MOLECULAR ENTOMOLOGY

John Law, Organizer

March 20 — April 6, 1986

Plenary Sessions

Poster Sessions

Late Additions

Olfaction and Gustation in Insects

K0 CHEMICAL DETERRANCE OF PLANTS, Elizabeth A.Bernays, Division of Biological Control, University of California, Berkeley, 1050 San Pablo Avenue, Albany, California 94706

It is usual for chemical deterrents to restrict host plant range of phytophagous insects. Even though specialist feeders are attracted to, and stimulated to feed by particular chemicals characteristic of the host, most non-hosts also yield deterrent compounds. The plant natural products are of very great variety and have miscellaneous physiological effects, but certain patterns are emerging. A view of the current situation will be presented which may influence the direction of research in evolutionary biology, behavior, methodology and crop protection. An attempt will be made to clarify the probable proximal and ultimate causes of rejection behavior caused by deterrent compounds in plants.

K1 FROM SEMIOCHEMICAL TO BEHAVIOR: OLFACTION IN THE SPHINX MOTH *MANDUCA SEXTA*, John G. Hildebrand, Arizona Research Laboratories, Division of Neurobiology, 603 Gould-Simpson Science Building, University of Arizona, Tucson, AZ 85721

Olfaction plays a major role in the regulation of insect behavior. Orientation and movement toward, and interaction with, sources of food, receptive mating partners, appropriate sites for oviposition, and hosts for parasitism usually invove olfactory signals that initiate, sustain, and guide the behaviors. We study insect olfaction both to contribute to understanding of the structure, function, and ontogeny of chemical-sensory systems in general and to advance understanding of the earth's most numerous and biologically successful fauna. Our research on the experimentally favorable insect, the sphinx moth *Manduca sexta*, probes the functional organization, cellular neurophysiology, neurochemistry, postembryonic development, and behavioral roles of the antennal olfactory system and other related sensory pathways. These efforts focus on the male-specific olfactory subsystem responsible for detection of, and integration of information about, the female's sex pheromones, as well as on elements involved in sensing host plants. We are especially interested in cell-by-cell processing of olfactory signals in the central nervous system, the transmitters mediating intercellular communication in the olfactory pathway, and the role of sensory inputs in the development of the olfactory centers in the brain and of behaviors regulated by olfactory information. This presentation will consider how biologically significant odor blends are detected and processed in insects and will provide background for the other papers in the session.

This research program has been supported by grants from NIH (NIAID) and NSF (BNS) and by a contract from the U.S. Army Research Office.

K2 CHEMO-ELECTRICAL TRANSDUCTION IN INSECT OLFACTORY CELLS,
Karl-Ernst Kaissling, Max-Planck-Institut für Verhaltensphysiologie, D 8131 Seewiesen, FRG

The topic concerns processes involved in the coding of odor stimuli into nervous signals. We report on electrophysiological and biochemical studies in the silkmoth Antheraea polyphemus using the ^{3}H-labelled pheromone component E-6,7-11 hexadecadienyl acetate. The pheromone molecules are first of all adsorbed on the olfactory hairs and migrate through the hair wall to the dendrites of receptor cells. Here they are recognized and elicit changes in electrical properties of the cell membrane leading to the generation of receptor potentials and nerve impulses. As a final step the stimulus molecules have to be inactivated. This process has to take place within the order of a second, as suggested from the decline of the receptor potential after end of stimulation. Hypotheses about the mechanism of inactivation will be discussed. It has been claimed that the pheromone molecules are rapidly degraded by the sensillar esterase because the isolated enzyme works at a high rate (2). However, enzymatic decomposition of the pheromone as monitored on intact antennae takes minutes and is, therefore, too slow for the postulated rapid inactivation. We favour the idea that the molecules, immediately after reacting at the cell membrane, bind to the pheromone binding protein present in the sensillum lymph (1). Using the concentration of this protein in the sensillum lymph (10^{-2}M), and the dissociation constant ($6x10^{-8}$M) (3), we calculate a reduction of free pheromone concentration in the lymph by a factor of $1.7x10^5$, under equilibrium conditions, compared with the protein-free situation (4). Therefore, binding could act as a mechanism of inactivation, if the equilibrium is reached quickly enough, and could also protect the pheromone and slow down its enzymatic degradation.

(1) R.G. Vogt and L.M. Riddiford (1981). Nature 293, 161-163
(2) -- (1984). Chemical Senses 8, 268
(3) K.-E. Kaissling, U. Klein, J.J. de Kramer, T.A. Keil, S. Kanaujia, J. Hemberger (1985) In: Molecular Basis of Nerve Activity. Proc. of the Int. Symp. in Memory of D. Nachmansohn, ed. J.-P. Changeux, F. Hucho, A. Maelicke, E. Neumann, Berlin/New York: W. de Gruyter & Co., 173-183
(4) K.-E. Kaissling (1986). Ann. Rev. of Neurosciences, in press.

K3 NEUROACTIVE SUBSTANCES AS PROBES OF CENTRAL THRESHOLDS IN SEX PHEROMONE PERCEPTION,
Wendell L. Roelofs and Charles E. Linn, Department of Entomology, Cornell University, Geneva, NY 14456

The sex pheromone-mediated flight behavior of male moths is a complex response that involves integration of visual and chemical inputs with a circadian rhythm that also regulates normal flight activity. Research in laboratory flight tunnels involving the full behavioral response sequence of oriented upwind flight and landing at the chemical source has shown that males are very sensitive to slight changes in the quantity and quality of the chemical signal. The normal profile of responses to a series of pheromone component blends and release rates can be changed by varying the temperature, the assay time in the photoperiod, and by injection of neuroactive substances. These substances include insecticides with specific modes of action affecting various pathways in the nervous system, and neurotransmitters and neuromodulators (1). We have been particularly interested in one class of compounds, the biogenic amines, which are widespread in the insect nervous system and have demonstrated roles as neurotransmitters and neuromodulators in a number of invertebrate systems. Our initial studies have concentrated on the effects of serotonin and octopamine on pheromone response thresholds and circadian rhythmicity (2). Further studies on the effects of various antagonists and agonists have been initiated. A long range goal of this project would be to define a neuroactive compound that can be used as a behaviorally active insect control agent.

1. Linn, C.E. and W.L. Roelofs. 1984. Sublethal effects of neuroactive compounds on pheromone response thresholds in male Oriental fruit moths. Arch. Insect Biochem. physiol. 1:331.

2. Linn, C.E. and W.L. Roelofs. 1986. Modulatory effects of octopamine and serotonin on male sensitivity and periodicity of response to sex pheromone in the cabbage looper moth, Trichoplusia ni. Arch. Insect Biochem. Physiol. In Press.

Peptides of the Insect Nervous System

K4 NEUROPEPTIDE CONTROL OF ECDYSONE BIOSYNTHESIS. L.I. Gilbert, W.A. Smith, W.L. Combest and D.B. Rountree, Department of Biology, University of North Carolina, Chapel Hill, NC 27514.

The cellular mechanism of action of the cerebral neuropeptide, prothoracicotropic hormone (PTTH), was investigated in vitro using prothoracic glands (PG) from the tobacco hornworm, Manduca sexta. An involvement of cAMP in PTTH-stimulated ecdysone synthesis was demonstrated as follows: (a) the steroidogenic effect of PTTH on PG of day 3 fifth instar larvae and day 0 pupae was mimicked by agents that act by increasing intracellular levels of cAMP (MIX; dibutyryl cAMP, and forskolin), and (b) PTTH stimulated the formation of cAMP in glands from both stages in a rapid, dose-dependent manner. However, a significant accumulation of cAMP in response to PTTH occurred only in larval PG. In pupal glands, effects of the neuropeptide on cAMP synthesis were seen only in the presence of a phosphodiesterase inhibitor. Although cAMP is involved in PTTH action at both stages, it appears that the developmental state of the PG influences the degree to which cAMP accumulates in response to the neurohormone. In addition to cAMP, it appears that Ca^{2+} plays an essential role in mediating the steroidogenic effects of PTTH, as indicated by the fact that: (a) PTTH-stimulated ecdysone synthesis was blocked by omission of Ca^{2+} from the incubation medium, and (b) ecdysone synthesis was stimulated by the calcium ionophore A23187. Agents that act by increasing intracellular levels of cAMP enhanced ecdysone synthesis equally well in the presence or absence of extracellular calcium. By contrast, both PTTH and A23187 stimulated cAMP formation in a manner absolutely dependent upon extracellular Ca^{2+}. The results suggest a primary role for calcium in mediating PTTH-stimulated synthesis of cAMP, with the cyclic nucleotide in turn stimulating ecdysone synthesis.

Further, PTTH-stimulated ecdysone synthesis is accompanied by activation of cAMP-dependent protein kinase (cAMP-PK). This occurs within 3 min of exposure of the PG to PTTH, clearly preceding the enhancement of ecdysone synthesis and is elicited by hormone concentrations that stimulate steroidogenesis. The predominant isozymic form of cAMP-PK in the PG is type II (90%) and both forms are inhibited by the specific inhibitor from bovine heart. In addition to the activation of cAMP-PK as determined by histone phosphorylation, the incubation of intact PG in the presence of PTTH revealed the selective phosphorylation of a single protein (M_r=34,000). The role of this phosphoprotein in ecdysone biosynthesis is being explored.

K5 PROTHORACICOTROPIC HORMONE OF BOMBYX MORI: PRIMARY STRUCTURE AND CRLLULAR LOCALIZATION AS REVEALED BY IMMUNOHISTOCHEMISTRY. Hironori ISHIZAKI, Biological Institute, Faculty of Science, Nagoya University, Nagoya, and Akinori Suzuki, Department of Agricultural Chemistry, The University of Tokyo, Tokyo, Japan.

The brain of the silkmoth, Bombyx mori, contains two distinct types of prothoracicotropic hormone(PTTH). One molecular form named 22K-PTTH(or PTTH-B, ca. 22,000 daltons) activates the prothoracic glands(PG) of Bombyx whereas the other named 4K-PTTH(or PTTH-S) activates PG of the saturniid moth, Samia cynthia ricini, but not the PG of Bombyx(1,2). Apparently 22K-PTTH is a genuine PTTH while 4K-PTTH, though it satisfies all the criteria as PTTH(3), must be referred to as "PTTH-like peptide" until the identity of this molecule with that derived from Samia is proved. We call here this molecule 4K-PTTH, however, simply for brevity.

A close homology in amino acid sequence of 4K-PTTH with vertebrate peptides of insulin family has been described(4). We now report the entire sequence of this molecule. Purification and partial sequences of 22K-PTTH will also be communicated.

We have been raising monoclonal antibodies against synthetic peptides corresponding to various parts of 4K- and 22K-PTTH. One of these, anti-4K-PTTH(1-10) specifically reacts with a native 4K-PTTH after cleavage of its S-S bonds. Immunohistochemical staining of brains using this antibody revealed 4 pairs in Bombyx and 8 pairs in Samia of immunoreactive neurosecretory cells in the pars intercerebralis. Axons distributing in the periphery of the corpora allata were also strongly positive, supporting the existing concept that the corpora allata are a neurohaemal organ of PTTH.

(1) H.Ishizaki et al.(1983) Devel. Growth & Differ. 25:593. (2) H.Ishizaki & A.Suzuki(1984) in "Biosynthesis, Metabolism, and Mode of Action of Invertebrate Hormones"(ed. J.Hoffmann & M.Porchet) Springer-Verlag, p.63. (3) H.Nagasawa et al.(1984) Gen. comp. Endocr. 53:143. (4) H.Nagasawa et al. (1984) Science, 226:1344.

K6 PEPTIDES, mRNAS, AND GENES OF THE AKH FAMILY, Martin H. Schaffer, Michael O'Shea, Barbara E. Noyes, Department of Psychiatry, University of Chicago, Chicago, Illinois 60637

There is a structurally related family of neuropeptides found in the corpora cardiaca (C.C.) and certain neurons of insects. Adipokinetic hormone (AKH) was the first of these peptides to be sequenced, but 5 more structures have been reported in the last two years, and each genus investigated has provided at least one new member. These peptides have a variety of humoral activities in different insects, but the lipid mobilizing effect of AKH in locust remains the clearest example of a physiologically important effect. The activities of these peptides in neurons remains to be explored, however studies of bioactivities suggest a role in excitatory motor neurons.

The synthesis of AKH and AKH-II and MI and MII can be readily studied in the C.C.'s of Schistocerca and Periplaneta respectively. Because of the high concentration of these peptides in this tissue, organ culture of C.C.'s with ^{3}H-labelled amino acids produces significant incorporation of radioactivity into fully processed peptides. Beyond documenting the C.C. as a site of synthesis, these studies demonstrate the potential of the C.C. as a model for the study of neuropeptide processing in insects.

Because of the interesting evolutionary, physiological, and biochemical questions raised by this family of neuropeptides we have begun to characterize their mRNA's and genes. To study the AKH mRNA we undertook primer extension studies using a mixture of four undecadeoxynucleotides. Unfortunately, primer extension with these oligonucleotides and C.C. total RNA from Schistocerca nitens produces a very complex mixture of cDNA's. To simplify the search for an AKH-related cDNA we repeated the experiment leaving out one of the four nucleotide triphosphate substrates. Inspection of the possible AKH mRNA sequences enabled us to predict which cDNA products were possible candidates for AKH cDNA. These products were sequenced, and in this way an AKH specific cDNA of 23 nucleotides was identified. Based on this sequence we have synthesized a 22 nucleotide probe which we are now using in further primer extension studies on the AKH mRNA and to screen a gene library for the AKH gene.

K6A ECLOSION HORMONE ACTION ON THE INSECT NERVOUS SYSTEM, James W. Truman and David B. Morton, University of Washington, Seattle WA

Eclosion hormone (EH) has diverse actions in insects ranging from triggering the death of certain muscles to releasing the stereotyped behaviors seen at ecdysis. An intriguing aspect of this peptide is that it is effective only during a narrow period late in each molt. These windows of sensitivity are brought about by the appearance and subsequent withdrawal of the ecdysteroids.

The mechanism by which the ecdysteroids render target tissues competent to respond to EH has been examined for the CNS of the hawkmoth, Manduca sexta. The first response of the CNS to EH is an elevation in intracellular cyclic GMP levels. This apparently results in the phosphorylation of two 54 Kd proteins which are thought to mediate the action of EH. Ecdysteroid exposure renders the CNS competent to respond to EH in a two step process. Initially after such an exposure, the CNS can respond to the peptide by elevating endogenous cyclic GMP levels, indicating that EH receptors are now present, but no motor response is observed, suggesting that an event distal to the second messender is blocked. Interestingly, the 54 Kd proteins cannot be detected at this time. They appear a number of hours later, and with their appearance the CNS now shows a full physiological response to EH.

Insect Hemolymph Proteins

K7 ACTION OF ADIPOKINETIC HORMONE ON LIPOPHORIN IN LOCUSTS. Haruo Chino, Biochemical Laboratory, Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan.

Lipophorin is the major lipoprotein in the hemolymph of most insects[1], and serves as a reusable shuttle to transport various lipids including diacylglycerol (DG), hydrocarbons, and cholesterol between tissues. Locust adipokinetic hormone (AKH)[2] released from the corpora cardiaca during flight stimulates the loading of DG by lipophorin from the fat body, resulting in the formation of large lipophorin particles with increased DG content.[3] AKH also causes the association of a low molecular weight, non-lipid-containing protein with lipophorin[4]. Similar observations have been extended to adult tobacco hornworm by Shapiro and Law[5] who proposed the term apolipophorin III (apo-III) for the low molecular weight protein that becomes associated with lipophorin in response to AKH.

We have extended these observations to the locust using various techniques including density-gradient centrifugation and electron microscopy. The major results are as follows: 1) The injection of AKH into adult locusts promotes the association of free apo-III (mol wt, 20,000) in hemolymph with lipophorin (8 moles apo-IIIs associate with each mole lipophorin). 2) AKH stimulates the loading of DG by lipophorin from the fat body, resulting in larger, lower density lipophorin particles. 3) These structural changes of lipophorin in response to AKH is completely reversible, so that within 24 hr of AKH injection, the apo-III dissociates from lipophorin and the size and density of lipophorin return to the original values observed in resting locusts.

We also propose a theory that the observed larger lipophorin particles may be formed by intermolecular fusion of the lipophorin"primarily" activated by AKH.

We have recently achieved the purification of apo-III from locust hemolymph, and characterized it extensively. Some physico-chemical properties of purified apo-III will also be reported briefly.

(1), Chino, H., Downer, R. G. H., Gilbert, L. I., and Wyatt, R. G. (1981) Insect Biochem 11: 491. (2), Mayer, R. J., and Candy, D. J. (1969) J. Insect Physiol 15: 611. (3), Van Der Horst, D. J., Van Doorn, and Beenakkers, A. M. (1979) Insect Biochem 9: 627. (4), Van Der Horst, D. J., Van Doorn, J. M., and Beenakkers, A. M. (1984) Insect Biochem 14: 495. (5), Shapiro, J. P., and Law, J. H. (1983) Biochem.Biophys.Res.Comm 115: 924.

K8 AFFINITY PURIFICATION AND CHARACTERISTICS OF JUVENILE HORMONE ESTERASE FROM LEPIDOPTERA, Bruce D. Hammock and Yehia A. I. Abdel-Aal, Departments of Entomology and Environmental Toxicology, University of California, Davis, CA 95616

The regulation of hormone titers in most endocrine systems examined to date is reported to be due exclusively to variations in rates of biosynthesis with a rather constant rate of degradation. In contrast, dramatic changes in the rates of both biosynthesis and degradation appear critical in regulating the titer of juvenile hormone (JH) in the Lepidopterous species so far examined. Since this degradation appears to be mediated by either a single esterase or a group of similar esterases, it becomes important to study their properties. Inhibition of these esterases has been shown to reduce the rapid decline in JH titers needed for metamorphosis and thus to result in the formation of giant larvae. Several classes of chemicals have been shown to be selective inhibitors of JH esterase, but among the most interesting are substituted 3-thio-1,1,1-trifluoropropanones. The structure activity relationships of these inhibitors indicate that the catalytic site of JH esterase is rather specific for JH I and II. Many of these compounds give slow-tight binding enzyme kinetics and are thought to act as transition state mimics at the enzyme catalytic site. The almost stoichiometric inhibition of JH esterase by these compounds allows one to estimate in crude hemolymph the concentration of catalytic sites and thus several valuable catalytic constants. Such data indicate that JH esterse is an exceptionally aggressive enzyme as determined by k_{cat}/K_m ratios. Thus, it becomes important to investigate pure preparations of the enzyme. To this end the trifluoroketones described above were bound to epoxy-activated Sepharose to yield affinity columns capable of purifying JH esterase over 1000 fold to apparent homogeneity high yield. Using this technique the JH esterase(s) from 3 strains of Bombyx mori, Heliothis virescens, and Manduca sexta were purified. Studies are underway on the properties of the pure enzyme and the biological effects of the pure enzyme when injected.

K9 THE CONTROL AND FUNCTION OF THE MAJOR LARVAL SERUM PROTEINS OF DROSOPHILA, David B Roberts, Genetics Laboratory, Department of Biochemistry, South Parks Road, Oxford, OX1 3QU, England

The major haemolymph protein of Drosophila, LSP-1, is synthesised by the fat body and only during the 3rd larval instar. At pupariation 0.7% of the wet weight of the larva is LSP-1. In Calliphora 5% of the wet weight of the larva immediately prior to pupariation is the homologous protein calliphorin. These proteins are coded for by a multi-gene family with the genes in tandem in some species, (Lucilia cuprina) and dispersed in other, (Drosophila melanogaster).

The synthesis of this protein stops because of some post-transcriptional event. In vivo transplantation and in vitro studies have shown that this stop is not dependent on time, nor is it dependent on the accumulation of the protein, nor, in a simple fashion, on the hormone ecdysone. The interaction of ecdysone with other hormone(s) does however seem to be involved.

We have found null alleles of all three Drosophila LSP-1 genes and from these we have constructed an LSP-1 null stock. These flies survive and by the criteria applied survive as well as the wild type control. The selection pressures that maintain this most abundant of dipteran proteins and prevents the DNA sequences from accumulating more random mutations, by genetic drift, than most dipteran genes, acts at the level of fertility. The quality of eggs laid, the quantity of eggs laid and the length of time for which eggs are laid are all reduced in the null stock compared with the wild type stock. Furthermore there are behavioural differences between the two stocks which exacerbate these effects.

