OBSERVATIONS ON STERILIZATION OF *ANOPHELES* (*C.*) *ALBIMANUS* WIEDEMANN BY X-IRRADIATION[1]

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ABSTRACT. Pupae of *Anopheles albimanus* Wiedemann, not more than 24 hours old, were exposed to a graded series of X-ray dosages, starting at 2,000 R and ending at 8,000 R. Mortality of adults emerging from these pupae was negligible. There was no reduction in the frequencies of insemination of treated or untreated females by treated or untreated males, except for the cross between males and females exposed to 8,000 R. Egg production by treated females was greatly reduced. Egg production by untreated females which had been inseminated by treated males was similar to that of the controls. The adjusted infertility rates of eggs from the above females which had been exposed to 5,000, 6,000, 7,000, and 8,000 R were, respectively, 84.3, 82.9, 88.7, and 100 percent.

It has been shown that anopheline as well as culicine mosquitoes can be sterilized by X- or gamma-irradiation, and that males emerging from treated pupae will inseminate and so sterilize normal females (Davis *et al.*, 1959; Abdel-Malek *et al.*, 1966; Tantaway *et al.*, 1967; Darrow, 1968; Smittle *et al.*, 1970, 1971 a, b). However, field tests with *Anopheles quadrimaculatus* (Weidhaas *et al.*, 1962; Dame *et al.*, 1964), *Aedes aegypti* (Morlan *et al.*, 1962), and *Culex quinquefasciatus* (Krishnamurthny *et al.*, 1962) have been disappointing. Weidhaas and his associates concluded that the males in their experiments, which were taken from a laboratory colony, had lost their ability to compete with wild males for wild females. Another possibility is that irradiation adversely affected longevity and competitiveness. During a discussion of this problem at a conference on anopheline biology and malaria eradication (see Ward and Scanlon, 1970), Dr. George Craig expressed the view that overtreatment of the males so as to bring about complete sterilization was a mistake. He stated, "What you are really trying to do is to put more bad sperm than good sperm into the field. You are trying to outweigh gametes, so that if you release 95%-sterilized males which have received a dose of 5000 R, you are going to have a better chance because these males are actually going to inseminate field females."

Anopheles albimanus continues to be a dangerous malaria vector in El Salvador and other parts of Central and Northern South America. Whether the sterile male technique will become practicable for control must be determined by field trials. Meanwhile, it would be of interest to know what dosage of irradiation would be capable of producing effective sterilization of this species without reducing the mating competitiveness or survival of the males. The object of these experiments was to observe the effect of a series of graded X-ray dosages, applied to the pupae, on survival of the emerging adults, and on the degree to which males from treated pupae inseminate and so sterilize normal females.

MATERIALS AND METHODS

The strain of *A. albimanus* originated in El Salvador and has been maintained in our insectary since 1959 (Hobbs, 1962).

Pupae not over 24 hours old were taken from the rearing pans, and divided at random into two groups. One of these was an untreated control group. The other group was subjected to the required dose of irradiation under a Keleket (Radiotherapy) X-Ray machine (250 KV, 15

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MA, constant potential, 1 mm Al. filtration, dose range from 100 to 300 R per minute). Dosages ranged from 2,000 to 8,000 R, in increments of 1,000 R.

Following treatment, the pupae were placed in groups of 2 to 5 in test tubes for emergence of the adults. The adults were separated according to sex and then placed in the desired combinations in cubic foot breeding cages. For each replicate experiment, there were four breeding cages, one containing treated females and treated males, the second with treated females and normal males, the third with normal females and treated males, and the fourth with normal females and normal males. Equal numbers of males and females were placed in each of the four cages. In most replicates there were 25 or 50 of each sex per cage. The adults were kept together for 4 or 5 days, and at the end of this mating period the females were allowed to engorge on human or guinea pig blood. Those appearing to be fully engorged were isolated in gauze-covered pint cartons or shell vials, in which a strip of moist paper toweling provided a surface for oviposition. During the mating and gestation periods, the adults were fed 10 percent sucrose solution. Eggs deposited by inseminated females were counted, flooded with water, and observed for hatching.

RESULTS

ADULT EMERGENCE. No significant difference occurred between emergence rates of adults from pupae exposed to the various dosages of X-irradiation and the respective control groups. Mortality at emergence ranged from 1.9 to 6.0 percent in the treated groups, and from 0.5 to 3.3 percent in the control groups.

Survival of adult females up to the time they had oviposited (about 8 to 10 days) varied between experiments, from a minimum of 54 percent to a maximum of 96 percent. Within a single replicate at a given dosage, however, there was no significant difference between treated and untreated groups. In most replicates, survival was over 90 percent.

INSEMINATION RATES. These are presented in Table 1. It is apparent that irradiation of the pupae had no effect, in the cubic foot cages, on the frequency of insemination of treated or untreated females by treated or untreated males, with one possible exception. Insemination appeared to be suppressed somewhat in the cross between females and males exposed to 8,000 R.

