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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

5.065

U.S. A.I.D

HORMONES AND PHEROMONES TO CONTROL REPRODUCTION IN TICKS OF MEDICAL IMPORTANCE

Semi-annual Report

1 October 1988 through 31 March 1989

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

Project Number: 936-5542

Period Covered: 10/01/88 - 03/31/89

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

DEPARTMENT OF DEFENSE

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Fiscal Data: PIO/T Oblig. No.: 7361080

Appropriation No.: 72-1171021.6

Allotment No.: DDSA-87-10600-KG11 (746-36-099-00-19-71)

Description of Budget Line Items	Current Billing	Cumulative Billing to Date
Salaries	$ 78,288.84	$ 116,254.84
Benefits		
Travel		
Transportation		
Supplies & Materials	$ 1,586.29	$ 3,648.45
Other (Services)	$ 1,528.33	$ 3,837.14
Overhead		
Totals	$ 81,403.46	$12[illegible],740.43

Cumulative Funds Authorized in PASA/RSSA through

Amendment Number original agreement

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

Rec'd in SCI JUN 2 2 1989

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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 activity of neurosecretory cells in the synganglion of mated female Argas (Argas) hermanni Audouin at different physiological states were studied. The number of neurosecretory cells, their size, and quantity of neurosecretions increased one hour after feeding, reached a peak on days two and three, and then declined. The decreases were greatest on day four but continued to decline gradually on the following days reaching levels similar to those in the unfed females by day 20. The neurosecretory cell changes were observed in all the synganglonic regions particularly those in protocerebral, pedal, and opisthosomal centers. The increase in the neurosecretory cell number, size, and neurosecretions suggests increased

production and storage of neurosecretions. The subsequent decrease suggests release of neurosecretions. The decline in neurosecretory cell parameters coincided with the period of vitellogenesis and oviposition initiation (days three to six after feeding) and termination (day 20 after feeding).

In mated female A. hermanni, a gonadotropic hormone is synthesized in the synganglion within two days after feeding and afterward is released into the hemolymph. Isolating the ovary from the synganglion by ligation during the period of hormone synthesis interfered with oocyte maturation. Injecting an extract of active synganglia from mated females on day two after feeding or hemolymph from mated females on day four after feeding into mated recently fed females induced oocyte maturation in their isolated ovaries. In the posterior body region isolated from the synganglion by ligation within one hour after feeding, the total protein level and protein fractions including hemolymph vitellogenins and ovary vitellins remained unchanged during vitellogenesis. The number, chemical nature, relative mobility and relative amounts of the protein fractions remained similar to those in the corresponding tissue in females one hour after feeding. These findings suggest that the lack of synganglion gonadotropic hormone prevents vitellogenesis and oocyte maturation by precluding protein production including preformed and new vitellogenins or their release into the hemolymph and by impeding endogenous vitellin production inside the ovary. The effect of the lack of the hormone on

obstructing the uptake of hemolymph vitellogenins by the ovary is not excluded as a factor preventing vitellogenesis and oocyte maturation.

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

Khalil, G.M., A.S. Marzouk & Z.E.A. Darwish. 1988. The effect of 20-hydroxyecdysone on the neurosecretory cell activity in female Hyalomma dromedarii Koch synganglion (Acari: Ixodidae). Exp. Appl. Acarol. 5: 101-119

Hagras, A.E. & G.M. Khalil. 1988. Effect of temperature on Hyalomma (Hyalomma) dromedarii Koch (Acari: Ixodidae). J. Med. Ent. 25: 354-359

Roshdy, M.A., L.B. Coons, A.S. Marzouk, W.H. Dees & H.Y. Wassef. 1988. Argas (Microargas) transversus Banks (Acari: Argasidae): spiracle surface morphology and structure. J. Med. Ent. 26: 37-40

Khalil, G.M. & A.E. Hagras. 1989. Effects of temperature on Hyalomma (Hyalomma) impeltatum Schulze and Schlottke (Ixodoidea: Ixodidae). Qatar Univ. Sci. Bull. (in press)