K10 THE DYNAMICS OF LIPOPHORIN INTERCONVERSIONS IN MANDUCA SEXTA, Robert O. Ryan, Department of Biochemistry, University of Arizona, Tucson, AZ 85721

Altered physiological demands for lipid transport which accompany the morphological changes associated with metamorphosis in Manduca sexta are reflected in lipophorin density, lipid composition and apoprotein content. At least six stage specific forms of lipophorin have been isolated and characterized. These different lipophorin species appear in hemolymph on a precisely timed developmental schedule, yet only one distinct species is present at any given time. In vivo experiments utilizing radioiodinated lipophorins or [^{3}H]-amino acids suggests that in the immature stage new lipophorin species arise from alterations of the lipid content and composition of preexisting lipophorins. Similarly, the adipokinetic hormone induced formation of low density lipophorin in adult M. sexta results from a specific uptake of diacylglycerol by adult high density lipophorin. It is concluded that a basic lipophorin matrix structure exists (composed of apolipophorin I, apolipophorin II and lipid) and that different lipophorin forms arise from alteration of the lipid content and composition of this basic matrix structure.

In vitro incubation of lipophorins of different density followed by density gradient ultracentrifugation has been used to assay hemolymph fractions for lipid transfer activity. Lipophorin-deficient hemolymph from immature and adult stages was found to possess a lipid transfer factor capable of catalyzing a redistribution of lipophorin lipids such that at equilibrium a single lipophorin species exists. Characterization of the lipid transfer activity reveals a direct relationship between lipid transfer factor concentration, donor and acceptor lipophorin concentration and transfer activity. In addition to net lipid transfer, evidence of diacylglycerol and phospholipid exchange between donor and acceptor lipoprotein was obtained. Furthermore, apoprotein exchange among lipophorins did not occur during the net lipid transfer reaction. Partial purification of the lipid transfer factor indicates a high molecular weight glycosylated protein (M_r > 500,000) of subunit size (M_r > 300,000) is responsible for the observed lipid transfer activity. The ability of M. sexta lipid transfer protein to catalyze a redistribution of lipid between liposomes and lipoprotein suggests a potential role of this protein in the observed shuttle mechanism of lipophorin lipid transport.

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K11 HORMONE-INDUCED LIPOPHORIN REARRANGEMENTS DURING FLIGHT OF THE LOCUST, Dick J. Van der Horst and Ad M.Th. Beenakkers, Department of Experimental Zoology, University of Utrecht, 3503 TB Utrecht, The Netherlands

Energy production in active locust flight muscles progressively relies on the utilization of *sn*-1,2-diacylglycerol (DG), which is released from the fat body by the action of the adipokinetic hormone (AKh). Lipid mobilization induced by flight activity results in significant changes of the hemolymph protein pattern due to lipoprotein-protein interactions. In the resting situation, DG is mainly carried by a high-density lipophorin (or Ay, MW approx. 450,000 by gel filtration chromatography). However, during flight (or after injection of AKH into resting locusts) the increased DG is primarily transported by a new, high lipid loaded, low-density lipophorin (A^+, MW about 3,500,000). Although the molecular mechanism is not yet known, immunological and radiolabeling experiments have evidenced that A^+ is formed in the hemolymph from existing lipophorin Ay and the non-lipid containing protein C_2 (MW 20,000), which has been confirmed *in vitro*. Functions of the association of protein C_2 with lipophorin (Ay) may include an increase in lipid loading capacity of the lipid carrier system and/or some specific interaction with the flight muscles, possibly resulting in activation of the flight muscle lipoprotein lipase. Therefore, molecular organization of A^+, which in view of the high DG content will be different from mammalian serum lipoproteins, is of vital importance. The high-density lipophorin (Ay) is composed of two prominent subunits, apo-I (MW approx. 240,000) and apo-II (MW approx. 77,000), which both contain carbohydrate as shown by FITC-lectin binding in electrophoretic blots of the lipophorin on nitrocellulose paper after SDS-polyacrylamide gel electrophoresis (PAGE). In the low-density lipophorin (A^+), both subunits are recovered along with the additional MW 20,000 subunit of protein C_2 (or apo-III). Although the latter subunit does not bind to the above lectins, it contains a large amount (12.5%) of carbohydrate as well consisting of a single biantennary N-linked oligosaccharide chain. Immunological probes using monoclonal antibodies specific for the three apoproteins revealed (both with enzyme-linked immunosorbent assays (ELISA) of apoproteins isolated by SDS-gel permeation chromatography and in electrophoretic blots of apoproteins after SDS-PAGE) that apo-I, apo-II and apo-III are not homologous. Monoclonal antibody specific for C_2 reacted strongly with intact A^+, indicating major localization at the lipophorin surface which is compatible with an involvement in flight muscle cell membrane recognition or enzyme activation. However, ELISA using native A^+ showed that (like in Ay) both apo-I and apo-II are additionally exposed despite the dramatic lipid loading and therefore may also reflect distinct recognition sites for target cells. Experiments in which isolated glycoprotein C_2, labeled with ^{35}S, was injected into locusts along with AKH resulted in recovery of labeled A^+. Since *in vitro* incubation of this A^+ with flight muscles yielded labeled C_2, it is proposed that formation of lipophorin A^+ provides a reversible and efficient shuttle mechanism for lipid transport during flight.

Molecular Aspects of Insect Reproduction I

K12 Regulation of the genes coding for Drosophila yolk proteins

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The three major yolk-polypeptides of D.melanogaster are coded for by single copy genes on the X-chromosome. We shall present sequence data for YP3 comparing it with the coding sequences for YP1 and YP2 which are already available.

Several factors which are important in regulating the expression of these genes will be discussed, including hormonal involvement, the importance of nutrition and the key role of genes which control sex determination.

Finally, a female sterile mutant has been analysed and found to have a single amino substitution in the coding region of YP1 which prevents its secretion from the fat body.

K13 HEMOLYMPH PROTEIN ENDOCTYOSIS IN SUPPORT OF REPRODUCTION AND DEVELOPMENT, William H. Telfer, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104

The three most concentrated proteins in pupal hemolymph of Hyalophora cecropia females include vitellogenin (15 mg/ml), and two hexameric glycoproteins--arylphorin (40 mg/ml), and a newly described riboflavin binding protein (18 mg/ml). All three are utilized during the second metamorphic molt, falling to less than 1 mg/ml by adult eclosion. Vitellogenin depletion can be ascribed to receptor-mediated endocytosis by oocytes during yolk deposition. The two hexamers occur only in traces in the eggs, however, and since they are also storage proteins of the male, another endocytotic mechanism must be responsible for the disappearance of arylphorin and the flavoprotein from the hemolymph. This paper will discuss the cellular physiology of the oocyte's endocytotic mechanism and the current status of the search for mechanisms that utilize arylphorin and the flavoprotein.

Molecular Aspects of Insect Reproduction II

K14 CELL AUTONOMOUS AND HORMONAL CONTROL OF SEX-LIMITED GENE EXPRESSION IN DROSOPHILA, Douglas R. Cavener, Michael Murtha, and Christopher Schonbaum, Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235

Sterility is a common adult phenotype associated with ecdysteroid deficiencies in Drosophila. This suggests that the major function of ecdysteroids at the adult stage is to modulate the expression of genes involved in reproduction. A temperature sensitive mutant of the ecd-1 gene exhibits reduced ecdysteroid titers and sterility in both sexes (1,2). We have studied the effect of the ecd-1 mutant on the expression of four genes: glucose dehydrogenase (Gld), alcohol dehydrogenase (Adh), sorbitol dehydrogenase (Sodh), and α-glycerophosphate dehydrogenase (Gpdh). Gld, Adh, and Sodh normally exhibit sexually dimorphic patterns of expression. At the adult stage Gld expression is limited to the male ejaculatory duct whereupon the GLD enzyme is transferred to females during copulation (3). SODH and ADH are expressed in both sexes but to a greater degree in males. GPDH levels are equivalent in the sexes. The levels of GLD and SODH are dramatically reduced in ecd-1 males reared at the restrictive temperature whereas ADH and GPDH are unaffected. In contrast the level of ADH is significantly increased in adult ecd-1 females reared at the restrictive temperature whereas GPDH, SODH, and GLD are unaffected. Thus, these effects are sex specific and are only observed in the three enzymes which normally exhibit some form of sexual dimorphism. The steady state levels of ADH mRNA is closely correlated with the ecd-1 dependent ADH increase in females. However, preliminary experiments indicate that GLD mRNA in ecd-1 males is not reduced despite a 3-4 fold reduction in enzyme activity. Temperature shift experiments demonstrate that the ecd-1 effect is reversible for ADH but irreversible for GLD. Our current hypothesis is that GLD secretion is blocked in the ecd-1 mutant. In order to test the cellular autonomy of GLD expression, we performed a series of genital imaginal disc transplantation experiments. Our results were as follows: (a) male D. melanogaster genital discs will differentiate normally in D. melanogaster or D. pseudoobscura females, (b) ejaculatory ducts in these females hosts express GLD, (c) ecd-1 ejaculatory ducts in wild type hosts retain their temperature sensitive reduction in GLD activity. Therefore, the expression of GLD does not require any sex specific or species specific hormones produced by tissues other than the genital disc derivatives. We have no evidence that observed effects of ecd-1 are directly a result of the reduced ecdysteroid titers. Experiments are in progress to examine this question.

[1]Garen, A., Kauvar, L., and Lepesant, J. (1977) Proc. Natl. Acad. Sci. USA 74: 5099-5103.

[2]Redfern, C. and Bownes, M. (1983) Mol. Gen. Genet. 189:432-440.

[3]Cavener, D. and MacIntyre, R. (1983) Proc. Natl. Acad. Sci. USA 80:6286-88.

K15 ESTROGENS AND ANDROGENS IN INSECTS, D. L. Denlinger[1], R. W. Brueggemeier[2], R. Mechoulam[2], N. Katlic[2], L. B. Yocum[1], G. D. Yocum[1], Department of Entomology[1] and College of Pharmacy[2], Ohio State University, Columbus, OH 43210

We recently found evidence for widespread distribution of androgens and estrogens in insects (1). Using radio immunoassays, we detected androgens and estrogens in Sarcophaga bullata, Periplaneta americana, Manduca sexta, Tenebrio molitor, Oncopeltus fasciatus, and Locusta migratoria. The identification of estradiol and estriol in samples of the flesh fly, S. bullata was confirmed by GC retention times of the derivatized estrogens and comparison of the MS spectra with those obtained from standard samples.

The flesh fly, which was examined most extensively, contains both androgens and estrogens in all developmental stages tested. Highest activity for both steroids was observed in 3-day old adults. Androgen and estrogen levels were roughly the same in males and females at each age of adult life. To test the possibility that the steroids may have originated from the food source, the larval food (beef liver) was also extracted and analyzed. No activity could be detected by RIA, thus implying that the androgens and estrogens are synthesized by the flies, presumably from steroid skeletons obtained from the food.

Hemolymph from third instar larvae of S. bullata has been analyzed for the presence of estrogen binding (or carrier) proteins. The estrogen binding affinity and protein concentration was determined by Scatchard plot analysis. We observe specific estradiol binding with an approximate K_D (dissociation constant) of 4.9×10^{-6} M. The concentration of binding sites is 3.6 sites/mg protein. High levels of aromatase activity have been detected in gonads and nervous tissue of adult flies, while flight muscle shows no activity.

The biological significance of this discovery is not yet clear, but preliminary experiments with cockroaches suggest a role for these steroids in modifying behavior. But, the identification of estrogens and androgens in insects does not necessarily imply that these compounds serve as sex hormones. If these steroids do play a role in insect reproduction, one should not presume that their function is necessarily identical to that in higher species. The presence of high levels of both androgens and estrogens in both males and females already indicates an important departure from the mammalian pattern.

(1) R. Mechoulam, R. W. Brueggemeier and D. L. Denlinger (1984), Estrogens in insects. Experientia 40:942-944.

K16 ACCESSORY GLAND DEVELOPMENT AND SPERMATOPHORE FORMATION IN MEALWORM BEETLES, George M. Happ, Department of Zoology, University of Vermont, Burlington, VT 05405

Male accessory glands of insects produce an array of secretory products that comprise the seminal fluids and package the semen. We will describe the structure of the accessory glands of Tenebrio molitor, the assembly of their products into the spermatophore, and the endocrine control of their development. There are two pairs of glands--the larger bean-shaped glands (BAGs) which produce semisolid products and the smaller tubular accessory glands (TAGs) which produce more fluid secretions.

The spermatophore consists of a multilayered outer wall and an inner core which surround the semen. The wall is largely derived from the products of eight kinds of secretory cells within the epithelium of the BAG. The cells of each type form a patch, and the products from each patch are secreted into the lumen where they form a coherent mass. By using monoclonal antibodies to the secretory proteins, we have traced secretions from some of the cells into the lumen and thence into the ejaculatory duct where the ordered secretory plug of the BAG is molded into the walls and core of the spermatophore.

The male accessory glands of Tenebrio originate as a mesodermal rudiment in the last instar larva. During the pupal stage, the glands grow mitotically and then differentiate in the post-ecdysial adult. Cell division continues through the pupal peak of 20-hydroxyecdysone and declines with the fall in titer of that hormone. In vitro, 20-hydroxyecdysone accelerates the flow of cells from G_2 to G_1, as demonstrated by increased mitotic indices and by accumulation in G_1 after hydroxyurea blockage. Physiological doses of hormone produce physiological responses. Calculation of cell cycle parameters and pulse-chase experiments with bromodeoxyuridine show that the hormone acts at a control point in early G_2 to truncate that phase. Brief pulses of hormone (30-60 minutes) produce an acceleration that persists for at least 24 hours. In the late pupa, the cells of the accessory glands arrest in G_2 and no longer divide in response to ecdysteroids.

The transfer of sperm within spermatophores is widespread among insects. The accessory glands and sperm sac of the mealworm beetle are useful models for study of the reproductive physiology of male insects as well as more general processes of macromolecular assembly and mechanisms of hormone action.

Supported by grants from the National Institutes of Health.

K17 REGULATION OF OOTHECIN SYNTHESIS BY JUVENILE HORMONE, Richard N. Pau, Agricultural and Food Research Council, Insect Chemistry and Physiology Group, University of Sussex, Brighton BN1 9RQ, England.

Cockroaches lay their eggs in batches which are enclosed in a protective egg case (ootheca) formed from the secretions of large accessory glands (colleterial glands). In Periplaneta americana the bulk of the egg case consists of a number of small glycine-rich structural proteins, which we have named oothecins. These are coordinately synthesised by the left colleterial gland, and are encoded by a multigene family. Synthesis of both cockroach yolk proteins and oothecins are regulated by juvenile hormone. We are using the synthesis of oothecins in the left colleterial gland of Periplaneta americana as a system to study at the molecular level how juvenile hormone regulates the expression of a family of genes involved in reproduction.

The primary sequences of 16 kDalton oothecins have been derived from nucleotide sequences of cDNAs. The 16 kDalton oothecins show a high degree of sequence homology with silkmoth chorion (egg shell) proteins. Unlike the oothecins, synthesis of the chorion proteins does not require a concurrent hormonal signal. We present an account of the characterization of oothecin genes.

Molecular Aspects of Ecdysone Action

K18 PROPERTIES OF GENES EXPRESSED DURING ECDYSONE-INDUCED IMAGINAL DISC MORPHOGENESIS IN DROSOPHILA, James W. Fristrom, Dianne K. Fristrom, Jeanette Natzle, David Osterbur, Stephenie Paine-Saunders, and Donald Withers, Department of Genetics, University of California, Berkeley, CA 94720

Metamorphosis in insects is a key developmental process regulated by ecdysone that includes the histolysis of juvenile tissues and the development of adult tissues. Drodophila imaginal discs commence morphogensis (evagination) to form legs and wings within 3-4 hrs after exposure to 20-hydroxyecdysone in vitro. Because evagination is mediated by cell movement within the disc epithelium and might, therefore, require changes in cell surface properties, we have cloned ecdysone-inducible genes (IMP's) whose transcripts associate with membrane-bound polysomes and presumably encoded secreted or membrane proteins. Transcripts from three of these genes, IMP-E1, IMP-E2, and IMP-E3, are expressed within 2 hrs of hormone exposure in vitro. These genes are cytologically localized in polytene chromosome regions 66C, 63E, and 84E, respectively. A fourth gene, IMP-L2, localized in 64B, is expressed only after 8 hrs of hormone exposure. Additionally, a gene localized in salivary band 2B5 that forms a puff in salivary gland polytene chromosomes after exposure to ecdysone, is also expressed in imaginal discs within two hours of incubation with 20-hydroxyecdysone in vitro. Mutants at the 2B5 locus are defective in imaginal disc morphogenesis. The expression of these ecdysone-responsive genes with respect to hormone levels, and tissue and temporal specificity during development will be compared and the properties of their transcripts characterized. For example, IMP-E1 encodes an 8-8.5 kb transcript that is expressed during embryogenesis, in 1st and 2nd instar larvae, and during puparium formation at the end of the third instar. At puparium formation the transcript is found in leg discs, wing discs, and antenna discs but not in the ommatidial region of eye discs. The transcript is also present in brain tissue in the optic lobes and near the neuropil. The temporal and spatial distribution of expression of IMP-E1 is consistent with its possible role in morphogensis of imaginal tissues.

K19 CONTROL ELEMENTS OF ECDYSONE INDUCIBLE GENES OF D. MELANOGASTER, J.-A. Lepesant[1], F. Maschat[1], H. Benes[1,2], T. Jowett[1,3], J. Roux[1], R. Pictet[1], J. Jami[1]. 1 Institut Jacques Monod, CNRS, Inserm et Universite Paris, 75251 Paris Cedex 05, France. 2 Division of Gerontology Research, University of Arkansas for Medical Sciences, Little Rock, AR 72205. 3 Department of Genetics, University of Newcastle upon Tyne, NE 1 7RU, England.

The P1 and LSP-2 genes are selectively expressed in the fat body of D. melanogester third instar larvae, under the positive control of ecdysone. The P-element mediated transformation was used to detect cis-acting regulatory sequences of these genes. A cloned LSP-2^{N} gene coding for the electrophoretic normal form of the unique subunit of the protein was introduced into a LSP-2^{Slow} background. This allowed the normal expression of the transformed gene to be detected at the protein level. The isolation of transformed lines containing one copy of constructs retaining various lengths of non coding sequences flanking the LSP-2^{N} gene showed that remote regulatory sequences are required for a normal expression of the LSP-2 gene.

Hybrid constructs were made by fusing to substitute coding sequences DNA fragments of various lengths directly adjacent to and including the first 25 transcribed bases of the P1 gene. Transformed lines of D. melanogester containing only one copy of these constructs were established and analysed for the expression of the substitute coding sequences. In this way control elements directing the spatial, temporal and hormonal specificities of transcription can be mapped in the 5' upstream region flanking the P1 gene.

K20 HORMONAL CONTROL OF SEQUENTIAL GENE EXPRESSION IN INSECT EPIDERMIS, Lynn M. Riddiford, Department of Zoology, University of Washington, Seattle, WA 98195

Insect growth and metamorphosis is controlled by 2 hormones, ecdysone causing a molt and juvenile hormone (JH) directing the type of molt. To learn how JH prevents a major switch in gene expression in response to ecdysone but allows the re-expression of the previously active genes, we have utilized the abdominal epidermis of the tobacco hornworm Manduca sexta which sequentially makes larval, pupal, then adult cuticle. Northern and dot blot hybridization analyses using cDNA clones coding for 3 larval endocuticular proteins (12.8, 13.3, and 14.6 kd) have shown that these mRNAs disappear by the time of head cap slippage during the larval molt, then reappear before ecdysis (1; Baeckmann, Hice, Rebers, and Riddiford, in preparation). They all disappear when the epidermis becomes pupally committed in response to 20-hydroxyecdysone (20HE) in the absence of JH. Trace amounts of the mRNA for the 14.6 kd protein are found during pre-ecdysial pupal cuticle deposition but none of the 3 mRNAs are found in the adult. In vitro these mRNAs disappear in response to 20HE at levels similar to those found in vivo. The addition of JH prevents this disappearance at low levels of 20HE but not at molting levels for 2 of the mRNAs. By contrast, the mRNA for the 14.6 kd protein is suppressed by JH alone. Sequencing of the corresponding genomic clones to look for common regulatory regions is in progress.

During the final day of feeding (day 3) of the 5th instar the cuticular lamellae become 5-10-fold thinner due to a change in cuticular protein synthesis involving a disappearance of the above mRNAs and the appearance of at least 3 new mRNAs (for 15, 17, and 27 kd proteins) (2). The application of JH on day 1 or early day 2 blocks this change by preventing an intermolt rise in ecdysteroid on day 2 to 30 ng/ml 20HE equivalents. JH also prevents the appearance of the new cuticular mRNAs in day 1 epidermis incubated with 25 ng/ml 20HE for 24 hrs. Thus, the sequential program of larval endocuticular gene expression is controlled primarily by ecdysteroid: high levels as during the molt causing cessation of transcription, low but fluctuating levels during the intermolt in the absence of JH causing a switch in the genes expressed leading to a change in cuticular structure. JH then modulates this expression, determining whether these larval-specific cuticular genes are turned off temporarily or permanently. Supported by NIH AI-12459 and NSF DCB 80-11152.