EGG PRODUCTION. The numbers of eggs produced by inseminated females, and the numbers of fertile eggs produced by the inseminated females that oviposited, are presented in Table 2 as the mean number of eggs per female and the standard deviation of the means. The large standard deviations reflect the great variability in the numbers of eggs produced by individual females. In almost every group, one or more females failed to lay eggs, al-

TABLE 1.—Effect of X-irradiation of pupae on insemination rates of *A. albimanus* adults.

Dosage (R)	Numbers of females dissected; percent positive for sperm							
	T♀ x T♂		T♀ x N♂		N♀ x T♂		N♀ x N♂	
	No.	%	No.	%	No.	%	No.	%
2,000	14	71.4	15	100	9	77.8	14	57.1
3,000	7	57.1	15	86.7	13	69.3	12	91.7
4,000	20	65.0	16	62.5	18	94.4	16	62.5
5,000	79	79.8	72	80.6	81	80.6	83	86.8
6,000	45	77.8	42	81.0	41	92.7	59	79.7
7,000	53	88.7	44	88.6	45	91.1	48	89.6
8,000	39	25.6	44	65.9	34	79.4	36	80.6

[1] T = treated; N = normal (untreated).

Table 2.—Fertile egg production of treated and untreated females inseminated by treated or untreated males.

Dosage (R)	T♀ x T♂			T♀ x N♂			N♀ x T♂			N♀ x N♂		
	No. ♀♀	M	SD	No. ♀♀	M	SD	No. ♀♀	M	SD	No. ♀♀	M	SD
2000 [3]												
Eggs [1]	10	9.0	14.6	15	15.9	22.3	7	66.6	40.4	8	75.0	15.1
Hatched [2]	5	12.0	14.3	8	22.9	18.1	6	38.7	16.8	7	73.6	19.9
3000 [3]												
Eggs	4	17.0	..	13	39.7	27.4	9	47.4	25.5	11	61.7	17.6
Hatched	2	13.5	..	11	30.8	20.9	9	28.3	24.8	11	44.2	20.6
4000 [3]												
Eggs	13	8.4	17.2	10	20.1	11.8	17	53.4	36.1	10	61.1	54.8
Hatched	4	5.3	12.4	7	13.4	9.6	12	30.5	15.7	9	30.1	22.4
5000 [4]												
Eggs	62	6.1	14.5	57	5.9	9.4	48	87.8	51.4	72	94.6	51.8
Hatched	17	2.9	6.9	19	8.3	8.2	39	12.0	12.7	61	78.7	32.6
6000 [4]												
Eggs	35	4.7	7.3	28	5.4	7.1	38	81.8	46.3	47	79.9	42.6
Hatched	16	0.4	..	13	1.9	..	32	12.3	16.9	41	68.1	27.1
7000 [4]												
Eggs	47	2.3	5.1	39	1.9	4.4	41	120.9	44.8	38	140.1	44.4
Hatched	14	0.4	..	9	1.3	2.6	39	12.9	22.0	37	126.2	44.1
8000 [3]												
Eggs	10	0	..	29	0	..	27	148.8	71.8	29	129.6	60.2
Hatched	0	..	..	0	..	..	23	0	..	26	67.6	26.6

[1] Numbers of eggs produced by inseminated females, expressed as means and standard deviations.
[2] Numbers of eggs hatching of those produced by ovipositing females, expressed as means and standard deviations.
[3] One test.
[4] Two replicate tests.

though they were positive for sperm and had been considered to have been fully engorged. There was only one observation at each of the three lowest dosages; hence the numbers of females examined were small. Nevertheless it is evident that egg production is markedly reduced, even by females treated in the pupal stage at the lowest dosages, and that these numbers decreased as the dosage was increased. Females exposed to 5,000 R laid an average of only about 6 eggs. However, even in the 6,000 and 7,000 R groups, a few fertile eggs were deposited by treated females that had been inseminated by treated males.

Untreated females inseminated by treated males produced normal numbers of eggs; however, in the 5,000, 6,000, and 7,000 R groups, the mean numbers of fertile eggs produced by the experimental and the respective control females were 12.0 *vs.* 78.7, 12.3 *vs.* 68.1, and 12.9 *vs.* 126.2.

Table 3 presents the total numbers of eggs laid and the percentages of hatching. Although females exposed to 2,000 and 3,000 R and inseminated by untreated males produced fewer eggs (Table 2,) the percent of hatching was comparable to that of the controls. At higher dosages, only a few of the eggs from these females were able to hatch.

What is of primary concern is the fertile eggs production by normal females inseminated by the treated males. Although there was some reduction at the three lower dosages, there was a marked drop to a 11.1 percent hatch in the 5,000 R group. The fertility rate did not diminish in the 6,000 and 7,000 R groups. A 100 percent sterility was achieved at the 8,000 R dosage.