Hagras, A.H. & G.M. Khalil. 1989. Effect of unfed female weight on the biology of Hyalomma (Hyalomma) impeltatum Schulze and Schlottke (Acari: Ixodidae). Qatar Univ. Sci. Bull. (in press)

Marzouk, A.S., M.A. Roshdy, M. Osman & A.J. Main. 1989. Vaginal evagination in the bat tick Argas (Chiropterargas) boueti Roubaud and Colas-Belcour (Acari: Argasidae). J. Med. Ent. 26: (in press)

Gadallah, A.I., M.A. Roshdy, A.S. Marzouk & A.J. Main. 1989. Egg ribosomes of Argas (Persicargas) arboreus (Acari: Argasidae). J. Med. Ent. 26: (in press)

Gadallah, A.I., G.M. Khalil, W.H. Dees, M.A. Roshdy, A.S. Marzouk & A.J. Main. 1989. Biochemical effects of juvenile hormone III on the tick, Argas arboreus (Acari: Argasidae) during embryogenesis. J. Med. Ent. 26: (in press)

Mohamed, F.S.A, Khalil, G.M., A.S. Marzouk & M.A. Roshdy. 1989. Sex pheromone regulation of mating behavior in the tick

Ornithodoros (Ornithodoros) savignyi (Audouin) (Acari: Argasidae). J. Med. Ent. 26: (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)

Khalil, G.M. & A.H. Hagras. Effect of unfed female weight on the biology of Hyalomma (Hyalomma) dromedarii Koch (Acari: Ixodidae). J. Egypt. Soc. Parasitol. (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)

Shanbaky, N.M., M. Mansour, A.J. Main, A. El Said & N. Helmy. Hormonal control of vitellogenesis in Argas (Argas) hermanni Audouin (Acari: Argasidae). 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). J. Med. Ent. (submitted)

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

Khalil, G.M., M.A. Roshdy & A.S. Marzouk. Morphological studies of Argas (Argas) hermanni (Acari: Argasidae): Coxal organ anatomy and histology. (in preparation)

PERSONNEL, INSTITUTIONS, AND COLLABORATING LABORATORIES:

Principal Investigator:

Dr. Andrew J. Main,

Medical Zoology Department, NAMRU-3, Cairo, Egypt

Consultants, On-site participants:

Dr. Ahmed I. Gadallah

Plant Protection Department, Faculty of Agriculture,

Al Azhar University, Cairo, Egypt

Dr. Aleya S. Marzouk

Zoology Department, Faculty of Science

Ain Shams University, Cairo, Egypt

Dr. Mohamed A. Roshdy

Head, Zoology Department, Faculty of Science

Ain Shams University, Cairo, Egypt

Deceased: 7 April 1989

Dr. Nadia Helmy

Entomology Department, Faculty of Science

Ain Shams University, Cairo, Egypt

Dr. Galila Khalil

Head, Zoology Department, Faculty of Science

Qatar University, Doha, Qatar

Consultants (unsalaried), Off-site:

Dr. Daniel E. Sonenshine

Department of Biological Sciences, Old Dominion Universit

Norfolk, Virginia, USA

Dr. Paul J. Homsher

Department of Biological Sciences, Old Dominion Universit

Norfolk, Virginia, USA

Dr. James H. Oliver, Jr.

Department of Biology, Georgia Southern College

Statesboro, Georgia, USA

Guest Investigators (unsalaried)

Dr. Wegdan Bassiouni

Department Zoology, Faculty of Science

Cairo University, Cairo, Egypt

Ms. Saher Ibrahim

Zoology Department, Faculty of Science

Benha, University, Benha

Ms. Zakia Ashmawy

Zoology Department, Faculty of Science

Ain Shams University, Cairo, Egypt

Ms. Fatma Mohamed

Zoology Department, Faculty of Science

Ain Shams University, Cairo, Egypt