PN-AB6-709
69242

DEPARTMENT OF MEDICAL ZOOLOGY
U.S. NAVAL MEDICAL RESEARCH UNIT NUMBER 3
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

Final Report

1 October 1987 through 30 September 1990

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

Project Number: 936-5542

Period Covered: 09/30/87 - 09/29/90

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

DEPARTMENT OF DEFENSE

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: 09/30/87 - 09/29/90

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

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

Appropriation No.: 72-1171021.6

Allotment No.: DDSA-87-1060G-KG11 (746-36-099-00-19-71)

Description of Budget Line Items	Current Billing	Cumulative Billing to Date
Salaries	694.90	116,949.74
Benefits		
Travel	22.23	22.23
Transportation		
Supplies & Materials	2,848.84	6,497.29
Other (Services)	340.00	4,177.14
Overhead		
Totals	3,905.97	127,646.40

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 Bethesda (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:

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 the hemolymph of males were studied. There was a decline in the total protein level in hemolymph of mated ticks for 1 to 2 days after feeding. This decrease was followed by an increase reaching a maximum on day 5-6 after feeding in both males and females. Protein levels in the hemolymph of female ticks during the period of vitellogenesis and onset of oviposition (3-6 days after feeding) was higher than that of the

male. At the end of the oviposition period (day 20), the protein level in the hemolymph decreased, approaching that of the male. These data suggest an increase in the demand 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 level, 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 A. hermanni were used to make antisera for immunoelectrophoretic analysis of vitellogenins and vitellins in the hemolymph and ovaries, respectively. Thirteen fractions were derived from extraovarian vitellogenins, seven were endogenous vitellins produced in the ovaries and two 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 hemolymph counterparts (vitellogenins) were haemoglycolipo-proteins with a relatively low M_r of 66.2 - > 200 k D. Endogenous vitellins exhibited a higher M_r of 22-59 k D and were of a more variable chemical nature. The hemolymph 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 hemolymph with similar electrophoretic mobilities and five common vitellogenins were immunologically identical. Eight female-specific vitellogenins were restricted to the hemolymph and two of those with high M_r of 14.4 and 16.5 k D, appeared at the onset of oviposition. Ovaries contained 28 protein fractions which exhibited great fluctuations in their number and concentration during the different physiological states examined. Five to 13 fractions were exogenous vitellins and six appeared during vitellogenesis and the onset of oviposition. One to seven endogenous vitellins were detected in the ovaries primarily during the period just prior to the initiation of vitellogenesis. The significance of these results are discussed.

Changes in the activity of neurosecretory cells in the synganglion of mated female A. 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.

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.

The biochemical effects on developing oviposited eggs from juvenile hormone III (JH III) treated mated females were studied in A. 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.

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.

The proteins and nucleic acids from the ovaries of Hyalomma (Hyalomma) dromedarii Koch were determined during different stages of oogenesis. They were separated by methylated albumin-Kielseguhr column chromatography and sucrose-density-gradient centrifugation. The concentrations of total protein, DNA and RNA increased during oogenesis. The peaks for protein and DNA were reached in the fully-fed females and slightly earlier for RNA. The ribosomal RNA (rRNA) is composed of 27.0s, 17.0s, and 4.1s particles. The nucleotides of total RNA and rRNA were determined and the ratio of purine:purimidine was close to 1 and remained constant during oogenesis.

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.

The biochemical effects of 20-hydroecdysone (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 effects of exogenous 20-HE on protein contents during H. dromedarii Koch oogenesis development were studied. Ovaries from 20-hydroxyecdysone treated female ticks exhibited a significant

increase in protein concentration. The amounts of protein from ovaries of treated ticks ranged between 27.2 ± 1.15 mg proteins/g fresh ovary weight for unfed females on the day of molting to 187.8 ± 3.62 mg proteins/g fresh ovary weight for fully-fed females. Protein content of ovaries from untreated 20-hydroxyecdysone ticks ranged between 22.4 ± 25 mg proteins/g fresh weight for unfed females on the day of molting to 147.2 ± 1.4 mg for fully-fed females.