1) Riddiford, L.M. (1986) Arch. Insect Physiol. Biochem, in press.
2) Wolfgang, W.J. and Riddiford, L.M. (1986) Devel. Biol., in press.

K21 PROTEIN PRODUCTS OF LOCI WITHIN THE ANTENNAPEDIA GENE COMPLEX OF DROSOPHILA, Matthew P. Scott and Sean B. Carroll, Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, CO 80309-0347

The Antennapedia Complex (ANT-C) is a cluster of homoeotic and segmentation genes that regulate embryonic development and metamorphosis in Drosophila. Current experiments are focused on two homoeotic genes, Antennapedia (Antp) and Sex combs reduced (Scr), in which mutations change one part of the fly into another, and on the segmentation gene fushi tarazu (ftz), in which mutations give rise to embryos with half the usual number of body segments. A major goal is to understand how the different homoeotic and segmentation genes (within and outside the ANT-C) interact to attain normal pattern formation during development. To this end, we have prepared antisera with which the localization of ANT-C-encoded proteins can be examined at all stages of development in wild type and mutant flies. cDNA clone sequences were introduced into vectors to express the Drosophila proteins in bacteria. The proteins were obtained fused to β galactosidase (βgal) and the βgal portion was used to purify each protein from bacterial extracts. Antibodies against the Drosophila portion of each protein were purified by affinity chromatography and used to localize the Drosophila proteins in whole mount embryos and in dissected tissues. The examination of the patterns of ftz expression in embryos mutant for various segmentation genes has revealed that some of the zygotically active segmentation genes alter the normal ftz pattern of seven transverse stripes of nuclei at the blastoderm stage. Mutations in genes that act during oogenesis to regulate segmentation also have dramatic effects on ftz expression in the embryo. Antp proteins are found in several patterns during development. The position-specific expression of Antp responds to the altered function of some segmentation genes and several homoeotic genes. Thus, a hierarchy can be envisioned in which maternally active genes provide the initial information needed for segmentation genes to be expressed in the proper places, which is further refined and elaborated through interactions between the different segmentation genes. The homoeotic gene program responds to these positional cues in setting the overall spatial parameters of gene expression which is in turn refined through homoeotic gene interactions. The nuclear localization of these developmental gene products and their structural homology suggests that these interactions may be mediated at the protein-DNA level.

Natural Strategies for Insect Control (Joint)

K22 PHYTOCHEMICAL DISRUPTION OF ENDOCRINE MEDIATED PROCESSES, William S. Bowers, The University of Arizona, Tucson, AZ 85721

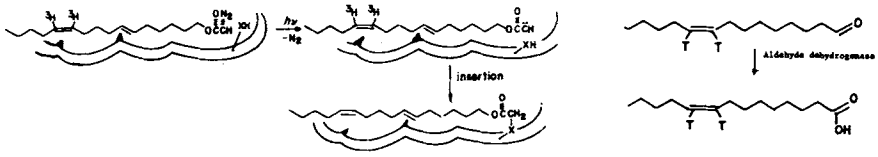
Plants deploy a variety of defensive strategies designed to limit predation by herbivores. Historically natural plant poisons have served as prototypic models for insecticide development. Other, less well understood, plant secondary chemicals are now being found to interfere with endocrine mediated processes in insects. Compounds with insect juvenile hormone activity interfere with insect morphogenesis; while anti-juvenile hormonal agents telescope immature development, induce sterilization and serve as anti-feedants. These biologically active agents are serving as useful probes with which to investigate the details of endocrine events in insects while their chemical optimization has now successfully commercialized and a new generation of Biorational agents for insect control is assured.

K22A CHEMICAL STUDIES OF PHEROMONE CATABOLISM AND RECEPTION, Glenn D. Prestwich, Department of Chemistry, State University of New York, Stony Brook, NY 11794

Biochemical studies of the binding and catabolism of pheromone molecules by specific proteins in antennal (and other) tissues of insects can be best studied with high specific activity ^{3}H-labeled pheromones and pheromone analogs. Inhibitors of catabolic enzymes and affinity labels for putative receptors provide new data on pheromone processing.

Tritium-labeled C_{14} and C_{16} pheromone components of Heliothis virescens were prepared at 58 Ci/mmol and were employed to study tissue specificity of acetate esterase, alcohol oxidase, and aldehyde dehydrogenase in male and female moths. The modest esterase activity was highest in legs of both sexes, but also occurs in antennal and glandular tissues. Oxidase activity required O_2 and was highest in female pheromone gland tissues, and in the male hairpencils. Aldehyde dehydrogenase activity was uniformly high in all tissues, but highest in antennal tissues of both males and females.

Sensory hair proteins from antennae of males of the wild silkmoth, Antheraea polyphemus contain an aggressive esterase and an abundant MW 15,000 binding protein, which interact to degrade labeled pheromone less efficiently in the presence of certain unsaturated acetate analogs of the natural pheromone. Enzymatic hydrolysis was examined for ^{3}H-labeled 6E,11Z-16:Ac and several pheromone analogs. Trifluoromethyl ketones were potent inhibitors of this esterase as well as the Heliothis-derived esterase(s). A high specific activity photoaffinity label, [11,12-^{3}H]-6E,11Z-hexadecadienyl diazoacetate (DZA), is behaviorally active and competes with natural substrate for the esterase but it is not hydrolyzed. Incubation of the photoaffinity analog with sensory hair proteins followed by irradiation at 254 nm results in covalent attachment of the labeled analog to the MW 15,000 binding protein. Pheromone-competable photoattachment to membrane-associated proteins of the male sensory hairs was also observed. This is the first report of selective photoaffinity labeling of proteins important in the pheromone detection/degradation system of an insect.



Insect-Specific Metabolism

K23 ANTIBACTERIAL DEFENSIVE RESPONSES OF THE TOBACCO HORNWORM, MANDUCA SEXTA, Peter E. Dunn, Department of Entomology, Purdue University, West Lafayette, IN 47907

Intrahemocoelic injection of bacteria into larvae of the tobacco hornworm elicits a complex, dose-dependent antibacterial defensive response. The initial component of this response is mediated by circulating hemocytes. In this phase of the response, bacteria are phagocytosed by hemocytes and/or entrapped within large melanized cellular aggregates called nodules. Nodules are composed of hemocytes and bacteria embedded in an extracellular matrix. Following a 6-8 hr lag period, these hemocyte responses are augmented by the synthesis and secretion of several hemolymph proteins by fat body. This set of proteins includes the bacteriolytic enzyme lysozyme, several bactericidal peptides and proteins, and a number of additional stage specific proteins with unknown biological activity. The induced lysozyme and two families of induced cecropin-like bactericidal peptides have been purified from hemolymph of bacteria-treated hornworm larvae. Partial amino acid sequences of several of these purified defensive proteins have been determined. A synthetic oligonucleotide probe whose sequence was deduced from the known amino acid sequence of the induced lysozyme has been used to identify fat body mRNA encoding lysozyme. Studies of defensive protein synthesis by fat body have identified peptidoglycan present in the bacterial cell wall as an elicitor of the response. Soluble fragments of peptidoglycan elicit defensive protein synthesis in vivo and stimulate cultured fat body to synthesize and secrete defensive proteins in vitro. Since cell wall peptidoglycan is not exposed on the surface of all bacteria that elicit defensive protein synthesis in vivo, it is hypothesized that hemocyte recognition and processing of bacteria may be a prerequisite to stimulation of fat body antibacterial protein synthesis.

K24 ISOLATION AND PARTIAL CHARACTERIZION OF GENOMIC DNA CLONES FOR CECROPINS ATTACINS AND LYSOZYME FROM HYALOPHORA CECROPIA.Ingrid Faye, J.Y. Lee, K.G. Xanthopoulos K. Kockum and H.G. Boman, Department of Microbiology, Stockholm University, S-106 91 Stockholm, Sweden

The three main groups of antibacterial proteins in hemolymph of Hyalophora cecropia are cecropins, attacins and lysozyme. They are all selectively induced and secreted into the hemolymph of diapausing pupae after a challenge with bacteria. Messenger RNA from such induced pupae was the basis for a cDNA bank, constructed in order to look for clones for antibacterial proteins.By the use of synthetic probes deduced from the amino acid sequences, clones for all three proteins were found[1].To find out the organization and if possible the regulation of these bacteria inducible genes, a genomic DNA bank has now been constructed. DNA from pupae was partially digested with Eco RI and insserted into the lamda cloning vector Charon 4A. The bank was screened by cDNA clones for the antibacterial proteins. A preliminary characterization by restriction enzyme mapping has been performed on a selected number of clones hybridizing to the different cDNA. Southern blots showed no crosshybridization between the clones for the three diffrent proteins. In the analysis of the attacin clones evidence was found that acidic and basic attacin are closely linked. Five cecropin clones showed different restriction patterns, but the structure gene was for all of them contained in two nabouring BglII fragments, 0.8 and about 3.5 kb long. These two fragments did not cross hybridize indicating the existance of only one gene in each clone. determinations of direction of transcription showed that the 5´- end of the structure gene is located in the 0.8 fragment. Sequenceanalysis of this fragment from one of the clones has revealed the 5´-part of a gene for the precursor of cecropin B. The coding sequence of cecropin B was interrupted in the triplet coding for amino acid 9 by an intervening sequence. A sequence homologous to the TATA box was idenified and a presumtive transcription initiation site was found 32 bp down stream from the TATA box.A possibly conserved pentameric secquence was found adjacent to the iniation of transcription site .

1. H.G. Boman et.al., On the primary structure of lysozyme, cecropins andattacins from Hyalophora.Paper presented in Denver Dec 1984. Dev. Comp. Immunol. Vol.9 pp 551-558 1985.

K25 MECHANISMS OF INSECT CUTICLE STABILIZATION, Karl J. Kramer, U.S. Grain Marketing Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture and Department of Biochemistry, Kansas State University, Manhattan,Kansas 66502, T. L. Hopkins, Department of Entomology, Kansas State University, and J. Schaefer, J. R. Garbow, G. S. Jacob and E. O. Stejskal, Monsanto Company, St. Louis, Missouri 63167.

Little information is available about the supramolecular structure of the insect exoskeleton or about the physical and chemical mechanisms that occur to stabilize it because the cuticle is an insoluble material and traditional methods aimed at its characterization have required partial or complete degradation. Many schemes for the hardening process have been proposed and five types of biochemicals are generally thought to contribute to cuticular stabilization including proteins, chitin, inorganic salts, catechols and lipids. We have been conducting chemical and physical studies on cuticles from moths, beetles, flies and cockroaches in order to determine the nature and contribution of sclerotization and mineralization to cuticular hardening. Sclerotization is the crosslinking of cuticular biopolymers by catecholic metabolites. Mineralization is the deposition of inorganic salts into the exoskeleton. The relative importance of these processes in cuticle stabilization is reflected by the quantitative differences in the amounts of protein (amino acids), catechols and inorganic salts. We have also been using ^{13}C- and ^{15}N-cross polarization magic angle spinning NMR (solid state NMR) and carbon-nitrogen double cross polarization NMR to sort out the crosslinking chemistry of cuticle hardening in tobacco hornworm pupae and adults. ^{13}C- and ^{15}N-labeled amino acids, carbohydrates and catechols are introduced into hornworms by ingestion or injection, and the subsequent metabolism of these compounds is studied in intact, lyophilized tissue. Our studies indicate that carbon-nitrogen crosslinking is indeed involved in the hardening process, with the side chain nitrogens of histidine and lysine playing significant roles. Correlation of the degree of crosslinking with the intensity of specific resonances in the ^{13}C and ^{15}N spectra of the cuticle points to the involvement of particular aromatic carbons and nitrogens in the crosslinks of the chitin-protein matrix. (Supported in part by research grants PCM-8411408 from the National Science Foundation and 85-CRCR-1-1667 from the USDA Competitive Research Grant Program).

K26 HEMOLYMPH PROTEINS PARTICIPATING IN THE DEFENSE SYSTEM OF SARCOPHAGA PEREGRINA, Shunji Natori, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

In this symposium, I am going to talk about two proteins active in defense system of insects. One is Sarcophaga lectin and the other is sarcotoxin I. There are several reports of the presence of humoral lectins in invertebrates. Such humoral lectins are believed to be important in scavenging invading parasites or unnecessary own-tissue fragments, but as yet there is no conclusive evidence to support this idea. We purified and characterized a lectin from the hemolymph of Sarcophaga peregrina larvae. The biological characters of this lectin are as follows: (1) The lectin is not found in the hemolymph of normal larvae, but it is promptly induced when the body wall of larvae is injured by pricking it with a hypodermic needle. (2) During normal development of Sarcophaga, this lectin is induced at the pupal stage when larval tissues are decomposed and eliminated extensively. (3) This lectin induces murine macrophages to produce tumor specific cytotoxic protein and human peripheral blood T-cells to produce interferon γ. (4) Treatment of tumor bearing mice with this lectin has a significant antitumor effect. These results indicate two important facts about Sarcophaga lectin. One is that it activates the defense system of Sarcophaga. The other is that vertebrate immune cells have a receptor(s) for this lectin and respond to it. From the aspect of comparative immunology, I would like to summarize the function of Sarcophaga lectin. Sarcotoxin I is a potent antibacterial peptide induced in the hemolymph of Sarcophaga larvae simultaneously with Sarcophaga lectin. Recently, we determined the primary structure of sarcotoxin I. Sarcotoxin I is a mixture of three peptides (sarcotoxin IA, IB and IC) with almost identical primary structures. These peptides were found to consist of 39 amino acid residues and to differ only 2 - 3 aminoacid residues, and all of them were toxic to bacteria. The amino-terminal half of the molecule was rich in charged amino acids and was hydrophilic, whereas the carboxy-terminal half was hydrophobic. When E. coli was treated with sarcotoxin I, potassium ion inside of the cells leaked out rapidly and ATP pool of the cells rapidly decreased. These results suggested that the bactericidal effect of sarcotoxin I was due to ionophore activity, and that it blocked the generation of ATP by inhibiting formation of the proton gradient essential for oxidative phosphorylation. In this symposium, I would like to talk about the recent progress on the structure and function of sarcotoxin I.

Invertebrate Sensory Systems and Neuroendocrinology

K27 IDENTIFICATION OF ACETYLCHOLINE RECEPTOR POLYPEPTIDES IN THE NERVOUS SYSTEM OF INSECTS, Heinz Breer, FB Biology, University Osnabrück, West Germany

The nervous system of insects contains high concentrations of cholinergic binding sites, being mostly of the nicotinic type. It is thus a suitable source for studying the properties of nACh-receptors in nerve cells and furthermore may offer some clues for understanding the course of evolution for ACh-receptors.

Analysis of uniformity and molecular size of the receptor by velocity sedimentation, gel permeation and electrophoresis revealed that the receptor represents a protein with a sedimentation coefficient of 10S and an apparent molecular weight of 250-300 kd; values very similar to the vertebrate receptor. On SDS-PAGE, however, the receptor protein, purified to homogeneity by affinity chromatography, gave only one band M_r=65,000; suggesting that the native receptor represents an oligomer of identical subunits.

After reconstitution in planar lipid bilayers, the purified receptor polypeptides displayed the functions of an ion translocating system activated by cholinergic agonists, indicating that the isolated protein is all that is needed to mimic the physiological response at the level presently achievable. Immunochemical approaches have shown that there are structural similarities between insect and vertebrate receptors and in immunocytochemical studies using anti-(locust AChR)-antibodies very distinct areas in the neuropile of insect ganglia were labelled. Poly A^+-RNA isolated from locust nervous tissue was expressed in the reticulocyte and oocyte systems. In Xenopus oocytes the expression of α-toxin binding sites and functional AChR directed by insect mRNA was observed.

K28 SYNAPTOSOMAL MEMBRANE PROTEIN KINASES IN THE BRAIN OF MANDUCA SEXTA. W.L. Combest M.J. Birnbaum, T.J. Bloom, and L.I. Gilbert, Dept. of Biology, University of North Carolina, Chapel Hill, NC 27514.

The intracellular actions of many neurotransmitters are mediated by Ca^{2+} and cAMP via protein kinase activation and subsequent protein phosphorylation. Four distinct integral membrane protein kinases (PK) in a lysed synaptosome preparation from the pupal brain of Manduca sexta have been characterized. Membrane associated cAMP-PK comprises 12% of the total brain cAMP-PK. Only the type II isozyme of cAMP-PK is present in the membrane fraction as determined by its elution from DEAE-cellulose and the specific labeling of the regulatory subunit (R II =55kDa) with 8-Azido-[^{32}P]-cAMP. Several endogenous substrates for cAMP-PK were identified when membranes were incubated with [γ ^{32}P] ATP and subjected to SDS-PAGE and autoradiography with the most prominent being a 34 kDa phosphoprotein. The major phosphoprotein in the membrane fraction is a 62kDa peptide whose phosphorylation is dependent upon Ca^{2+} and calmodulin presumably via a Ca^{2+}calmodulin-PK.

Two cAMP and Ca^{2+}-independent PK which utilize casein as an exogenous substrate were separated by DEAE-cellulose. Casein kinase II (CK II) comprises 70% of the total membrane CK activity and is activated by K^+ ions, the polyamines spermine and spermidine, and inhibited by heparin. CK I is also activated by K^+ but is insensitive to polyamines and heparin. All four protein kinases along with their endogenous protein substrates can be solubilized from the membrane by Triton X-100 and 0.5M NaCl. The membrane localization of these protein kinases and their substrates may relate to their involvement in basic neuronal functions such as ion conductance and neurosecretion.

K29 AN IN VITRO ASSAY FOR THE PROTHORACICOTROPIC HORMONE(PTTH) OF DROSOPHILA MELANOGASTER Vincent C. Henrich and Lawrence I. Gilbert, University of North Carolina, Chapel Hill N.C. 27514

In several insects, prothoracic glands will synthesize dramatically increased levels of ecdysone in vitro when challenged with extract prepared from homogenized brains. Two or more peptides (i.e. small and big PTTH) evoke this response in Manduca. A D. melanogaster ring gland (which contains the prothoracic gland) dissected from a wandering third instar larva and placed in vitro will produce ecdysone at three to four times basal levels when challenged with a Drosophila brain extract. This response is dose dependent and does not occur with a similar extract prepared from imaginal discs. The peptide(s) responsible for accelerated synthesis is(are) heat stable in Drosophila, as in Manduca. This in vitro assay is being utilized to characterize those peptides responsible for ring gland stimulation. In addition, we have attempted to identify the lesion sites of several mutations known to affect ecdysone synthesis and/or metabolism, notably ecdysoneless temperature-sensitive (ecd1-ts) and Dominant Temperature-Sensitive (DTS3).

K30 Basis for the development of sensitivity to eclosion hormone in the CNS of Manduca sexta. David B. Morton & James W. Truman, Department of Zoology, University of Washington, Seattle WA 98195.

The peptide, eclosion hormone (EH) acts on the CNS of the hawkmoth Manduca sexta to trigger ecdysis behavior at the end of each instar. However the CNS will only respond to EH for a period of several hours prior to ecdysis. The biochemical basis for the development of sensitivity has been investigated.

Evidence suggests that the development is a two stage process, the final event being the ability of EH to stimulate the phosphorylation of two 54 Kd proteins via the action of cGMP. Results are presented showing why phosphorylation is not observed before the CNS is sensitive to EH and how ecdysone acts to make these proteins available for phosphorylation. Evidence is also presented that one of these proteins is present in both soluble and membrane fractions and the other is found purely in the membrane fractions of CNS homogenates. This presents the possibility that at least one of these proteins is associated with an ion channel.

K31 INFORMATION ENCODING IN PRIMARY CHEMOSENSORY NEURONS OF PERIPLANETA, Dale M. Norris Univ. of Wisconsin, Madison, WI 53706.

An informational code simply is a set of rules which governs the mapping of an input variable onto an output variable. In the studied chemoreception by Periplaneta americana, this input variable was the amount (e.g., moles) of 1,4-naphthoquinone repellent required to elicit a prescribed behavioral response (e.g., >99% avoidance). The primary output variable in such chemoreception was the maximum millivolt electrochemical ($E_{\frac{1}{2}}$) shift inducible in the receptor redox-complex protein of the dendritic membrane of involved primary chemosensory neurons by a saturating amount of a given 1,4-naphthoquinone. A linear relationship was found between the input variable and the primary output variable. Correlation between this primary output variable and the secondary output variable, maximum % inhibition of a standardized EAG by a given 1,4-naphthoquinone repellent, was such that only one of these two outputs is required to predict the elicited whole insect behavior. Our results clarify why EAGs have been successful investigative parameters for screening candidate messenger chemicals. Exchange of energy between repellent 1,4-naphthoquinones and cockroaches such that avoidance behavior results is reasonably described in an equation for a linear relationship. Information for this behavior thus is encoded in the primary sensory neuron. CNS only must connect this primary neuron to the muscles required to effect the animal behavior.

