PN-ABA-533

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 6/10/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 5.065

semi-ann - 30 Sept 87 - 31 Mar 88

rec'd 6/10/88

Attachment

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5.065

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

30 September 1987 through 31 March 1988

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

Project Number: 936-5542

Period Covered: 09/30/87 - 03/31/88

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

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

Project Name: Hormones and pheromones to control reproduction in ticks of medical importance

Project Number: 936-5542

Period Covered: 10/01/87 - 03/31/88

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

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	694.90	694.90
Benefits	-0-	-0-
Travel (Field Trip)	22.23	22.23
Transportation	-0-	-0-
Supplies & Materials	1,371.41	1,371.41
Other (Minor Equipment)	340.00	340.00
Overhead	-0-	-0-
Totals	$ 2,428.54	$ 2,428.54

Cumulative Funds Authorized in PASA/RSSA through

Amendment Number original

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 is 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:

The effects of 20-hydroecdysone (20-HE) on the lateral segmental organs and on the neurohemal organs were observed in the emerging unfed, semifed and fully engorged female *Hyalomma dromedarii* and compared with those in untreated ticks. The effect induced by 20-HE included (a) The lateral segmented organs in each group of treated female were comparatively larger than that in the untreated ones, and (b) the dorsal lobes of the neurohemal organs were comparatively smaller. The results suggest that the lateral segmented organs play a role in ecdysone production.

The effects of 20-HE on neurosecretory cell activity in adult female *Hy. dromedarii* was investigated. In emerging adult females, some neurosecretory cells (NSC) in certain synganglion centers exhibited changes in size and/or neurosecretory material (NSM) shape, distribution and/or quantity. These changes were compared with those normally occurring in untreated unfed, semifed virgin and mated, and engorged females. 20-HE effects included (a) accelerating the changes induced by mating and/or feeding in certain NSC, (b) reducing, to various extents, NSC

response to mating and feeding, and (c) inducing changes in some NSC which do not normally exhibit any changes in untreated females. The results suggest that (a) most female NSC respond more or less similarly to indigenous 20-HE, (b) 20-HE may have a role as a positive feedback regulator for NSM synthesis and/or release by certain NSC, (c) the response to 20-HE may be primarily a function of NSC location in the synganglion, and/or (d) NSC considered to be of one type may actually belong to different cell types.

The biochemical effects of 20-HE on embryogenesis were examined in Hy. dromedarii. The exogenous ecdysteroid stimulated significant increases ($p < 0.05$) in protein and RNA synthesis. Glucose 6-phosphate dehydrogenase increased and alkaline phosphatase decreased significantly ($p < 0.05$) in activity in eggs from 20-OH-ecdysone treated ticks. Only a slight increase (1-3%) in DNA synthesis occurred in these eggs. No observable differences were detected in total lipid and phospholipid concentrations, malate dehydrogenase, lactic dehydrogenase, acid phosphatase and acetylcholinesterase between eggs from treated and control ticks. Stimulation of protein and RNA synthesis in eggs from treated ticks suggests possible exogenous ecdysteroid conjugation and release during oogenesis and early embryogenesis, respectively.

The effects of 20-HE on some isozymes were examined in Hy. dromedarii. Eggs were collected 0-20 days postoviposition and the changes in isozyme patterns of acetylcholinesterase, lactic

acid dehydrogenase, malate acid dehydrogenase, acid phosphatase, alkaline phosphatase and glucose 6-phosphate dehydrogenase were studied in eggs deposited by 20-HE treated and control females. There were no detectable differences in acetylocholinesterase, lactic acid dehydrogenase, malate acid dehydrogenase, acid phosphatase, and alkaline phosphatase isozyme patterns between eggs from untreated and treated females. Glucose 6-phosphate dehydrogenase shows differences in isoenzyme mobility ratio. The development of the 7 and 5 multiple forms of esterases and lactic acid dehydrogenase respectively was evident during late embryogenesis. No change in malate acid dehydrogenase, glucose 6-dehydrogenase, acid phosphatase and alkaline phosphate isozyme patterns occurred during embryogenesis.

The biochemical effects on developing oviposited eggs from juvenile hormone III (JH III) treated mated females were studied in Argas arboreus. The exogenous JH III caused a decrease in total proteins ($P<0.001$) only during the first 2 days of embryogenesis. No significant difference ($P>0.05$) between RNA and DNA contents from control and JH-eggs. No significant differences ($P>0.05$) were observed between control and JH eggs in their lipid or phospholipid contents throughout embryogenesis. A total of 14-17 protein bands and 6-8 glycoprotein bands was separated by electrophoresis during embryogenesis of A. arboreus with some differences in mobility ratio between bands from control and JH eggs. Change in activity and isozyme patterns of esterases, malate acid, lactic acid, glucose 6-phosphate dehydrogenase and

acid phosphatase, alkaline phosphatase were studied during embryogenesis of control and JH treated A. arboreus. No differences were detected in either the activity nor the isozymes studied between eggs from treated and control mated females A. arboreus.

PUBLICATIONS:

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. 4: (in press)

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

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)

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. (submitted)

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

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