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THE INFLUENCE OF ANTIBODIES TO SELECTED MOSQUITO IMMUNOGENS ON MOSQUITOES FOLLOWING INGESTION OF BLOOD FROM AN IMMUNE VERTEBRATE HOST

FINAL REPORT

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Our objective in this pilot project has been to examine the effects of mouse antibodies directed against "concealed" antigens on the life processes of the mosquito Aedes aegypti. We immunized mice with mosquito antigens prepared from sugar-fed and blood-fed mosquitoes as follows: heads and thoraces, abdomens, and dissected midguts. After determining the immune status, using an ELISA, of the immunized mice, we allowed samples of mosquitoes to obtain blood meals and then determined survivorship, fecundity; and egg viability! Our results did not indicate a deleterious effect of antibodies from an immune mouse on mosquito survivorship, the number of eggs deposited (fecundity), or the viability of eggs deposited.

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SUMMARY

A particularly interesting aspect of blood-feeding arthropod/vertebrate host interaction involves the host's immune reactions to the arthropod, and the effects of these reactions on arthropods which take blood meals from immunized hosts. Under natural circumstances the only arthropod antigens to which a vertebrate has the opportunity to react are those in the arthropod's saliva. Recently, interest in the effects of antigens derived from other than salivary gland secretions has emerged. These so-called "concealed" antigens are significant in that the vertebrate host's immune systems have not "seen" them and consequently there has not been the opportunity for the evolution, in the blood-feeding arthropod, of defenses against the possible deleterious effects of antibodies to these antigens. In contrast, it is likely that, even though deleterious effects of antibodies to salivary gland antigens have been recorded, natural selection has operated such that these negative effects on blood-feeding arthropods have been minimized.

Our objective in this pilot project has been to examine the effects of mouse antibodies directed against "concealed" antigens on the life processes of Aedes aegypti.

We have immunized mice with mosquito antigens prepared from both sugar-fed and blood-fed mosquitoes as follows: whole heads & thoraces, whole abdomens, and dissected midguts. After determining the immune status, using an ELISA, of the immunized mice, as well as Freund's-injected and uninjected, control mice, we allowed samples of mosquitoes to obtain blood meals and then determined survivorship, fecundity, and egg viability. Our results did not indicate a deleterious effect of antibodies from an immune mouse on mosquito survivorship, the number of eggs deposited (fecundity), or the viability of eggs deposited.

FOREWORD

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William S Rogers 5 August 1992

PI Signature Date

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I. Introduction

A. Background

The interactions of blood-feeding arthropods with their hosts have been of long-standing interest. Aspects of these interactions include host location, nutritional considerations, host specificity, feeding mechanisms, etc. One particularly interesting aspect of blood-feeding arthropod/vertebrate host interacion involves the host's reactions to the arthropod. These reactions range from behavioral to various physiological/immunological responses to the piercing of the skin, injection of saliva and withdrawal of blood. The immunological responses of the vertebrate host, and especially the effects of these responses on arthropods which have fed on immune hosts, are of interest here.

Immune responses of vertebrate hosts to blood-feeding arthropods have been studied extensively in ticks, apparently beginning with the research of Trager (1939a, 1939b, 1940). Reviews and bibliographies with regard to research done since the work of Trager include: Ackerman (1980); Brown (1985, 1988a, 1988b); Brown & Askenase (1986); Kaufman (1989); McGowan & Barker (1980); Nelson et al. (1977); Tatchell (1987); Willadsen (1980; 1987); Wikel (1982); Wikel & Allen (1982). In some cases the host immune responses to tick feeding have been shown to have a negative effect on aspects of the tick's physiology, with a consequent negative effect on vectorial capacity (Kaufman, 1989).

The tick antigens that have received the most attention have been those derived from the salivary glands (Ben-Yakir, 1986). This is not surprising since salivary gland produce the antigens to which hosts are exposed naturally and provide the route by which both pathogens and toxins are introduced into the vertebrate blood stream. Host responses to salivary gland antigens consist of infiltration of the bite site by inflammatory cells due to chemotaxis, and the development of antigen-antibody complexs associated specifically with salivary gland secretions (Ackerman et al., 1980). In some cases the salivary gland antigen responsible for resistance has been identified (Brown, 1988c; Brown et al., 1984; Gordon & Allen, 1987). There is evidence that extracts from the salivary glands of Dermacentor andersoni (Stiles) and Rhipicephalus sanguineus (Latreille) afford a degree of protection against tick infestation when used as vaccines (research cited in Ackerman et al., 1980; Brown et al., 1984)

Recently, the concept of using antigens derived from other than salivary gland secretions has emerged (Kaufman, 1989). These so-called "concealed" antigens (Willadsen & McKenna, 1991) are significant in that the vertebrate host's immune systems have not "seen" them and consequently there has not been the opportunity for the evolution, in the blood-feeding arthropod, of defenses against the possible deleterious effects of antibodies to these antigens. In contrast, it is likely that, even though deleterious effects of antibodies to salivary gland antigens have been recorded, natural selection has operated such that these negative effects on blood-feeding arthropods have been minimized. Ackerman et al. (1980) evaluated the effects of antibodies directed against antigens extracted from midguts of Dermacentor variabilis on individuals of this species with regard to the "...temporal dynamics of adult, female tick attachment/detachment; body weights of engorged female ticks; egg production and egg hatching..." They reported delays in attachment, reduced engorgement weights, lengthened preoviposition periods and disturbances in egg production of ticks fed on immunized versus non-immunized hosts respectively. On the other hand, no significant differences in "biological performance," including mean engorgement weight, were found in Amblyomma americanum and Dermacentor variabilis fed on rabbits immunized with tick hemolymph and non-immunized rabbits (Ben-Yakir & Barker, 1987).

Recently, Mongi & Aganyo (1986) have isolated and characterized tick midgut antigens from Rhipicephaus appendiculatus which are recognized by immune rabbit IgGs. However, in most cases the composition of immunogens from non-salivary tick tissues is not known; nor are the mechanisms by which antibodies to non-salivary tick antigens adversely affect tick physiology understood. Suggestions have included inhibition of a given enzyme, neuralization of a hormone in the hemocoel (Ben-Yakir et al., 1986), impaired absorption of materials from the midgut due to effects of antibodies on the midgut epithelium, interference with the incorporation of materials once they have been absorbed into the hemocoel, or possibly some direct effect of antibodies on ovarian tissue (Ackerman et al., 1980). Antibody-associated pathology in the tick hemocoel seems likely since a number of investigators have shown that immunoglobulin G (IgG) taken in a blood meal passes through the midgut epithelium into the hemocoel (Ben-Yakir, 1986; Tracey-Patte et al. 1987). For example, Ackerman, et al. (1981) fed Dermacentor variabilis on rabbits which had been immunized with extracts from tick salivary glands and ovaries. Thus, resistance due to ingestion of antibodies to non-salivary gland antigens may be acting internally within the tick hemocoel (Ackermann et al., 1980).

The adverse effects of anti-tick antibodies on tick physiology suggest the feasibility of the development of anti-tick vaccines. In particular, antibodies to non-salivary gland antigens hold promise as potential vaccines (Kaufman, 1989; Ben-Yakir et al., 1986). For example, vaccines prepared from Boophilus microplus gut and gut plus synganglion tissues gave high levels of protection against infestation with larval ticks (Opedebeek et al., 1988). With the techniques of antigen identification, gene cloning and expression of non-microbial antigens in microbial systems that are available today, it is feasible to consider the identification and ultimate mass production of antigen(s) for use as vaccines for tick control. The promise of such vaccines is especially important in view of the current concerns with environmental pollution withinsecticides and acaricides and the development of acaricide- and insecticide-resistant strains of arthropods (Ben-Yakir et al, 1986).

The hard ticks (Order Acari; Family Ixodidae) which have been the subjects of most of the research on host-acquired immunity are comparatively slow feeders, ingestion of a meal being measurable in days. Fast-feeding hematophagous insects, in which ingestion is measurable in minutes, have also been examined with regard to host-acquired immunity, but to a much lesser extent (Brown, 1988). As with the hard ticks, vertebrate host immune defenses have been shown to have deleterious effects such as interference with feeding, fecundity, and survival in a number of different insect species and the possibility for the development of useful vaccines exists.

In the mosquito Anopheles stephensi, females fed on rabbits previously injected with a mosquito midgut homogenate displayed greater death rates than mosquitoes fed on control rabbits injected with homogenized whole mosquitoes (Alger & Cabrera, 1972). However, such an increase in death rate was not observed in Ae. aegypti fed on immunized rabbits (Ramasamy et al., 1988).

Decreased fecundity was observed in female Aedes aegypti mosquitoes which fed on rabbits or guinea pigs which had previously been injected with whole mosquito body homogenates (Sutherland & Ewen, 1974). However, fecundity was not reduced in Culex tarsalis females allowed to feed on the same rabbits or guinea pigs, implying that the the mechanism involved with fecundity reduction is specific to Ae. aegypti. Likewise, Ramasamy et al. (1988, 1989) report a decrease in fecundity in Ae. aegypti fed on rabbits immunized with antigens from various body parts dissected from specimens that had taken a blood meal 24 hours prior to killing and freezing.

A variety of pathological effects were observed in Stomoxys calcitrans after feeding on rabbits previously injected with various fly tissues, including homogenized thoracic muscles, antibodies to which caused an increase in mortality in comparison to controls (Schlein & Lewis, 1976). The pathological effects were apparently non-specific, since feeding on rabbits injected with Stomoxys calcitrans also increased mortality in the tsetse fly Glossina morisitans.

Tsetse flies (Glossina morsitans) fed on human blood, which contains albumin, and subsequently allowed to ingest rabbit serum that contained anti-human albumin antibodies died within 2 hours (Nogge & Giannetti, 1980). The pathological mechanism apparently involved a disturbance of osmoregulation in the flies, probably due to the formation of antigen-antibody complexes.

Krinsky (1985) studied fifth-instar triatominae reduviid bugs Rhodnius prolixus fed on: (1) mice that had previously been used once as blood meal sources for other bugs of the same species a week earlier ("primed" hosts); or (2) that had repeatedly been used as blood meal sources; or (3) unexposed, control mice. Bugs (both nymphs and adults) fed on "primed" hosts or control hosts showed longer engorgement times than those fed on repeatedly exposed hosts. Although egg viability was unaffected, female bugs fed on control mice deposited many more eggs than bugs that fed on exposed mice.

As described above with ticks, insects which feed repeatedly on the same host species are sometimes adversely affected, apparently by the host's immune response. For example, repeated feeding on guinea pigs by the phlebotomine sand fly Lutzomyia longipalpus was associated with a decrease in fertility (Brown & Rosalsky, 1984). Likewise repeated feeding on guinea pigs by the tsetse fly Glossina morsitans decreased longevity of the fly (Brown & Cipriano, 1985).

As demonstrated in ticks, immunoglobulin G (IgG) has been shown to pass from a fresh blood meal through the midgut epithelium and into the hemocoel of Anopheles stephensi Liston, An. gambiae Giles, and An. albimanus Wiedemann and to persist in the hemocoel for 18-24 hours following ingestion of blood from typhus-immune rats (Vaughn & Azad, 1988). However, IgG was not found in the hemocoel of similarly treated An. freeborni Aitken and Ae. aegypti (L.). In contrast, Ramasamy et al. (1988) reported the passage of IgG into the hemocoel of Ae. aegypti after feeding on rabbits immunized with antigens from blood-fed mosquitoes. Serum immunoglobulins have also been found to pass through the midgut of the flesh fly Sarcophaga falculata Pand. (Schlein et al., 1976). The mechanism of

IgG passage across the midgut epithelium is apparently not known.

B. Objective

Our objective has been to carry out a pilot study designed to evaluate the effects of vertebrate antibodies on selected life processes in females of the mosquito Aedes aegypti.

II. Materials & Methods

A. Species studied

Female Balb/c mice were used in this study since in the event of positive results, it would be desirable to isolate monoclonal antibodies which produced deleterious effects on mosquitoes. Mice were immunized with antigen prepared from mosquito parts.

Female Aedes aegypti (Rockefeller Strain) mosquitoes were used in this study. They were reared from eggs hatched in deoxygenated water ("boiled" at room temperature in a partial vacuum for at least 20 minutes). Larvae were maintained in plastic pans which contained approximately 1300 milliliters of water. Larvae were fed an equal parts mixture of ground rabbit chow, brewer's yeast, and liver powder. Pupae were placed in cages (0.9 liter "icecream" cartons with screen lids and openings occluded by approximately 18 inches of orthopedic stockinete) and allowed to emerge as adults. Moist gauze pads placed on the screen lids of the individual cages and enclosure in plastic bags were used to maintain high humidity. Additional gauze pads soaked in 10% sucrose were provided, via the screen lids, as a carbohydrate source. All mosquito life stages were kept in a Kysor-Sherer, walk-in, controlled environment room (6' x 8' x 6') held at 28°C with a 16 hour light/8 hour dark periodicity.

B. Antigen Sources & Preparation

Antigen was prepared from whole heads & thoraces; whole abomens; and dissected miguts obtained both from sugar-fed and from blood-fed mosquitoes. Preparations from mosquitoes that had not blood fed and from mosquitoes which have blood fed (24 hours prior to freezing) were made in order to obtain antibodies to proteins which are expressed at different times during the digestion and assimilation of a blood meal (Briegel & Lea, 1975; Van Handel & Romoser, 1987) and during the process of vitellogenesis (Raikel, 1984; Raikel & Lea, 1983).

Four milliliters of Chapso solution (10mM Chapso in 0.05% Tris-HCl, pH 8.0, with 1:100 enzyme inhibitor) were

added to a collection of mosquito body parts (as described above). This mixture was homogenized with an electric grinder for 2-3 min and vortexed for 30 min. The homogenate was left overnight at 4°C, vortexed for about 5 min and then centrifuged at 17,500 rpm (in Sorvall) for 90 min. The supernate was carefully removed and dialysed against PBS (pH 7.2) for 24 hr.

C. Immunization of Mice

The total protein content of a given antigen preparation was determined using the Lowry method. Based on the total protein in a given sample, dilution with PBS was adjusted so that 0.1 mg of protein was injected, except in the case of midguts dissected from sugar-fed mosquitoes in which case approximately 0.06 mg of protein was injected. The volume injected was typically between 0.6 and 0.8 ml. Mice were injected, intraperitoneally, three times as follows: (a) initially with Complete Freund's and Chapso solubilized mosquito antigen preparation (1:1); (b) four weeks later, Incomplete Freund's and mosquito antigen (1:1); (c) 3 to 4 days before a given experiment, mosquito antigen only, i.e. a "boost."

Mice to be used as controls were treated in two ways. One kind of control was composed of mice that received no injections. The other control consisted of mice that received the same schedule of 3 injections as the experimental mice, but phosphate buffer (PBS) was substituted for mosquito antigen.

To determine the immune status of each mouse to be used in an experiment, samples of blood were drawn from the tail vein (10 - 50 ul) periodically and tested using an enzyme linked immunosorbant assay (ELISA) in order to follow the development of immunity. The sera for the titer determinations presented in the "Results" section of this report were taken within three days prior to the beginning of a given experiment.

Ninety-six (96) well PVC microtiter plates were coated with mosquito antigen in PBS (1:5) Following incubation for 2 hours at room temperature (RT), or overnight at 4°C, plates were washed 4 times in PBS containing 0.05% Tween-20 (PBS-Tween) and blocked with 200 ml of 5% non-fat dry milk in PBS (PBS-milkfor 1 hour at RT. After 4 washes in PBS-Tween, 50 ul of primary antibody (mouse serum) diluted 1:500 and 1:1000 in PBS-Tween was added to the wells. After 1 hour at RT, the plates were washed 3 times in PBS-Tween and then 50 ul of secondary antibody-peroxidase conjugate (goat-anti-mouse) diluted 1:5000 in 5% PBS-Milk was placed in each well. Plates were then incubated for 1 hour at RT, washed 3 times in PBS-Tween and "developed" as follows: (1) 50 ul of substrate (o-phenylenediamine = OPD) was added to

each well. (2) Following a 15 min incubation at RT in the dark, the enzyme rections were stopped by the addition of 50 ul of 12 % H_2SO_4. The optical density of the contents of each well was determined using an automated ELISA reader at a wavelength of 490 nm.

For most experiments, immune sera from each mouse were tested against the antigens used in the immunization and against antigens other than those used to immmunize. Sera from unimmunized, control mice and from Freund's injected, control mice were tested against all antigens used in the immunizations. The number of replications of each antigen/antiserum combination varied from experiment to experiment. In several experiments, in order to check for a dose-response effect antibody was diluted 1:500 and 1:1000.