K32 BIOSYNTHESIS OF LOCUST ADIPOKINETIC HORMONE: DEVELOPMENTAL CONTROL AND IDENTIFICATION OF PRECURSOR. M. O'SHEA and S. HEKIMI, University of Geneva

The adipokinetic hormones (AKH I and AKH II) of the locust (Schistocerca) are structurally related, sequenced (AKH I : pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2, AKH II = pGlu-Leu-Asn-Phe-Ser-Gly-Thr-Trp-NH_2) hormones which function in the adult to mobilize lipid metabolism during prolonged flight. Each is contained in the glandular lobe of the corpus cardiacum (CC) from which they are released into the heamolymph. We have examined the developmental acquisition of these hormones. Both are present throughout postembryonic development. The AKH II/AKH I ratio however changes markedly. Thus in the 1st instar (first stage of postembryonic development) AKH I and II are present in approximately equal amounts (0.8 ratio) but in adults there is a dramatic increase in AKH I levels producing an adult ratio of 0.2. In order to understand this shift we are studying the biosynthesis of AKH by in vitro culture of the CC and by following the incorporation of ^3H amino acids. Using molecular sieving HPLC we have identified a 17Kd protein as a putative precursor of AKH. This protein (PAKH) is present in the glandular lobe of the CC but not the storage lobe. It first appears to incorporate ^3HTrp after 20 mins incubation whereas ^3HAKH appears only after 2 hrs. Pulse-chase and cycloheximide-translation block experiments confirm the transfer of label from PAKH to both AKH I and II. The HPLC method (TSK 2000) does not rule out the existence of two forms of PAKH of approximately equal molecular weight. We will report on our further findings concerning the nature of the precursor(s) and the regulatory mechanisms producing shifts in AKH I and II levels during development.

K33 ADIPOKINETIC HORMONE OF HELIOTHIS ZEA, Ashok Raina and Howard Jaffe, Department of Entomology, University of Maryland, College Park, MD 20742 and Livestock Insects Laboratory, USDA, Agricultural Research Service, Beltsville, MD 20705

Reverse phase high-performance liquid chromatography of brain-corpora cardiaca complex of adult Heliothis zea (Lepidoptera: Noctuidae), revealed a peptide that closely resembled locust adipokinetic hormone. Subsequently, the peptide was isolated and purified from the corpora cardiaca. Amino acid analysis revealed the following: 2 Ser, 2 Thr, Glx, Gly, Leu, Phe and Trp. Injection of the purified hormone at 5 pmol/adult caused a 70% increase in the total hemolymph lipid over saline injected control in a one hour period.

K34 BIOCHEMICAL MECHANISMS OF PEST RESISTANCE IN PASTURE LEGUMES
G.B. Russell, D.R. Biggs, G.A. Lane, O.R.W. Sutherland, DSIR, Palmerston North, New Zealand

Several pasture legumes are resistant to root-feeding scarab larvae (Costelytra zealandica and Heteronychus arator). Behavioural studies have shown that the insects are deterred from feeding by the isoflavonoid constituents of the roots. The most active compounds are those that have also been recognised as phytoalexins in the foliage of legumes. The feeding deterrent activity of these compounds is highly structure dependent and relates to their stereochemistry.

Besides isoflavonoids, the resistant plant Lotus pedunculatus contains high yields of glucose nitropropionic acid esters which are feeding deterrent and toxic at the concentrations (0.2% ww) found in the root. The nitro esters do not have a particularly high biological activity but they make a substantial contribution to the total feeding deterrent activity of the plant because of their high concentration. By contrast, the isoflavonoids are very active and account for a significant part of the feeding deterrent activity of the plant in spite of their low yield (ca. 10 ppm). This is an example of "quantitative and qualitative resistance". The investigation of the mechanisms of resistance will lead to the application of biochemical techniques in selection or genetic manipulation to develop a resistant white clover.

K35 INDUCED CHANGES IN SEX PHEROMONE BIOSYNTHESIS OF MOTHS, Peter E.A. Teal, University of Guelph, Guelph, Ontario, N1G 2W1 and James H. Tumlinson, Insect Attractants Behavior and Basic Biology Research Laboratory, USDA, Gainesville, Florida, 32604

In vivo application of large amounts of alcohols (normally present in small amounts) to the female sex pheromone glands of 3 species of Heliothis moths resulted in the preferential conversion of the alcohols to the corresponding pheromonal aldehydes. The enzyme responsible has been characterized as an alcohol oxidase which is specific for primary alcohols but has no specifity for chain length, or number or geometry of double bonds. Using this technique we have induced female H. zea to produce the sex pheromone blend used by, H. virescens, by applying tetradecanol and (Z)-9-tetradecenol. Treated H. zea females were attractive to H. virescens males and caused males to attempt interspecific copulation. Females of H. subflexa possess an esterase which converts acetates to alcohols. The esterase does not apparently function in pheromone biosynthesis by female H. virescens but is responsible for the terminal step in pheromone biosynthesis by males. The esterase present in the gland of female H. subflexa converts a portion of the acetate pheromone components to alcohols which are converted to aldehydes via the oxidase. Thus the female produced pheromone of H. subflexa differs from that of its sibling species, H. virescens, in that both acetates and aldehydes are used. Therefore, it appears that differential responses to blends of aldehydes, acetates and alcohols are due to differential perception and central processing of the molecules by both sexes of each species.

K36 **ANTENNAL SENSILLA DISTRIBUTION AS RELATED TO SEXUAL DIMORPHISM IN PERIPLANETA AMERICANA.**

A. Usha Rani, Regional Research Laboratory, Hyderabad 500 009, INDIA

The morphology, density and distribution of various sensilla on the antennal flagellum of male and female American cockroach, Periplaneta americana (L), were examined with light microscope and scanning electron microscope. The antenna contains chemosensitive sensilla on every segment. Sensilla basiconica, which could be categorised morphologically into four different types, and grooved pegs were found scattered all over the antennal segments. Morphologically there was no difference in the size or shape of each segment. However, on the male antenna there is a variation in the density and distribution of sensilla. Sensilla basiconica type III with long shaft walls appear more frequent on all antennal segments of the males. At the basal region they are much more abundant and constitute nearly 50 percent of the sensillum population of the male antennae. This could perhaps indicate the possibility of their involvement in perception of the female sex pheromones.

K37 AKH AND GPAH ARE NOT IDENTICAL IN MANDUCA SEXTA. R.Ziegler*, GKegel[+] and R.Keller[+]

*Inst. Tierphysiologie, Freie Universitaet Berlin, [+]Inst.Zoophysiol.Universitaet Bonn

In the corpora cardiaca (CC) of Manduca sexta there is a glycogen phosphorylase activating hormone (GPAH) which activates fat body glycogen phosphorylase in larvae during starvation (Siegert and Ziegler, 1983, Nature, 301, 526) and an adipokinetic hormone (AKH) which mobilizes lipids during flight in adults (Schulz and Ziegler, in preparation). Extracts of CC from adults and from larvae showed, when injected, both activities. In the adults both activities were associated with a nonapeptide of known sequence (Ziegler et al., in press). These hormonal activities (AKH and GPAH) then could be associated with a single peptide having different roles in different life stages of the insect. Comparison of dose-resonse curves, however, showed that more than one peptide was involved. Preliminary chromatographic separation indicated that larval CC contain more than one peptide, which can influence the regulation of lipid and carbohydrate metabolism. The main peptide peak which had both activities, had a slightly different retention time from that of AKH when analyzed on reversed phase HPLC. A preliminary amino acid analysis indicated a difference in the amino acid composition. We therefore conclude that AKH and GPAH are different peptides with overlapping activities.

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Peptides and Proteins in Invertebrates

K38 CHITINASE ACTIVITY (I.): ISOLATION OF NATIVE CHITIN. M.L. Bade, A. Stinson, Nehad A.-M. Moneam, Dept. Biology, Boston College, Chestnut Hill, MA 02167

We have published conditions for measuring molting fluid chitinase activity in vitro (Arch. Biochem. Biophys. 206, 213-221 (1981); Insect Biochem. 11, 599-604 (1981)) which specify as substrate chitin made from insect larval cuticles. Insect chitin made as there specified is fibrous under moderate magnification in the light microscope, and properties are predictable and reproducible. these properties include ready recognition by specific enzymes which, regardless of biological origin, generate product rapidly and linearly with time. Data thus obtained behave normally in conventional kinetic analyses. We have demonstrated persistence of chitin fibrous structure seen in electronmicrographs of the intact exoskeleton (where it is stabilized by structural proteins) via purified compacted chitin (in which fibers, though latent, can nevertheless be demonstrated) to the fibers ultimately isolated (where the stabilizing functions is carried out by sparse covalently linked inorganic ester functions).

We have now devised techniques for reproducibly isolating fibrous chitins from other chitinous animal carapaces such as those from shrimp, lobster, and crab. Enzymatic, chemical, and photomicrographic evidence indicates that fibrous chitins so isolated maintain their native fine structure. This permits not only valid kinetic and mechanistic characterization of enzymes not thus characterized until now, but also accesses 100 billion tons of annually renewed biomass for important technological utilization. (Work supported by Boston College).

K39 PARASITISM-SPECIFIC HEMOLYMPH PROTEINS IN TOBACCO HORNWORMS PARASITIZED BY Cotesia congregata. N.E. Beckage*, T.J. Templeton*, B.E. Nielson*, D.I. Cook**, and D.B. Stoltz**. *Issaquah Health Research Institute, Issaquah, WA., 98027 and **Dalhousie University, Halifax, Nova Scotia.

Parasitism of tobacco hornworm larvae by the braconid wasp Cotesia congregata induces the synthesis of a new, parasitism-specific, protein in the host. In vivo labeling of the protein with ^{35}S-methionine indicates its synthesis begins 2 to 4 hr post-parasitization, with abundant amounts detectable by 12 hr. SDS-PAGE analysis shows the approximate molecular weight of this protein is 33,000 daltons. The same protein is inducible in unparasitized larvae of the same age by the injection of ovarian calyx fluid from adult female wasps, which is known to contain virus particles normally inserted into the host during parasitization. Incubation of preparations of calyx fluid with psoralen in the presence of long wave UV-light prevents induction of the protein, presumably due to inactivation of the viral nucleic acids prior to injection. During the final phases of the parasite's development within the host, this 33 kd protein is not obviously present but at least two other major, and one minor, parasitism-specific bands are detectable in hemolymph from terminal stage fifth instar hosts. The same proteins are also present in terminal stage third, fourth, and supernumerary sixth instar hosts, indicating their synthesis, like that of the early stage 33 kd protein, is correlated with the stage of development of the parasites rather than the host. Hemolymph phenoloxidase activity is also affected by parasitism, indicating there are multiple changes in hemolymph proteins.

K40 ADIPOKINETIC HORMONE INDUCED HYPERGLYCEMIA AND HYPERLIPEMIA IN LOCUST LARVAE, Ad M. Th. Beenakkers, Dick J. Van der Horst and Wil J.A. Van Marrewijk, University of Utrecht, Utrecht, The Netherlands

Injection of adipokinetic hormone I (AKH) into adult Locusta migratoria results in elevation of the lipid concentration in the haemolymph, whereas the carbohydrate concentration remains unaffected. In fifth instar locust larvae, however, AKH induces both hyperglycemia and hyperlipemia.
The increase of the carbohydrate level in larval hemolymph is hormone-dose dependent in the range of 0.2-20 pmol AKH. The hormone appears to activate fat body glycogen phosphorylase, the percentage active phosphorylase increasing dose dependently in the range of 0.04-20 pmol AKH. The dose-response relationships for elevation of carbohydrate concentration and activation of glycogen phosphorylase are well correlated.
The hyperlipemic response in larvae remains substantially below that in adults. In the adult locust hormone-induced diacylglycerol release from fat body is attended by the association of hemolymph lipophorin and glycoprotein C_2 to form a larger lipoprotein particle with high lipid loading (A^+). Although also in larvae the mobilized lipid is diacylglycerol, the amount of lipoprotein A^+ is very low. Elevation of the very low level of C2 in larval hemolymph by injection of C_2 from adults nor injection of adult lipophorin did result in increased lipid mobilization, showing that C_2 concentration or lipophorin characteristics are not the only factors responsible for the weak hyperlipemia in larvae. With in vitro assays it was demonstrated that the low hyperlipemic response of larval fat body to AKH is a significant additional factor in the imcomplete adipokinetic overall response in larval locusts.

K41 ONLY 3 GENES ENCODE ACTINS IN DROSOPHILA VIRILIS, Laura Elsenboss-Pena, Ann Sodja, Dept. of Biological Sciences, Wayne State University, Detroit MI 48202

Actin genes are encoded by multigene families in most organisms studied to date. In Drosophila melanogaster the 6 actin genes code for 6 functional proteins. The expresssion of these 6 genes has been followed through development using 3' transcribed but untranslated sequences specific for each gene. The expression of each gene appears to be tissue and temporal specific.

From genomic Southern blot analysis only 3 actin genes have been detected in Drosophila virilis. These genes appear to be more divergent in sequence from each other than they are from the D. melanogaster actin genes. Screening of a genomic D. virilis library resulted in isolation of only 3 recombinant phage clones containing unique actin sequences. Restriction enzyme maps of these 3 clones show little or no similarity to the restriction enzyme maps of D. melanogaster actin genes. Data relating to further characterization of these genes including the temporal and tissue expression will be presented. Given that in D. virilis 3 and not 6 genes satisfy all the functional demands for actin, we speculate as to what constitutes a truly tissue/temporal specific gene or gene product.

K42 AMINO ACID SEQUENCE OF INSECT APOLIPOPHORIN-III
Germain J. P. Fernando-Warnakulasuriya, John H. Law and Michael A. Wells, University of Arizona, Tucson, AZ 85721

The amino acid sequence of Manduca sexta apolipophorin-III has been determined. Apo-III is a non-glycosylated protein of 165 amino acids and a molecular weight of 17,500. Apo-III is found in high concentration in adult hemolymph. It associates with lipophorin when diacylglycerol is released from the fat body in response to the action of adipokinetic hormone. Apo-III helps to stabilize the diacylglycerol rich lipophorin. The sequence reveals the presence of amphipathic helices which may be responsible for the lipid binding properties of Apo-III. The sequence homology of Apo-III to other known protein sequences in the National Biomedical Research Foundation library was determined. There is a 28% identity in a 29 amino acid overlap with human apolipoprotein E. The region of overlap (ApoE(131-157)) corresponds to the receptor binding region of ApoE. Although Apo-III, like mammalian apoproteins, is high in Glu/Gln and Asp/Asn and lacks Cys, no other apparent similarities were seen with other mammalian apoproteins.

K43 PURIFICATION OF JUVENILE HORMONE ESTERASE FROM THE LAST LARVAL STADIUM OF Trichoplusia ni AND ITS DETECTION AND PARTIAL PURIFICATION FROM EARLIER STADIA. Terry N. Hanzlik, Yehia Abdel-Aal and Bruce Hammock, University of California, Davis, Ca.

Juvenile Hormone Esterase (JHE) activity was detected in the hemolymph of T. ni in 3 stadia prior to the last stadium. This activity occured towards the end of molting, peaked at ecdysis, then declined rapidly thereafter. Protein(s) associated with this activity were partially purified from whole-body homogenates using a novel method of affinity chromatography based upon trifluoromethyl ketone ligands. When preparations from the 4^{th} to 5^{th} and 3^{rd} to 4^{th} ecdyses were analyzed by SDS-PAGE, a band was found to have an identical mobility to JHE purified to apparent homogeneity from preparations containing the 2 peaks of activity that occur in the last stadium. This protein(s) has an M_r of 64 kD and is shown to be responsible for the majority of JH hydrolysis that occurs in whole-body extracts by an experiment involving preincubation of the extracts with specific inhibitors prior to loading the affinity gel. Using rabbit polyclonal antibodies raised against JHE purified from prewandering hemolymph, Western blotting revealed these proteins to be immunogenically related. Analysis of the purified proteins from the the last larval stadium with IEF-PAGE revealed 4 seperate bands from JHE purified from a prepupal whole-body extract while only 2 of these were evident in JHE from prewandering hemolymph with 1 being predominant. This is the 1^{st} report of JHE presence in early stadia of a lepidopteran species during which, JH is thought to be maintained at a continual high level.

K44 IMMUNOCHEMICAL DETECTION OF HEMOLYMPHATIC HEMAGGLUTININ IN THE ACRIDIDAE (GRASSHOPPERS). Kenneth D. Hapner, Department of Chemistry, Montana State University, Bozeman, MT 59717.

The hemagglutinin present in the hemolymph of Melanoplus differentialis and M. sanguinipes has been isolated and purified and shown to be inhibited by low concentrations (<5mM) of either D-glucosidic or D-galactosidic carbohydrates (M. R. Stebbins and K. D. Hapner, Insect Biochemistry (1985) 15:451-462). Monoclonal antibodies that are specific for the hemagglutinin were characterized from several mouse hybridoma supernatant solutions. Molecular isotyping showed that monoclonal antibodies of both the IgG and IgM classes were produced. SDS-polyacrylamide gel electrophoresis resolves whole hemolymph into numerous protein bands, two of which are detectable, when transferred to nitrocellulose and overlaid with monoclonal antibodies and enzyme-labeled secondary antibody. The two positive bands correspond to identical bands observed with purified hemagglutinin, however their relative intensity is reversed, suggesting the existence of two interconvertible forms of the grasshopper agglutinin. Several acridid and orthopteran specimens were examined for the presence of identical or cross-reacting hemolymphatic antigens and results suggest that the agglutinin present in M. differentialis is narrowly distributed.

K45 **A BLUE CHROMOPROTEIN FROM THE CORN EARWORM, *Heliothis zea*.**
Norbert H. Haunerland and William S. Bowers, Department of Entomology, The University of Arizona, Tucson, AZ 85721.

During the development of the corn earworm, *Heliothis zea*, the color of its hemolymph changes: In early larval instars the hemolymph appears colorless to yellow, while in late larval stages an intense green or blue color predominates. Pupal and adult hemolymph again appear yellow. We find that the blue color of the blood from late larvae is due to the appearance of a blue chromoprotein. This protein, a glycolipoprotein, was purified to homogeneity. It has the molecular weight of 560,000 and is composed of 4 apparently identical subunits (M_r - 150,000). The protein contains 7 % lipid and has an equilibrium density of 1.24 g/ml. One molecule biliverdin is bound noncovalently to the tetramer. The changes in its concentration during different life stages are responsible for the visual color changes in the hemolymph: The concentration rises from less than 1 mg/ml in early larvae to 12 mg/ml in 5^{th} instar larvae, but drops rapidly to below 1 mg/ml in pupae. Adult moths completely lack the blue protein. Unlike other known biliverdin containing insect proteins, the *H. zea* chromoprotein seems to be specific for the late larvae. Antibodies raised against the *H. zea* chromoprotein do not crossreact with the insecticyanin from *Manduca sexta* or cyanoproteins from grasshopper species.

K46 CLONING OF THE GENE FOR APOLIPOPHORIN III FROM LOCUSTA MIGRATORIA, M.R. KANOST, H.L. McDonald, J. Locke, J.Y. Bradfield, and G.R. Wyatt, Queen's University, Kingston, Canada K7L 3N6

Apolipophorin III (apoLp-III) becomes associated with lipophorin upon stimulation by adipokinetic hormone and is present at greater concentrations in mature adult insects than in larvae. We have purified apoLp-III from Locusta migratoria, and have cloned a gene for this protein, as steps in studying the regulation of its synthesis. Our purified locust apoLp-III has properties similar to those reported by other workers (protein C_2 of Van der Horst et al., 1984, Insect Biochem. 14: 495; C-I of Goldsworthy et al., 1985, Physiol. Entomol. 10: 151). The gene was isolated from a library of Locusta genomic DNA, using cDNA to RNA from adult female fat body as a probe. Clone lambda SauC contains DNA which hybridizes to a 1200 nt fat body RNA that is abundant in adult male and female locusts and present at low levels in fifth instars. The gene was identified as apoLp-III by hybrid select translation. RNA dot hybridization has shown that the juvenile hormone (JH) analog, methoprene, stimulates accumulation of apoLp-III mRNA in fat body of adult female locusts. Immunodiffusion assay has shown that the apoLp-III protein is present in fifth instar and day 1 adult hemolymph at approximately 7 mg/ml, and increases during adult maturation to 35 mg/ml by day 10, when increasing JH levels stimulate expression of the vitellogenin genes in females. These data suggest that the apoLp-III gene is constitutively expressed at low levels and is positively regulated by high JH titer in adult locusts. (Supported by NSERC Canada and U.S. NIH)

K47 MICROVITELLOGENIN, A LOW MOLECULAR WEIGHT FEMALE SPECIFIC PROTEIN. PURIFICATION AND CHARACTERIZATION, John K. Kawooya, Ellie O. Osir and John H. Law, Department of Biochemistry, University of Arizona, Tucson, AZ 85721

Microvitellogenin belongs to a new class of low molecular weight female specific proteins found in insects. The protein has been purified from the hemolymph of the adult female tobacco hornworm, Manduca sexta. Microvitellogenin is synthesized by the fat body and first appears in the hemolymph 18 days before eclosion. The protein is sequestered from the hemolymph into the eggs where it accumulates at relatively higher concentrations. Data from molecular weight estimates, amino acid compositions, isoelectric points, circular dichroic spectra, immunological studies, and N-terminal amino acid sequence residues suggest that microvitellogenin does not undergo any modifications before or after its sequestration into the eggs. The protein has neither immunological, chemical, nor physical identity to vitellogenin. Microvitellogenin is most probably involved in the reproduction processes of the female insect.

