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DEPARTMENT OF MEDICAL ZOOLOGY

U.S. NAVAL MEDICAL RESEARCH UNIT NUMBER 3

U.S.NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND

DEPARTMENT OF DEFENSE

U.S. A.I.D

HORMONES AND PHEROMONES TO CONTROL REPRODUCTION IN TICKS OF MEDICAL IMPORTANCE

Semi-annual Report

April 1988 through 30 September 1988

PASA No.: DPE-5542-P-DZ-7063

Project Number: 936-5542

Period Covered: 04/01/88 - 09/30/88

PASA Agreement Period: 09/30/87 - 09/29/90

DEPARTMENT OF DEFENSE

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Description of Budget Line Items	Current Billing	Cumulative Billing to Date
Salaries	-0-	$ 694.90
Benefits	-0-	-0-
Travel	-0-	22.23
Transportation	-0-	-0-
Supplies & Materials	$ 2,062.16	3,433.57
Other (Services, including IPA)	40,274.81	40,614.81
Overhead	-0-	-0-
Totals	$42,336.97	$44,765.51

Cumulative Funds Authorized in PASA/RSSA through

Amendment Number orginal agreement

Billings For services rendered under this agreement should be billed to: USAID through NMRDC Bethsda (code 1031)

SUMMARY:

The relationships between hormones and sex pheromones produced by ticks for communication during reproductive processes are being investigated. Natural (tick-originated) ecdysteroids and juvenile hormones are to be identified and characterized chemically and biologically. Hormones of known identity are being applied to different developmental stages of ticks to determine whether these treatments disrupt sexual communication. Insect hormones and natural tick hormones are to be tested comparatively by the same biological parameters. The hormone-producing organs and major biosynthetic steps are under investigation.

When natural tick hormones are characterized and their regulatory roles clarified, it may be possible to interrupt or suppress hormone synthesis and production or to apply antagonists to disrupt hormone action on sex pheromone activity. Biological control, using natural tick hormones or proven bioactive analogues, is especially attractive. These compounds may be used as systemics in vertebrate hosts of ticks with little or no effect on the mammals. This study will serve as a useful model for developing pest control strategies employing innovative biological control mechanisms.

SPECIFIC AIMS:

The specific aims of the investigation are to:

1. Determine the nature of interactions between natural tick hormones and sex pheromones, specifically the roles of ecdysteroids and juvenile hormones (JH) in the development and regulation of sex pheromone systems.

2. Identify the chemical nature of tick ecdysteroids, especially those that stimulate the development and activity of sex pheromone glands.

3. Investigate the source and biosynthesis of tick ecdysteroids associated with sex pheromone system(s) as well as compounds that may disrupt their production.

4. Investigate the role of juvenile hormones (JH), using tick-originated JH and/or JH analogues, on the development and activity of sex pheromone system(s).

5. Investigate the role of sex pheromone(s) other than 2,6-dichlorophenol (DCP) and/or allomones in the ixodid tick mating processes.

6. Develop the knowledge of specific tick hormone-pheromone interactions for use in biological control of important tick species.

BENEFITS TO EGYPT:

This project will provide immediate and long lasting benefits to Egypt. The basic research is at the forefront of contemporary scientific investigation. Egyptian personnel will be trained to use the most advanced biochemical and molecular techniques, which they may use in turn to train colleagues at local institutions. Specifically, Egyptian technical personnel will acquire skills in High Performance Liquid Chromatography (HPLC), Gas Chromatography, Radioimmunoassay (RIA) and Enzyme Linked Immunosorbent Assay (ELISA). They will learn biological techniques (e.g., tissue culture) and acquire an understanding of current knowledge in major scientific disciplines related to this project. This investigation, operating with Egyptian consultants and graduate students, will also have a ripple effect, attracting advanced students and faculty for graduate and postgraduate training.

ARTHROPOD CONTROL:

In numerous developing nations, ticks are a major problem affecting public health and livestock production. This research, directed toward tick control using the camel tick, *Hyalomma dromedarii*, as the model species, combines basic research with immediate application of new techniques to control these and other pests.

NEW AND INNOVATIVE CONCEPTS:

This project proposes to control ticks by using hormones or hormone analogues to disrupt the mating process, thereby diminishing reproduction and limiting population growth. The use of pheromones to control arthropod pest populations to prevent mating is not new; the use of hormones or hormone analogues to prevent mating is unique. Hormones may also be administered (as systemics) to the host mammal to minimize tick feeding and development. These molecules can be used without harming livestock. Other innovative techniques include (1) controlled release of tick sex pheromones, so that the sexes do not mate and reproduce, and (2) natural tick repellents.