D. Mosquito Feeding and Holding

Prior to placement in a cage of mosquitoes a mouse was anesthestized by intraperitoneal injection of sodium pentobarabital. The anesthetized mouse was left in the mosquito cage for 60 minutes. Prior to the termination of anesthesia, the mouse was be removed from the mosquito cage and returned to its own cage with food and water. An oviposition dish (a small plastic cup approximately one-third full with water) with a strip of paper towelling placed on the inside circumferance was placed in the cage of mosquitoes on the day following blood-feeding and regularly checked for eggs. Eggs were collected, left under moist conditions, and dried for two days prior to counting and testing for viability.

E. Parameters Measured

After blood-feeding, samples of mosquitoes were set aside for determination of daily survival (# alive & # dead daily), fecundity (average number of eggs per sample of females or number of eggs per female), and egg viability (percent hatching among eggs deponsited per female). Counts of the same strip of eggs were made two or three times under a dissecting scope and the mean number of these counts recorded. For viability determinations, eggs were hatched in water which had been placed in partial vacuum for 20 minutes to reduce oxygen content, and held until the larvae reached the second instar, at which time they were counted. To count larvae, they were removed in small numbers from the rearing container with a small dropper and counted.

F. Statistical Analysis

Survivorship curves were analyzed and compared using the Kaplan-Meier product limit estimator and the Mantel-Cox P values. Fecundity and egg viability data were analyzed using single classification analysis of variance (ANOVA) or

two-level nested ANOVA as appropriate to the experimental design. All percent data were arsin transformed. The square root transformation was applied to all discrete count data. The level of significance was taken at 0.05.

G. Controls

Samples of female mosquitoes were allowed to feed on immune or control mice which had been treated identically except for the injection of mosquito antigen preparations.

In order to see if there is an effect due to injection of Freund's antigen additional samples of female mosquitoes were allowed to feed on mice which had recieved no injections whatsoever.

H. Experiments

We carried out seven experiments which are summarized in Table 1.

EXPERIMENT 1 (Table 2). This experiment was designed to test the effects, on mosquito survival and fecundity, of ingestion of mouse blood containing antibodies directed against antigen prepared from mosquito body parts (heads & thoraces pooled; abdomens) obtained from mosquitoes that had been maintained on sucrose, but that had not yet been given a blood meal. Four immunized mice were used, 2 had been injected with mosquito head-thorax antigen and 2 with abdomen antigen. Four non-immunized "control" mice were also used, two which had been treated the same as the immunized mice, but had not received mosquito antigens, i.e. they had been injected with Freund's complete and incomplete adjuvant (Freund's controls) and two which had received no injections whatsoever (untreated controls). For use in both survival and fecundity determinations, samples of female mosquitoes, ranging from 29 to 50, were allowed to blood-feed on each of the mice as shown in Table . The mosquitoes were checked daily for mortality for ten days and the deposited eggs were removed from each cage and counted.

EXPERIMENT 2 (Table 3). For the purpose of determining the effects, on mosquito survival and fecundity, of ingesting mouse blood containing antibodies against antigens prepared from whole body parts of mosquitoes that had been fed sucrose only, the design of Experiment 2 was identical to Experiment 1. In addition, the immunized mice were given booster injections with mosquito antigen. Mortality was checked daily for 19 days. For fecundity determinations this experiment was designed differently than Experiment 1. Individual female mosquitoes from samples of 5 mosquitoes that had fed on each mouse were placed in small cages along with 2 male mosquitoes and allowed to oviposit. In this way the number of eggs deposited by each female

Table 1. Summary of Experiments

Experiment	Treatment of Antigen-source Mosquitoes	Antigen Injected into Mice	Parameters Measured
1	Sucrose only	HT & ABD	Survival, Fecundity
2	Sucrose only	HT & ABD	Survival, Fecundity
3	Blood-fed Sucrose only	HT & ABD MG	Survival, Fecundity, Egg Viability
4	Sucrose only	MG	Fecundity
5	Sucrose only	MG	Fecundity, Egg Viability
6	Blood-fed	MG	Survival, Fecundity
7	Blood-fed	MG	Survival, Fecundity

1 HT, whole heads & thoraces pooled; ABD, whole abdomens; MG, dissected midguts.

Table 2. Experiment 1.

	Mosquitoes Fed on Mice	
	Survival & Fecundity[2]	
Injected into Mice[1]	Replicate	Sample Size
ABD	1	40
	2	29
HT	1	42
	2	29
Freund's only	1	50
	2	50
Nothing	1	30
	2	45

[1] ABD, antigen prepared from abdomens of sucrose-fed mosquitoes; HT, antigen prepared from pooled heads and thoraces of sucrose-fed mosquitoes; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Daily mortality determined for 10 days following ingestion of blood; fecundity determined by counting total eggs deposited by each sample.

Table 3. Experiment 2.

	Mosquitoes Fed on Mice			
	Survival[2]		Fecundity[3]	
Injected into Mice[1]	Replicate	Sample Size	Replicate	Sample Size
ABD	1	30	1	5
	2	30	2	5
HT	1	30	1	5
	2	30	2	5
Freund's only	1	30	1	5
	2	30	2	5
Nothing	1	30	1	5
	2	30	2	5

[1] The mice used were the same as those used in Experiment 1, but each received an additional, "booster," injection of mosquito antigen three days before the beginning of this experiment. ABD, antigen prepared from abdomens of sucrose-fed mosquitoes; HT, antigen prepared from pooled heads and thoraces of sucrose-fed mosquitoes; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Daily mortality determined for 19 days following ingestion of blood.

[3] Mosquitoes held individually; the number of eggs deposited by each female were counted.

would be determined and thus the variation in egg number between females determined.

EXPERIMENT 3 (Table 4). This experiment was designed to test the effects, on mosquito survival, fecundity and egg viability, of (1) ingestion of mouse blood containing antibodies directed against antigen prepared from whole body parts (heads & thoraces; abdomens) obtained from blood-fed mosquitoes; and (2) mouse antibodies directed against antigens prepared from midguts that had been dissected from mosquitoes which had been given only sugar. Six immunized mice were used in this experiment, 2 had been immunized with mosquito abdomen antigen, 2 with head-thorax antigen, and 2 with midgut antigen. In addition, 4 non-immunized, control, mice were used, 2 which had been treated identically with the immunized mice except for injection of mosquito antigen (Freund's controls) and 2 which had received no injections whatsoever (untreated controls). Samples of 30 mosquitoes were allowed to blood feed on each of the mice and mortality was determined daily for 17 days. For determinations of fecundity and egg viability, individual mosquitoes from samples of 5 mosquitoes that had fed on each mouse were placed in small cages along with 2 male mosquitoes and allowed to oviposit. The eggs deposited by each female were counted and then an attempt to hatch them was made.

EXPERIMENT 4 (Table 5). This experiment weas designed to test the effects, on mosquito fecundity, of ingesting mouse blood containing antibodies against antigen prepared from dissected midguts obtained from mosquitoes that had been maintained on sugar only. The mice immunized with midgut antigen from Experiment 3 were boosted with and additional injection of midgut antigen three days before the test mosquitoes were allowed to feed on them. The Freund's injected "control" mice from Experiment 3 were also used. Samples of female mosquitoes were allowed to blood-feed on the mice and then individual female mosquitoes, along with 2 male mosquitoes, were held in small cages. Subsequently, eggs were removed and counted.

EXPERIMENT 5 (Table 6). This experiment was designed in a fashion identical to Experiment 4 and the same mice were used. However, in addition to fecundity, egg viability was determined.

EXPERIMENT 6 (Table 7). This experiment was designed to test the effect, on mosquito survival and fecundity, of ingesting mouse blood containing antibodies directed against antigen prepared from midguts dissected from blood-fed mosquitoes, approximately 24 hours following ingestion of blood. Five mice were used in this experiment, 2 which had been immunized with the blood-fed midgut antigen and 3 which had been injected with Freund's only. Samples

Table 4. Experiment 3.

	Mosquitoes Fed on Mice			
	Survival[2]		Fecundity/Viability[3]	
Injected into Mice[1]	Replicate	Sample Size	Replicate	Sample Size
ABD	1	30	1	5
	2	30	2	5
HT	1	30	1	5
	2	30	2	5
MG	1	30	1	5
	2	30	2	5
Freund's only	1	30	1	5
	2	30	2	5
Nothing	1	30	1	5
	2	30	2	5

[1] ABD, antigen prepared from abdomens of blood-fed mosquitoes; HT, antigen prepared from pooled heads and thoraces of blood-fed mosquitoes; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Daily mortality determined for 17 days following ingestion of blood.

[3] Mosquitoes held individually; the number of eggs deposited by each female were counted and then eggs were placed in low O_2 water for hatching.

Table 5. Experiment 4.

Injected into Mice[1]	Mosquitoes Fed on Mice-Fecundity[2]	
	Replicate	Sample Size
MG	1	20
	2	11
Freund's only	1	15
	2	15

[1] The mice used were the same as those in Experiment 3, but received an additional "booster" injection of midgut antigen. MG, antigen prepared from midguts dissected from mosquitoes fed sucrose only; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Mosquitoes were held in cages individually and the number of eggs deposited by each female were counted.

Table 6. Experiment 5.

Mosquitoes Fed on Mice-Fecundity/Viability[2]		
Injected into Mice[1]	Replicate	Sample Size
MG	1	14
	2	11
Freund's only	1	15
	2	10

[1] The mice used were the same as those in Experiment 4. MG, antigen prepared from midguts dissected from mosquitoes fed sucrose only; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Mosquitoes held individually; the number of eggs deposited by each female were counted and then eggs were placed in low O_2 water for hatching.

Table 7. Experiment 6.

	Mosquitoes Fed on Mice			
	Survival[2]		Fecundity[3]	
Injected into Mice[1]	Replicate	Sample Size	Replicate	Sample Size
MG	1	28	1	10
	2	37	2	10
Freund's only	1	35	1	10
	2	35	2	10
	3	45	3	10

[1] MG, antigen prepared from midguts dissected from mosquitoes approximately 24 hours following a blood meal; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Daily mortality determined for 17 days following ingestion of blood.

[3] Mosquitoes held individually; the number of eggs deposited by each female were counted.

of female mosquitoes were allowed to blood feed on the mice and daily mortality was checked for 17 days. For fecundity determinations, individuals from samples of 10 female mosquitoes which had fed on the mice were placed, along with 2 male mosquitoes, in small cages and the number of eggs deposited were counted.

EXPERIMENT 7 (Table 8). This experiment was designed in a fashion identical to Experiment 6 and the same immunized and two of the same Freund's control mice were used. In addition, two new Freund's control mice plus two new untreated controls. The immunized mice were each given a booster injection 3 days prior to use as blood sources. Two uninjected control mice were also included in this experiment. Samples of female mosquitoes were allowed to blood-feed on the mice and mortality was recorded daily for 17 days. Fecundity was determined from samples of 10 females fed on each mouse identical to Experiment 6.

Table 8. Experiment 7.

	Mosquitoes Fed on Mice			
	Survival[2]		Fecundity[3]	
Injected into Mice[1]	Replicate	Sample Size	Replicate	Sample Size
MG	1	30	1	10
	2	30	2	10
Freund's only	1	30	1	10
	2	30	2	10
Nothing	1	30	1	10
	1	30	2	10

[1] The mice used were the same as those in Experiment 6, but received an additional "booster" injection of blood-fed midgut antigen. MG, antigen prepared from midguts dissected from mosquitoes approximately 24 hours following a blood meal; Freund's only, mosquitoes injected only with complete and then incomplete Freund's antigen, but not injected with mosquito antigen.

[2] Daily mortality determined for 17 days following ingestion of blood.

[3] Mosquitoes held individually; the number of eggs deposited by each female were counted.

III. Results

A. Response of Mice to Immunization

The responses of the mice to immunization are shown in Figures 1-6.

With three minor exceptions, all sera from mice injected with Freund's adjuvant (FC) and sera from untreated mice (UC) showed very low reactivity with all mosquito antigens (HT, AB, and MG). The three exceptions were as follows: FC2 antiserum and HT antigen in Experiment 2; FC1 and FC2 antisera and HT antigen in the repeat of the test of the mice used in Experiment 2. However, even in these cases reactivity was substantially below that displayed by the immune sera. With the exceptions just noted, no differences in reactivity were seen among the control sera, that is when sera from Freund's injected mice were compared to untreated, non-immune (NMS, normal mouse serum) mice. The reactivity of sera from mice immunized with mosquito antigen was consistently many magnitudes greater than the reactivity displayed by the control sera.

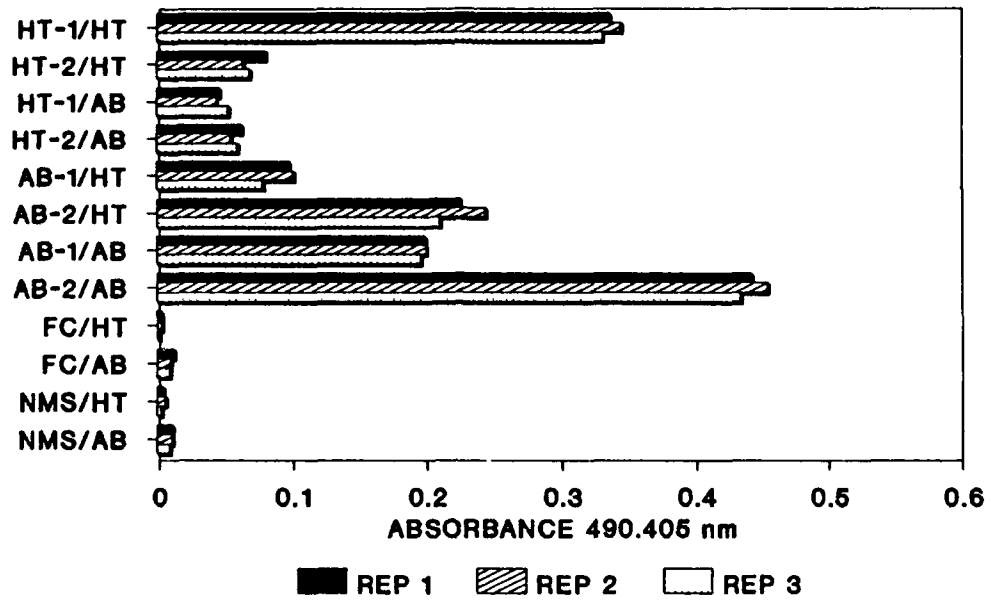
Different immune mouse sera often displayed different degrees of reactivity to the same antigen, e.g. mouse #1 in Experiment 1 showed a higher reactivity to HT antigen than did mouse #2. Likewise in Experiment #1 the sera from mouse #2 that had been immunized against AB mosquito antigen showed higher reactivity than the sera from mouse #1. It is interesting to note, however, that the differences in reactivity between mouse #1 and mouse #2 immunized with AB antige nearly disappeared following the "booster" injection of antigen given prior to Experiment #2.

In Experiments #6 and #7, the same mice were used and a "booster" injection of antigen was given between these two experiments. As can be seen by comparing the patterns of reactivity in Figs. 5a versus 5b and Figs. 6a versus 6b are very consistent. Likewise ELISAs were run twice on the 1:500 dilutions of antisera and no differences in patterns of reactivity were revealed.

In most ELISA runs two or more replicates of the same antiserum/antigen combination were included. These replicates displayed very little variation.