Supported by grants from the National Institutes of Health and the National Science Foundation.

K48 LIPOPHORIN BIOSYNTHESIS DURING THE LIFE CYCLE OF THE TOBACCO HORNWORM MANDUCA SEXTA, Sarvamagala V. Prasad, Kozo Tsuchida, Kenneth D. Cole and Michael A. Wells, University of Arizona, Tucson, Arizona 85721

Lipophorin, the major hemolymph lipoprotein, is present during all stages of development. During the larval and pupal stages, lipophorin contains two apolipoproteins, ApoLp-I and ApoLp-II, while in the adult, an additional apolipoprotein, ApoLp-III, is present. During the change from larvae to pupae, lipophorin undergoes dramatic changes in lipid content and composition, but these changes do not involve new ApoLp-I or ApoLp-II synthesis. In vivo experiments show that biosynthesis of lipophorin only occurs during the first 3 days of the fifth instar and in vitro experiments show that isolated fat body secretes lipophorin during this same time period. In vivo experiments show that lipophorin synthesis does not occur during the pupal stage, but recommences just prior to adult eclosion. mRNA was isolated from the fat body at various stages of development and translated in the reticulocyte lysate system. The results show that ApoLp-I and ApoLp-II mRNA is present at times when the fat body is not actively secreting lipophorin. It appears that lipophorin biosynthesis in M. sexta may represent an interesting example of post-transcriptional control of protein synthesis.

K49 VERY HIGH DENSITY LIPOPROTEIN (VHDL) FROM LARVAL HONEYBEE, Justin O. Schmidt, Barbara A. Shipman, Robert O. Ryan and John H. Law, Carl Hayden Research Center and University of Arizona, Tucson, AZ 85719

A novel honeybee hemolymph protein consisting of two identical apoproteins plus lipid and carbohydrate was isolated from late fifth instar worker larvae. Larvae were removed from combs, bled via punctures in the lateral posterior aspect, and the blood (in phosphate buffered saline with glutathione and diisopropylphosphorofluoridate) spun briefly to remove particulates. A two step procedure consisting of successive 16 h density gradient centrifugation at 242,000 g yields pure protein. The first centrifugation involved overlaying the hemolymph in KBr of density 1.31 g/ml with an equal volume of KBr of density 1.16 g/ml. The fractions containing VHDL were pooled, dialyzed and centrifuged in KBr of density 1.13 g/ml overlayered with an equal volume of KBr of density 1.23 g/ml. The resultant protein is pure by SDS-PAGE. VHDL contains approximately 10% lipid, mainly phospholipids, and has a density of 1.26 g/ml. VHDL contains 2.6% carbohydrate in the form of high mannose oligosaccharide as determined by affinity for concanavalin A. The protein has a single apoprotein of 160,000 daltons by SDS-PAGE and based on crosslinking experiments with dimethylsuberimidate, exists in a dimeric form. The intact protein exhibits an apparent MW of 450,000 daltons by both gel permeation chromatography and pore limiting polyacrylamide gel electrophoresis. Amino acid analysis revealed no similarity of this protein to either honeybee arylphorin or the honeybee lipophorin apoproteins. Rabbit anti-VHDL is being generated. VHDL is present in both honeybee worker and drone late fifth instar and in early pupae. It is absent in younger larvae and in adults.

K50 IDENTIFICATION OF GUANYL NUCLEOTIDE BINDING PROTEINS IN DROSOPHILA, Mark A. Stamnes, Rosemary S. Hopkins, Moira B. Glaccum, Melvin I. Simon and James B. Hurley, HHMI, University of Washington, Seattle, WA 98195

G-proteins, a class of guanyl nucleotide binding proteins involved in signal transduction, are substrates for pertussis or cholera toxin catalyzed ADP-ribosylation. Drosophila G-proteins were identified by labeling head homogenate with [α-^{32}P]-NAD and pertussis toxin. SDS-gel electrophoresis of the labeled Drosophila head membrane fraction, followed by autoradiography, demonstrated that a 39kD protein was ADP-ribosylated. This protein corresponds to the alpha subunit of previously characterized mammalian G-proteins which range in size from 39 to 52kD. When the pertussis toxin treated homogenate is partially digested with trypsin, three labeled fragments form that range in size from 8 to 17kD. After complete digestion, only the 17kD fragment remains. The fact that several bands form during limited proteolysis may indicate that there are several types of G-proteins which comigrate at the 39kD position. The pattern of proteolysis of the 39kD Drosophila protein is affected by the presence of GTPγS (a non-hydrolyzable GTP analog). A similar result was shown with the mammalian G-proteins, transducin and G_o (Hurley, et al., Science 226:860, 1984). A 37kD tryptic fragment becomes stable when GTPγS is added to the digestion reaction. Proteolytic fragments have been partially purified in preparation for amino acid sequence determination and for the preparation of antibodies.

K51 HEAT SHOCK AND GENE TRANSFER IN LOCUSTS, Virginia K. Walker, Steven Whyard, Gerard R. Wyatt and Michael R. Kanost, Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

Locusta migratoria is native to regions of equatorial Africa where the average air temperature is 32°C and exposed sand can reach temperatures above 50°C. We have found that the optimal induction temperature for heat shock proteins (hsp) is 45°C, the highest eukaryotic hs temperature known. Pretreatments at 45°C enhance survival of insects exposed to lethally high temperatures, suggesting that the hsps may contribute to thermoprotection. Although the molecular weights of the 6 locust hsps correspond to those of Drosophila proteins, there appears to be little of the translational control that is characteristic of the fly's hs response. Vitellogenin synthesis at 45°C is 55% of that observed at 30°C. Thus, while the hs response in locusts has some similarities to that of Drosophila, it also possesses features that may be adaptive to the locust's native environment. We were curious to observe the results of transfer of a Drosophila hs gene to locusts. A construct containing the Drosophila hsp 70 promoter and bacterial chloramphenicol acetyltransferase (hsp-CAT; DiNocera and Dawid, 1983, PNAS 80, 7095) was injected into 1 h locust embryos. After 7 days, CAT enzyme activity could be detected at 25°C, but was significantly higher after short heat shocks at 37°C (the Drosophila hs temperature) and at 45°C. (Supported by NSERC Canada and U.S. NIH).

K52 REGULATION OF DROSOPHILA HMG-CoA REDUCTASE ACTIVITY, John A. Watson, Christopher M. Havel, Ernest Rector II, Michael Garfinkel and Tracey Ripmaster, University of California San Francisco, San Francisco, CA 94143.

Kc cell's 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase activity is modulated by mevalonate availability. We have proposed that a product distal to isopentenyll-pyrophosphate (IPP) served as the regulatory signal molecule for mevalonate mediated suppression of Kc cell HMG-CoA reductase activity. Unfortunately, no direct correlation was obtained between radioactive mevalonate conversion to IPP, total isopentenoid lipids, or specific water soluble isopentenoid pyrophosphate esters. However, ≥ 40% of the mevalonate carbon converted to IPP was shunted through polyprenols to n-fatty acids. We isolated and characterized a NAD dependent "prenol" dehydrogenase and aldehyde oxidase activity which served to oxidize polyprenols to prenoic acids. Our results suggest a possible regulatory role for shunted, post IPP carbon in the control of Kc cell HMG-CoA reductase activity.

K53 STORAGE PROTEIN PROFILES AND SYNTHESIS IN Manduca sexta LARVAE. Bruce A. Webb and Lynn M. Riddiford. Dept. Zoology, Univ. of Wash. Seattle, WA 98105

Manduca arylphorin(MA) is present at low levels in the hemolymph early in the fifth instar[1]. The titer of MA rises rapidly until wandering. Midway through the prepupal period the titer of MA declines in the hemolymph and concurrently reappears in the fat body. Our studies of protein synthesis by the fat body show that MA is not synthesized after wandering. Consequently, the reappearance of MA in the fat body must be due to uptake from the hemolymph.

The female-specific protein(FSP) first appears in the hemolymph of females on day 2 of the fifth larval instar and rises to high titers by wandering[2]. When the mRNA for the prepupal specific protein(PSP) appears late on the day before wandering[3] we find that the protein is being synthesized and that some is present in the hemolymph. The hemolymph titers of FSP and PSP begin to decline rapidly the second day after wandering and are undetectable by 12 hrs before pupal ecdysis. Studies of protein synthesis during the prepupal stage have been complicated by the presence of greatly increased protein content but preliminary results suggest that protein synthesis is suppressed relative to the feeding stage fat body. Preliminary in vitro studies suggest that MA is turned off by 20-hydroxyecdysone but that FSP is not.

1.Kramer, S.J., Mundall, E.C., Law, J.H. (1980) Insect Biochem., 10:279.
2.Ryan. R.O., Keim, P.S., Wells, M.A., Law, J.H. (1985) J Biol Chem (in press)
3.Riddiford, L.M. and R.H. Hice (1985) Insect Biochem., 15:489.
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K54 ACTIVITY AND DEVELOPMENTAL BIOLOGY OF HEMOLYMPH HEMAGGLUTININS OF A NORTH AMERICAN SILKMOTH, HYALOPHORA CECROPIA, Robin W. Yeaton and William H. Telfer, Molecular Biology Institute, University of California, Los Angeles, CA 90024, and Biology, University of Pennsylvania, Philadelphia, PA 19104

Hyalophora cecropia hemolymph contains proteins which agglutinate mammalian erythrocytes by specifically binding multivalently to plasma membrane oligosaccharides. The functions of these lymphatic hemagglutinins, commonly occurring in invertebrates, have yet to be discovered. Hypotheses include immune defense, tissue-specific cell adhesion during development, sugar and glycoprotein transport during oogenesis, and anti-predator/parasite defense. To correlate the occurrence of hemagglutinins with important developmental events, over 500 samples of hemolymph and egg extracts were collected from cecropia of different sexes, genetic backgrounds and all stages of metamorphosis, and hemagglutinin titers measured. Embryonic onset of hemagglutinin synthesis, increase in titer throughout the larval instars, and maintenance of high titer from fifth instar, through pupation and adult development suggest that this is a unique and important set of proteins, constituting both larval and adult protein cohorts.

Invertebrate Reproduction

K55 ENDOCRINE INTERACTIONS AND VITELLOGENIN LEVELS IN Musca domestica, T. S. Adams, USDA-ARS, Fargo, ND, 58105

Vitellogenin levels were measured in Musca domestica without ovaries and/or the corpus allatum (CA) - corpus cardiacum (CC) complex and in unoperated controls over time. These insects were treated with methoprene (JHA) and/or 20-hydroxyecdysone (ECD). It was found that ECD did not induce vitellogenin synthesis in flies without the CA-CC complex but JHA did. ECD did increase vitellogenin levels in ovariectomized and in unoperated control flies. JHA and ECD treatments produced significantly elevated vitellogenin levels in ovariectomized and unoperated controls.

This data suggests the following: 1) ECD alone cannot induce vitellogenin synthesis 2) JHA did induce vitellogenin synthesis in all cases 3) There is an apparent JHA*ECD and ECD*CA-AA complex interaction.

K56 CANTHARIDIN BIOSYNTHESIS: EXTENT, TIMING AND INHIBITION, James E. Carrel, J.P. McCormick, J.P. Doom and K. E. Smith, University of Missouri, Columbia, MO 65211.

The simple, symmetric structure of cantharidin belies the complex transformation that accounts for its biosynthesis from a farnesyl precursor. Also intriguing are the biological roles played by this defensive substance, to which are generally ascribed the toxic, vessicant, and aphrodisiac properties of blister beetles, including the "Spanish fly". As a foundation for chemical and biological investigations addressing these questions, we have studied the extent and timing of cantharidin biosynthesis in adult blister beetles. Our experimental approach features development and exploitation of (1) protocols for complete laboratory rearing of several blister beetle species and (2) an inhibitor of cantharidin biosynthesis at the pre-farnesyl stage. Using an efficient quantitative analytical method, we have found that cantharidin apparently is not biosynthesized by adult females but is actively synthesized by adult males. Males transfer large portions of their cantharidin, present both systemically and stored in a reproductive tract accessory gland, to females during mating. 6-Fluoromevalonate, administered either by injection or in the diet, effectively inhibits cantharidin biosynthesis in adult male beetles. Financial support from the National Institutes of Health is gratefully acknowledged.

K57 PURIFICATION AND SOME BIOCHEMICAL PROPERTIES OF VITELLINS FROM Rhipicephalus appendiculatus EGGS, AND THEIR USE AS ANTIGENS TO INDUCE TYPE II IMMUNE RESISTANCE IN RABBITS , Tarlochan S. Dhadialla , The International Centre of Insect Physiology and Ecology , P.O. Box 30772 , Nairobi , Kenya.

The two major egg yolk proteins, vitellins (Vn), have been purified from freshly deposited eggs of the brown ear tick, Rhipicephalus appendiculatus, by gel permeation and anion-exchange chromatography. The two proteins, which have native molecular weights of about 480,000 each, could not be further separated by varying the conditions for ion-exchange or by chromatofocussing techniques. However, with prolonged running times of native - PAGE , the two proteins resolved as distinct, closely migrating, bands. The vitellins were found to be hemeglycolipoproteins by spectrophotometric and histochemical methods. Under reducing conditions, they separated into eight sub-units by SDS - PAGE. The sub-units which range in molecular weights from 43,000 to 160,000 , are present in unequal stoichiometric amounts as indicated by their relative staining intensities on electropherograms.

Antisera to the Vn prepration has been raised in rabbits as well as by hybridoma technology. An experiment was conducted to bioassay the potency of rabbit-anti-Vn serum to disrupt egg-production in adult female R. appendiculatus which were allowed to feed on rabbits immunized with Vn. However, there were no significant differences in engorgement weights or weights of deposited egg-batches of ticks fed on either experimental or control rabbits.

K58 A MONOCLONAL ANTIBODY AGAINST AN EPITOPE IN BOTH THE SPERMATOPHORE AND CUTICLE OF TENEBRIO MOLITOR. Karin A. Grimnes, Connie S. Bricker and George M. Happ, University of Vermont, Burlington, Vermont 05405

The reproductive accessory gland complex of the male mealworm beetle contains two pairs of glands: the bean-shaped (BAGs) and the tubular accessory glands (TAGs). Both pairs contribute material to the spermatophore, which is assembled in extracellular space and used to transfer sperm to the female. Monoclonal antibodies were produced against the secretory products of the glands. Antibodies produced by one clone, designated PL15.2, recognized several high molecular weight proteins (115-170Kd) and one low molecular weight protein (about 20Kd) in homogenates of the TAGs and the spermatophore.

Immunohistochemical staining with antibody PL15.2 in wax sections was distributed throughout the secretory epithelium and in the lumen of the TAGs but it was restricted to the lumen of the spermatophore. At the ultrastructural level, the epitopes were detected primarily within the secretory granules of the TAG. Antigen was also detected within the secretory material which mixes with the sperm in the lumen of the spermatophore.

Antibody PL15.2 also recognized an epitope present in the epicuticle of the ejaculatory duct (where endocuticle seems to be absent), along the efferent ductules of the pit glands, and the subcuticular zone between the inner surface of the cuticle and the epidermal cells.

Both spermatophore and cuticle are assembled and stabilized in extracellular space. The existence of a common epitope suggests that there may be common structural features and perhaps common mechanisms of assembly in these two large extracellular structures.

K59 TRANSPOSABLE ELEMENTS IN THE ANOPHELES GAMBIAE COMPLEX, H. H. Hagedorn, B. Tyler, R. A. Reiss, R. J. MacIntyre, J. M. Calvo, Departments of Entomology, Genetics and Biochemistry, Cornell University, Ithaca, NY 14853

The Anopheles gambiae complex includes the major vectors of malaria in Africa. Most members of this complex can be distinguished only by inversion patterns on their polytene chromosomes, but they do show behavioral and ecological differences that result in considerable differences in their efficiency as vectors. The observed chromosomal inversions could have been caused by the action of transposable elements. To investigate whether transposable elements exist in the mosquitoes of this complex, genomic libraries of A. gambiae s.s., A. melas, and A. arabiensis were prepared and screened using total genomic DNA probes under conditions in which only repetitive DNA will hybridize. Eight clones were identified that hybridized strongly with DNA of one species but not with the DNA of other species. One of these was chosen for further study. It showed marked hybridization with DNA from several strains of A. gambiae s.s. but little or no hybridization with DNA from A. melas or A. arabiensis. This DNA was mapped and subcloned. In situ hybridization techniques are being developed to determine the chromosomal positions of probe-related sequences in different subspecies and crosses between them. This approach to identifying transposable elements has been used successfully for Drosophila.

We thank Dr. Alan Clements from the London School of Hygiene and Tropical Medicine for the mosquitoes used in these studies.

K60 SEX-SPECIFIC REGULATION OF YOLK PROTEIN GENE EXPRESSION IN DROSOPHILA Alfred M. Handler, USDA/ARS, Gainesville, FL 32604, John Belote, Mariana Wolfner, Kenneth Livak, and Bruce Baker, University of California, San Diego, La Jolla, CA 92093

Expression of the yolk protein (YP) genes is subject to temporal, spatial, and sex-specific regulatory cues, as well as being subject to hormonal control. We have defined the sex-determination regulatory gene, transformer-2 (tra-2), and the insect steroid hormone, 20-hydroxy-ecdysone, as major YP regulatory factors. Experiments with the temperature sensitive $tra\text{-}2^{ts}$ allele indicate that the sex-determination genes are necessary to initiate and maintain YP gene expression throughout adulthood. Northern blot experiments indicate that this regulation is at the level of RNA accumulation, possibly to allow mRNA synthesis, stabilization, or both. The sexual commitment of the fat body to YP synthesis can be reversed at any time during development, even in the differentiated adult in the the absence of cell division. Titers of 20-hydroxyecdysone do not differ between adult males and females, yet females respond to a 1000-fold lower concentration of hormone to produce YP, as compared to males. We have therefore measured ecdysterioid-receptor levels in whole animal homogenates, and in the fat body, using the high-affinity ecdysteroid ligand ^{3}H-ponasterone A. Receptor levels are nearly equivalent in males and females at eclosion, but after three to four days when yolk protein synthesis is maximal, a nearly four-fold higher level of ecdysteroid-receptor is found in females, and the female adult fat body. It is proposed that the sex-determination gene heirarchy acts primarily to permit YP gene transcription in females, and secondarily modulates the level of transcription by regulating hormone-receptor concentration.

K61 COORDINATION OF MACROMOLECULAR AND POLYAMINE SYNTHESES DURING THE OOGENIC CYCLE OF THE MOSQUITO, AEDES AEGYPTI, Philip H. Kogan, Cornell University, Ithaca, NY 14853

Polyamines are ubiquitous, amino acid derived, small cations. They are essential for optimal DNA replication, cell cycle traversal, and maximum and efficient protein synthesis. Polyamines may be involved in facilitating transcription, assembling ribosomes, and conferring biological activity to transfer RNA. A strong correlation is often found between RNA and polyamine syntheses. Also, translation of large proteins is dependent on polyamines in vitro. However, it is less well documented that the coordination of polyamine and RNA production is absolutely necessary for normal protein synthesis in vivo. The oogenic cycle in Aedes aegypti is initiated by a blood meal and procedes via regulated and consecutive hormonal and metabolic events, including synthesis of DNA, RNA, and of the yolk protein — vitellogenin. This system thus presents a good model for investigating the role of polyamine production during specific defined periods of macromolecular syntheses. Ornithine decarboxylase (ODC), one of the rate-limiting enzymes of polyamine synthesis, undergoes a substantial increase in activity within a few hours of the blood meal and again during the time of continuous vitellogenin production. ODC converts ornithine to putrescine, which is subsequently converted to spermidine and spermine. Labelled ornithine injected into blood-fed mosquitoes is converted to putrescine and spermidine in a pattern that correlates well with DNA, RNA, and vitellogenin syntheses. Administration of a suicide inhibitor of ODC, α-difluoromethyl ornithine (DFMO) inhibits polyamine, RNA, and protein syntheses in vivo. The effect of DFMO is not permanent and can be partially reversed by exogenous polyamines.