Topical application of 20-HE to female ticks influenced the level of DNA content during oogenesis of H. dromedarii Koch. There was a significant difference in DNA contents of ovaries from treated ticks on the day of molting, 7 days after molting, semi-fed and fully-fed females. DNA concentrations from ovaries of treated females ranged between 13.4 ± 1.36 to 44.6 ± 1.43 ug/g weight while DNA from ovaries of untreated females ranged between 11.8 ± 1.06 to 43.0 ± 2.88 ug/g female weight.

The effects of exogenous 20-HE on RNA level during oogenesis of H. dromedarii Koch were examined. Ovaries from 20-HE treated female ticks exhibited significant increase in RNA concentration. RNA contents in ovaries of treated females followed the same pattern of RNA content of ovaries of the untreated ticks, but with higher concentrations. The amount of RNA found of ovaries from treated females ranged between 29.8 ± 2.39 - 222.2 ± 3.18 ug/g female weight and 28.0 ± 1.41 - 167.6 ± 3.61 ug/g female weight for ovaries of untreated females.

Overall lipid concentrations in ovaries from treated and untreated ticks were significantly different. Significant changes were associated with the individual physiological stage of the ovaries.

Effects of 20-HE on protein synthesis of salivary glands of adult females during different development stages of H. dromedarii were examined. No change was observed in the total protein concentrations of salivary glands from treated female ticks except for those glands from semi-fed and fully-fed females which exhibited a significant increase.

DNA content was not significantly different although 2 different developmental stages (semi-fed and fully-fed females) showed significant increases in DNA with the greatest concentration of DNA occurring in salivary glands of the fully-fed females. A great decrease was observed in DNA content of the salivary glands of the ovaries of ovipositing stages.

Topical application of 20-HE significantly influenced RNA concentrations in salivary glands of treated female ticks. The greatest amount of RNA was found in the salivary glands of fully-fed females (58.6 ± 3.25 ug RNA/g salivary glands for 20-hydroxyecdysone treated and 49.72 ± 3.54 for the control). These

amounts were decreased to 50.16% and 57.1% in the ovipositing stages for the treated and untreated ticks, respectively.

The effect of 20-HE on the lipid content of salivary glands in adult female of H. dromedarii was studied. No significant changes occurred in the lipid content of salivary glands of treated ticks on days 0, 3, and 5 post-molting compared with untreated control. However, topical application of 20-HE significantly effected the lipid content of salivary glands at 7 days after molting and of semi-fed, fully-fed and ovipositing females. The amount of lipids increased in salivary glands from both treated and untreated with maximum levels being reached in fully-fed adults. A significant reduction in the lipid content occurred during the ovipositing stage.

Biochemical effect of 20-HE on ovaries and salivary glands of the camel ticks, H. dromedarii Koch were studied. The exogenous ecdysteroid stimulated significant increase ($P < 0.001$) in protein, DNA and RNA synthesis in ovaries of treated female ticks. Lipid were also significantly different ($P < 0.005$). Only salivary glands from 20-HE treated semi-fed and fully-fed females exited a significant increase in protein and DNA concentrations ($P < 0.05$). 20-HE influenced significantly ($P < 0.05$) in RNA of salivary glands of female ticks in all physiological stages. Topically application of 20-HE significantly ($P < 0.05$) effect lipid content of salivary glands of female ticks at 7 dm, semi-fed, fully-fed and ovipositing females.

The hemolymph proteins of normal and 20-HE of H. dromedarii females were separated electrophoretically into 21 bands. Three hemoglycolipoprotein bands were detected in the normal and treated females with different mr and there were 6 hemoglycoprotein bands in the normal female hemolymph, while only 2 hemoglycoproteins bands were observed in the treated female hemolymph, this might represent the breakdown products of preformed vitellogenins when the females treated with 20-HE. The glycoprotein was found in 12 bands of the normal female hemolymph of H. dromedarii, while it was found in 16 bands of the treated female hemolymph. Considerable interest is naturally aroused by the part which glycoproteins play in the process of morphogenesis and differentiation occurring in insects at the key stages of their development, in particular those regulated by 20-HE.

No significant differences were detected in MDH, LDH, Acp and ALP activity between hemolymph of normal of 20-HE treated females H. dromedarii ticks. However, AchE and G 6pDH increased in treated female hemolymph than that of the normal female hemolymph. Also, there are differences in densities of the bands of the esterase isozyme and great difference in mobility between G 6pDH isozymic bands from normal and 20-HE treated females hemolymph. It was noticed that esterases activity increased

throughout the developmental stages.

Ecdysteroids (20-HE) may be function in controlling H. dromedarii tick ovarian development, vitellogenin synthesis and vitellogenesis.

The effects of 20-HE on the lateral segmental organs and on the neurohemal organs was observed in the emerging unfed, semifed and fully engorged female H. 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 H. 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.

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

Khalil, G.M. & A.E. Hagras. 1988. Effects of temperature on Hyalomma (Hyalomma) impeltatum Schulze and Schlottke (Ixodoidea: Ixodidae). Qatar Univ. Sci. Bull. 8: 187-204

Roshdy, M.A., L.B. Coons, A.S. Marzouk, W.H. Dees & H.Y. Wassef. 1989. Argas (Microargas) transversus Banks (Acari: Argasidae): spiracle surface morphology and internal structure. J. Med. Ent. 26: 37-40

Marzouk, A.S., M.A. Roshdy, M. Osman & A.J. Main. 1989. Vaginal eversion in a bat tick Argas (Chiropterargas) boueti Roubaud

and Colas-Belcour (Acari: Argasidae). J. Med. Ent. 26: 349-353

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 (Persicargas) arboreus (Acari: Argasidae) during embryogenesis. J. Med. Ent. 26: 360-367

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: 414-420

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)

Al Thani, H.Y.J. 1989. Developmental Morphology of the Salivary Glands in Argas (Argas) hermanni (Ixodoidea: Argasidae). MSc Thesis, Ain Shams University

Gadallah, A.I. 1990. Biochemical effects of 20 hydroxyecdysone on Hyalomma (Hyalomma) dromedarii ticks. BOSTID/AID Wrkshp. (April, Nairobi) (abstract)

Abou El-Khair, S.M. 1990. Anatomy and histology of certain internal body systems of the ticks Ornithodoros (Ornithodoros) savignyi (Audouin) (Ixodoidea: Argasidae). MSc Thesis, Faculty of Science, Zagazig University

Mohamed, F.E-S.A. 1990. Study on the pheromonal sites in the tick Ornithodoros savignyi (Audouin) (Ixodoidea: Argasidae). PhD Thesis, Faculty of Science, Al Azhar Girls University

Darwish, Z.E.A. 1990. Effect of (20-hydroxy) ecdysone on the internal tissues of the tick Hyalomma (H.) dromedarii Koch (Ixodoidea: Ixodidae). PhD Thesis, Faculty of Science, Al Azhar Girls University

Khalil, G.M. & A.H. Hagras. 1990. Effect of unfed female weight on the biology of Hyalomma (Hyalomma) dromedarii Koch (Acari: Ixodidae). J. Egypt. Soc. Parasitol. 20: 69-83

Mohamed, F.S.A, Khalil, G.M., A.S. Marzouk & M.A. Roshdy. 1990. Sex pheromone regulation of mating behavior in the tick Ornithodoros (Ornithodoros) savignyi (Audouin) (Acari: Argasidae). J. Med. Ent. 27: 288-294

Shanbaky, N.M., A.J. Main, A. El Said & N. Helmy. 1990. Hormonal control of vitellogenesis in Argas (Argas) hermanni (Acari: Argasidae). J. Med. Ent. 27: (in press)

Gadallah, A.I., G.M. Khalil, W.H. Dees, M.A. Roshdy, A.S. Marzouk & D.E. Sonenshine. 1990. Biochemical changes in Hyalomma (Hyalomma) dromedarii (Acari: Ixodidae) embryos and effect of 20-hydroxyecdysone applied to the mother. J. Med. Ent. 27: (in press)

Shanbaky, N.M., M. Mansour & N. Helmy. 1990. Changes in total hemolymph and ovary proteins during oogenesis in Argas (Argas) hermanni (Acari: Argasidae). J. Med. Ent. 27: (in press)

Shanbaky, N.M., M. Mansour, A. El Said & N. Helmy. 1990. Changes in neurosecretory cell activity in female Argas (Argas) hermanni (Acari: Argasidae). J. Med. Ent. 27: (in press)

Shanbaky, N.M., M. Mansour, A.J. Main & N. Helmy. 1990. Vitellogenic and non-vitellogenic proteins in hemolymph, ovaries and egg of the argasid tick, Argas (Argas) hermanni (Acari: Argasidae). J. Med. Ent. 27: (in press)

Gadallah, A.I., G.M. Khalil, A.J. Main & A.S. Marzouk. Nucleic acid levels in the ovaries of Hyalomma (Hyalomma) dromedarii (Acari: Ixodidae). J. Med. Ent. (in press)

Bassiouni, W.M. Histological study in the salivary glands of the unfed female Argas (Chiropterargas) boueti (Ixodoidea: Argasidae). New Egypt. J. Med. (in press)

Darwish, Z.E.A., A.I. Gadallah, A.S. Marzouk & A.J. Main. Biochemical effect of 20-hydroxyecdysone on salivary glands and ovaries of the tick Hyalomma (Hyalomma) dromedarii (Acari: Ixodidae). (submitted)

Helmy, N, A.I. Gadallah, A.J. Main & A.S. Marzouk. Effect of 20-hydroxyecdysone on hemolymph proteins of the female camel tick, Hyalomma dromedarii (Acari: Ixodidae). (submitted)

Gadallah, A.I., G.M. Khalil & A.S. Marzouk. Effect of juvenile hormone III on proteins in the tick Argas (Persicargas) arboreus (Acari: Ixodidae). (in preparation)

PERSONNEL, INSTITUTIONS, AND COLLABORATING LABORATORIES:

Principal Investigator:

Dr. Michael E. Kilpatrick, CAPT, MC, USN
Commanding Officer, NAMRU-3, Cairo, Egypt

Consultants, On-site participants:

Dr. Andrew J. Main,
Head, Medical Zoology Division, NAMRU-3, Cairo, Egypt

Dr. Ahmed I. Gadallah
Head, Plant Protection Department, Faculty of Agriculture,
Al Azhar University, Cairo, Egypt

Dr. Mohamed Roshdy (died 6 April 1989)
Head, Zoology Department, Faculty of Science
Ain Shams University, Cairo, Egypt

Dr. Aleya S. Marzouk
Zoology Department, Faculty of Science
Ain Shams University, Cairo, Egypt

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 University
Norfolk, Virginia, USA

Dr. Paul J. Homsher
Department of Biological Sciences, Old Dominion University
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. Hessa Y. J. J. Al Thani
Zoology Department, Faculty of Science
Ain Shams University, Cairo, Egypt

Ms. Saher Ibrahim
Zoology Department, Faculty of Science
Benha, University, Benha

Ms. Zakia Ashmawy
Zoology Department, Faculty of Science
Al Azhar University, Cairo, Egypt

Ms. Fatma Mohamed
Zoology Department, Faculty of Science
Al Azhar University, Cairo, Egypt

Mr. Mohamed Elbassal,
Department Zoology, Faculty of Science
Cairo University, Cairo, Egypt