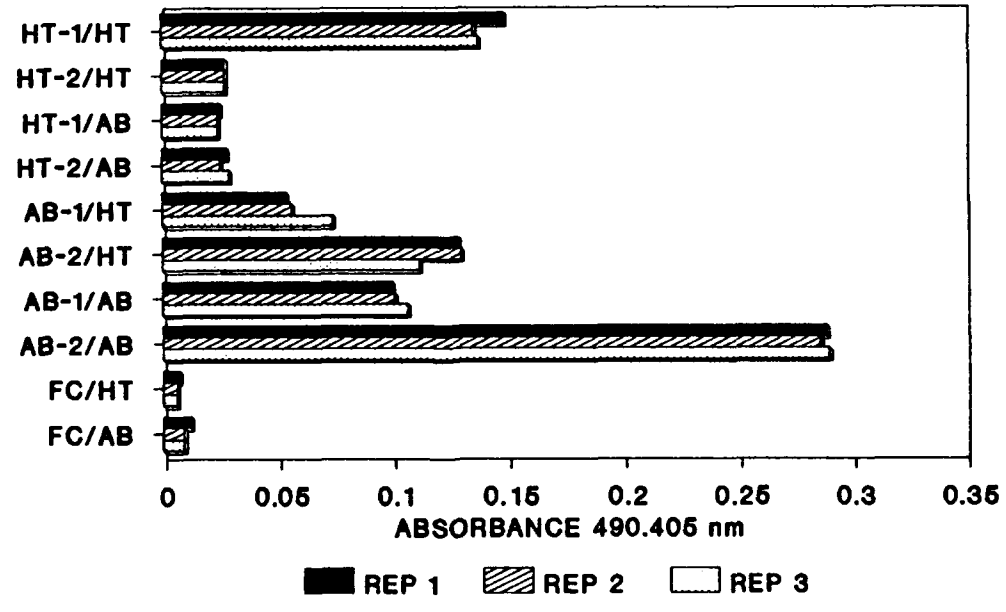
With very few exceptions, there was significant cross-reactivity between heterologous antisera/antigen combinations. Cross-reactions occurred between all possible combinations of antisera and antigens used in this study. In some cases, e.g. serum from mouse #2 immunized with AB antigen and MG antigen in Experiment #4 (AB2/MG), cross-reactions were as strong as in the homologous

Figures 1 - 6. Response of mice to immunization based on enzyme-linked immunosorbent assay (ELISA) readings. AB, HT, & MG, mice injected with antigen prepared from mosquito abdomens, heads & thoraces, and dissected midguts, respectively; FC, Freund's control, i.e. mice injected with Freund's adjuvant; UC or C or NMS, uninjected controls, i.e. uninjected mice, or normal mouse serum. Numbers 1 & 2 associated with a given antigen designate individual mice; 1 + 2, survivorship curves for the two mice were not significantly different and therefore the data have been pooled; REP 1, 2, 3, replicates of same antiserum/antigen combination.



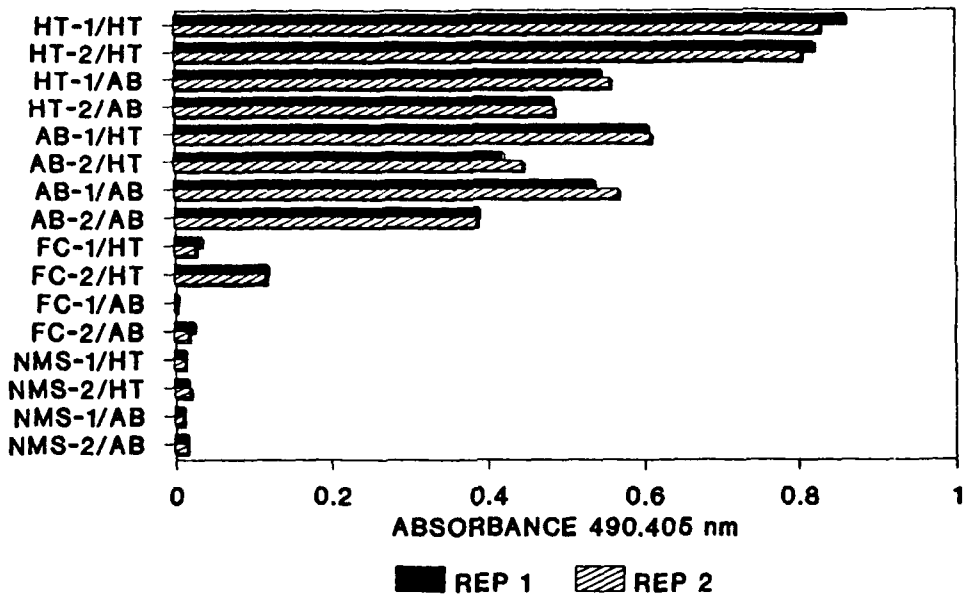
EXP. 1 - ANTIBODY 1:600

Figure 1a



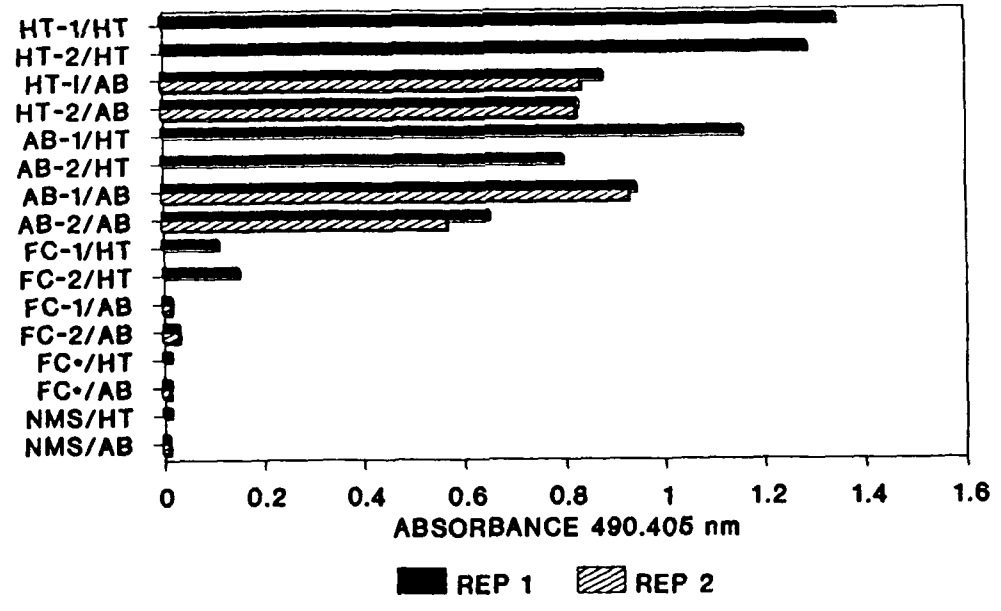
EXP. 1 - ANTIBODY 1:1000

Figure 1b



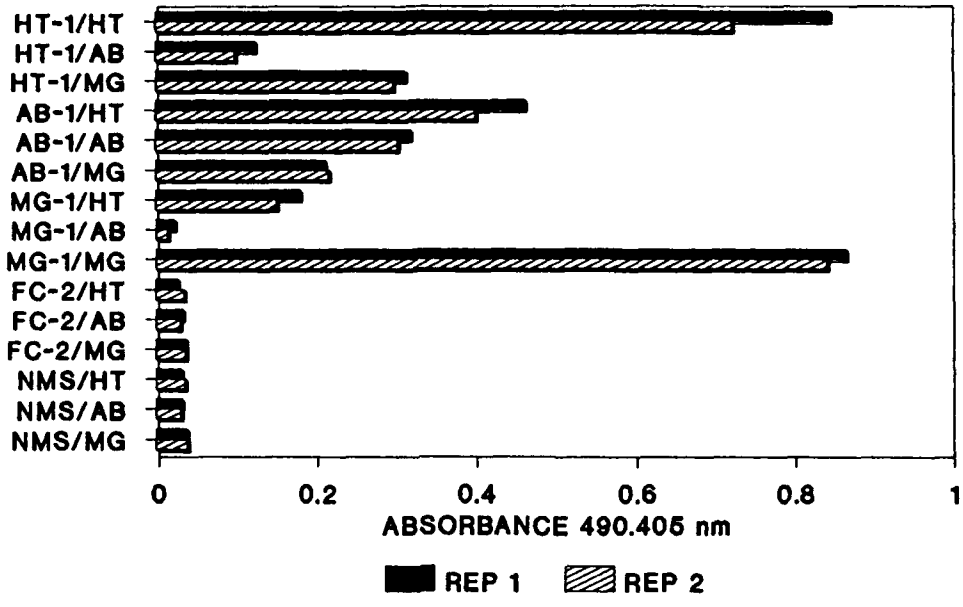
EXP. 2 - ANTIBODY 1:600

Figure 2a



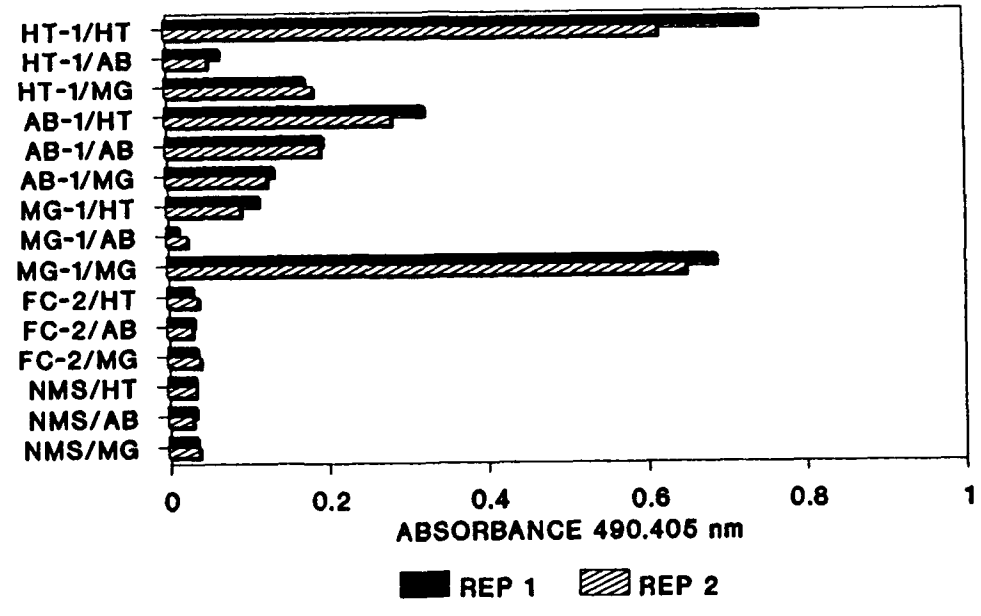
EXP. 2 REPEAT - ANTIBODY 1:600

Figure 2b



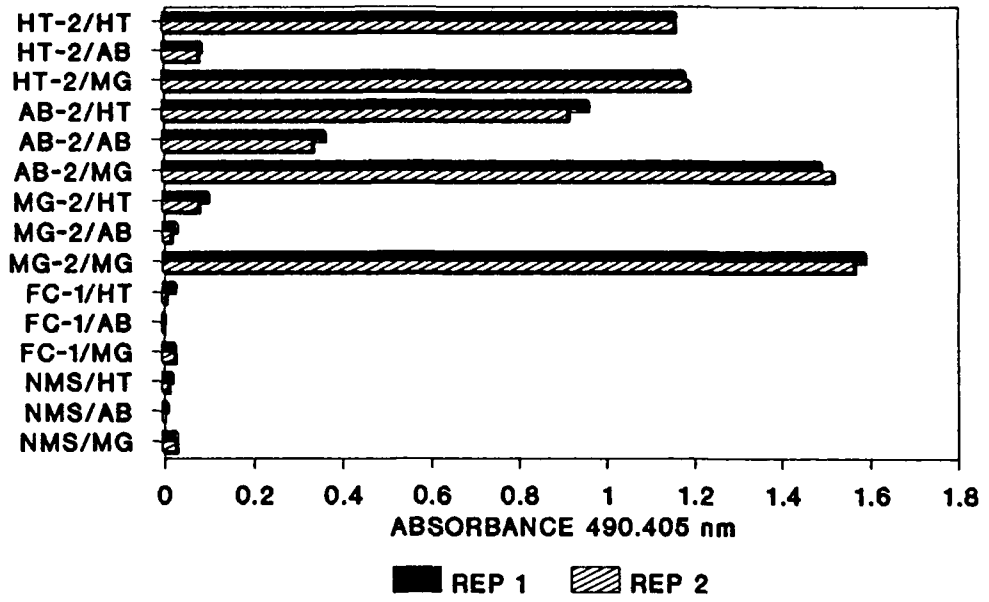
EXP. 3 - ANTIBODY 1:500

Figure 3a



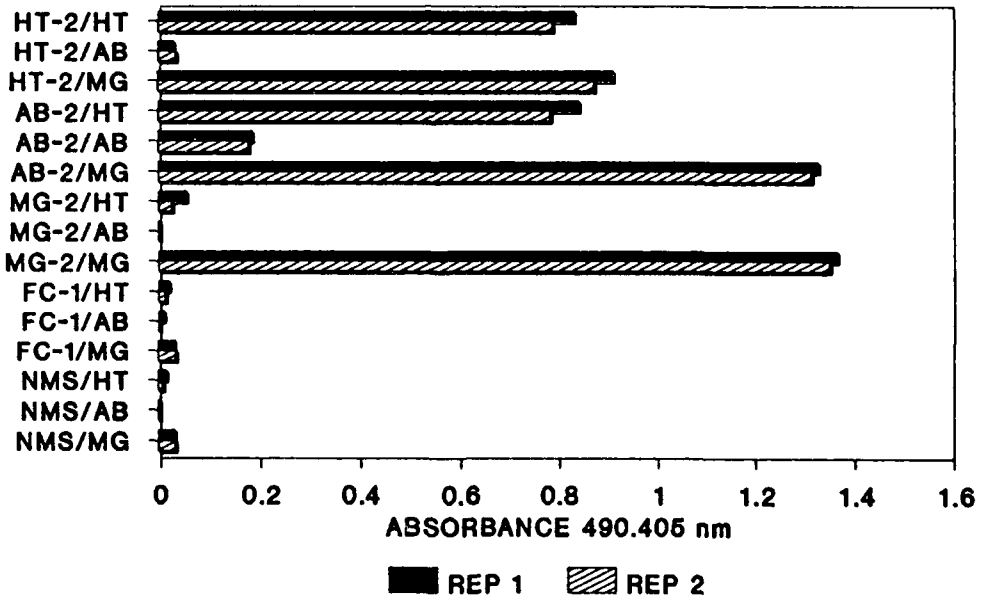
EXP. 3 - ANTIBODY 1:1000

Figure 3b



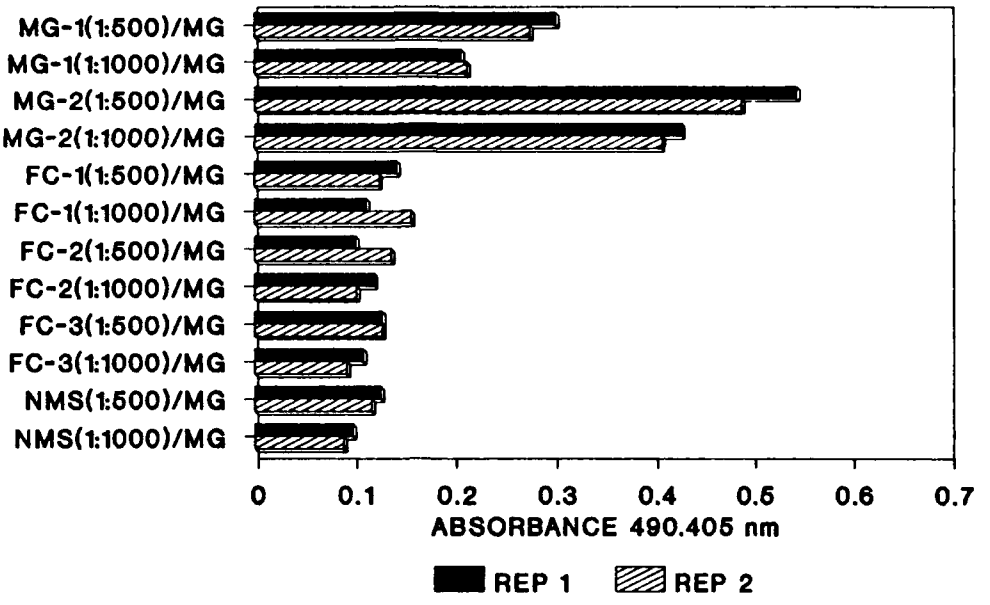
EXP. 4 - ANTIBODY 1:500

Figure 4a



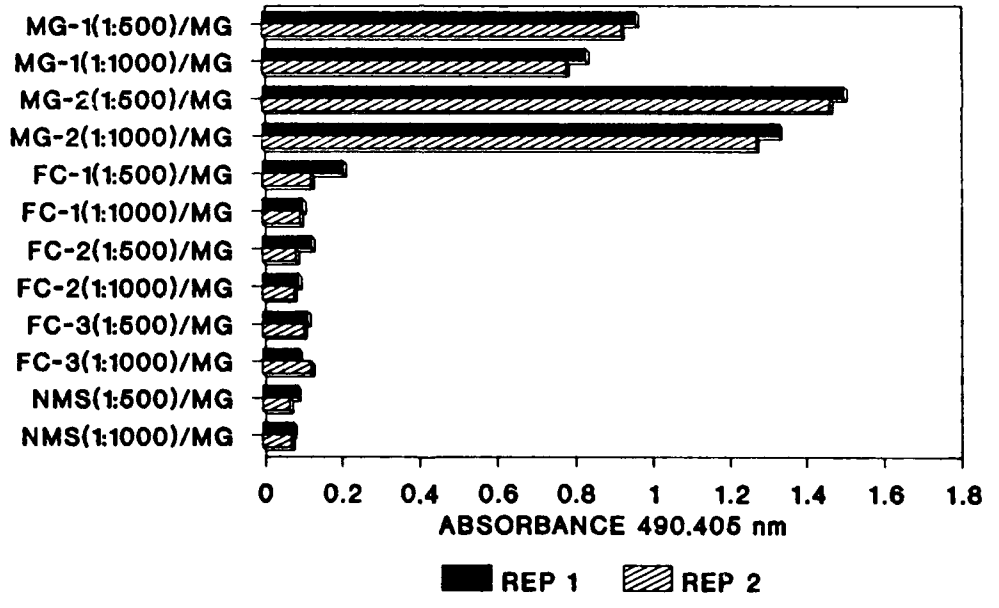
EXP. 4 - ANTIBODY 1:1000

Figure 4b



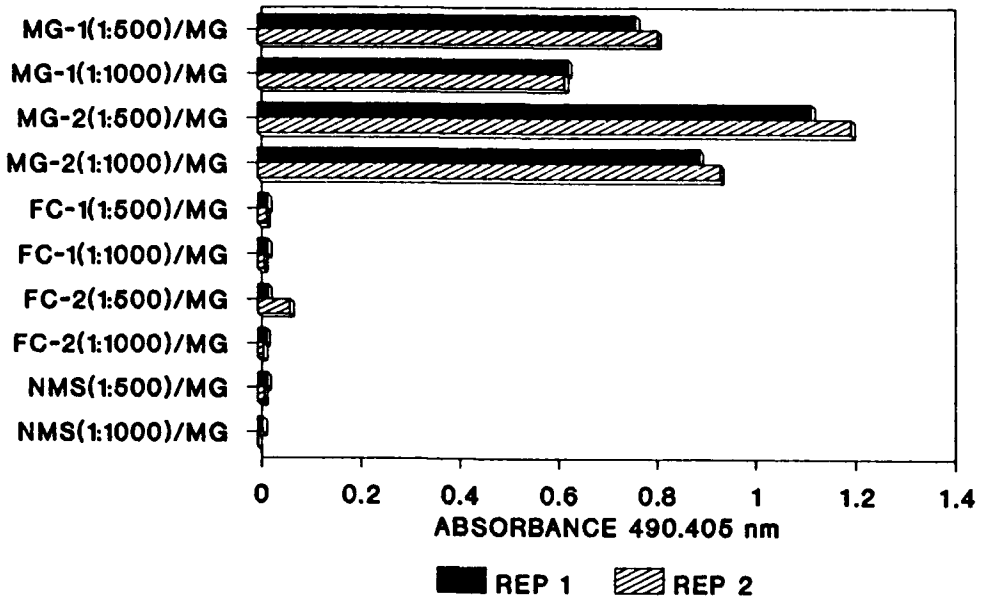
EXP. 6 - FIRST BLEED

Figure 5a



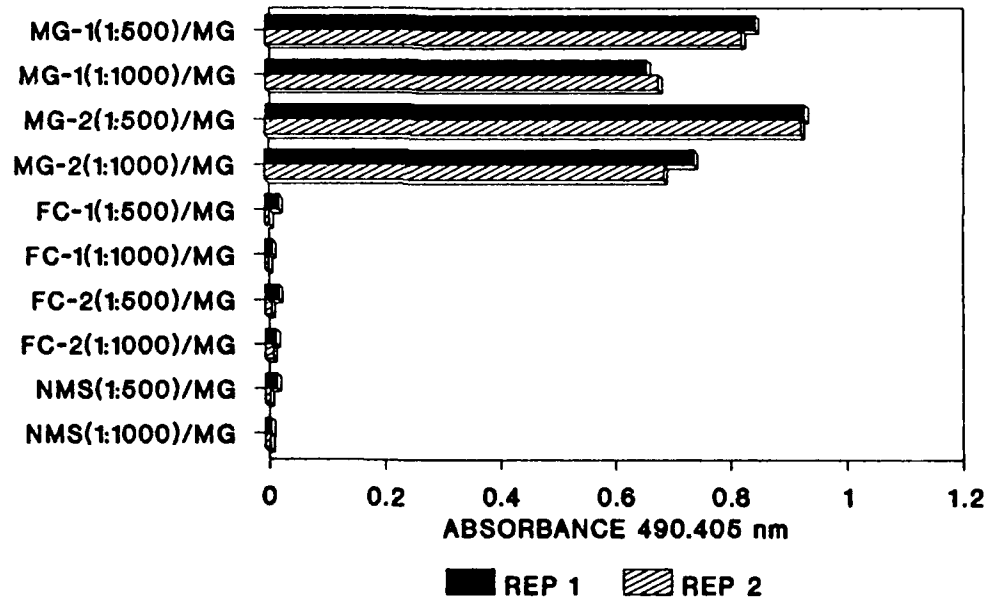
EXP. 6 - SECOND BLEED

Figure 5b



EXP. 7 - FIRST BLEED

Figure 6a



EXP. 7 - SECOND BLEED

Figure 6b

reactions (In Experiment #4, MG2/MG). It is interesting to note the magnitude of cross-reactivity displayed by heterologous serum/antigen combinations in Experiment #1 showed a substantial increase relative to the reactivity of the homologous serum/antigen combinations in response to the "booster" injections of mosquito antigen given in preparation for Experiment #2.

Antiserum dilutions of 1:500 and 1:1000 were used in the ELISAs run in association with Experiments 1, 3, 4, 6 and 7. Not surprisingly sera from immunized mice displayed clear dose-responses, while there was little evidence of dose-response among the non-immunized, "control" mice.

B. Survivorship of Mosquitoes Fed on Immune Hosts

The survivorship data is presented graphically in Figures 7-12. Data are represented as curves and bars in order to facilitate comparison between experimental (i.e. mosquitoes that fed on mice immunized with mosquito antigen) and controls (i.e. mosquitoes that fed on mice injected with Freund's adjuvant, FC, and uninjected mice, UC) or to facilitate comparison of controls with one another, i.e. FC versus UC or C. Tables 9-13 summarize the results of the statistical analyses of the survivorship curves.

There were four instances where the survivorship curve for mosquitoes that fed on mice immunized with mosquito antigen decayed at a rate significantly greater than the survivorship curves for mosquitoes that fed on non-immunized, control mice: Experiment #1, AB1+2 versus FC1+2 and HT2 versus FC1+2 (Table 9) and Experiment #3, AB2 versus FC1 and AB2 versus UC 1+2 (Table 11). The immune mice in Experiment #1 had been injected with antigen from sugar-fed mosquitoes and the immune mice in Experiment #2 had been injected with antigen from blood-fed mosquitoes.

In two cases (Experiment #2, AB1+2 versus UC2 and HT1 versus UC1), the survivor shop curves for the mosquitoes fed on untreated control mice decayed at a greater rate than the survivorship curves for mosquitoes which fed on immunized mice.

In all experiments in which mosquito midgut antigen, from sugar-fed or blood-fed mosquitoes, was used to immunize mice, no significant differences in survivorship were detected between experimental and control groups.

There were four cases where the control curves were significantly different from one another: Experiment #1, UC1+2 versus FC1+2; Experiment #2, UC1 versus UC2 and FC1+2 versus UC2; and Experiment #3, FC1 versus FC2.

Figures 7 -12. Survivorship curves for mosquitoes fed on immune mice or control mice. Data are represented as curves and bars to facilitate comparison between experimental (i.e. mosquitoes that fed on mice immunized with mosquito antigen) and controls (i.e. mosquitoes that fed on mice injected with Freund's adjuvant, FC or uninjected mice, UC or C) or to facilitate comparison of controls with one another, FC versus UC or C.

(AB, HT, & MG, mice injected with antigen prepared from mosquito abdomens, heads & thoraces, and dissected midguts, respectively; FC, Freund's control, i.e. mice injected with Freund's adjuvant; UC or C or NMS, uninjected controls, i.e. uninjected mice, or normal mouse serum. Numbers 1 & 2 associated with a given antigen designate individual mice; 1 + 2, survivorship curves for the two mice were not significantly different and therefore the data have been pooled.)

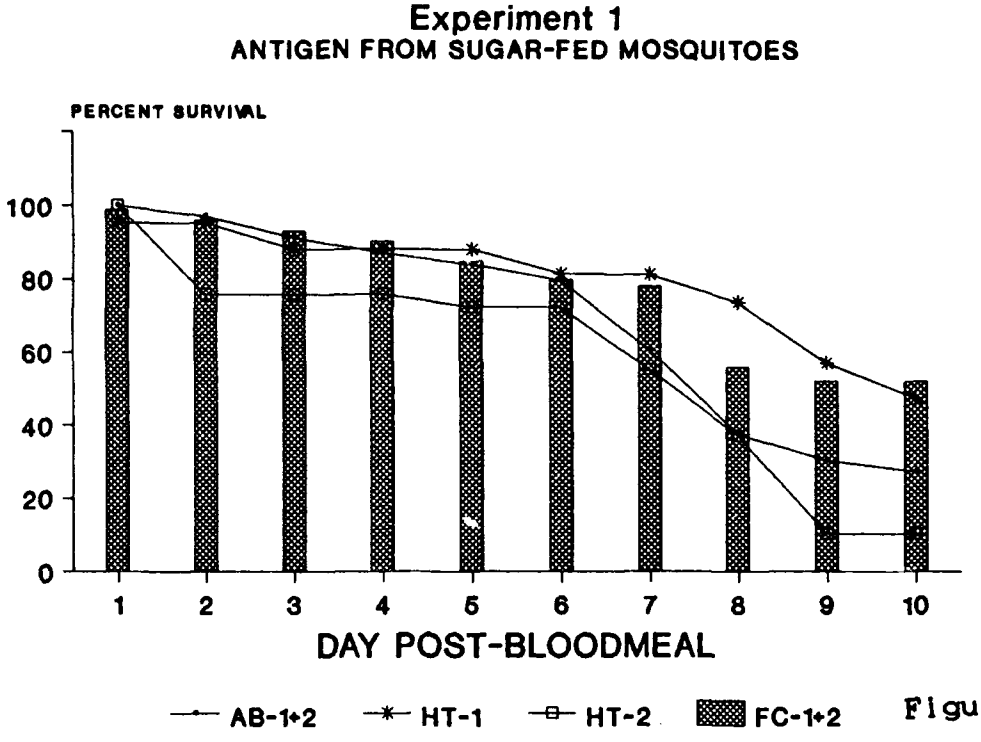


Figure 7a

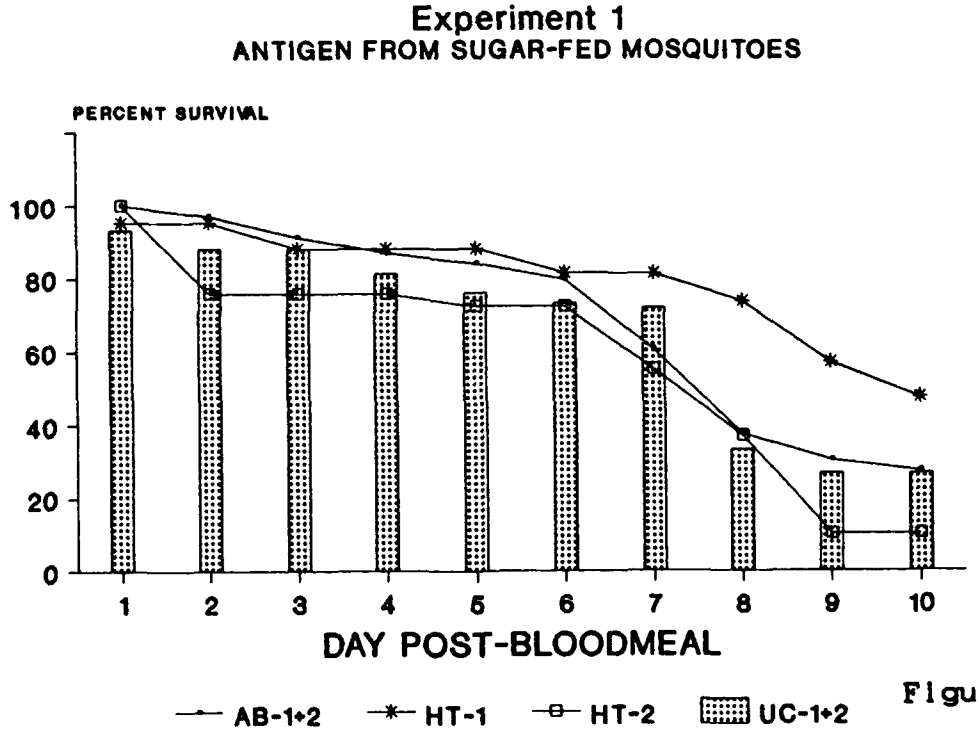


Figure 7b

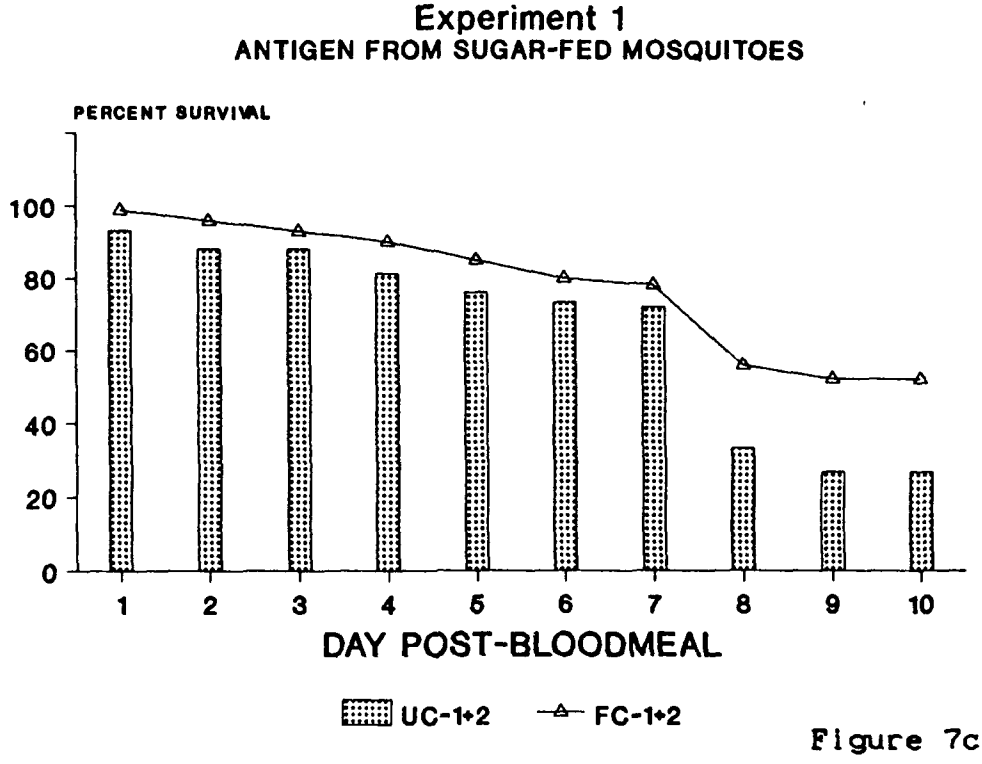

Experiment 1
ANTIGEN FROM SUGAR-FED MOSQUITOES
PERCENT SURVIVAL
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
DAY POST-BLOODMEAL
UC-1+2
FC-1+2

Figure 7c

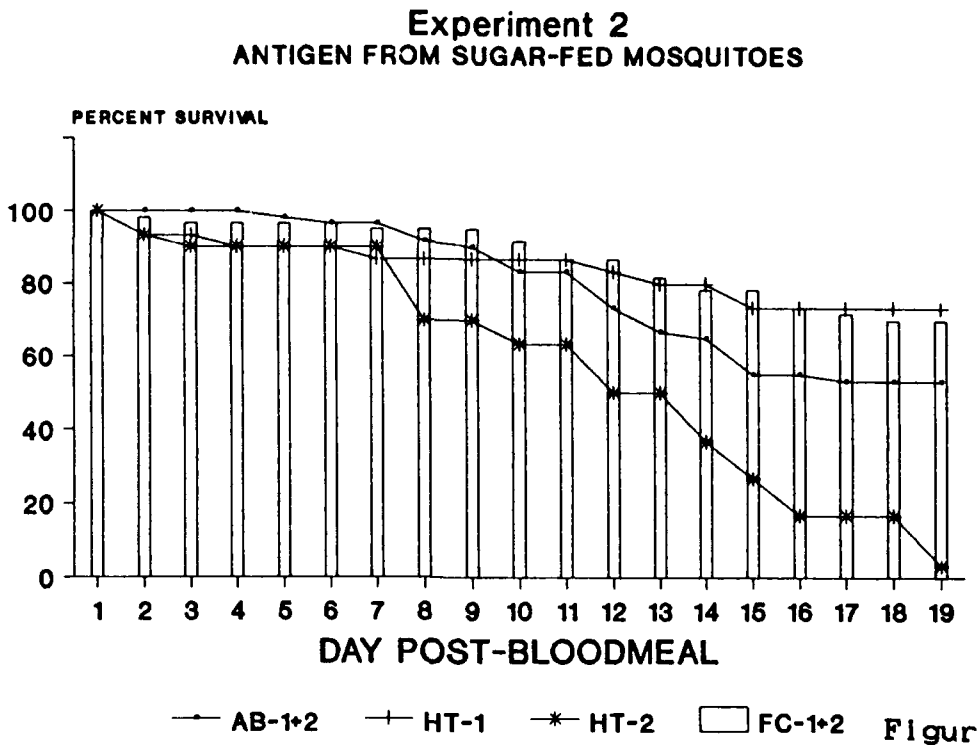


Figure 8a

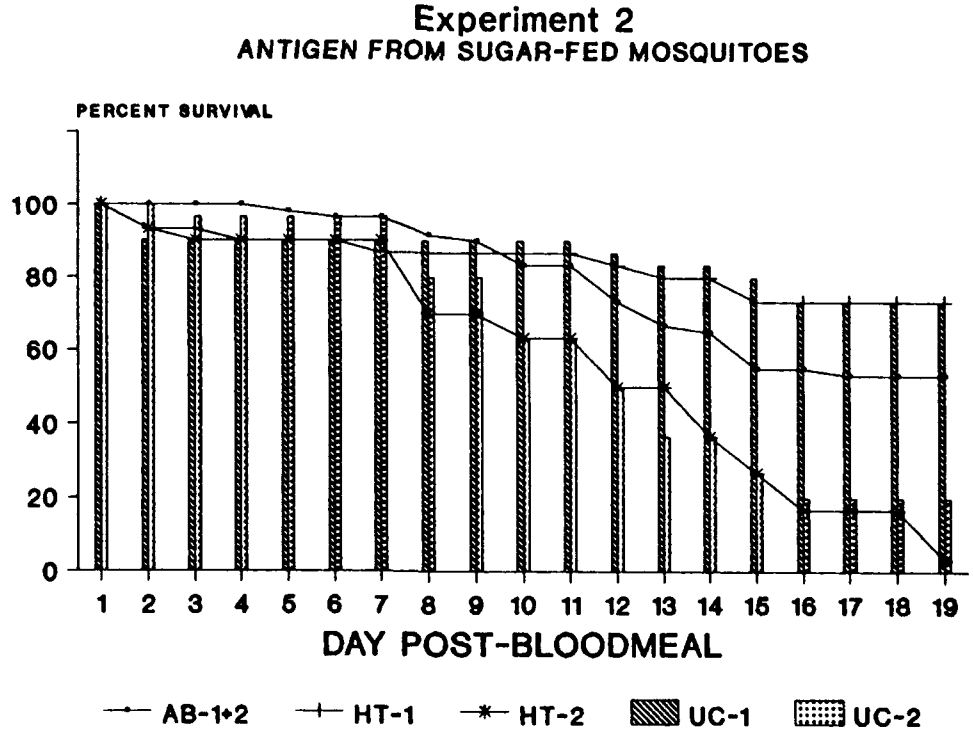


Figure 8b

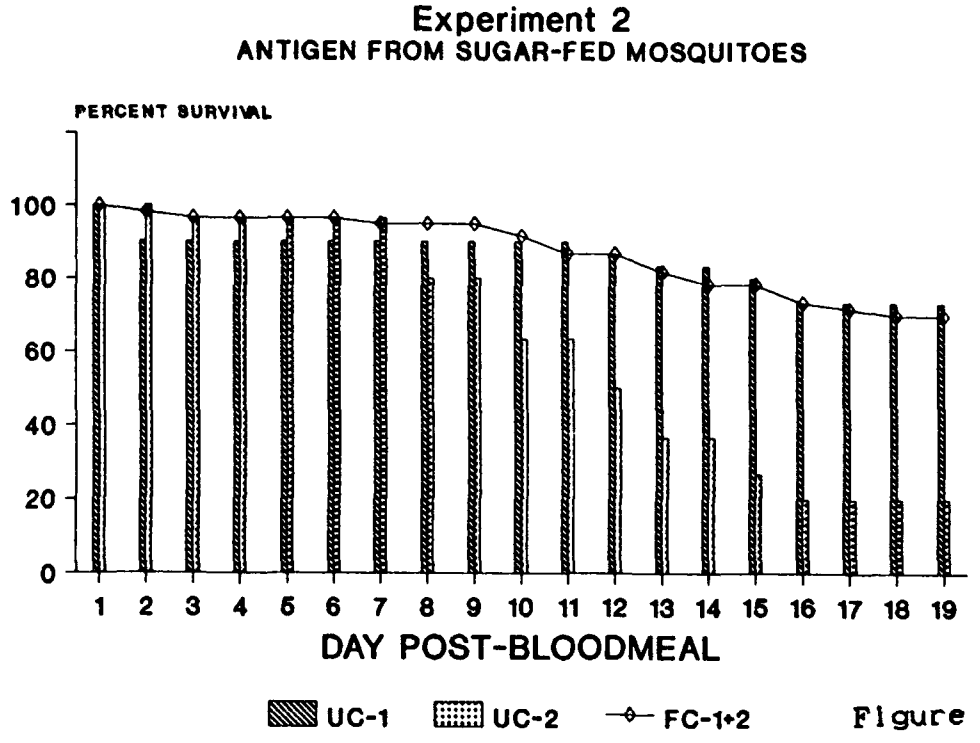

Experiment 2
ANTIGEN FROM SUGAR-FED MOSQUITOES
PERCENT SURVIVAL
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAY POST-BLOODMEAL
UC-1
UC-2
FC-1+2

Figure 8c

Experiment 3
ANTIGEN FROM BLOOD-FED MOSQUITOES

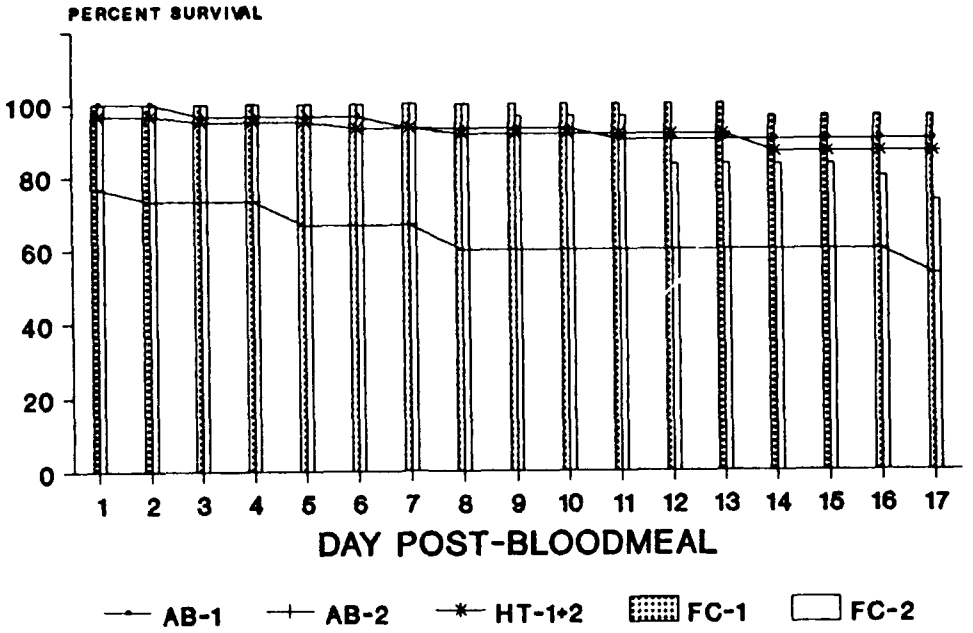


Figure 9a

Experiment 3
ANTIGEN FROM BLOOD-FED MOSQUITOES

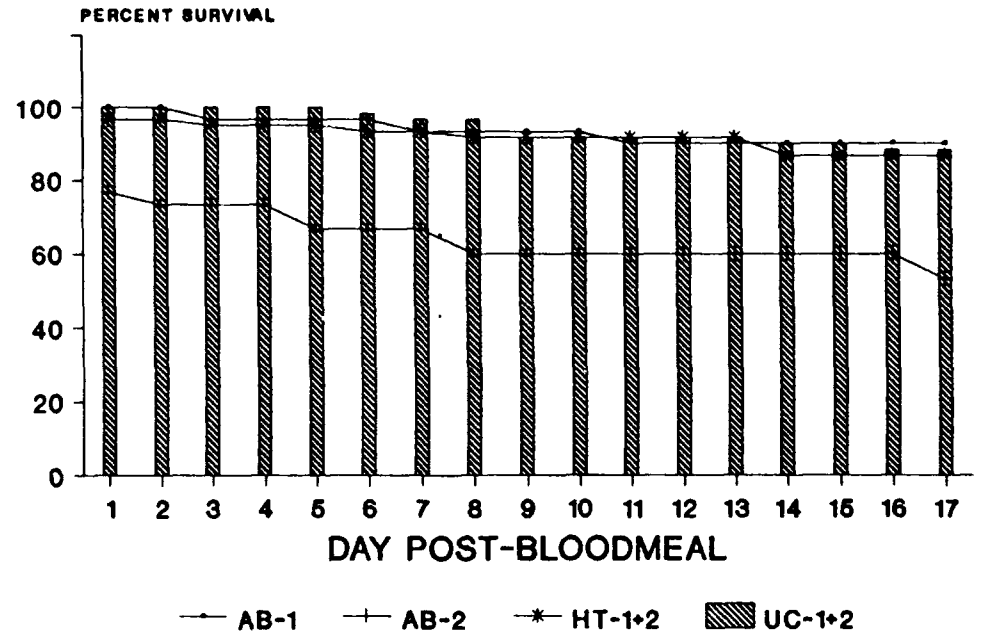


Figure 9b

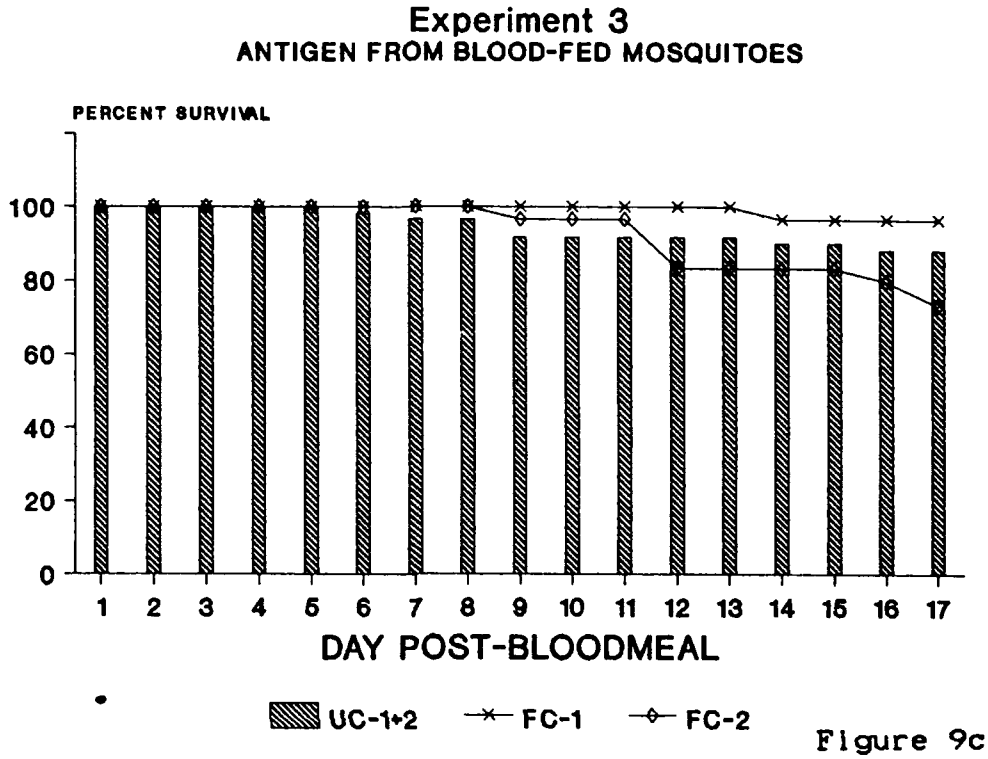

Experiment 3
ANTIGEN FROM BLOOD-FED MOSQUITOES
PERCENT SURVIVAL
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
DAY POST-BLOODMEAL
UC-1+2
FC-1
FC-2

Figure 9c

Experiment 3
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES

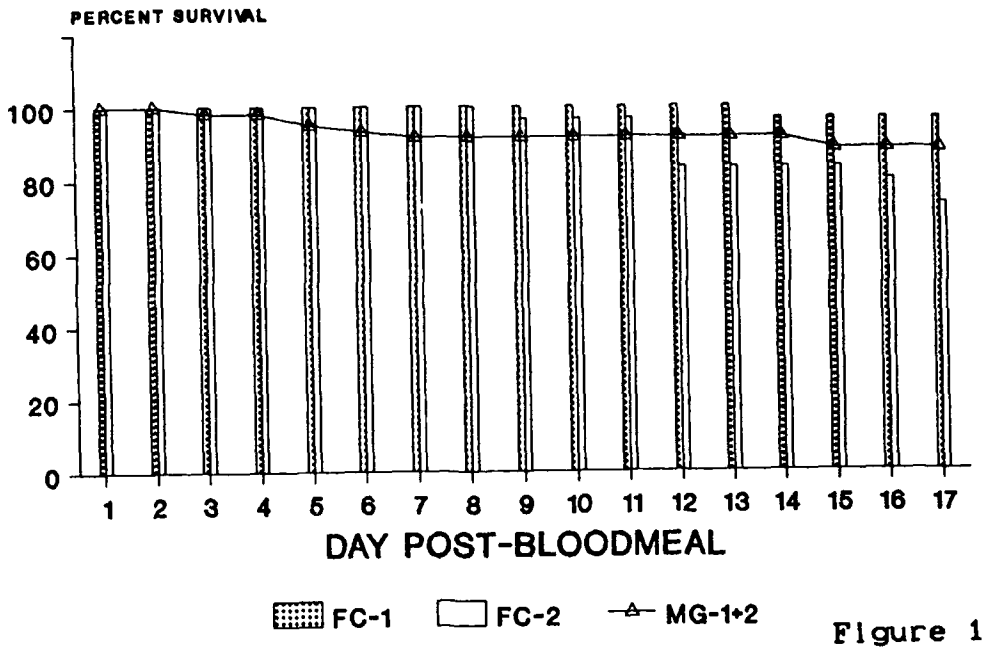


Figure 10a

Experiment 3
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES

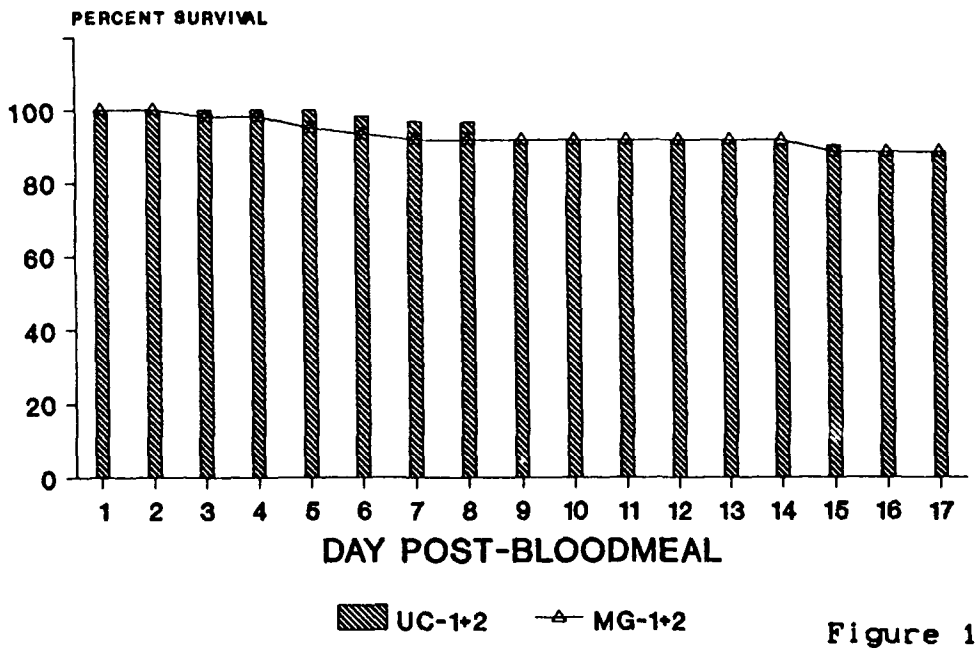


Figure 10b

Experiment 6
ANTIGEN: MIDGUTS FROM BLOOD-FED FEMALES

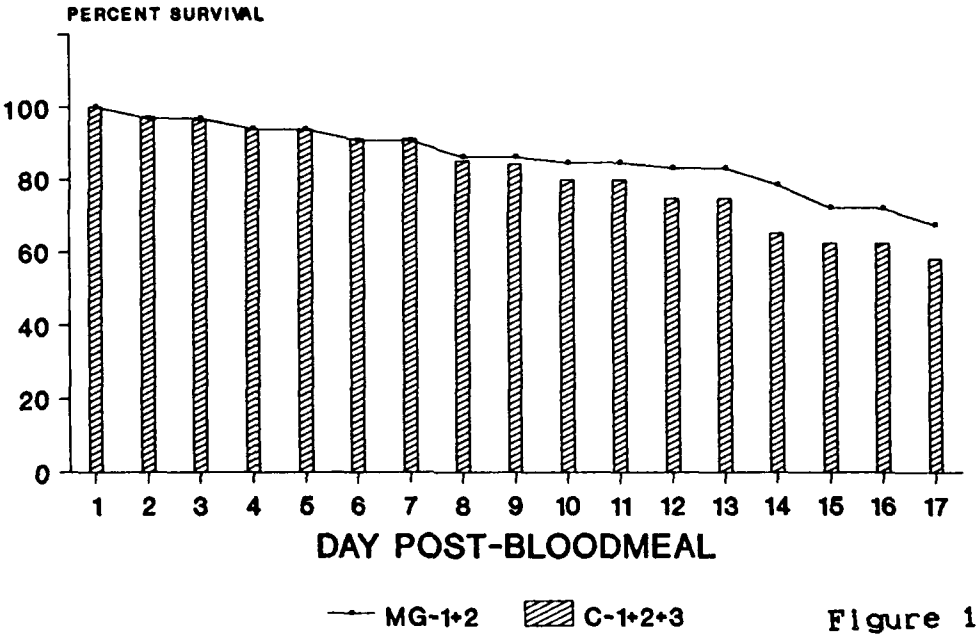


Figure 11

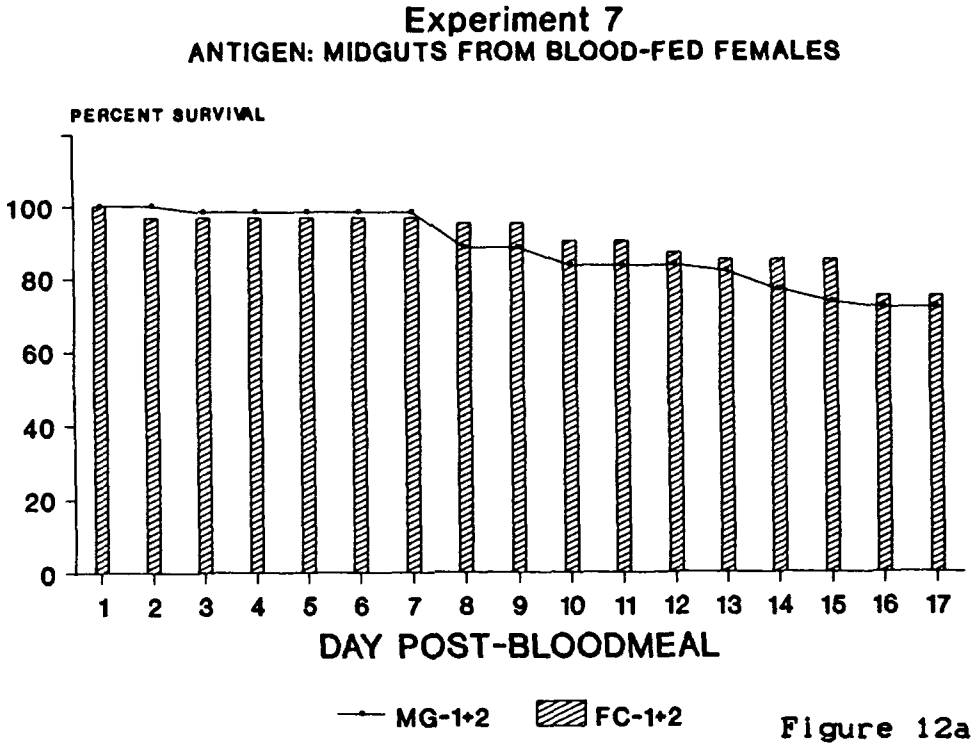


Figure 12a

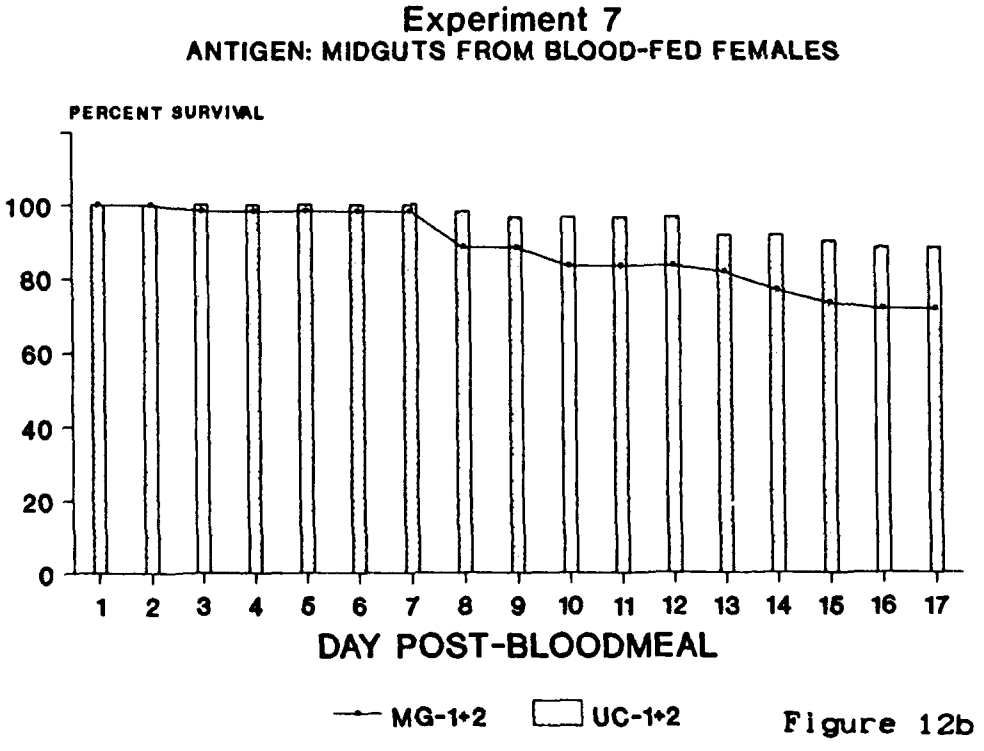


Figure 12b

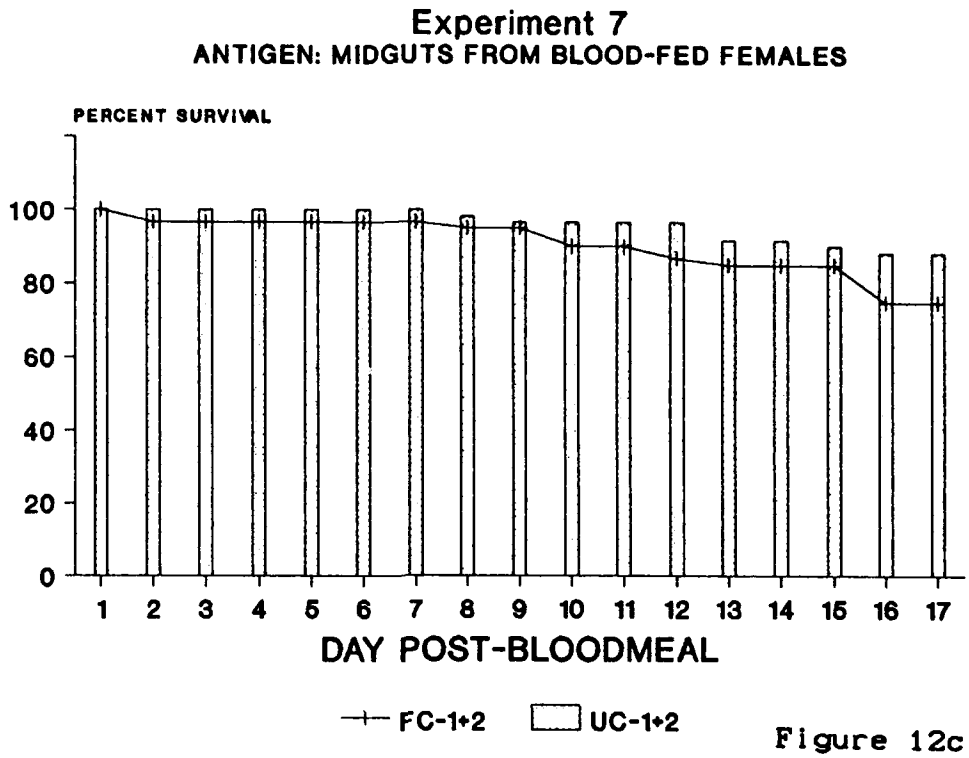

Experiment 7
ANTIGEN: MIDGUTS FROM BLOOD-FED FEMALES
PERCENT SURVIVAL
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
DAY POST-BLOODMEAL
FC-1+2
UC-1+2

Figure 12c

Table 9. Results of Statistical Analysis of Survivorshop Curves for Experiment 1 (Mice immunized with antigen from Sugar-fed Mosquitoes).

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
AB1		AB2		x	
HT1		HT2			x
FC1		FC2		x	
UC1		UC2		x	
AB1+2		HT1		x	
AB1+2		HT2		x	
AB1+2		FC1+2	x		
AB1+2		UC1+2		x	
HT1		FC1+2		x	
HT2		FC1+2	x		
HT1		UC1+2		x	
HT2		UC1+2		x	
UC1+2		FC1+2	x		

[1] AB, HT, mice injected with antigen prepared from mosquito abdomens, heads & thoraces, and dissected midguts, respectively. FC, Freund's control, i.e. mice injected with Freund's adjuvant. UC, uninjected control, i.e. uninjected mice. Numbers 1 and 2 associated with a given antigen designate individual mice; 1+2, survivorship curves for the two mice were not significantly different and therefore the data have been pooled.

[2] (A > B, A = B, A < B), mosquitoes that fed on mice injected with antigen A died at a significantly greater rate, the same rate, or a significantly slower rate, respectively, over the time period of the experiment than did mosquitoes that fed on a mouse or mice injected with antigen B.

Table 10. Results of Statistical Analysis of Survivorshop Curves for Experiment 2 (Mice immunized with antigen from Sugar-fed Mosquitoes).

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
AB1		AB2		x	
HT1		HT2			x
FC1		FC2		x	
UC1		UC2			x
AB1+2		HT1		x	
AB1+2		HT2			x
AB1+2		FC1+2		x	
AB1+2		UC1		x	
AB1+2		UC2			x
HT1		FC1+2		x	
HT2		FC1+2		x	
HT1		UC1			x
HT1		UC2		x	
FC1+2		UC1		x	
FC1+2		UC2			x

1,2 See Table 9.

Table 11. Results of Statistical Analysis of Survivorshop Curves for Experiment 3 (Mice immunized with antigen from Blood-fed Mosquitoes, HT & AB and from sugar-fed mosquitoes, MG).

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
AB1		AB2			x
HT1		HT2		x	
FC1		FC2			x
UC1		UC2		x	
MG1		MG2		x	
AB1		HT1+2		x	
AB1		FC1		x	
AB1		FC2		x	
AB1		UC1+2		x	
AB1		MG1+2		x	
AB2		HT1+2	x		
AB2		FC1	x		
AB2		FC2		x	
AB2		UC1+2	x		
AB1+2		MG1+2	x		
HT1+2		FC1		x	
HT1+2		FC2		x	
HT1+2		UC1+2		x	

Table 11 (continued)

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
HT1+2		MG1+2		x	
FC1		UC1+2		x	
FC1		MG1+2		x	
FC2		UC1+2		x	
FC2		MG1+2		x	
UC1+2		MG1+2		x	

1 See Table 9; MG, mice injected with antigen prepared from dissected mosquito midguts.

2 See Table 9.

Table 12. Results of Statistical Analysis of Survivorshop Curves for Experiment 6 (Mice immunized with antigen from Blood-fed Mosquitoes).

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
MG1		MG2		x	
FC1		FC2		x	
FC1		FC3		x	
FC2		FC3		x	
MG1+2		FC1+2+3		x	

1 See Table 9; MG, mice injected with antigen prepared from dissected mosquito midguts.

2 See Table 9.

Table 13. Results of Statistical Analysis of Survivorshop Curves for Experiment 7 (Mice Immunized with antigen from Blood-fed Mosquitoes).

Antigen Injected[1]			Survivorship Curve Profiles[2]		
A	versus	B	A > B	A = B	A < B
MG1		MG2		x	
UC1		UC2		x	
FC1		FC2		x	
NFC1		NFC2		x	
MG1+2		UC1+2		x	
MG1+2		FC1+2		x	
MG1+2		NFC1+2		x	
UC1+2		FC1+2		x	
UC1+2		NFC1+2		x	
FC1+2		NFC1+2		x	

1 See Table 9; MG, mice injected with antigen prepared from dissected mosquito midguts; NFC, new Freund's controls.

2 See Table 9.

The results of all statistical analyses of survivorship curves generated by mosquitoes fed on immune versus non-immune control mice and all comparisons of survivorship curves made among mosquitoes which fed on non-immune control mice are summarized in Table 14. In 80%(24/30) of the statistical comparisons of survivorship curves between mosquitoes fed on immune mice versus mosquitoes fed on non-immune control mice, no significant differences were detected. In 20% (6/30) of the comparisons between mosquitoes fed on immune mice versus mosquitoes fed on non-immune control mice, significant differences in survivorship curves were found. Among all statistical comparisons of survivorship curves generated by mosquitoes that fed on non-immune mice (FC versus FC, UC versus UC, and FC versus UC), 79% (15/19) revealed no significant differences, while in 21% (4/19) of the comparisons, the survivorship curve profiles were significantly different.

C. Fecundity

The results of the experiments in which mosquito fecundity was measured are shown graphically in Figures 13-20. No statistically significant differences were found between the fecundity of mosquitoes which fed on mice immunized with mosquito antigens and the fecundity of mosquitoes which fed on Freund's-injected, or uninjected, control mosquitoes.

D. Egg Viability

The results fo the experiments in which mosquito egg viability was determined are shown graphically in Figures 21-23. No statistically significant differences were found in eg viability between the mosquitoes that had fed on mice immunized with mosquito antigen and the mosquitoes that had fed on mice infected with Freund's adjuvant or, non-injected, control mice.

Table. 14. Results of All Statistical Analyses of Survivorship Curves Generated by Mosquitoes Feeding on Immune versus Non-Immune Control Mice and All Comparisons of Survivorship Curves of Mosquitoes Fed on Non-Immune Control Mice.

Source of Mosquito Blood	No significant difference	Significant difference
Immunes vs. Controls[1]	80% (24/30)	20% (6/30)
Controls vs. Controls[2]	79% (15/19)	21% (4/19)

[1] All comparisons of HT, AB, & MG versus FC & UC.

[2] All comparisons of FC vs. FC, UC vs. UC, and FC vs UC.

Figures 13 - 20. Mosquito fecundity as a function of immune status of blood source (mouse). Note that for Experiment #1, bars are based on number of eggs per female calculated from total number of eggs deposited/total number of females. For the remaining experiments, the short horizontal lines represent mean number of eggs per female while vertical bars represent the range of eggs per female for a given sample.

(AB, HT, & MG, mice injected with antigen prepared from mosquito abdomens, heads & thoraces, and dissected midguts, respectively; FC, Freund's control, i.e. mice injected with Freund's adjuvant; UC or C or NMS, uninjected controls, i.e. uninjected mice, or normal mouse serum. Numbers 1 & 2 associated with a given antigen designate individual mice.)

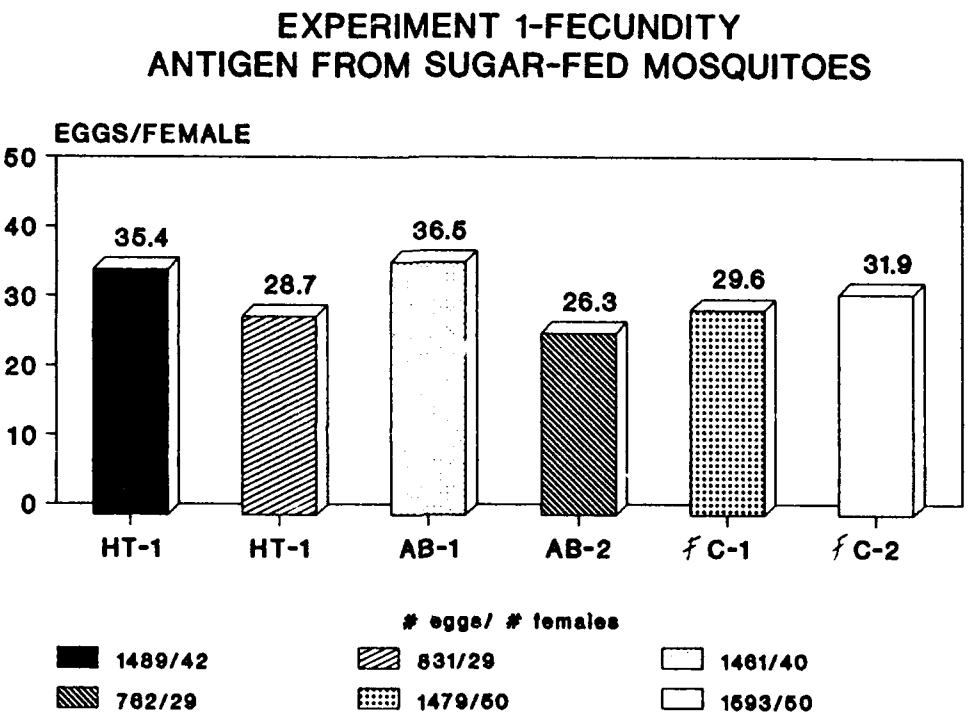

EXPERIMENT 1-FECUNDITY
ANTIGEN FROM SUGAR-FED MOSQUITOES
EGGS/FEMALE
50
40
30
20
10
0
35.4
28.7
36.5
26.3
29.6
31.9
HT-1
HT-1
AB-1
AB-2
♂ C-1
♂ C-2
eggs/ # females
1489/42
831/29
1461/40
782/29
1479/50
1693/50

Figure 13

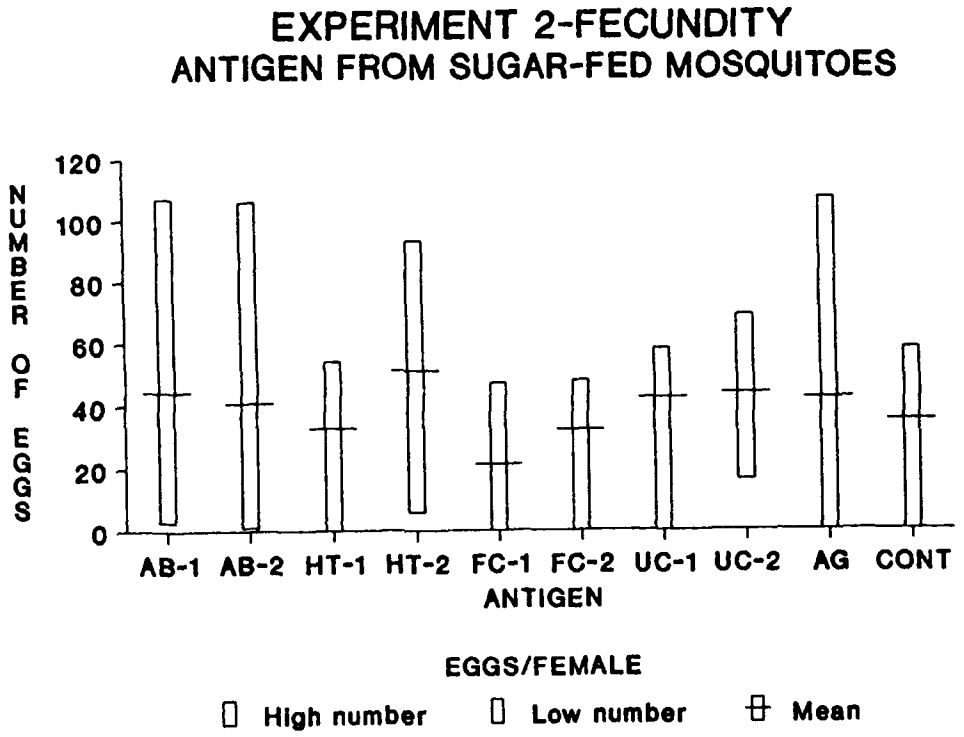

EXPERIMENT 2-FECUNDITY
ANTIGEN FROM SUGAR-FED MOSQUITOES
NUMBER OF EGGS
120
100
80
60
40
20
0
AB-1
AB-2
HT-1
HT-2
FC-1
FC-2
UC-1
UC-2
AG
CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 14

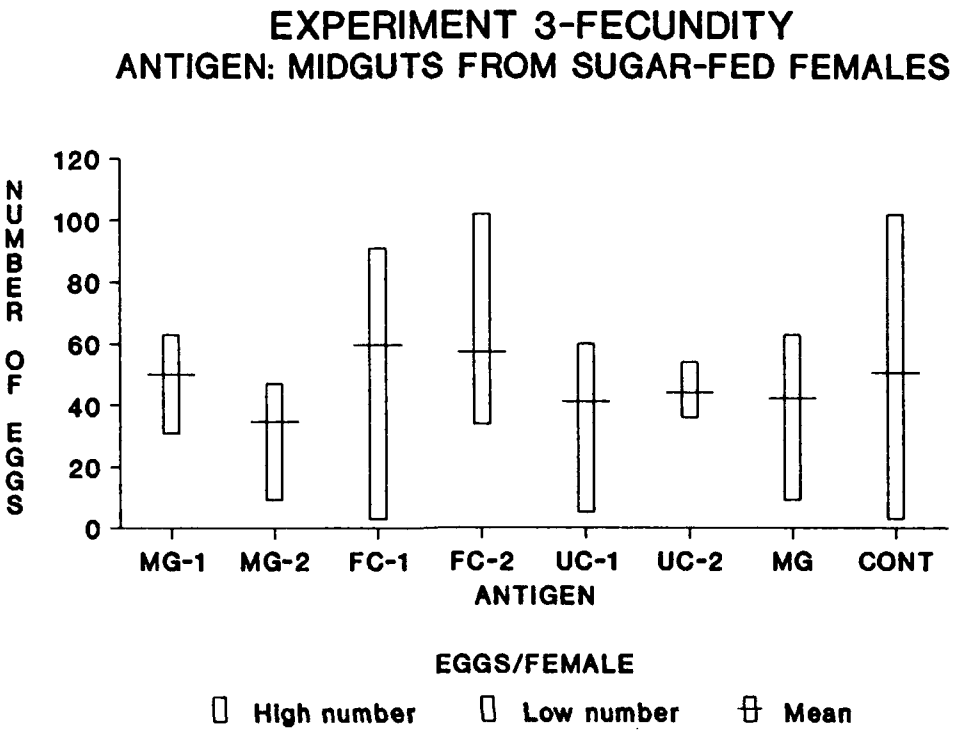

EXPERIMENT 3-FECUNDITY
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES
NUMBER OF EGGS
120
100
80
60
40
20
0
MG-1
MG-2
FC-1
FC-2
UC-1
UC-2
MG
CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 15

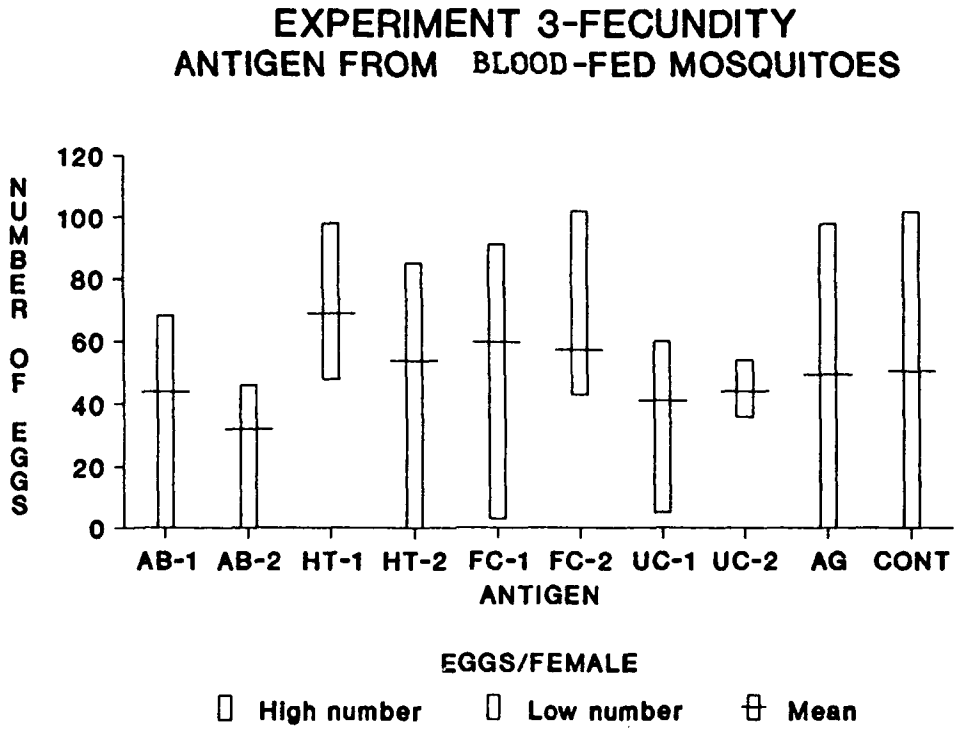

EXPERIMENT 3-FECUNDITY
ANTIGEN FROM BLOOD-FED MOSQUITOES
NUMBER OF EGGS
120
100
80
60
40
20
0
AB-1 AB-2 HT-1 HT-2 FC-1 FC-2 UC-1 UC-2 AG CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 16

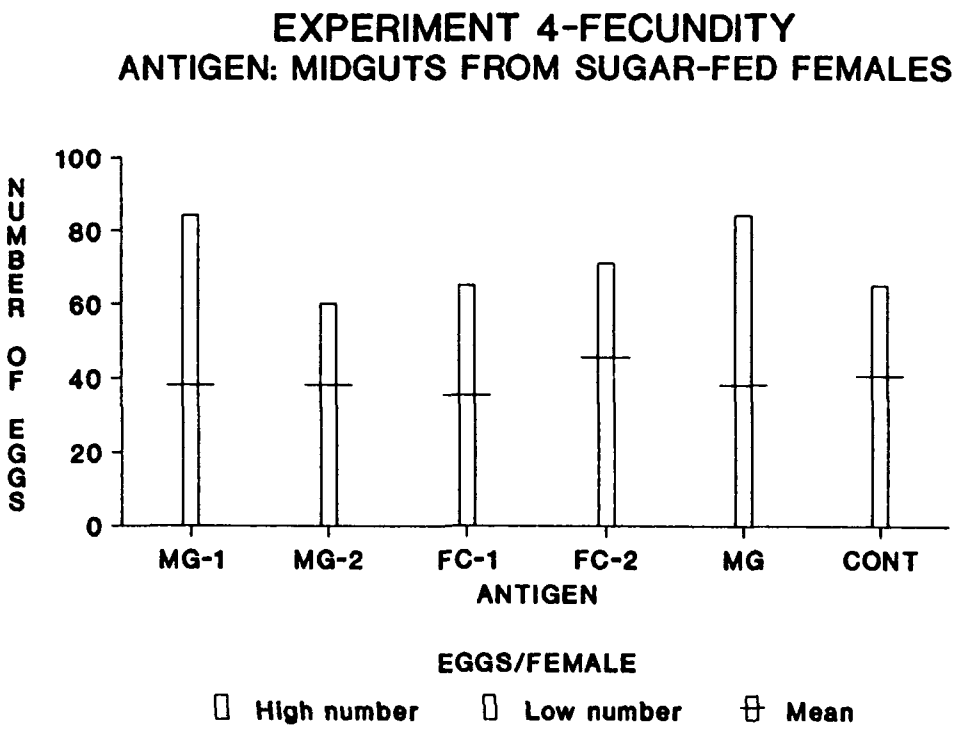

EXPERIMENT 4-FECUNDITY
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES
NUMBER OF EGGS
100
80
60
40
20
0
MG-1
MG-2
FC-1
FC-2
MG
CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 17

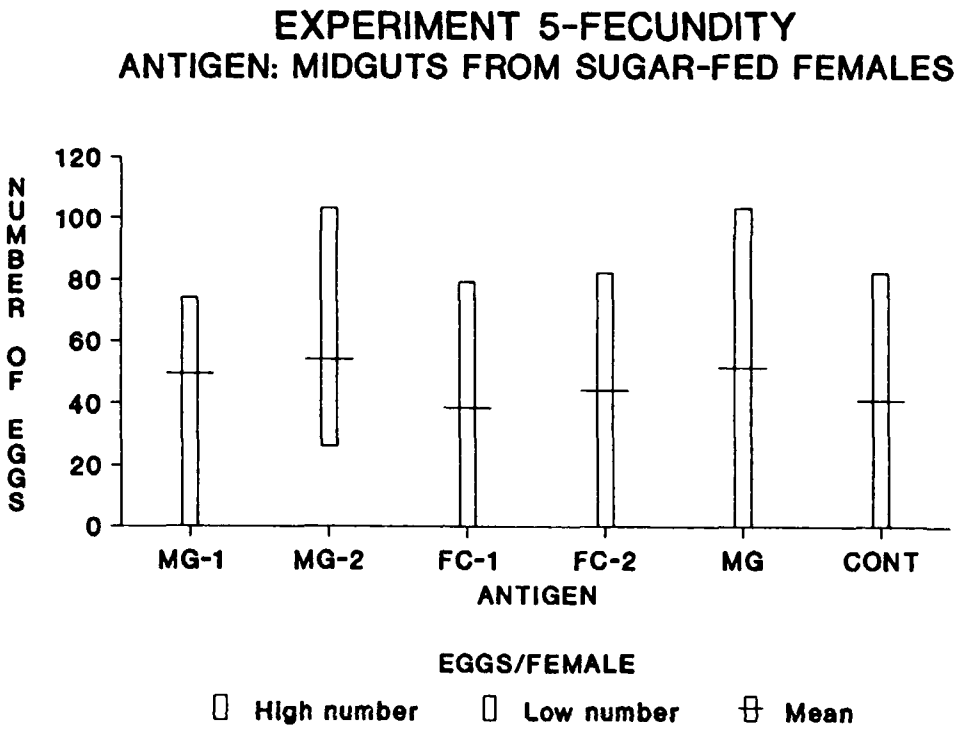

EXPERIMENT 5-FECUNDITY
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES
NUMBER OF EGGS
120
100
80
60
40
20
0
MG-1
MG-2
FC-1
FC-2
MG
CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 18

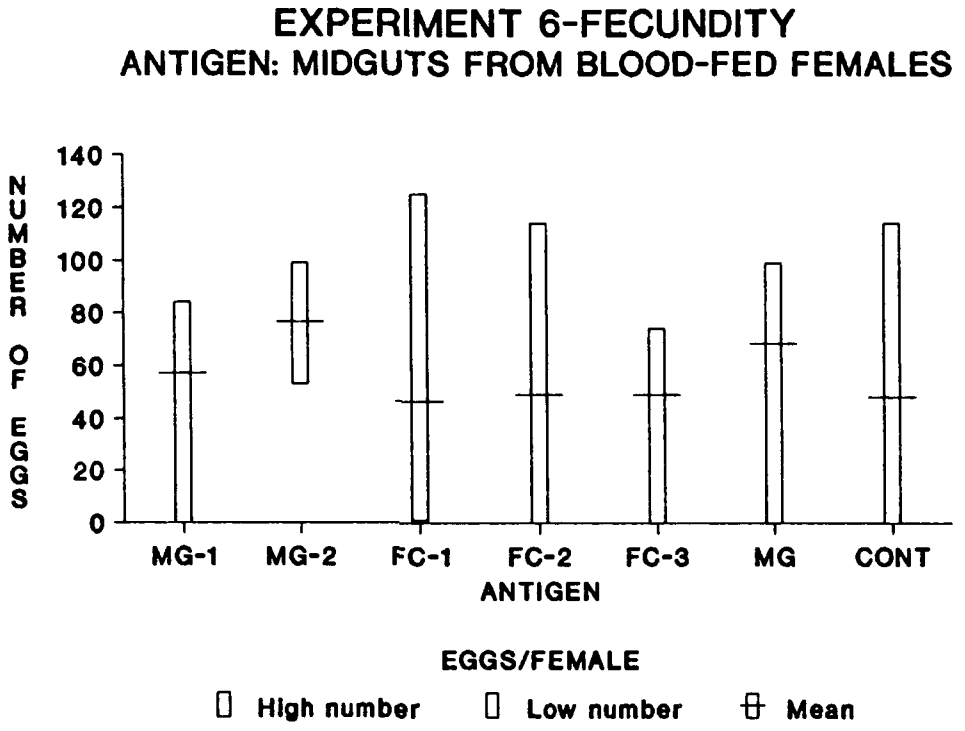
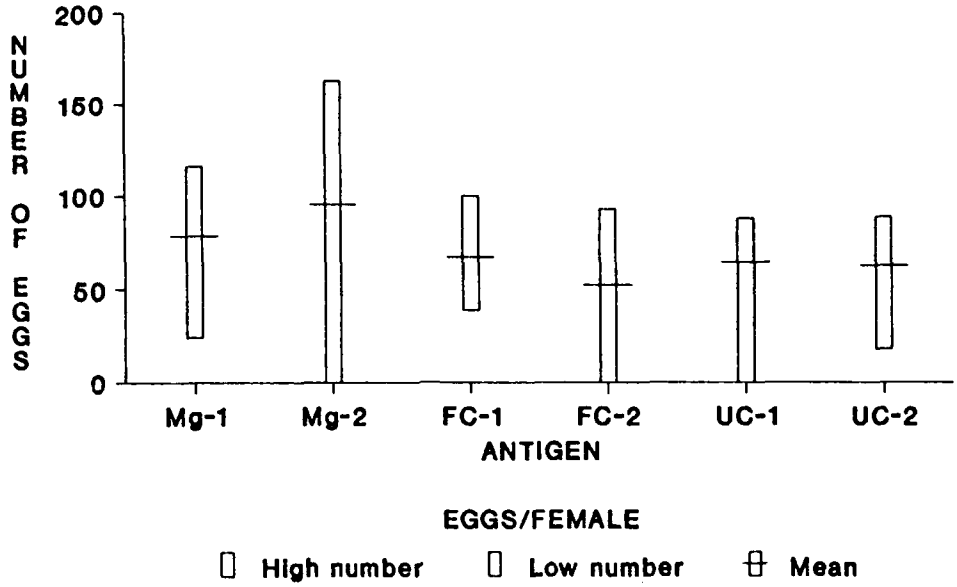

EXPERIMENT 6-FECUNDITY
ANTIGEN: MIDGUTS FROM BLOOD-FED FEMALES
NUMBER OF EGGS
140
120
100
80
60
40
20
0
MG-1
MG-2
FC-1
FC-2
FC-3
MG
CONT
ANTIGEN
EGGS/FEMALE
High number
Low number
Mean

Figure 19

EXPERIMENT 7-FECUNDITY
ANTIGEN: MIDGUTS FROM BLOOD-FED FEMALES



n = 10 for each group

Figure 20

Figures 21 - 23. Mosquito egg viability as a function of immune status of blood source (mouse). For each experiment represented, the short horizontal lines represent mean percent hatch per batch of eggs deposited per female, while vertical bars represent the range of percent hatch per batch of eggs deposited per female for a given sample of blood-fed female mosquitoes.

(AB, HT, & MG, mice injected with antigen prepared from mosquito abdomens, heads & thoraces, and dissected midguts, respectively; FC, Freund's control, i.e. mice injected with Freund's adjuvant; UC or C or NMS, uninjected controls, i.e. uninjected mice, or normal mouse serum. Numbers 1 & 2 associated with a given antigen designate individual mice.)

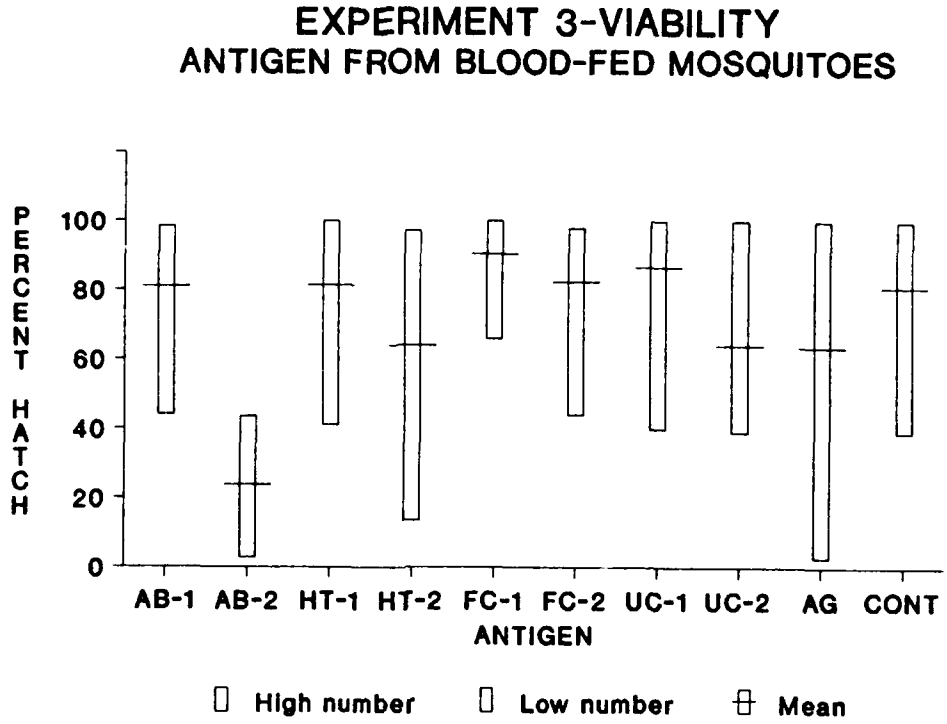

EXPERIMENT 3-VIABILITY
ANTIGEN FROM BLOOD-FED MOSQUITOES
PERCENT HATCH
100
80
60
40
20
0
AB-1
AB-2
HT-1
HT-2
FC-1
FC-2
UC-1
UC-2
AG
CONT
ANTIGEN
High number
Low number
Mean

Figure 21

EXPERIMENT 3-VIABILITY
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES

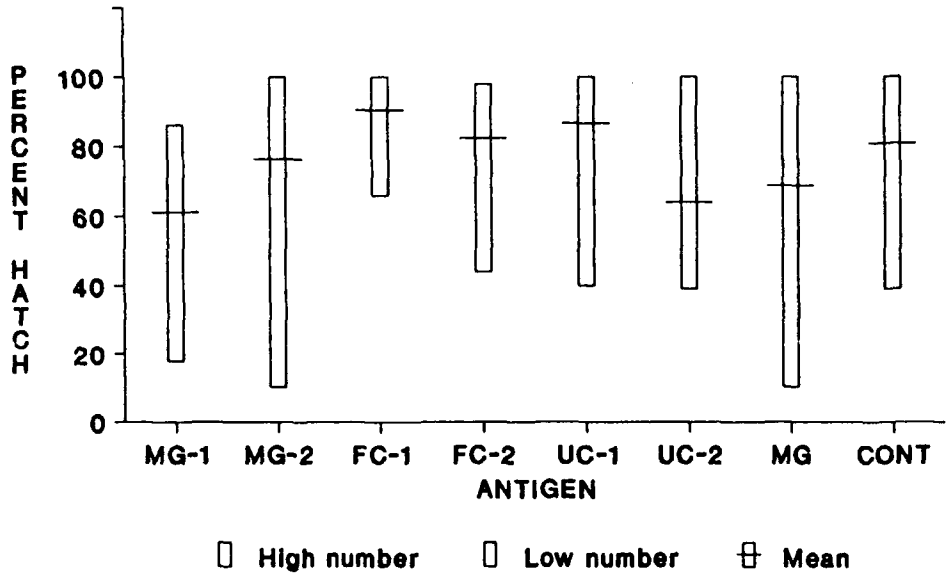


Figure 22

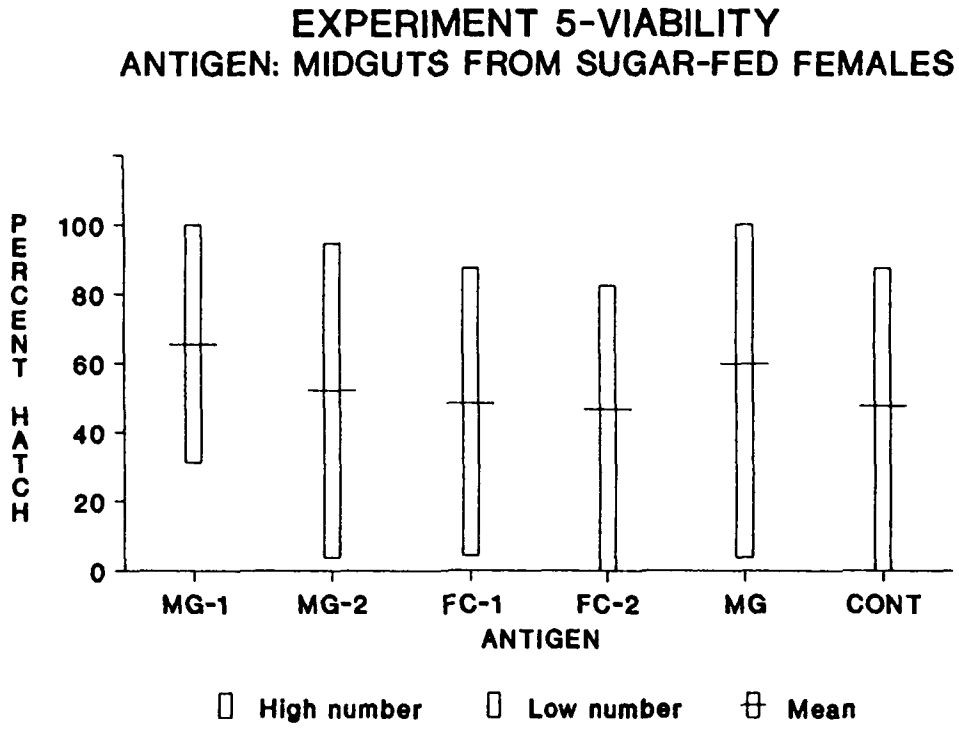

EXPERIMENT 5-VIABILITY
ANTIGEN: MIDGUTS FROM SUGAR-FED FEMALES
PERCENT HATCH
100
80
60
40
20
0
MG-1
MG-2
FC-1
FC-2
MG
CONT
ANTIGEN
High number
Low number
Mean

Figure 23

IV. Discussion

A. Responses of Mice to Immunization

Because the reactivity of sera from mice immunized with mosquito antigen was consistently many magnitudes greater than the very low reactivity displayed by the control sera, we are confident that mosquitoes which fed on immunized mice ingested antibodies to mosquito antigen. The distinct dose-response of antisera/antigen combinations (1:500 versus 1:1000), the consistency of results among replicates of a given antiserum/antigen combination, among repeated ELISAs on sera from the same group of immune mice, and consistency of patterns of reactivity between different antiserum dilutions and between different bleeds of the same mouse all lend credence to our determinations of the immune status of the mice with the ELISA method.

The cross-reactivity of mouse antisera with the various mosquito antigens is not surprising since all antigen preparations were relatively crude and all the mouse antisera were polyclonal.

B. Mosquito Survivorship

The survivorship curves generated by all mosquitoes, regardless of whether they fed upon immune or non-immune mice, were the Type I of Slobodkin (1962) which describes a situation in which mortality acts most heavily on older individuals. Considering the four instances where the survivorship curves for mosquitoes that fed on immune mice decayed at a rate significantly greater than the mosquitoes that fed on non-immune control mice, one might be tempted to conclude that one or more antibody species in host mouse blood had some kind of pathological effect. Or, considering the two cases where the mosquitoes that fed on non-immune control mice decayed at a faster rate than the mosquitoes that fed on immune mice, one might conclude that one or more antibody species in host mouse blood had, in some way, a beneficial effect on the mosquitoes. However, viewing all the survivorship curves together (Table 14) shows that significant differences occurred among the survivorship curves of all control mosquitoes at essentially the same frequency as significant differences occurred among the mosquitoes that fed on immunized mice. On this basis, and the fact that in the majority of cases there were no significant differences between the survivorship curves of mosquitoes that fed on immune versus non-immune mice, we conclude that, within the context of our experiments, mosquitoes obtaining a blood meal from a mouse immunized with mosquito antigens have as good a chance of surviving as mosquitoes that feed on non-immune mice. Our results are in contrast with those of Alger & Cabrera (1972) who describe an increased death rate in <u>Anopheles stephensi</u> after feeding

on rabbits immunized with mosquito midgut homogenate. However, both the mosquito species and vertebrate host species they studied were different than those we studied. Relative to Ae. aegypti results of other investigations vary. Ramasamy et al. (1988) did not observe an increased death rate in mosquitoes fed on immunized rabbits, but Hatfield (1988) showed an increase in daily mortality rates in mosquitoes fed on immunized mice.

C. Mosquito Fecundity and Egg Viability

As shown by Ramasamy et al. (1988) in Ae. aegypti antibodies may somehow pass the midgut epithelium and into the hemocoel and thereby potentially exert an effect on a variety of physiological process. One of these processes could be vitelligenesis. However, our results did not indicate a deleterious effect of antibodies from an immune mouse on the number of eggs deposited (fecundity) or the viability of eggs deposited. Our results are in contrast with other investigators who studied Ae. aegypti, namely Sutherland & Ewen (1974) and Ramasamy et al. (1988) who found decreased fecundity and in the case of Ramasamy and wo-workers, decreased egg viability in response to feeding on an immunized host. But both teams of investigators used rabbits, and Sutherland & Ewen also used guinea pigs, as the host vertebrates, whereas we used mice. It is possible, therefore, that the species of vertebrate host from which a blood meal is taken can affect the results. We used mice because they would have been easily amenable to monoclonal antibody preparation had we obtained positive results. It is also possible that strain differences among colonies of Ae. aegypti could account for differences in results. Different results have been shown to occur when different mosquito species are used (Sutherland & Ewen, 1974).

D. Future Studies

Our experiments and results must be viewed as preliminary and many more studies need to be done. Such additional studies might include the following: (1) Determination of the effects of anti-mosquito-antigen antibodies on various parameters such as survivorship, fecundity, and egg viability as a function of: the 2nd and even later gonotrophic cycles, age, temperature, nutritional state, etc.; (2) Development and testing of polyclonal and monoclonal antibodies directed against vitellogenic eggs and against mosquito cell lines, e.g. Aedes albopictus C6/36 cells; (3) Testing of various species and strains of mosquitoes, using the methods in this study and those studies in #1. and #2. above; (4) Carrying out of similar studies using different vertebrate species, e.g. rabbits, guinea pigs, hamsters, etc.

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VI. Presentation (AMCA paper)

A paper entitled "The effect of vertebrate antibodies directed against mosquito antigens on Aedes aegypti females" was presented to the American Mosquito Control Association in March, 1992 in Corpus Christi, Texas. E. Lucas, M.R. Powell, E.C. Rowland and W.S. Romoser were co-authors.

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