K62 IN VITRO INVESTIGATION OF YOLK PROTEIN ENDOCYTOSIS,
P.C. Kulakosky and W.H. Telfer, Department of Biology, University of Pennsylvania, Philadelphia, PA, 19104-6018

We have demonstrated saturable uptake, in vitro, of two radiolabed proteins by vitellogenic follicles from Hyalophora cecropia. Autoradiographic analysis confirmed stable incorporation of [35S]-vitellogenin (VG) and [125I]-microvitellin (MVN) into yolk spheres. No significant labeling of the follicular epithelium or nurse cells was observed. In vitro uptake kinetic experiments yielded Kuptake and Vmax estimates that are close to the concentration of yolk precursors in the hemolymph, and the rates of yolk protein deposition in vivo, respectively. Competition experiments indicated that VG and MVN do not compete for the same binding site; on the contrary, we found that VG increased the MVN uptake rate. This is probably due to an increase in endocytotic activity, since VG stimulates a similar increase in retention of fluid phase markers.

Paravitellogenin, a protein secreted by the follicular epithelium, is also endocytosed by the vitellogenic oocyte. Inhibition of follicular protein synthesis had no effect on the extent or the rate of VG, MVN or fluid phase uptake. These experiments suggest that paravitellogenin, and other proteins secreted by the follicular epithelium, are not essential to the endocytotic mechanism.

We have also investigated the uptake, in vitro, of two hemolymph proteins not significantly sequestered by vitellogenic oocytes in vivo. Autoradiography showed that small amounts of 125I-labeled arylphorin and flavoprotein are found within the yolk spheres, where they are probably trapped in the fluid phase during uptake of yolk precursors. In contrast to VG and MVN, arylphorin and flavoprotein bind in large quantities to the basement membrane.

K63 SYNTHESIS OF VITELLOGENINS BY THE FAT BODY AND THE OVARY OF THE COLORADO POTATO BEETLE, AND THEIR IMMUNOCYTOCHEMICAL LOCALIZATION IN THE OVARY. M. Feferoen* and A. De Loof@, *University of Vermont, Department of Zoology, Burlington, Vermont 05405, USA. @Catholic University of Leuven, Zoological Institute, Naamsestraat 59. 3000 Leuven, Belgium.

In the haemolymph of the Colorado potato beetle, there are two vitellogenins which are immunologically unrelated to each other. Although isolated ovaries of reproductive females synthesize and secrete both vitellogenins, the contribution of ovarian tissue to total yolk production is very small. The major site of synthesis of the vitellogenins is the fat body. There is a time lag between vitellogenin synthesis and yolk sequestration which suggests that at the time of yolk protein synthesis, the follicle epithelium and/or the oolemma are not yet capable of accumulating proteins. Although both vitellogenins are present in the haemolymph at the same time, endocytosis of vitellogenin 1 starts somewhat later than the uptake of vitellogenin 2. This suggests that each yolk protein has its own, specific pathway for uptake. The immunocytochemical localization of both vitellogenins during yolk formation shows that the vitellogenins are dispersed throughout the ooplasm and are intermingled with each other. At the end of vitellogenesis, the smooth ooplasm is transformed into large spheres, containing the vitellins. Yolk proteins cannot be detected neither in follicle cells nor in the trophariun.

K64 MONOCLONAL ANTIBODIES AGAINST MOSQUITO YOLK PROTEINS, Alexander S. Raikhel and Arden O. Lea, University of Georgia, Athens, GA 30602

Insect vitellogenesis requires the coordinated activity of the fat body and oocytes, and provides a wealth of problems from endocrine control of gene expression to receptor-mediated endocytosis of yolk protein precursors. We have produced a library of monoclonal antibodies (mAB) against yolk proteins of Aedes aegypti, which will serve as molecular probes for studying vitellogenesis. After the initial screening, 45 hybridoma cell lines were selected and cloned. Immunoblot analysis revealed three groups of mABs recognizing either a 200 kDa, a 68 kDa, or both of these polypeptides. While the degree of binding by different mABs varied widely, all mABs recognized these polypeptides only in extracts from vitellogenic fat bodies and oocytes. The mABs were further characterized by video-enhanced immunofluorescence. The immunolocalization in trophocytes of the fat body suggested that mABs may recognize different steps in the processing of yolk protein precursors. Similar analysis carried out in oocytes strongly indicated that, for both the 200 kDa and 68 kDa polypeptides, we have obtained panels of mABs recognizing conformational modifications of these molecules from the precursor forms, at the beginning of their internalization by the oocyte, to the final crystalline forms in mature yolk bodies.

K65 DEVELOPMENTAL AND EVOLUTIONARY CHANGES IN SILKMOTH CHORION GENE EXPRESSION, Jerome C. Regier and Antonis K. Hatzopoulos, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201

Regionalized protein synthesis occurs at the very end of choriogenesis in the Polyphemus silkmoth follicle to form prominent surface structures called aeropyle crowns, missing from the corresponding flat region. The genetic basis of this cell-specific pattern of chorion gene expression has been analyzed using cDNA and chromosomal clones representing two cell-specific sequences, called E1 and E2. We can make the following conclusions: 1) E1 and E2 RNAs are transcribed at similar times. 2) Rates of transcription are substantially greater in the aeropyle crown region. 3) E1 and E2 gene copy numbers are equivalent in both regions throughout development, and thus cannot explain differences in transcription. 3) E1 and E2 genes are closely linked. 4) The 5' flanking regions of E1 and E2 genes share short nucleotide sequences which are potential cis-acting control elements. By contrast, their coding regions show no homology.

In the Cecropia silkmoth synthesis of new proteins at the very end of choriogenesis is minimal and aeropyle crowns are absent. What is the genetic basis for the evolutionary change in this developmental program? From analysis with cloned Cecropia sequences, we can make the following conclusions: 1) Amounts of E1 and E2 RNAs are greatly diminished in Cecropia relative to Polyphemus. 2) There are 1-3 E1 and E2 genes per haploid genome in Cecropia, equivalent to that in Polyphemus. 3) E genes in Cecropia are paired as in Polyphemus. Thus, evolutionary changes in E gene transcription have occurred despite constant gene copy numbers and organization.

K66 **Interaction of penfluron at molecular level in the embryos of D. cingulatus, K Satyanarayana and Kumuda Sukumar, Regional Research Laboratory, Hyderabad 500 007, India**

Penfluron, the trichloro derivative of N-phenyl-N-benzoyl urea, when treated to females of Dysdercus cingulatus showed interferences on embryogenesis at lower dose and adverse effects on oocyte growth at higher dose. The tritiated thymidine incorporation was low in the non-viable eggs deposited by the adults which received a low dose of penfluron. The study on DNA protein synthesis and LDH activity at different intervals during the course of embryogenesis indicated gradual increase in both the treated and normal eggs, but in the treated eggs even the initial amount of the three cell constitutents as well as the increase were lower order. The inhibition of DNA synthesis was very high than protein synthesis and LDH activity. This suggests that the penfluron interacts with other biochemical processes besides the chitin biosynthesis, and this may be the factor attributable to its sterilant action. At higher dose, although no adverse effects were noticeable in the mating behaviour, the effect on the histopathology of the oocyte was very evident since the oocyte development was completely arrested.

K67 20-HYDROXYECDYSONE SUPPRESSES YOLK PRODUCTION IN THE INDIANMEAL MOTH, Paul D. Shirk, IAB&BBRL, USDA, ARS, Gainesville, FL 32604.

As in other moths that have short-lived adult stages, egg maturation takes place during pharate adult development in the Indianmeal moth, Plodia interpunctella. Vitellogenesis was found to begin on day 5 of pharate adult development which coincided with the synthesis of 4 major yolk polypeptides (YPs) and was maintained through adult eclosion on day 8. Synthesis of the YPs was divided between two tissues; YP1 and YP3 were produced by the fat body, and YP2 and YP4 were produced in the ovaries. Twice daily treatment of pharate adult females with 20-hydroxyecdysone (20HE) blocked vitellogenesis and resulted in the suppression of YP synthesis in the fat body and the ovaries. Treatment of females with 22-hydroxyecdysone, a hormonally inactive ecdysteroid analogue, had no effect on vitellogenesis or YP synthesis. Total RNA was isolated from fat body and ovaries of 20HE treated females and translated in a cell-free reticulocyte lysate. Immunoprecipitation of the translation products with antiserum specific to yolk proteins showed that 20HE suppressed the accumulation of YP mRNAs in both tissues. Inclusion of varying concentrations of 20HE in the culture medium for ovarioles and fat body showed that maximal YP synthesis occurred at 10^{-8} M 20HE. Concentrations of 20HE higher than 10^{-8} M suppressed YP synthesis in both tissues. Translation of RNA isolated from ovarioles or fat body cultured in vitro with 20HE showed that the hormone concentration was correlated with the accumulation of YP mRNA. These data suggest 20HE exerts a negative control over vitellogenesis, and the initiation of YP synthesis and vitellogenesis requires decreasing titres of 20HE.

Invertebrate Hormones and Development

K68 INFLUENCE OF A PARASITE ON THE ECDYSTEROID TITER OF ITS HOST, Douglas L. Dahlman and Danise Coar, Dept. of Entomology, University of Kentucky, Lexington, KY 40546

The development of the lepidopterous larvae, Heliothis virescens, that are parasitized by the braconid wasp, Microplitis croceipes, is halted at a specific point prior to the release of the ecdysteroid pluse that triggers larval-pupal ecdysis. The parasite remains in the host for an additional one to four days after cessation of host development. After the parasite exits the host and spins its cocoon, the host remains alive for an additional number of days but never develops further. Host tissues remain responsive to exogenous 20-hydroxyecdysone but ecdysteroid titer in parasitized larvae does not increase beyond that observed in the specific physiological stage at the time host development ceased. Information on the activity of ecdysone 20-monooxygenase, which converts ecdysone to 20-hydroxyecdysone, the availability of sterol substrate for synthesis of ecdysone, the titers of ecdysteroids throughout the development of the parasite, and the result of various manipulative procedures will be presented.

K69 MILLER-TYPE CHROMATIN SPREADS OF LARVAL EPIDERMIS FROM MANDUCA SEXTA, Catherine M. Fittinghoff, Aimee H. Bakken, and Lynn M. Riddiford.

An improved method for making Miller-type chromatin spreads from larval epidermis from Manduca sexta has been devised. A previous method involved forcing tissue through a narrow gauge needle followed by filtration to separate epidermis from other tissues (Dyer and Riddiford, 1983), but was overly harsh. The new method relies on manual dissection of the epidermis and cuticle away from the fat body, trachea, muscle, and basement membrane. The epidermal cells are then scraped away from the cuticle and placed in saline. The suspension is then further disrupted by several passages through a Pasteur pipet. After settling for thirty minutes on ice, the solution will be a suspension of single cells with clumps of cells at the bottom of the tube. Cells from the upper portion of the suspension can then be treated with a mild detergent to break cells and nuclei. Centrifugation on to carbon-coated copper grids produces well-spread chromatin with easily identifiable nucleosome strctures and replication bubbles. Importantly, the chromatin is visible after protein staining.

The presence of protein suggests that the spreads may be useful for hormone-chromatin binding studies, both with endogenous chromatin and injected plasmid. Studies have been initiated to test binding of ^{3}H-Ponasterone A to Drosophila EIP gene and of ^{3}H-juvenile hormone to Manduca cuticle genes.

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K70 HOMONAL REGULATION OF PHENOLOXIDASE AND DOPA DECARBOXYLASE IN MELANIZATION OF MANDUCA. K. Hiruma & L. M. Riddiford, Dept. Zool., Univ. Washington, Seattle, WA 98195

During a larval molt the absence of juvenile hormone (JH) around the time of head capsule slippage (HCS) causes the deposition of premelanin granules containing prophenoloxidase into the newly forming cuticle 12-13 hr later. When the ecdysteroid titer declines, the enzyme is activated and melanization occurs 3 hr before ecdysis. Treatment of cuticular homogenates with 0.1% SDS followed by electrophoresis showed 4 phenoloxidase (110, 105, 100, and 94 kD) unique to cuticle and one (68 kD) common with cuticle lacking premelanin granules. Developmental studies showed that the high MW phenoloxidases appear in the cuticle coincident with the deposition of the granules at 14 hr. These enzyme complex appeared earlier at 10 hr in the epidermis, then disappeared when it appeared in the cuticle. The relationship of the onset of this epidermal synthesis to the time of JH regulation is being explored. A second enzyme, dopa decarboxylase (DDC), is required for melanization since dopamine is the primary precursor for melanin. DDC activity increases due to increased DDC synthesis just before the onset of melanization. This increase is inhibited by infusion of 20-hydroxyecdysone (20-HE) or by 20-HE *in vitro*. Thus, the onset of DDC synthesis at the larval molt is cued by the fall of ecdysteroid. When JH is absent at the peak of the ecdysteroid titer just after HCS, the later DDC synthesis is enhanced two-fold. Study of the regulation of the synthesis of these 2 enzymes thus provides a good system in which to study the role of JH in directing ecdysteroid action on the genome. Supported by NSF DCB 80-11152 and NIH AI 12459.

K71 ISOLATION AND CHARACTERIZATION OF AN ECDYSONE INDUCIBLE CUTICLE GENE OF MANDUCA SEXTA, Frank M. Horodyski and Lynn M. Riddiford, University of Washington, Seattle, WA 98195

Cuticular proteins are major products of the epidermal cells in Manduca sexta. On day 3 of the 5th larval instar, a new class of cuticular proteins is synthesized while others are turned off under the control of juvenile hormone and ecdysone. We have constructed a cDNA library in λgt10 from day 3 mRNA, screened this library by differential hybridization, and isolated several clones whose expression is specific to day 3. A class of cross-hybridizing clones was selected for further analysis. To determine the identity of these clones, day 3 mRNA was hybrid-selected and translated *in vitro* to yield two proteins of MW 15kD and 16kD which are precipitable with antiserum to larval cuticle. This suggests the presence of cross-hybridizing mRNAs. Multiple bands are seen in genomic Southerns. Several genomic clones have been isolated and their organization is currently under study. Northern blot analysis revealed the presence of a 540 nucleotide-long mRNA at day 3 but not at day 1. This mRNA was also abundant in Northern blots when day 1 epidermis was cultured for 24 hours in the presence of 25 ng/ml 20-hydroxyecdysone, but not following culture in the absence of hormones. This is similar to the hormonal state of the animal when this mRNA begins to be expressed *in vivo*. Sequencing of the cDNA and genomic clones is currently in progress. This work was supported by NIH A512459 to LMR and ACS Fellowship PF2150 to FMH.

K72 CONTROL OF GROWTH AND DIFFERENTIATION OF THE DERMAL GLANDS (VERSON'S GLANDS) OF MANDUCA SEXTA. Kathleen L. Horwath and Lynn M. Riddiford, University of Washington, Seattle, WA 98195.

The pair of epidermally derived Verson's glands (VG) on each segment secrete at ecdysis a proteinaceous product which coats the epicuticle. These products of a single secretory cell are different for the larva and the pupa. Moreover, there are distinct segmental differences in the pupal proteins, with the borderline between the 1st and 2nd abdominal segment. Documentation of VG cell growth during the final (5th) larval instar by assessment of secretory cell area, protein content, and an electrophoretic analyses of VG proteins indicates growth commences post-wandering during the prepupal period and at a time coincident with the molting surge in endogenous ecdysteroid levels. Also, studies of protein synthesis by the gland show that the pupal proteins appear as the gland grows. Twenty-hydroxyecdysone (20HE) (0.83ug/ml) *in vitro* stimulates growth and pupal protein synthesis by the wandering stage gland. The time of the change from larval to pupal commitment of the gland was found by juvenile hormone application and transplantation experiments to occur during the mid-feeding period. This change occurs before that of the dorsal abdominal epidermis and appears to be associated with the decline of juvenile hormone.

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K73 EFFECTS OF TOPICAL APPLICATION OF JUVENILE HORMONE AND ANTI-JUVENILE HORMONES ON PREPUPAL DEVELOPMENT AND ECDYSTEROID TITER IN LAST STADIUM LARVAE OF TRICHOPLUSIA NI. Rick A. Newitt and Bruce D. Hammock, UC,Davis, Davis,Ca. 95616

The hemolymph ecdysteroid titer during the late last stadium of the cabbage looper moth, Trichoplusia ni, was monitored in the presence of experimentally altered JH levels to test the hypothesis that the appearance of a prepupal burst of JH enhances prothoracic gland activity. Two peaks of ecdysteroid activity were detected in the hemolymph of last stadium larvae with 20-hydroxyecdysone more prevalent. Treatment of post feeding last stadium larvae with JH-I, methoprene, or epofenonane had no effect on the timing or character of pupation. Application of JH-I to neck ligated larvae failed to restore peak ecdysteroid levels to those of unligated controls nor were the levels different from those found in neck ligated controls. Application of the anti-JH compounds, fluoromevalonolactone and 3,3-dichloro-2-propenyl hexanoate, disrupted metamorphosis causing delays in tanning and the presence of malformed pupae. Yet, corresponding deviations in the timing or magnitude of the ecdysteroid peak were not observed. Shifts in the timing of peak ecdysteroid levels, coincident with delayed pupation, were observed in earlier aged larvae treated with the JH esterase inhibitor, 3-octylthio-1,1,1-trifluoro-2-propanone. These results suggest that the prepupal prothoracic glands of T. ni may be considerably less sensitive to changes in the endogenous JH titer by the time larvae have begun to wander, even though treatment at this time may produce teratogenic effects that influence ecdysis and/or pupal morphology.

K74 20-HYDROXYECDYSONE ALTERS THE PATTERN OF EPIDERMAL PROTEIN SYNTHESIS IN THE LARVAE OF TENEBRIO. Y. Ouellette and S. Caveney, Department of Zoology, University of Western Ontario, London, Ontario, CANADA.

Epidermal cells of the last-instar larvae of Tenebrio molitor were labelled in vitro with [35-S] -methionine in the presence or absence of 20-hydroxyecdysone (20-HE). Cells incubated with the hormone incorporate 28% more label into non-cuticular proteins. The cells undergo a marked change in their pattern of protein synthesis as detected by one- and two-dimensional gel electrophoresis followed by fluorography. 20-HE affects the synthesis of at least 56 polypeptides, of which 31 show a decrease in their relative rates of synthesis. The pattern of polypeptide synthesis typical of 20-HE induction can be recognized at a hormone concentration as low as 0.1 µg/ml, but 2 µg/ml was typically used. An early and late phase in response to hormone can be distinguished. The early response (3-6 hours) is characterized by the de novo synthesis of a polypeptide with a molecular weight of 43000 with a pK of 7.2. Its synthesis is not detectable after 12 hours of hormonal incubation. The synthesis of 43 kD polypeptide is selectively blocked by 10^{-2} µg/ml of cycloheximide (CHX). At this concentration, CHX inhibits 56% of overall cellular protein synthesis. The late response to 20-HE (12-16 hours) was investigated by examining newly synthesized polypeptides in subcellular fractions. The nuclear, mitochondrial/lysosomal, microsomal and cytosol fractions were isolated and their polypeptides separated by gel electrophoresis. All four fractions show altered polypeptide content in response to 20-HE. Some of the 20-HE sensitive polypeptides are being characterized in order to elucidate the mechanism by which the hormone modulates cell differentiation. (Supported by NSERC of Canada)

K75 BRAIN FACTOR INHIBITS CORPORA ALLATA IN LARVAE OF THE COCKROACH DIPLOPTERA PUNCTATA, Charles R. Paulson and Barbara Stay, University of Iowa, Iowa City, IA 52242

Regulation of the corpora allata has been investigated by manipulating the glands in vivo and measuring the resulting changes in juvenile hormone synthesis using an in vitro radiochemical assay. We have previously shown that the corpora allata are inhibited via a humoral pathway during the final stadium in that: 1) activity of larval corpora allata declines during the final stadium whether the glands are innervated or denervated, 2) activity of corpora allata from late final-instar males increases following transplantation into adult females compared to similar glands denervated in situ, and 3) activity of adult female corpora allata declines following transplantation into late final-instar males [J. Insect Physiol. (1985) 31,625]. We have also shown that inhibition does not occur in adult corpora allata implanted into decapitated larval hosts, and that aqueous extract of protocerebra from larval and adult males inhibits corpus allatum activity in vitro. This inhibitory activity is retained following boiling of the extract for 10 m but is lost following treatment with trypsin [Amer. Zool. (1985) 25(4)].

We now report that implantation of single protocerebra from late final-instar or adult males into decapitated larval hosts restores the normal inhibition of juvenile hormone synthesis. However, implantation of protocerebra from early final-instar males does not cause inhibition of corpora allata. These results together with the earlier findings suggest that a brain peptide acts via the haemolymph to inhibit juvenile hormone synthesis during the final stadium.