There was some variation in fertility rates in the several control groups, possibly because of differences in environmental conditions or vigor of the populations used in the replicates. However, in a given test, these variables should apply to all of the four populations, which were treated alike. The experimental sterility rates should thus be compared with those of the controls in

TABLE 3.—Egg production by treated and untreated females inseminated by treated or untreated males.

Dosage (R)	T♀ x T♂	T♀ x N♂	N♀ x T♂	N♀ x N♂
2000				
Total eggs	90	239	466	600
% hatched	66.7	76.6	49.8	85.8
3000				
Total eggs	68	516	427	679
% hatched	39.7	65.7	59.7	71.6
4000				
Total eggs	109	201	908	611
% hatched	19.3	46.8	40.3	80.7
5000				
Total eggs	376	336	4,215	6,809
% hatched	13.3	46.7	11.1	70.5
6000				
Total eggs	163	152	3,107	3,757
% hatched	0.4	16.5	12.7	74.3
7000				
Total eggs	109	72	4,957	5,322
% hatched	4.6	16.7	9.9	87.8
8000				
Total eggs	0	0	4,017	3,757
% hatched	..	..	0	46.8

TABLE 4.—Fertility rates for eggs deposited by untreated females inseminated by treated males.

Dosage in R	2000	3000	4000	5000	6000	7000	8000
Adjusted * % sterility	42.0	16.6	50.0	84.3	82.9	88.7	100

* Adjusted by Abbot's formula.

the respective tests. These rates, adjusted by Abbott's (1925) formula, for the eggs deposited by untreated females inseminated by treated males, are shown in Table 4.

DISCUSSION

In the present experiments, X-irradiation of 24-hour old pupae of *A. albimanus* through the 8,000 R dosage had no effect on the ability of the treated males to inseminate normal females. Whether males treated at this dosage have lower competitiveness with wild males in nature can be determined only by field trials. Developing ovaries were extremely sensitive to X-irradiation; microscopic examination of the ovaries dissected from the adult females showed that most of the follicles were in various stages of degeneration. Even at the lower dosages, only a few of the follicles seemed to have apparently normally developing ova. Thus, separation of the sexes in a release program would not have to be rigid; the females would not be able to reproduce, and their only effect would be to divert some males from normal females.

Normal females, inseminated by treated males, produced normal numbers of eggs. Treatment with 5,000 R caused about an 85 percent reduction in fertility, and this reduction remained the same at the 6,000 and 7,000 R dosages. The 8,000 R dosage caused a 100 percent sterility.

The dieldrin-resistant strain of *A. albimanus* from El Salvador which has been reared in our insectary since 1959 undoubtedly has undergone modifications resulting from adaptations to laboratory conditions. However, if it has not changed with respect to its susceptibility to X-irradiation, then the relatively mild 5,000 R dosage should come close to the goal suggested by Dr. Craig.

It is recognized that the current emphasis in insect control through use of sterilized males is on the chemosterilants. However, if technical and other difficulties cause a renewal of interest in sterilization through irradiation, the responses of this El Salvador strain of *A. albimanus* to X-irradiation may serve as a guide-line.

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ESTABLISHMENT OF A LABORATORY COLONY OF *ANOPHELES (MYZOMYIA) ANNULIPES* WALKER 1856

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INTRODUCTION. *Anopheles annulipes* is the most common and widespread anopheline species in southern Australia. Many attempts to colonise it have failed, the refusal of the adults to copulate in the laboratory being the principal cause of failure of Hill (1919), Wallace (Lee and Woodhill 1944) and Mackerras and Lemerle (1949). Cannibalism among larvae affected Lee's attempt in 1935, and the refusal of adult females to take a blood meal hindered Rudeforth in 1950.

These difficulties and others were encountered in the present attempt, the purpose of which was to provide vector anophelines for a malaria research programme. They were overcome in various ways, particularly by the use of a technique of induced copulation (McDaniel and Horsfall, 1957).

COLLECTION OF MOSQUITOES. In coastal areas of New South Wales *A. annulipes* adults are scattered among so many suitable resting places in low vegetation and rock crevices that collection of adults was usually unsuccessful. They were collected once in a large humid drainpipe near a creek in which a few larvae were present; the progeny of these gravid females supplemented the larger collections of larvae made elsewhere over a long period.

The larvae live in clean shallow water, in both temporary and permanent ground pools; they were scarce in Sydney in 1967 but a reliable breeding site was found late in 1967 at the Nattai River near Mittagong, 80 miles from Sydney. In the summer months 500–1500 larvae could be collected in 2–3 hours from the filamentous green algae at the margins of the slow-flowing river.

LABORATORY CONDITIONS. All field-collected larvae and adults were taken to a 14′ x 16′ insectary, which was maintained at a temperature of 78±2° F, and a relative humidity of 70–90 percent. Two large windows facing west provided natural light which could be supplemented by fluorescent light.

REARING METHODS. Larvae were reared in white plastic trays measuring 4″ x 6″ x 2″, filled with river water to a depth of half an inch. River water gave con-

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