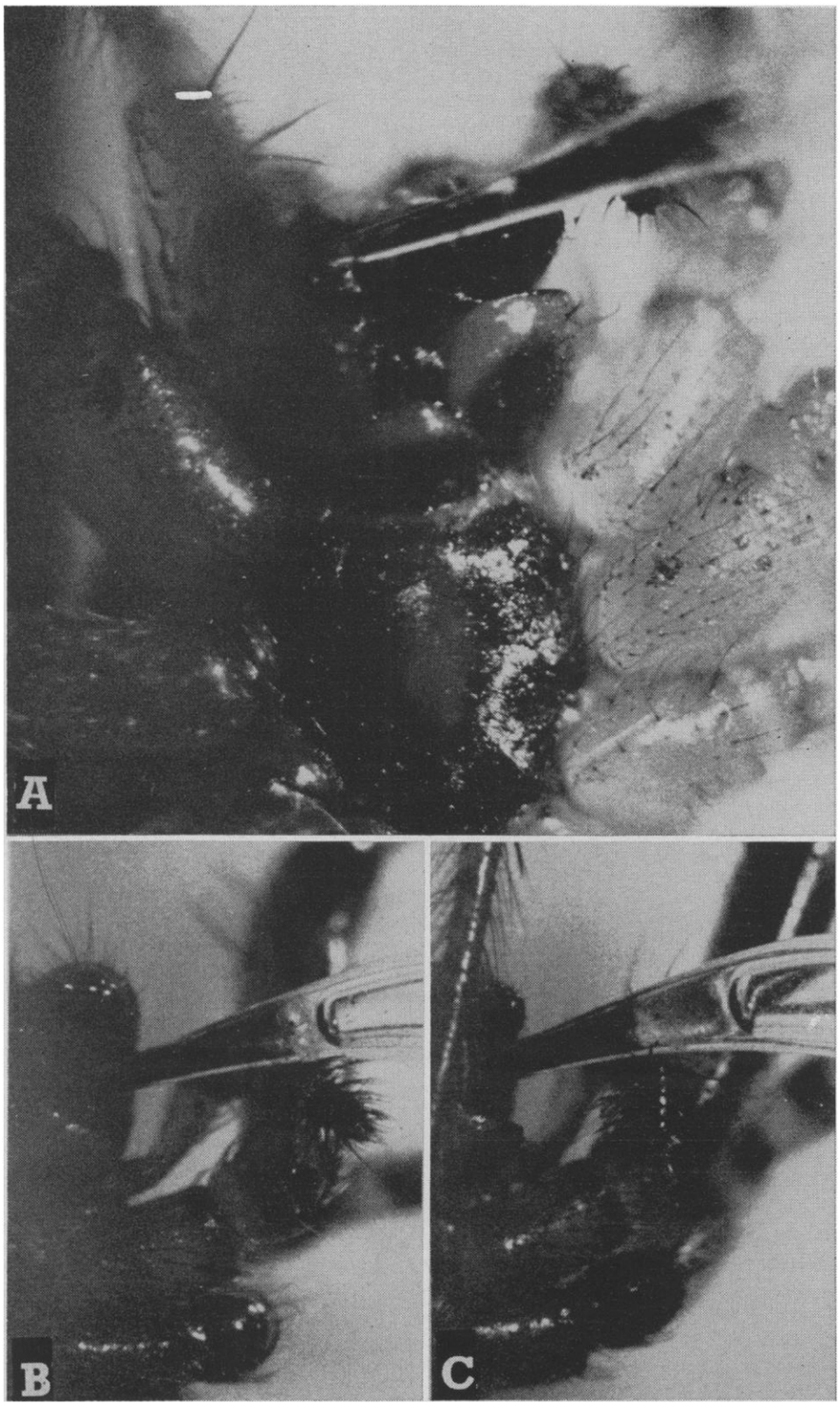

Fig. 1. (*A*) Typical sampling and feeding pipette in relation to spider (× 48 at *f*/24); electronic flash. (*B*) Pipette in normal animal filling by capillarity to approximately 0.1 mm^3 (× 32 at *f*/24); electronic flash. (*C*) Same pipette as (*B*) 2 minutes later (× 32 at *f*/24); electronic flash.