K76 POTENTIATION OF 20-HYDROXYECDYSONE AND VITELLOGENIN GENE EXPRESSION IN THE MOSQUITO, Aedes aegypti, Jeffrey V. Racioppi and Henry H. Hagedorn, Harvard School of Public Health, Boston, MA 02115, Cornell University, Ithaca, NY 14853.

Endocrine studies suggested that 20-hydroxyecdysone was one of several factors influencing vitellogenin gene expression. A subcloned fragment (403-1c) of vitellogenin DNA, isolated from an Aedes aegypti genomic library, was used in dot-hybridization assays to quantitate vitellogenin mRNA. Following a blood meal, juvenile hormone titers declined rapidly while titers of 20-hydroxyecdysone paralleled the accumulation and degradation of vitellogenin message. Endocrine rescue experiments established that both a blood meal and exogenous 20-hydroxyecdysone were required for substantial accumulation of vitellogenin message. Topical application of the juvenile hormone analog methoprene 6 hr after a blood meal, decreased the rate of vitellogenin protein synthesis. Fat bodies from non-blood-fed females incubated with physiological concentrations of 20-hydroxyecdysone and methoprene contained twice as much vitellogenin message as those incubated with 20-hydroxyecdysone alone. Methoprene alone had no effect. Although 20-hydroxyecdysone appears to stimulate accumulation of message, its effect was substantially augmented by a blood meal or methoprene. We suggest that juvenile hormone may potentiate 20-hydroxyecdysones effect on vitellogenin transcription; the blood meal, in turn, influences both these hormones.

K77 GENOMIC CLONING OF DEVELOPMENTALLY REGULATED LARVAL CUTICLE GENES FROM THE TOBACCO HORNWORM, MANDUCA SEXTA, John Rebers, Frank Korodyski, Robert Hice and Lynn M. Riddiford, University of Washington Seattle, WA 98195

The epidermis of holometabolous insects sequentially produces larval, pupal, and adult cuticle under the control of ecdysteroid and juvenile hormone. The regulation of three larval cuticle proteins produced early in the fifth instar of Manduca sexta at the levels of translatable mRNA (1) and protein synthesis (2), and more recently with cDNA clones encoding these polypeptides of 12.8, 13.3, and 14.6 kilodaltons. The RNAs for the two lower molecular weight proteins are larval-specific, whereas the RNA for the 14.6 kilodalton protein is also found in low amounts during pre-ecdysial pupal endocuticle formation. We have used cDNA clones for these RNAs to select the corresponding genomic regions from a Manduca genomic library. Work is in progress to map the transcripts for the larval cuticle genes to determine possible common regulatory regions, and to compare the genes with Drosophila larval cuticle genes, which have already been well characterized (3).

1. Riddiford LM (1982) Dev Biol 92:330-342.
2. Kiely ML, Riddiford LM (1985) Wilhelm Roux's Arch 194:325-335.
3. Snyder M, Hunkapiller M, Yuen D, Silvert D, Fristrom J, Davidson N (1982) Cell 29:1027-1040.

Supported by NIH grant # 5F32GM09419-02 to JR and NIH grant # 12459 to LMR.

K78 TERMINAL DIFFERENTIATION OF THE INTERSEGMENTAL MUSCLES OF THE TOBACCO HAWKMOTH Manduca sexta, L.M. Schwartz and K.S. Bloom, Biol. Dept., University of North Carolina, Chapel Hill, N.C. 27514

During the course of adult development, the intersegmental muscles (ISM) of Manduca undergo a developmentally programmed cell death. Three days before adult eclosion, the ISM enter the atrophy phase which is characterized by a 40% loss of mass without a signifcant change in physiological properties. Then coincident with eclosion, the muscles begin to degenerate and completely disappear during the subsequent 36 hrs. Progression through these developmental stages is controlled by the declining haemolymph ecdysteroid titer. We have sought to define the molecular events associated with these changes. Using the Drosophila actin gene, which hybridizes to Manduca DNA under highly stringent conditions, we have identified an abundant poly A^+ transcript. We are measuring its levels during the course of adult development. We will make cDNA's to identify cell death specific sequences.

K79 A DROSOPHILA MUTANT RESISTANT TO A CHEMICAL ANALOGUE OF JUVENILE HORMONE, Thomas G. Wilson and Judit Fabian, University of Vermont, Dept of Zoology, Burlington,VT 05405

Methoprene is a chemical analogue of juvenile hormone which is toxic when applied to late third-instar larvae of Drosophila melanogaster. We have selected a mutant in an ethyl methane sulfonate mutagenesis screen which is nearly 100X resistant to either methoprene or juvenile hormone III topically applied or incorporated into the diet. The mutation, named methoprene tolerant (met), also confers resistance to JH III- or methoprene-induced vitellogenic oocyte development in adult females. met Adults show little or no cross-resistance to four other insecticides. The mutation was mapped by recombination to a location 35.5 on the X-chromosome and uncovered by chromosomes deficient for the region 10C2-10D4. The characteristics of met are consistent with an altered juvenile hormone binding or receptor protein mutant.

General Topics

K80 Two amylase isozymes from adults of the rice weevil, Sitophilus oryzae, were purified by ammonium sulfate precipitation, formation of an insoluble glycogen-amylase complex, ion exchange chromatography on DEAE-Sephacel, and HPLC on a Synchropak AX-300 analytical ion exchange column. The isozymes, designated RW-1 and RW-2, had similar pH optima between pH 4.5 and 5.0, molecular weights of about 56,000, comparable amino acid profiles that included high concentrations of aspartic acid and low levels of cystine and methionine, and identical action patterns for hydrolysis of amylose that were typical of alpha-amylases. However, the isozymes differed significantly in their kinetic constants for hydrolysis of starch. Km values were 0.246% for RW-1 and 0.066% for RW-2. Vmax values for starch were 837 AU/mg protein for RW-1 and 308 AU/mg protein for RW-2. [One amylase unit (AU) produces 1.0 mg maltose hydrate/min at 30°C]. Partially-purified naturally-occurring alpha-amylase inhibitors were prepared from a saline extract of whole wheat flour by ammonium sulfate fractionation and HPLC on a preparative Synchropak AX-300 column. Significant differences were found in the sensitivity of RW-1 and RW-2 to several major inhibitor fractions. Understanding the biochemical interaction of weevil amylases with the inhibitors from wheat may provide the basis for a more thorough analysis of the utility of these inhibitors as cereal resistance factors against these major insect pests.

K81 NEUROTOXIC ACTION OF BACILLUS THURINGIENSIS SUBSP. ISRAELENSIS δ-ENDOTOXIN. Peter Y.K. Cheung, Dan Buster, and Bruce D. Hammock, University of California, Davis, Ca 95616; R. Michael Roe, N. Carolina State Univ., Raleigh, NC 27695; A. Randall Alford, Univ. of Maine, Orona, ME 04469.

The neurotoxic, cytolytic, and insecticidal properties of the δ-endotoxin of Bacillus thuringiensis subsp. israelensis (BTI) were compared with those of a cytolytic protein (25Kd) isolated from the δ-endotoxin and the cytolytic enzyme phospholipase A_2. Using ventral nerve cord preparations of larval Trichoplusia ni, electrophysiological studies showed that of the three toxin preparations only the whole extract of the toxin induced hyperexcited activity in the nerve cord preparations. Using lactate dehydrogenase activity in the haemolymph of toxin injected larvae to quantitate cell lysis, we cannot account for the high insecticidal activity of the BTI toxin by general cytolysis alone. Bioassays at 28, 17 and 9° demonstrated a positive temperature correlation for the BTI toxin, but inverse correlation for the two cytolytic materials.

This and previous studies indicate that insect mortality is not solely due to the cytolytic activity of the BTI δ-endotoxin. Unidentified neurotoxic factor(s) exists within the BTI δ-endotoxin which induces hyperexcited activity in insect nervous system. This factor may inpart the δ-endotoxin with biological and physiological activities different from the isolated cytolytic component.

K82 REGULATION OF EXPRESSION OF JUVENILE HORMONE ESTERASE, Anita Click, Grace Jones and Davy Jones, Department of Entomology, University of Kentucky, Lexington, KY 40546.

Juvenile hormone esterase (JHE) activity in Trichoplusia ni hemolymph increases to high abundance twice during the final larval stadium, the first peak under the control of stimulatory and inhibitory neurohormones and the second peak apparently directly induced by JH itself. Thus, JHE is interesting for studies on hormonal regulation of gene activity. The hemolymph activity during both peaks is due to three electrophoretic variants (pI 5.5, 5.3, 5.1). An in vitro fat body tissue culture system was developed to study the action of the neurohormone and JH on transcription, translation, processing and secretion of JHE. JH acted directly on the fat body to increase and sustain the rate of appearance of active JHE in the medium. The ratio of the activity represented in the electrophoretic forms was apparently changed by a JHA in vivo, indicating that putative fat body processing of the variants may be a level of JH action. Fat body and medium content of JHE activity indicates the appearance of more JHE activity in vitro than can be accounted for by mere release of JHE activity already present in the fat body. Co-culture of brains and fat body from larvae during the 1st JHE decline showed a clear brain suppression of appearance of JHE activity in the medium The ratio of the electrophoretic variants was unchanged by the brain. Molecular studies on JHE from the two peaks have been initiated. JHE from both periods of JHE activity has been purified to homogeneity. Each electrophoretic variant, and JHE from both periods, have molecular weights of 66,000. The amino acid composition for the pI 5.5 variant has been obtained, and antisera in preparation will be used in future studies on the regulation and cloning of gene(s) for JHE. (Supported, in part, by NIH GM 33995).

K83 POSTMEIOTIC EXPRESSION OF A TESTIS-SPECIFIC PUTATIVE ß-TUBLLIN GENE IN HELIOTHIS, Minh-Tam B. Davis and Stephen G. Miller, Insect Attractants, Behavior, and Basic Biology Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida 32604

A putative ß-tubulin DNA sequence has been isolated from a Heliothis testis cDNA library using a testis-specific ß-tubulin probe from Drosophila. The Heliothis ß-tubulin cDNA hybridized strongly to a single transcript of approximately 1,700 nucleotides from testis $(A)^+$ RNA, but failed to hybridize any $(A)^+$ RNA from fat body, ovaries, embryos or ovarian-derived cultured cells. The steady-state level of the ß-tubulin transcript was low in larvae, increased dramatically in testes from newly eclosed pupae, and declined thereafter in later stages of pupal development. This pattern of ß-tubulin RNA accumulation suggests that the relative level of expression of this gene increases following meiosis since testes from day-1 pupae contain a population of cells which have essentially all (>90%) completed meiotic cell divisions.

As a potential for molecular biological control of Heliothis, an antisense testis-specific ß-tubulin construct with a heat shock promotor could be introduced into Heliothis by germ-line transformation. Expression of the antisense ß-tubulin RNA, induced by heat shock, could interfere with spermatogenesis by its binding to testis-specific ß-tubulin RNA, and thus produce sterile males.

K84 Na^+, K^+ - ATPase INHIBITION BY DELTA ENDOTOXIN, Leigh English and Lewis Cantley, Tufts University School of Medicine, Boston, MA 02111.

A 68 KDa protein, delta endotoxin, produced by Bacillus thuringiensis ssp. Kurstaki inhibits ion transport, (Na,K) ATPase and K^+ - p-nitrophenylphosphatase (K^+-pNPPase) activity catalysed by the Na^+ pump. The K_i for inhibition of the K^+-pNPPase activity of purified dog kidney (Na,K) ATPase was ~0.37 uM. Delta endotoxin had a similar K_i for inhibition of (Na,K) ATPase activity when assayed at low Na^+ concentration (10 mM) but the inhibition was reversed when high concentrations of Na^+ (100 mM NaCl), were added to the assay. Phosphorylation of the active site aspartyl residue with $^{32}PO_4^{3-}$ was also blocked by delta endotoxin. Ouabain sensitive $^{86}Rb^+$ uptake into intact human red blood cells was not inhibited by externally added toxin; however, strophanthidin-inhibitable $^{22}Na^+$ uptake into inside-out vesicles from red blood cells was completely blocked by delta endotoxin (K_i~).73 uM). These data suggest that delta endotoxin must enter the cell before it can inhibit the Na^+ pump.

K85 HISTONE GENE COPY IN DROSOPHILA IS NOT CONSTANT, D. Fitch and L. Strausbaugh, Dept. of Molec. and Cell Biol., Univ. of Connecticut, Storrs, CT 06268.

The copy number of various genes has been shown to change on evolutionary as well as physiological time scales. Generally, such changes are assumed to be in response to developmental (or other) demands on the organism. For example, the high level of reiteration (approx. 100 copies per haploid genome) of histone genes in D. melanogaster has been attributed to high demands for histone proteins during early embryogenesis. We have discovered, however, that high levels of reiteration of histone genes are not a general characteristic of species in the genus Drosophila. Comparison of histone gene copy numbers in D. melanogaster and D. hydei by quantitative hybridization of diploid genomic DNA with a highly conserved histone probe have shown that histone genes are present in considerably lower numbers in D. hydei. Results of in situ hybridization to polytene chromosomes have identified a fundamental difference between these two species: D. melanogaster histone genes are located adjacent to the quasiheterochromatic base of 2L (39DE); D. hydei histone genes are located in an euchromatic region on 4 (80C3-5). These observations raise the possibility that chromosomal location of these genes (not only developmental demands) may play a major role in reiteration levels. Histone DNA in D. melanogaster is underreplicated in polytene DNA when compared with levels in diploid DNA. This physiological change in histone gene copy number that occurs during development is not merely a consequence of chromosomal underreplication of centromeric region since D. hydei histone DNA is also underrepresented in polytene tissues. These results suggest that disproportionate replication is an inherent property of histone DNA.

K86 **H. Höfte, H. Vanderbruggen, M. Zabeau and M. Vaeck**
Plant Genetic Systems, Gent, Belgium.

A crystal protein gene (bt2) has been cloned from plasmid DNA of Bacillus thurigiensis (B.t.) berliner 1715. E. coli containing this clone (pGI502) produce large amounts of M_r 130000 protein (Bt2) which is as toxic against Pieris brassicae and Manduca sexta as the original crystal proteins of B.t. berliner 1715.
The comparison of the deduced amino acid sequence of the Bt2 protein with the B.t. kurstaki HD-Dipel, B.t. kurstaki HD73 and B.t. sotto crystal protein sequences suggests that homologous recombination between the different crystal protein genes has occured.
Trypsin treatment of the Bt2 protein yields a M_r 60000, protease resistant and fully toxic polypeptide with the N-terminus starting at AA 29. This minimal portion of the Bt2 protein that is required for toxicity has been mapped by analysing the polypeptides produced by deletion derivatives of the Bt2 gene. The minimal toxic polypeptide coincides with the M_r 60000 protease resistant Bt2 fragment. From a set of monoclonal antibodies against B.t. berliner crystal proteins, one was identified which specifically recognizes a conformational determinant associated with toxic Bt2 fragments only.

K87 N-ACYLCATECHOLAMINE METABOLISM AND HORMONAL REGULATION FOR SCLEROTIZATION OF INSECT CUTICLE. T. L. Hopkins[1], T. D. Morgan[1], K. J. Kramer[2,3], C. R. Roseland[1], T. H. Czapla[1] and R. A. Elonen[1]. Departments of Entomology[1] and Biochemistry[2], Kansas State University, Manhattan, KS 66506 and U.S. Grain Marketing Research Laboratory[3], ARS, USDA, Manhattan.

N-Acetyldopamine (NADA) and N-β-alanyldopamine (NBAD) are the primary precursors of cuticular sclerotizing agents in species so far examined in Orthoptera, Lepidoptera, Coleoptera and Diptera. They are sequestered in hemolymph as phenolic glucosides or other conjugates prior to ecdysis and subsequently decrease as transport into tanning cuticle occurs. Our studies on the tobacco hornworm, Manduca sexta, have shown that synthesis of NBAD is regulated by 20-hydroxyecdysone which also brings about pupal cuticle formation and tanning. Larval fat body cultured in vitro in Grace's medium synthesized NBAD and lesser quantities of NADA when dopamine is added. cAMP and octopamine stimulated higher levels of synthesis. We have found that NADA is more associated with hard colorless cuticle in M. sexta while NBAD predominates in dark brown cuticle. High levels of free dopamine are associated with melanization. Similar correlations exist in other species, but some cuticles involve a complexity of sclerotization and melanization reactions as evidenced by the diversity of catecholic metabolics observed during hardening and darkening. The β-hydroxylated metabolites of NBAD and NADA, N-β-alanylnorepinephrine and N-acetylnorepinephrine appear to be important sclerotization products arising from side-chain oxidation by an insoluble enzyme(s) in cuticle. (Supported in part by National Science Foundation Grant PCM-8411408.)

K88 A SYSTEM FOR TESTING MOVEMENT OF GENES FOR PRODUCTION OF NONPROTEIN (E.G. ALKALOIDAL) INSECT RESISTANCE FACTORS, Joseph Huesing, Craig Heim, Erno Zador and Davy Jones, Department of Entomology, University of Kentucky, Lexington, Kentucky 40546

Little attention has been given to plant genetic engineering for insect resistance except in situations where a protein is itself toxic. Our alternative approach is to initiate a 4 phase program of 1) evaluation of 22 Nicotiana species for resistance to Manduca sexta and Heliothis virescens, 2) identification of nicotinoid alkaloids involved, 3) purification of protein(s) controlling their synthesis and 4) cloning and movement of genes for the enzyme(s) into N. tabacum. Species in the section Repandae were highly toxic to M. sexta, and an unrelated species, N. gossei, was highly toxic to H. virescens. The biochemical basis of resistance to M. sexta was in the leaf exudate. TLC analysis of Repandae exudate extracts showed a high concentration of a novel nicotinoid alkaloid. This acylated alkaloid (Severson et al. 1985) was tested topically on 1st instar M. sexta. An LD 50 in dramatic contrast to nicotine was found (2 vs. 500 ug). The new alkaloid was not toxic to H. virescens. A wide species sexual hybrid N. repanda x N. tabacum possessed an intermediate level of the novel alkaloid (TLC). Analysis of N. gossei exudate, and that from a hybrid with N. tabacum resistant to H. virescens, showed a preponderance of nicotine. Identification of the enzyme(s) involved in synthesis of the Repandae alkaloid has begun, the synthesis likely being an additional acylation step. Since wide species hybridizations are difficult, and N. tabacum is amenable to recombinant DNA techniques, we propose this system as optimal for testing a new approach to genetic engineering for non-protein resistance factors (supp.,in part, USDA 58-43YK-5-0034).

K89 MOLECULAR ANALYSIS OF VIRAL PARTICLES IN PARASITIC WASPS, CHELONUS SPP., Davy Jones and Erno Zador, Dept. of Entomology, University of Kentucky, Lexington, Ky 40546.

The nucleic acids in the viruses from ovaries of C. insularis and C. near curvimaculatus have been characterized and compared. Electrophoretic fractionation of the DNA from each virus shows the presence of a number of bands, suggesting multipartite virus genomes. The molecular weights of the (15) bands from C. near curvimaculatus ranges from 5.6-18.1 x 10^6, while that for C. insularis (12 bands) range from 6.5-25.7 x 10^6. Hybridization studies are under way to test for homology among bands. Purification of virus from C. near curvimaculatus on a continuous sucrose gradient yields a single viral band, the DNA from which shows the same electrophoretic pattern as that obtained above from preparations of ovariole homogenate. Sedimentation of extracted viral DNA in CsCl gradients results in two DNA bands (relaxed and superhelical forms). Fractionation of the DNA in each form provides the same number and pattern of bands as directly extracted DNA. Restriction enzyme analysis of viral DNA from the two wasps shows distinct differences between the two genomes (Fig.1). Stung host Lepidoptera receive the virus, and precociously initiate metamorphosis, including 'pseudoparasitized' hosts which contain no live parasite. mRNA from normal and pseudoparasitized insects is being studied. (NIH GM 33995)

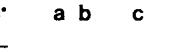

Fig. 1 Eco RI digest
a- superhelical pBR322
b- pBR 322 cut
c- C. insularis cut
d- curvimaculatus uncut
e- curvimaculatus cut

K90 MOLECULAR BIOLOGY OF A JH SUPPRESSABLE PROTEIN IN T NI, Grace Jones Dept. of Entomology, University of Kentucky, Lexington, KY 40546

A basic protein with a M.W. of 76,000 can be suppressed by JH analog treatment in vivo. This phenomenon was investigated further by using in vitro fat body culture. The JH analog was found to suppress the appearance of the protein in the medium (Fig 1). We have demonstrated the amenability of the in vitro system for radiolabelling the newly synthesized basic protein by supplying 35S-methionine to the medium (Fig. 1). The average isoelectric point of this protein is 8.3. Under high resolution electrofoc-using, this basic protein focuses into 4 bands. In T ni, there is no protein detectable by Coomassive blue staining with an isoelectric point higher than 7.5, except for the JH suppress-able protein. This situation was used to purify the protein on preparative isoelectric focusing. Antibodies to be made from this protein will be used in molecular studies. The suppress-ive effect of JH on translatable mRNA for this protein was also demonstrated (Jones, Hellman and Rhoads 1985). (NIH GM 33995).

Fig. 1 In vitro suppsuppression/labelling of basic protein (arrow).
a- JHA treatment
b- Control
c- 35S-Methionine labelled protein

K91 MOLECULAR APPROACHES TO PUPAL DIAPAUSE IN FLESH FLIES (SARCOPHAGA). Karl H. Joplin, David R. Bezanson and David L. Denlinger. The Ohio State University, Columbus, Ohio 43210.

Diapause in Sarcophaga crassipalpis and Sarcophaga bullata has been well studied from a physiological and ecological standpoint. Pupal diapause in these Diptera is an optional developmental state used to circumvent adverse environmental conditions. The diapause state results in hormonal and physiological changes that are distinct from those in non-diapause pupae. This state is induced by photoperiodic clues received by the late embryo and early larval stages. In addition, a maternal effect prevents diapause in offspring from females that have been exposed to diapause-inducing conditions during her embryonic and larval development.

Thus pupal diapause in Sarcophaga is an interesting developmental model system, which we are beginning to examine using molecular techniques. We are exploring involvement of the circadian rhythm locus (period) in pupal diapause of Sarcophaga. We are studying this locus, which has been characterized and isolated from Drosophila, by Southern and Northern analysis of Sarcophaga lifestages, cloning period homologous region from a Sarcophaga library and mapping these loci on Sarcophaga polytene chromosomes. This will lead to other studies for isolating diapause-specific and diapause-controlling loci by competitive hybridization of a Sarcophaga genomic library from diapause and non-diapause stages.

K92 CONSERVATION OF ENGRAILED SEQUENCES, Judith A. Kassis, Deann Wright, Steven Poole, Claude Desplan and Patrick H. O'Farrell, University of California, San Francisco, CA 94143

engrailed is a gene involved in pattern formation in Drosophila. Physical localization of mutations in the engrailed gene suggest that at least 70kb of genomic sequences contribute to the normal function of this gene. Despite the large size of the genetic unit, molecular studies suggest that engrailed function is encoded in a small, 4.5kb, primary transcript. We propose that the remaining 65kb are involved in regulating the complex expression of this transcript. In order to locate functional regions within the engrailed locus we have identified sequences conserved between D. melanogaster and D. virilis (estimated divergence time 60 million years). Electron microscopic analysis indicated that in the 70kb there are 20kb of conserved DNA in 33 different regions dispersed throughout the engrailed locus, of which only two are known to encode protein. Seqencing studies have identified potential regulatory sequences, some of which are highly conserved and repeated in the genomes of many Drosophila species and even in the house fly, Musca domestica. Studies to test whether regulatory sequences in virilis and melanogaster engrailed DNA's are functionally equivalent are underway.

K93 THE EFFECT OF PRECOCENE ANALOGUES ON INSECT PESTS, WITH SPECIAL REGARD TO THE TOXICITY OF 7-PROPARGYLOXI DERIVATIVES ON PIERIS BRASSICAE AND LEPTINOTARSA DECEMLINEATA, Peter Kulcsar, Béla Darvas and László Varjas, Plant Protection Institute, Budapest, Hungary, P.O.Box 102, H-1525.

Topical treatments and feeding bioassays with precocene 1, 2, 3, as well as with further 37 cromene-analogues on Mamestra brassicae, Hyphantria cunea, Pieris brassicae and Leptinotarsa decemlineata, resulted in highly variable levels of toxicity. The level of toxicity produced by 6-methoxi-7-isobutyloxi-2,2-dimethylchromene and 5-methyl-7-sec.buthyloxi-2,2-dimethylchromene on P. brassicae and 3,4-dichlor-7-propargyloxi-2,2-dimethylchromene on both P. brassicae and L. decemlineata /susceptible species/ was 5-8 times higher than that of the precocene 2. In this respect M. brassicae and H. cunea may be regarded as resistant species. On the basis of results, it seems reasonable to conclude that the toxic effect is strongly related to the position 7 of chromene ring. The toxicity of derivatives substituted by a propargyloxi group at this position, was significantly enhanced. The 3,4-dichlor derivatives also produced a somewhat higher toxicity as compared to the unsubstituted ones. In all cases, the toxicity exerted by the above compounds was not accompanied by any morphogenetic abnormalities.

K94 THE UNIQUENESS OF ARTHROPOD GOLGI COMPLEXES, Michael Locke, The Cell Science Laboratories, Department of Zoology, U.W.O., London, Ontario, N6A 5B7, CANADA

The smooth surface of the RER near the forming faces of GC's has rings of beads 10-12 nm in diameter that are arranged in rings around the base of transition vesicles. Although the beads can, with difficulty, be resolved in many organisms, arthropod GC beads are unique in being readily identifiable by their staining with bismuth. Something about their chemistry separates arthropods from all other organisms, raising the hope that an understanding of the beads may lead to the discovery of a correspondingly unique aspect of arthropod metabolism. The beads are probably the morphological counterpart of the energy dependent step in the transfer of material from the RER to the outer saccule of the GC, since the only reagents that interfere with the integrity of the rings also lower cellular ATP levels. The beads remain on their membranes after some kinds of fractionation and react with bismuth in vivo. Bismuth staining is being used to assay for the beads in GC fractions purified by free flow electrophoresis. The aim is to prepare a pure fraction for analysis as a first step in determining GC bead function. This paper reports some of the results using the GCs of insect fat body a) by the differential centrifugation of heavy (ie bismuth stained) beads, and b) by the separation of GC components using free flow electrophoresis.

K95 Host metabolites essential for two species of parasitoids. W. C. Nettles, Jr., K. Irie, Z. N. Xie, R. K. Morrsion, A. C. Chen, G. M. Holman, and S. B. Vinson. USDA-ARS and Texas A&M University, College Station, TX 77841

Low molecular weight host metabolites are essential for growth and development of two species of parasitoids reared in vitro on artificial diets. Eleven free amino acids and asparagine are essential for Eucelatoria bryani Sabrosky (Diptera: Tachinidae), a larval parasitoid of Heliothis spp. larvae. Host feeding by first instar larvae of E. bryani prior to feeding on artificial diet significantly increases percentage survival to puparia and adults. Two or more host metabolites have been partially purified and are essential for growth and development in vitro to the pupal stage of the egg parasitoid Trichogramma pretiosum Riley (Hymenoptera: Trichogrammatidae). Liquid obtained from Manduca sexta (L.) eggs contain factor(s) of undetermined molecular weight(s) which are essential for high percentage survival to the adult stage of T. pretiosum reared in vitro. Identification of host metabolites and incorporation of synthetic chemicals into artificial diets is needed for the economical production and large scale use of parasitoids in augmentation programs.

K96 Characterization of epoxide metabolism by epoxide hydrolase and glutathione S-transferase in Drosphila melanogaster.
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ABSTRACT

Epoxides are a group of chemicals that are ubiquitous in our environment. They are of public concern because they have the potential to react with important biological macromolecules causing mutation and cancer. Risk assessment of chemical hazards such as epoxides is becoming increasingly important as public awareness of the detrimental effects of chemicals in the environment is heightened. The use of insects to test toxic chemicals offers a number of advantages over the currently used bacterial and mammalian models.

Data are presented that confirm the existence of epoxide metabolizing enzymes in Drosophila melanogaster. Assays for epoxide hydrolase and glutathione transferase using trans- and cis-stilbene oxides were optimized and used with four strains of Drosophila. Similarities between the enzyme profiles of this insect and the corresponding mammalian activities are discussed. In addition, interstrain differences in substrate selectivity, subcellular distribution and inducibility were measured suggesting that the expression of these activities is genetically diverse. These genotypic differences may be used to complement existing tests for chemically induced genetic damage (e.g. Amés assay and recessive lethal tests) for evaluating the roles of these enzymes as deterents of the genotoxicity of epoxides. Finally, the genetic diversity of these activities may facilitate the development of tester strainswith enzyme profiles similar to those of man.

K97 LACK OF HUMORAL IMMUNITY TO SERRATIA MARCESCENS AND PHENOLOXIDASE ACTIVITY BY HELIOTHIS VIRESCENS LARVAE, Donald D. Ourth, Memphis State Univ., Memphis, TN 38152

Serratia marcescens is a bacterial insect pathogen, and Heliothis virescens is an insect pest of cotton and other crops. No bactericidal humoral immunity to S. marcescens could be detected in cell-free hemolymph from 5th instar Heliothis larvae. This was true for hemolymph obtained from uninoculated larvae and for hemolymph obtained from inoculated larvae at 24 hours post-inoculation of heat-killed S. marcescens. C-Reactive protein, an inflammatory protein, was demonstrated only in hemolymph from inoculated larvae. Only a 20% difference in phenoloxidase activity was found between hemolymphs obtained from uninoculated and inoculated larvae again indicating lack of immunity and larval recognition of S. marcescens. Oral infection of larvae with S. marcescens increased larval death 17 times when comparing uninfected larvae (1.1% dead) with infected larvae (18.5% dead).

K98 HUMORAL IMMUNITY IN LARVAL AND ADULT DROSOPHILA, Mark Robertson, Doug Kolstoe, and John Postlethwait, University of Oregon, Eugene, OR 97403

Inoculation of adult Drosophila melanogaster with live Enterobacter cloacae causes proteins to appear in the hemolymph that inhibit the growth of Escherichia coli. This humoral antibacterial immunity is evident within two hours after inoculation, levels off at 48 hours, and continues for at least sixty days. Analysis of hemolymph proteins by radiolabeling and SDS-polyacrylamide gel electrophoresis revealed that inoculated flies synthesized at least 8 new polypeptides, called antibacterial response polypeptides (AR). The time course of AR appearance paralleled the acquisition of antibacterial activity. ARs ranged from 5 to 70 kd, the most prominent being AR24, AR22, and AR19, at 24, 22, and 19 kd, respectively. Non-denaturing gel electrophoresis and isoelectric focusing separated the antibacterial activity into four protein species, called antibacterial proteins (ABs), with isoelectric points (pIs) of 7.1, 8.7, 9.0, and 9.2. Two dimensional gel electrophoresis showed that AR24, AR22, and AR19 had pIs corresponding to the basic ABs. Similar proteins were found both in inoculated larvae and adults.

K99 CONSERVED ASPECTS OF HISTONE GENE REPEATS IN DROSOPHILA, L. Strausbaugh, D. Fitch, A. Borgida, A. Frimberger, Dept. of Molec. and Cell Biol., Univ. of Connecticut, Storrs, CT. 06268

The vast majority of the histone genes of Drosophila melanogaster are organized into two sizes of repeating units, 5.0 kb and 4.8 kb in length. The repeating units each contain one coding region for each of the five histone subtypes and differ in the size of the large spacer region between the genes for H1 and H3. By examining properties of the histone gene repeat that are conserved in Drosophila species, we expect to gain insight into those elements that are important for regulation of expression and copy number. Comparisons of whole genome blots of DNA from a number of species have shown the ubiquitous presence of a 5.0 kb sized repeating unit. Although this length is highly conserved between species extensive sequence heterogeneity is detected by differences in restriction enzyme recognition sites. Coding regions from D. melanogaster histone repeating unit have high homology to histone sequences in other species; the large spacer between the genes for H1 and H3, however, has little homology to more distant relatives. These observations support the suggestion that length of this repeating unit is a critical parameter. -- In addition to the majority of histone sequences that are arranged in a 5.0 kb unit, there may be less abundant arrangements in the genome. One such minor class of sequences has a size of 4.0 kb and has been found in other species, including those with different copy numbers and chromosomal locations from D. melanogaster. The conservation of this class of histone arrangement among different species suggests it as a candidate for future studies.

K100 QUINONE METHIDE SCLEROTIZATION, Manickam Sugumaran, Department of Biology, University of Massachusetts at Boston, Boston, Mass 02125

Two molecular mechanisms have been proposed for sclerotization reaction involving o-diphenols as cross linkers. Quinone tanning mechanism calls for the generation and subsequent reactions of highly reactive o-benzoquinones with cuticular components. The alternate mechanism known as beta sclerotization requires the participation of unknown intermediate and is characterized by the presence of covalently bound o-diphenols in cuticle. Earlier we have reported the characterization of this intermediate to be a quinone methide derivative A different compound,namely 1,2-dehydro-N-acetyldopamine,has also been suggested to be the putative intermediate by other workers. In order to test the involvement of the latter intermediate in sclerotization reaction, we have synthesised this compound from 3,4-dimethoxycinnamic acid. Trapping experiments reveal that this dehydro compound may not involve in beta sclerotization reactions. In addition, they confirm our contention that quinone methides are the reactive intermediates in beta sclerotization. In analogous to quinone tanning where quinones participate, we wish to rename beta sclerotization as quinone methide sclerotization.

K101 CHITIN TURNOVER: NOT AN ALTERNATIVE TARGET FOR MILBEMYCIN/AVERMECTIN ACTIVITY, S.W. Tanenbaum, P.M. Gordnier and J. Brezner, SUNY College of Environmental Science & Forestry, Syracuse, N.Y. 13210.

The milbemycins/avermectins (MBD/AVM) represent novel antibiotics with high specificity for invertebrates. We have begun to examine the spectrum of activity of MBD against selected forest pests and laboratory insects. Contact studies over wide concentrations(10^{-3} to 2.0 mg in acetone) applied to intersegmental dorsal thorax areas were negative for toxic effect. Oral administration evinced moderate activities against the house cricket, gypsy moth, and mealworm larvae. Field colonies of the European saw fly(Neodiprion sertifer) succumbed when infested Scots pine were sprayed with 0.03% MBD. To test a recent claim (Calcott and Fatig, J. Antibiotics 37,253: 1984) that the mode of action of AVM lies in chitin turnover, we have used 4th and 5th instars of T. molitor which contain chitinase and chitin synthase. Addition of either AVM or MBD (10^{-5} to 10^{-7} M) showed no antagonistic effects towards these enzyme complexes. Replicate synthase controls with polyoxin D were inhibited 53-90% at comparable drug concentrations. Additionally, neither congener inhibited chitinase from Acheta or from Streptomyces. It would therefore appear that these agents intefere, as more generally believed, with insect neurotransmission. We are currently in the process of synthesizing potential affinity-labelling species of MBD in order to probe for such receptors.

(Supported by an institutional McIntire-Stennis grant from the USDA).

K102 PRODUCTION AND USE OF MONOCLONAL ANTIBODIES SPECIFIC FOR CRYSTAL PROTEIN OF BACILLUS THURINGIENSIS VAR. ISRAELENSIS, Kevin B. Temeyer, Maurice Haufler, and John Pruett, U.S. Livestock Insects Laboratory, USDA-ARS, Kerrville, TX 78028

Murine hybridomas were constructed by fusion of the myeloma cell line SP2/0-AG14 with spleen cells of BALB/c mice which had been immunized with crystal protein of Bacillus thuringiensis var. israelensis (BTI). A direct ELISA (enzyme-linked immunosorbent assay) was modified to produce 100-1000 fold increased sensitivity, and was used to screen the hybridomas to identify and subclone cell lines secreting monoclonal antibody specific for BTI crystal protein. Immunoblot analysis demonstrated specificity of the monoclonal antibody (subclass IgG3) for the 68000 protein component of BTI crystals, which is believed to be the insecticidal δ-endotoxin. Use of monoclonal antibodies enables molecular cloning and genetic engineering of the BTI gene specifying production of the δ-endotoxin, development of immunochemical assays to quantitate the BTI δ-endotoxin for industrial process development and quality control, and provides improved methods for supervision of microbial pesticide production and use by regulatory agencies.

K103 DROSOPHILA CYTOCHROME P-450: PARTIAL PURIFICATION OF INSECTICIDE RESISTANCE-RELATED FORMS. Larry C. Waters and Carroll E. Nix, Oak Ridge Natl. Lab., Oak Ridge, TN 37831

By SDS-PAGE the cytochrome P-450 of Drosophila melanogaster is resolved into two distinct protein bands [Biochem. Biophys. Res. Communs., 123, 907-913 (1984)]. Apparent molecular masses are 59.3 kDa (band A) and 55.8 kDa (band B). Expression of band A is ubiguituous among the strains tested, whereas band B is observed in only a few strains. Dimethylnitrosamine demethylase activity is expressed only in strains that are expressing band B. Strains expressing band B are relatively more resistant to killing by phenylurea. Furthermore, expression of band B is controlled by a locus on chromosome 2 that maps at or near a major locus for insecticide resistance. Expression of band B is also under trans regulatory control by loci on chromosome 3, at least one of which maps at another major resistance locus. We have used successive chromatography on octylamino-Sepharose, DEAE-cellulose and hydroxylapatite to partially purify the proteins in bands A and B. Preparations enriched for the band B protein (SA $\geq$ 4.5 nmol/mg) were obtained from microsomes of the resistant Hikone-R strain. These preparations contain significant amounts of the band A proteins ($\sim$20-30%), but are apparently free of other proteins as judged by SDS-PAGE. Band A proteins were purified from either Hikone-R or the sensitive Oregon-R strain to homogeniety as judged by SDS-PAGE. Specific activities of these preparations are $\geq$ 6.9. These preparations are being used to make polyclonal and/or monoclonal antibodies to be used to study the genetic regulation of cytochrome P-450 expression in insects, particularly as it relates to resistance to insecticides. (Research sponsored by the Office of Health and Environmental Research, U.S. Dept. of Energy under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.)

K104 ULTRASTRUCTURAL DISTRIBUTION OF PUPAL CUTICLE PROTEINS IN *DROSOPHILA MELANOGASTER*, William J. Wolfgang, D. Fristrom, J.W. Fristrom, K. Grimnes*, and G.M. Happ*; Univ. of Ca., Berkeley. *Univ. of Vt., Burlington.

Although much biochemical information exists on insect cuticular proteins, little is known about their distribution within the cuticle. Using a variety of primary antibodies we were able to localize by EM immuno-gold labelling specific pupal cuticle proteins (PCP's) within the cuticle of *Drosophila melanogaster*. The morphologically distinct inner and outer lamellae of the pupal cuticle were found to contain distinct sets of PCP's. None of our antisera against detergent extractable PCP's reacted with the basal assembly zone. In contrast two independently produced monoclonal antibodies react strongly with a component of the assembly zone but not with the lamellar PCP's. Western blot analysis indicates that the two antibodies bind to a single cuticular antigen which has an apparent molecular weight between 250-400 kD, is probably a glycoprotein, and is extracted only by detergent plus mercaptoethanol. The antigen accumulates as cuticle deposition proceeds suggesting that it is present in the lamellate cuticle but is masked from the antibody. To account for these observations we envision that cuticle formation is a two step process in which a relatively insoluble scaffold, exposed in the assembly zone, becomes embedded in a matrix of soluble PCP's during formation of lamellae. NIH 5 F32 GM09647

Late Additions

K105 MODULATION OF NOVEL LENGTH DOPA DECARBOXYLASE TRANSCRIPTS BY 20-OH-ECDYSONE IN A DROSOPHILA K_c CELL SUBLINE, John D. O'Connor and Ruth E. Swiderski, Department of Biology, University of California, Los Angeles, CA 90024

The induction of DOPA decarboxylase (DDC) activity by 20-OH-ecdysone in a subline of Drosophila K_c cells was investigated. Cells cultured in the continuous presence of the steroid hormone first exhibited a detectable Ddc transcript after 48 hours of exposure. The concentration of Ddc RNA increased 8-fold to its peak value 96 hr after initial exposure to hormone and was correlated temporally with a 26-fold increase in DDC enzyme activity. The K_c Ddc primary transcript, processing intermediates, and mature mRNA all were approximately 500 nucleotides longer than the corresponding transcripts observed in newly eclosed adult Drosophila. The novel length Ddc mRNA of K_c cells was capable of directing the in vitro synthesis of a polypeptide which was immunologically identical to larval Drosophila DDC.

K106 EARLY EMBRYO STAGE SPECIFIC GENES OF DROSOPHILA, Judith A. Lengyel, Department of Biology, University of California, Los Angeles, CA 90024

We are using a system of "reverse genetics" to study genes important at a particular developmental stage. The blastoderm stage in Drosophila embryogenesis is a time of dramatic transitions in nuclear division and RNA transcription, as well as of cellularization and cell determination. To understand these unique processes, we are studying genes which are expressed only at this stage. Using a combination of competition and differential hybridization screens, we identified 3 blastoderm stage-specific genes, mapping to chromosomal loci 25D3, 75C1,2, and 99D4, 8[1]. Sequence analysis and transcription mapping indicate that all three loci contain more than one transcription unit[2,3,4]. