THE HISTOPATHOLOGY OF THERMALLY INDUCED STERILITY IN AEDES AEGYPTI (DIPTERA: CULICIDAE)\(^1\)

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Abstract: Rudimentary gonads can be produced in both sexes of *Aedes aegypti* (Tampa strain) if eggs are heat stressed at 43℃. Exposure during hours 6–18 of embryonic development produces anomalies at a rate of 10–30%. No effect is seen after that time period and exposure during the first 6 hr of embryogenesis is lethal. Neither oocytes nor nurse cells are present in sterile ovaries; the follicular epithelium is also significantly reduced. Testes are essentially agametic with a few spermatogonia that lack nucleoli. It is postulated that thermal stress deleteriously affects pole cell differentiation or migration with the germ band.

The usual pattern of adult sexual dimorphism in several species of *Aedes* mosquitoes can be appreciably skewed by exposing larvae to abnormally high temperatures, as was first shown by Horsfall & Anderson (1961). Owing to its heterogametic constitution (Hickey & Craig 1966), the masculine genotype is affected so that both primary and secondary sexual characters can be intergraded or nearly feminized by constant or fluctuating temperatures (Anderson & Horsfall 1963, Brust & Horsfall 1965, Olson & Horsfall 1972, Horsfall 1974). Further, thermal stress selectively applied during imagogenesis may also produce supernumerary male genital appendages (Voorhees & Horsfall 1971).

Excessively high temperatures applied during embryogenesis of certain aedine species induce anomalies generally different from those that occur if thermal pressure is applied during larval development. The range of sensitivities may vary at the subgeneric level, with both larval and imaginal primordia affected (Anderson & Horsfall 1965, Cupp & Horsfall 1970a). Gonads of *Aedes stimulans* and *Ae. sierrensis* may be hypertrophied or sterilized and portions of the tract considerably deleted. Larval anomalies noted for both species include malformation of abdominal segments and the respiratory siphon.

*Aedes aegypti* (L.), a circumtropical mosquito, is considerably more heat resistant than either *Ae. stimulans* or *Ae. sierrensis*. However, adults that arise from embryos heat stressed at 38.5℃ may possess gonads that are rudimentary and appear agametic (Cupp & Horsfall 1970b). The purpose of this paper is to describe the histopathology of thermally induced sterility in this species, noting the cellular and tissue conditions of the gonads and remainder of the reproductive tract. An explanation for the probable cause of sterility will be given based on interpretations of recent embryological observations for this species (Raminani & Cupp 1975).

MATERIALS AND METHODS

The Tampa strain of *Aedes aegypti* was used. Inseminated females were given a human blood meal and, following ovarian maturation, allowed to oviposit on moist filter paper. Oviposition was synchronized by placing adults under a light-dark regimen (12:12), so that all eggs obtained for experimentation were less than 2 hr old (Cupp & Horsfall 1970a).

In order to obtain sterile adults, eggs were thermally stressed at every hour starting from 1 hr...
up to 24 hr of embryogeny. Duration of stress was either 4 or 6 hr. The temperatures employed ranged from 38.5° to 43°C. Fifty eggs were used per treatment group as well as for controls. Following the stress period eggs were returned to room temperature (25°C) and allowed to complete development.

Hatching was induced by placing eggs in shell

FIG. 1-4. (1) Whole mount preparation of a normal ♀ reproductive system (48 x). (2) A bilaterally sterile ♀ reproductive system (48 x). (3) Whole mount preparation of a normal ♂ reproductive system (48 x). (4) A bilaterally sterile ♂ reproductive system (96 x). Af = anterior filament; AG = accessory gland; fb = fat body; LO = lateral oviduct; MO = median oviduct; O = ovary; S = spermatheca; SV = seminal vesicle; T = testis; Tr = trachea; Vd = vas deferens; Ve = vas efferens.
vials containing tap water. For rearing, larvae were transferred to enamel pans containing 200–300 ml of tap water and fed a live yeast suspension. Pupae were collected into small jars and placed in cages for adult emergence.

Newly emerged adults of both sexes were dissected and the condition of the gonads and reproductive tract was noted. Permanent slides of the reproductive system were made by staining tissues in aceto-orcein and mounting in permount. For histologic examination, the gonads from stressed individuals and controls were dissected and treated with Kahle’s fixative (Demerec 1950) for approximately 24 hr, embedded in paraplast and sectioned at 8 μ. Sections were stained with Delafield’s hematoxylin, using eosin as the counter stain. Photomicrographs were taken with an Olympus phase contrast microscope equipped with a PM-6 camera.

RESULTS

Gonadal abnormalities occurred in the Tampa strain of *Ae. aegypti* when embryos were heat stressed by placing eggs at 43°C. A 12-hr period of sensitivity was detected. Eggs that were subjected to thermal pressure during hours 6 through 18 produced adults with sterile gonads, but no effects were noted after that. Heat stress during the first 6 hr of embryogeny was lethal. The rate of deformity ranged from 10–30% during the sensitive period with no real pattern or trend evident during this time. All males were bilaterally sterile, whereas unilateral involvement was occasionally noted in females. Adults were normally vigorous and exhibited no differences in external morphology or mating behavior from control mosquitoes.

**Cross morphology**

**Females:** Normal ovaries of newly emerged *Ae. aegypti* are translucent, ellipsoid structures located in the 5th and 6th abdominal segments (FIG. 1). Anteriorly, each organ tapers into a filament which extends to the 2nd abdominal segment where it connects with the alary muscles of the heart (Ronquillo & Horsfall 1969). The posterior ends of the ovaries are joined to lateral oviducts which are connected to a common or median oviduct in the region of the 7th abdominal segment. Three spermathecae are located dorsally to this structure.

Anomalous ovaries from thermally stressed *Ae. aegypti* were devoid of germinal cells (i.e., oocyte and nurse cells) (FIG. 2) and were reduced to 1/2 to 1/4 the length of normal ovaries and 1/8 the width. However, in several instances sterile ovaries were nearly as long as the normal ones. All aberrant ovaries were white in contrast to normal gonads. Tracheation was normal. The anterior filament of the sterile ovaries appeared to be normal, but the lateral oviducts were compressed. The median oviduct was normal. Three spermathecae were always observed in sterile females.

**Males:** Normal male gonads are located dorsolaterally in the 6th abdominal segment. Each testis is nearly enveloped by a thick fat body which contains fine, red-brown granules (FIG. 3). An apical filament extends anteriorly to the 2nd abdominal segment where it inserts into the ventral wall of the heart (Hodapp & Jones 1961). Posteriorly, the testes are joined by the vasa efferentia which connect to the vasa deferentia to form common ducts. These, in turn, empty into 2 long seminal vesicles that are fused medially. On either side of the seminal vesicles are 2 large pear-shaped accessory glands. A thin connective tissue filament extends from the anterior end of each gland and attaches to the testicular duct precisely where the vasa efferentia and vasa deferentia join.

Anomalous testes are very much reduced in size and, like the female gonads, germinal cells appeared to be essentially absent (FIG. 4). The vasa efferentia were occasionally abnormal in that they were shorter than those seen in normal males and also appeared somewhat contorted. A frequent type of abnormality observed consisted of the testes being divided into 2 equal-sized lobes, with the vasa efferentia arising ventrally between the subdivisions. Vasa deferentia were normal. Both the vasa efferentia and vasa deferentia were devoid of spermatozoa. The seminal vesicles were colorless in contrast to the distinct bluish color of the normal ones and were devoid of spermatozoa. These structures were covered with fat body as in the normal condition. The accessory glands were normal in appearance.

**Histology**

**Females:** The ovary is enveloped by a membrane, the tunica externa, which also covers the remainder of the reproductive system with the exception of the spermathecae (FIG. 5). This membrane extends forward to enwrap the anterior filament. Each ovary is composed of an aggregate of ovarioles, the proximal ends of which are connected to a calyx by a funicle. A well-developed follicular epithelium surrounds each primary follicle and a thin membrane, the tunica propria, is loosely adherent to the entire structure (FIG. 7). Both primary and secondary follicles are evident. The primary follicle consists of an oocyte and a complex of 7 nurse cells enclosed by the follicular epithelium (Parks &
The oocyte is located closest to the pedicel and can be distinguished from the nurse cells in that its nucleus stains more lightly and is generally smaller. The nurse cells are also usually smaller than the oocyte.

Epithelial cells of sterile ovaries were extensively collected along the lumen of the calyx. A tunica externa was present as in the normal ovary (FIG. 6). The calyx was rather conspicuous, extending throughout the length of the ovary. The number of ovarioles was reduced, with a concomitant reduction in their size, but they remained attached to the calyx. However, the pedicel was much longer than that seen in a normal ovary. The tunica propria appeared to be thicker compared to a normal ovariole. The follicular epithelium was poorly developed, occurring as a thin membranous lining, and was devoid of epithelial cells (FIG. 8).

The cellular components were seen only in the part of the ovariole corresponding to the primary follicle. Eight cells were observed. These were concentrated along a medial axis. The follicles were devoid of oocytes and nurse cells. A series of small cells with indistinct boundaries filled the core of each follicle. These cells had not differentiated but were parenchymal in appearance. There were no cells in the ovariole corresponding to a secondary follicle. A funicle was present however.
Males: The normal testis is enclosed in fat body and surrounded by a composite sheath made up of a membrane underlain by elongate cells (Fig. 9). Germ cells are divided into compartments, termed spermatocysts. Gamete formation is completed in the posterior spermatocyst so that mature spermatozoa are formed shortly after emergence. Spermatocysts in the middle region usually contain spermatids and spermatozoa. Spermatocytes and spermatogonia are located anteriorly. The spermatogonia are spherical cells containing large nuclei. The nucleoplasm is marbled with strands of chromatin and the nucleolus appears as a darkly stained spot. Both testicular ducts and the seminal vesicles are filled with mature spermatozoa.

The sterile testis, like the normal, had a well-developed fat body covering (Fig. 10). However, the testicular sheath was poorly developed and distinct compartments were not evident. Each testis contained a few spermatogonia which, in some cases, had differentiated to the pyriform stage of spermatid formation but not beyond. The nuclei of the spermatogonia were reduced in size and the chromatin material was concentrated at the periphery of the nucleoplasm, forming a ring. Nucleoli, which were distinctly observed in normal spermatogonia, were absent. Spermatogonia were never observed in the testes or the testicular ducts and seminal vesicles.

DISCUSSION

The response to thermal stress by the Tampa strain of *Ae. aegypti* is generally similar to that noted in a previous study utilizing the Rockefeller strain of this species (Cupp & Horsfall 1970b). However, a threshold difference of 5°C (±0.5) is observed between the 2 strains, with anomalies occurring at a higher temperature in the stock most recently isolated from the field. Thus, it would seem that colonization for some 25 to 30 years has served to lower thermal resistance in the Rockefeller strain.

Earlier studies describing the occurrence of sterility in *Ae. aegypti* were based essentially on the gross morphology of the gonads (Jones 1961, 1963, Cupp & Horsfall 1970b). The present investigation details the cellular conditions of sterility, as well as notes certain other general conditions of histopathology. Of interest is the fact that spermatogonia, while occasionally seen in anomalous testes, remained undifferentiated and possessed small nuclei with no nucleoli. Also, the chromatin material was concentrated at the periphery of the nucleoplasm, forming a ring. This is unlike the nuclei of normal spermatogonia, where the chromatin material is evenly distributed in the nucleoplasm. Primary follicles in sterile ovaries were devoid of both nurse cells and oocytes. Instead, medial concentrations of small, epithelial-like cells, perhaps representing undifferentiated oogonia, were present. A prominent histological change also seen concerned the very poor development of the follicular epithelium, an otherwise distinct structure in the mosquito ovary.

Anomalous gonads have been produced in Diptera by the use of other mutagens, particularly irradiation. Treatment of the posterior pole plasm of *Culex pipiens* eggs with an ultraviolet source prior to blastoderm formation results in sterile imagines of
both sexes (Oelhafen 1961). Also, agametic gonads have been produced in Drosophila sp. by irradiation of either the polar plasm of newly deposited eggs or the pole cells prior to their migration (cf Counce 1973). Sterility was attributed in both groups of insects to the destruction of polar granules or the pole cells. Cupp & Horsfall (1970b) noted that sterility in the Rockefeller strain of Ae. aegypti could probably be attributed to the failure of pole cells to form or locate properly due to thermal stress. It would appear to be the same in the Tampa strain. A recent embryological account describing pole cell ontogeny in Ae. aegypti indicates that these cells are extrablastodermal or migratory with the germ band during much of the thermally sensitive period noted in this study (Raminani & Cupp 1975).

Ultraviolet irradiation at the late blastoderm or early gastrular stages in Drosophila sp. produces a high rate of unilateral sterility. Geigy (1931) and Aboim (1945) attributed this to an unusual adherence of irradiated pole cells so that migration occurred as a group rather than singly. A similar explanation can be advanced to account for unilateral sterility in anomalous females seen in this study, as well as the unilateral castrates of both sexes described previously for the Rockefeller strain (Cupp & Horsfall 1970b). A high degree of lethality was also noted when Drosophila eggs were irradiated during the early phases of blastoderm formation (Aboim 1945). A comparable situation was evident in this investigation when eggs were heat stressed during the first 6 hr of embryogenesis. This period corresponds to the time during which the syncytial blastoderm is formed in Ae. aegypti embryos (Raminani & Cupp 1975).

Rudimentary testes similar in general appearance to those described for Aedes aegypti have been reported as the result of interspecific crosses among sibling species of Anopheles mosquitoes (Davidson & Horsfall 1964, Kreutzer & Kitzmiller 1971) and a variety of crosses in Drosophila sp. (Dobzhansky 1970). Also, agametic testes and ovaries are produced in Drosophila subobscura as a result of the gene grandchildless. No pole cells are formed in this mutant due to the early degeneration of the pole plasm (Fielding 1967).

Rai (1964) described the histopathological aspects of chemically induced sterility in Ae. aegypti. Chronic exposure of early larvae to apholate produced gonadal derangements in adult flies, but the histopathology differed considerably from anomalous gonads induced by thermal stress. Ovarioles exhibited varying degrees of degeneration, but some maintained a normal follicular integrity, producing eggs. Such a graded response was not seen in the present study. Chemosterilized males exhibited no reduction in testicular size, but dominant lethal mutations were routinely detected in their sperm.

LITERATURE CITED


THE SUBGENUS PERSICARGAS (IXODOIDEA: ARGASIDAE: ARGAS)

28. Argas (P.) arboreus: Effect of blood meal weight on nympha l instar numbers

By Isaac S. Isaac

Abstract: The weight of the blood meal taken by the 1st-instar nymph (N₁) and not the prefeeding body size of N₁ determines the number of molts before the nymph reaches the minimum blood meal-quantity threshold necessary for molting to the adult stage. During the same number of developmental nymphal instars, females ingest more blood than males. Nymphs reaching the adult stage after 3 or 4 instars ingest more blood than those reaching this stage after 2 instars.

In previous studies of the life cycle of Argas (Persicargas) arboreus Kaiser, Hoogstraal & Kohls, Hafez et al. (1971) and Hajjar (1972) weighed each nymphal instar before and after feeding. They determined that an additional instar is required for the lightest nymphs to molt to the adult stage and that females and males emerge from relatively heavy and light-weight nymphs, respectively. Balashov (1963) reported similar results in Ornithodoros papillipes but expressed his data in terms of ingested blood volume rather than weight. The inverse relationship between blood meal size and number of nymphal instars was proven by Balashov (1968) by limiting the size of blood meals ingested by argasid ticks. Hafez et al. (1971) also reported that the ratio between blood meal weight and unfed body weight of 3 nymphal instars (N₁-N₃) of A. (P.) arboreus did not vary significantly.

In this study, I investigated the unfed body weight,