SCIENTIFIC PROGRESS:

Changes in the total protein concentrations in the hemolymph and ovaries of female Argas (Argas) hermanni Audouin during oogenesis and in males were studied. There was a decline in total protein level in hemolymph of mated ticks for 1 or 2 days after feeding. This decrease was followed by an increase reaching a maximum on day 6 or 5-6 in males and females, respectively. The level of protein in the hemolymph of female ticks was higher than that of the male during the period of vitellogenesis and onset of oviposition (3-6 days after feeding). At the end of oviposition period (day 20), the level of protein in the hemolymph decreased, approaching that of the male. These data suggest increasing demands and utilization of proteins in

the hemolymph of females by the developing oocytes. The increase of the hemolymph proteins in the females coincided with an increase of the ovary protein titer which was linearly correlated to the ovary weight and the number of mature oocytes inside it. This suggests an uptake of hemolymph proteins by the ovaries and/or increasing protein synthesis within the ovaries.

Twenty-two egg vitellins of Ar. hermanni were used as antisera for immuno-electrophoresis assay of vitellogenins and vitellins in the haemolymph and ovaries, respectively. Thirteen fractions were derived from extraovarian vitellogenins, 7 were endogenous vitellins produced in the ovaries and 2 vitellins with relative mobilities (M_r) of 35.5 and 47.2 k D existed only in the deposited eggs. Almost all exogenous vitellins and their haemolymph counterparts (vitellogenins) were haemoglycolipoproteins with relatively low M_r of 66.2 - > 200 k D. Endogenous vitellins had higher M_r of 22-59 k D and variable chemical nature. The haemolymph of the female had more protein fractions (22-24) than that of the male (15). All proteins in the male had corresponding fractions in the female haemolymph with similar electrophoretic mobilities and 5 fractions were immunologically identical common vitellogenins. Eight female specific vitellogenins were restricted to the female haemolymph and 2 of them had high M_r of 14.4 and 16.5 k D appeared at the onset of oviposition. Ovaries had 28 protein fractions which showed great fluctuations in number and percent concentration at the different physiological states studied. Five to 13 were exogenous

vitellins, 6 appeared during vitellogenesis and onset of oviposition. One to 7 endogenous vitellins were detected in the female ovaries mostly during the period prior and up to vitellogenesis initiation. The significance of the obtained results were discussed.

The ribosomes from Argas (Persicargas) arboreus eggs were isolated and their ability to initiate protein synthesis in a cell-free system was investigated. A greater proportion of polymeric forms was detected in the ribosome fractions at late embryogenesis. Synthesis of RNA, ribosomal RNA, and ribosomal protein occurred during embryogenesis. The ratio of rRNA to rprotein was 1:1.02-1.15, of purines to pyrimidines was 1:1.06-1.10, and of 6-keto groups to 6 amino groups was 1:1. Twenty one different ribosomal proteins were separated by disc electrophoresis. The requirements for an amino acid-incorporating system from A. arboreus eggs were established. The pH 5 enzymes prepared from the tick eggs successfully supported the incorporation of C^{14} leucine by isolated ribosomes from A. arboreus eggs. RNase but not DNase inhibited the protein activity. The functioning of the A. arboreus ribosomes requires Mg^{++}, GTP and/or ATP.

Bioassays performed on Ornithodoros (Ornithodoros) savignyi (Audouin) demonstrated the presence of a female sex pheromone. Unfed and fed adults, after various postfeeding periods, were cross-tested. Two independent criteria were used: the percentage of positive male responses and the time elapsed before

these responses. Females were least attractive when unfed and on the feeding day. Their attractiveness increased afterwards reaching the highest values 6 days postfeeding (confirmed attractive, CA, females) then decreased gradually thereafter. Males were least responsive during the first 2 postfeeding days and became more responsive afterwards reaching a peak 8 days postfeeding (sexually active, SA, males). Unfed male responses to CA females were strong but were lower than those of SA males. The fastest responses in all bioassays were those of SA males to CA females. The maximum range of SA male response to CA females was ca 9 cm. SA males were not attracted to nymphs, CA females with sealed coxal orifice or dead CA females. However, they responded to nymphs coated with coxal fluid collected from CA females. Nymphs and females were not attracted to CA females.

Changes in DNA, RNA, total lipids, phospholipids and proteins and the activity of certain enzymes and pattern of their isozymes were studied during Hyalomma (Hyalomma) dromedarii Koch embryogenesis (20 incubation days). Total proteins fluctuated with a net increase on day 20 and total lipids increased during the first half of embryogenesis and then declined with a net decrease to ca 3/5 of the original level. Total phospholipds decreased to 1/2 its original level. DNA content increased greatly during cleavage, gastrolation, and organogeny. RNA increased during cleavage and blastula formation. Glucose-6-phosphate dehydrogenase (G6PD) activity decreased reaching 1/5 its original level on day 12 with no further change thereafter.

Malic acid dehydrogenase (MDH) activity remained nearly unchanged throughout embryogenesis. Lactic acid dehydrogenase (LDH) activity increased greatly with the highest level on day 14 with no further change. Acetyl choline esterase (ACHE) activity increased greatly during the first 12 days with no significant change thereafter. Acid (ACP) and alkaline phosphatase (ALP) activity increased during the first 12 days and declined returning to the original level on day 20. Seven ACHE and 5 LDH isozymes were detected during different embryogenesis stages. Two MDH, 2 ACP, 1 ALP and 2 G6PD isozymes were detected throughout embryogenesis. Treating the mother with 20-hydroxyecdysone was associated with different degrees of increase in protein and RNA contents and G6PD and ACHE activity, and with a decrease in ACP and ALP activity in the embryos.

PUBLICATIONS:

Marzouk, A.S., G.M. Khalil & Z.E.A. Darwish. 1987. Neurosecretory cell types and distribution in unfed female *Hyalomma dromedarii* (Acari: Ixodoidea: Ixodidae) synganglion. J. Egypt. Soc. Para. 17: 41-61

Marzouk, A.S., M.K. Abdel Moez & Z.E.A. Darwish. 1987. Effect of feeding and mating on the neurosecretory activity in female *Hyalomma dromedarii* (Acari: Ixodoidea: Ixodidae). I. Changes in neurosecretory cell types in semifed virgin and mated females. J. Egypt. Soc. Parasit. 17: 547-570

Marzouk, A.S., G.M. Khalil, M. El Tunsy & Z.E.A. Darwish. 1987. Effect of feeding and mating on the neurosecretory activity in female Hyalomma dromedarii (Acari: Ixodoidea: Ixodidae). II. Changes in neurosecretory cell types in fully engorged females. J. Egypt. Soc. Parasit. 17: 657-669

Marzouk, A.S., G.M. Khalil, F.S.A. Mohamed & N. Farid. 1987. Hyalomma dromedarii (Acari: Ixodoidea: Ixodidae): Central and peripheral nervous system anatomy. Exp. Appl. Acarol. 3: 145-161

Helmy, N. 1988. Neuroendocrine reproduction interrelationships in the female tick Argas (Argas) hermanni Audouin. PhD Thesis, Faculty of Science, Ain Shams University

Marzouk, A. S. 1988. The effect of 20-hydroxyecdysone on the neurohemal endocrine organs in Hyalomma dromedarii Koch (Ixodoidea: Ixodidae). J. Egypt. Soc. Parasit. 18: 471-480

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Gadallah, A.I., M.A. Roshdy, A.S. Marzouk & A.J. Main. Egg ribosomes of Argas (Persicargas) arboreus (Acari: Argasidae). J. Med. Ent. (accepted)

Gadallah, A.I., G.M. Khalil, W.H. Dees, M.A. Roshdy, A.S. Marzouk & A.J. Main. Biochemical effects of juvenile hormone III on the tick, Argas arboreus (Acari: Argasidae) during embryogenesis. J. Med. Ent. (accepted)

Gadallah, A.I., W.H. Dees, D.E. Sonenshine, M.A. Roshdy, A.S. Marzouk & A.J. Main. Biochemical effect of 20-HO-ecdysone on embryogenesis in the camel tick, Hyalomma dromedarii Koch (Acari: Ixodidae). J. Med. Ent. (submitted)

Gadallah, A.I., G.M. Khalil, W.H. Dees, A.S. Marzouk, H.A. Hanafi & A.J. Main. Effect of 20-hydroxyecdysone on some isozymes in the camel tick, Hyalomma dromedarii Koch (Acari: Ixodidae). J. Med. Ent. (submitted)

Mohamed, F.S.A, Khalil, G.M., A.S. Marzouk & M.A. Roshdy. Sex pheromone regulation of mating behavior in the tick Ornithodoros (Ornithodoros) savignyi (Audouin) (Acari: Argasidae). J. Med. Ent. (submitted)

Shanbaky, N.M., M. Mansour, A.J. Main & A. El Said. Vitellogenic and non-vitellogenic proteins in hemolymph, ovaries and egg of the argasid tick, Argas (Argas) hermanni Audouin (Acari: Argasidae). J. Med. Ent. (submitted)

Shanbaky, N.M., M. Mansour, A.J. Main & N. Helmy. Changes in total hemolymph and ovary proteins during oogenesis in Argas (Argas) hermanni Audouin (Acari: Argasidae). J. Insect Physiol. (submitted)

Gadallah, A.I., G.M. Khalil, W.H. Dees, M.A. Roshdy, A.S. Marzouk, D.E. Sonenshine, H.A. Hanafi & A.J. Main. Biochemical changes in Hyalomma (Hyalomma) dromedarii Koch (Acari: Ixodidae) embryos and effect of 20-Hydroecdysone applied to the mother. J. Med. Ent. (submitted)

Shanbaky, N.M., N. Helmy, M. Mansour & A. El Said. Effect of feeding on neurosecretory cell activity in female Argas (Argas) hermanni Audouin (Acari: Argasidae). (in preparation)

Shanbaky, N.M., N. Helmy, M. Mansour & A. El Said. Effect of feeding on hormonal control of vitellogenesis in Argas (Argas) hermanni Audouin (Acari: Argasidae). (in preparation)

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