In order to sample digestive fluids or to administer solutions, micropipettes with a mean diameter of 0.1 mm in the terminal 1 mm were suitable. Samples were obtained on contact by capillarity, and variations in secretory activity were observed directly under the microscope and recorded photographically (Fig. 1). For this a 35-mm camera provided with a mirror reflex housing, viewing magnifier, and bellows extension fitted with a 25-mm Micro Summar (Leitz) was used; an electronic flash unit (Zeiss) combined with fast film enabled satisfactory photographs to be obtained. Delivery of the contents of a pipette tip (drawn up in controlled amounts by capillarity) was effected by the micromanipulator syringe; contents in contact with secretory fluids in a pipette tip over 20 μ in diameter may be easily expelled. Pipettes were marked for measured volumes and delivered quantities of the order of 0.01 mm^3. By such means the effects of feeding and the injection of possible test substances on the secretion of digestive glands may be estimated.

The techniques described may thus be capable of extension to further experimental studies on the reactions of these animals to substances affecting tissue components other than those of the central nervous system.

C. A. ERSKINE
School of Anatomy, Trinity College, University of Dublin, Dublin, Ireland

References

H. M. Peters and P. N. Witt, *Experientia* **5**, 161 (1949).
2. P. N. Witt and R. Weber, *Monatsschr. Psychiat. Neurol.* **132**, 193 (1956).
D. Wolff and U. Hempel, *Z. vergleich. Physiol.* **33**, 497 (1951).
4. E. Dejdar, *Z. wiss. Mikroskop.* **46**, 361 (1929).
H. H. Hillemann, *Anat. Record* **84**, 343 (1942).

6 October 1960

Multi-Resistant Aedes aegypti in Puerto Rico and Virgin Islands

Abstract. The Isla Verde, Puerto Rico, laboratory colony, highly resistant to DDT and dieldrin in 1959, became even more so by 1960; resistance to organic phosphates was also greater than before. In laboratory tests Bayer 29493 was best for killing resistant larvae and adults. It is improved with piperonyl butoxide at the ratio of 10:1.

The theory that *Aedes aegypti* can be resistant to DDT or dieldrin but not to both (*1*) was disproved by the Isla Verde strain (*2*, *3*). This dual resistance occurs not only in the laboratory but along the whole north coast of Puerto Rico (*4*). When there is danger of the spread of yellow fever in the Caribbean (*5*), the presence of vigorous races recalcitrant to control has ominous implications for public health. To find an insecticide for overcoming resistant strains is imperative and for this purpose the Isla Verde strain is obviously more useful than the susceptible strains of most laboratories (*6*). The original toxicological tests (*2*) on this strain were made from March through July 1959 on about the F_{10} generation of a colony started in November 1958. By July 1960 the colony was estimated to be at the F_{50} generation after continuous inbreeding. At that time, in the field not far from the original site, more specimens were collected (*7*), and the F_1 and F_2 generations were obtained for tests to compare with the original data. In 1959, 2.5 parts of malathion, diazinon, and dipterex per million gave 100 percent mortality; therefore, this concentration was used as a standard (*8*). Table 1 shows that the colony became even more resistant to DDT, lindane, dieldrin, and chlordane; in the field, except for lindane, the same high resistance occurs. As larval resistance to the chlorinated hydrocarbons increased in the colony, so did the resistance to the organic phosphates, but field resistance to the latter was not so marked. Resistance developed also to Bayer 21/199, the best larvicide in 1959. For specific use in mosquito control, Bayer 21/199 has been replaced by the manufacturers with Bayer 29493 (*9*), which is more effective against adult mosquitoes. Bayer 29493 was the most toxic in our tests and was made more so by synergism with piperonyl butoxide (*10*) at the ratio of 10:1 rather than the reverse ratio (*11*).

To prove further the resistance to organic phosphates, tests were made on the Isla Verde colony larvae at 0.5 part per million (ppm). In 1959 (*2*) dipterex, diazinon, and malathion only gave 72, 74, and 85 percent dead, and the same for dead plus moribund mortality; Bayer 21/199 gave 87 and 100 percent mortality. In 1960 the results were as follows (average of two replicates, dead and dead plus moribund mortality): Bayer 21/199, 65 and 100 percent; dipterex, 5 and 37 percent; malathion, 41 and 68 percent; diazinon, 34 and 73 percent; Bayer 21/199 plus piperonyl butoxide (10:1), 64 and 100 percent; Bayer 29493, 80 and 100 percent; Bayer 29493 plus piperonyl butoxide (10:1), 92 and 100 percent.

Females of the Isla Verde colony, which were exposed for 1 hour to bond paper impregnated with the following concentrations of insecticides at the rate of 3.6 mg/cm^2, gave the following percentage mortalities after 24 hours (average of two replicates): 4 percent DDT, 81 percent; 4 percent dieldrin, 47 percent; 3 percent Bayer 21/199 alone and with piperonyl butoxide (10:1 and 1:10), 18 to 22 percent; 0.4 percent malathion, 100 percent; 0.1 percent diazinon, 100 percent; 0.1 percent Bayer 29493, 100 percent; 0.05 percent Bayer 29493 plus piperonyl butoxide (10:1), 100 percent.

That DDT-dieldrin resistance already occurs in another island of the Caribbean was evident from tests on a St. Croix strain (*12*). Table 2 indicates moderate resistance to all insecticides listed except Bayer 29493. The lack of complete mortality with DDT and dieldrin at 0.5 ppm with this strain must be considered in relation to 100 percent mortality of susceptible strains at 0.02 ppm of these insecticides. It is likely that in about a year's time the same high resistance of Puerto Rico strains will occur in St. Croix, if DDT continues to be used there.

In conclusion, the following points are emphasized: (i) DDT-dieldrin resistance is not simply a laboratory phenomenon but is general in Puerto Rico and perhaps in other islands of the Caribbean where DDT resistance has been reported. (ii) A DDT-dieldrin resistant strain under the conditions of our laboratory did not lose its resistance after 18 months' colonization (*13*), but in fact became more resistant. (iii) The evidence is against the idea that genetic differences separate resistance to insecticides into distinct types.

IRVING FOX
ILEANA GARCIA-MOLL
School of Tropical Medicine, University of Puerto Rico, San Juan

Table 1. Percentage mortality of *Aedes aegypti* fourth instar larvae, Isla Verde, P.R., strain after 24 hours of exposure to insecticides at 2.5 ppm (average of four replicates, dead only). The effect of 18 months of colonization is compared with data from field material.

Insecticides	Laboratory colony		Field
	1959 ($F_{10\pm}$)	1960 ($F_{50\pm}$)	1960 (F_{1-2})
DDT	62*	25†	20†
Lindane	77*	30*	67*
Dieldrin	77*	0†	6†
Chlordane	25†	0†	2†
Dipterex	100‡	55§	94‡
Malathion	100‡	64§	94§
Diazinon	100‡	62‡	96‡
Bayer 21/199	100‡	58‡	97‡
Bayer 21/199 + piperonyl butoxide (10:1)		90‡	96‡
Bayer 21/199 + piperonyl butoxide (1:10)		97‡	55‡
Bayer 29493		91‡	94‡
Bayer 29493 + piperonyl butoxide (10:1)		100‡	100‡
Bayer 29493 + piperonyl butoxide (1:10)		95‡	36‡

Dead plus moribund: *79–89 percent; †0–44 percent; ‡100 percent; §98–99 percent.

Table 2. Percentage mortality of *Aedes aegypti* fourth instar larvae of a colony (F3–4) from material collected at Christiansted, St. Croix, in October 1959 (average of two replicates, dead and dead plus moribund).

Insecticide	0.5 ppm		2.5 ppm
	Dead	Dead plus moribund	Dead*
DDT	77	88	90
Lindane	45	77	95
Dieldrin	33	45	88
Malathion	66	88	95
Diazinon	60	78	95
Bayer 29493	92	100	85

*Dead plus moribund, 100 percent mortality for all.

References and Notes

1. A. W. A. Brown, *Insecticide Resistance in Arthropods* (World Health Organization, Geneva, 1958), p. 21.
2. I. Fox, *Bol. ofic. sanit. panam.* **48**, 375 (1960).
3. A. W. A. Brown, *Mosquito News* **20**, 114 (1960).
4. I. Fox, A. H. Boike, Jr., I. García-Moll, *Amr. J. Trop. Med. Hyg.* **9**, 428 (1960).
5. I. Fox, *Mosquito News* **18**, 124 (1958).

6. This work was supported partly by research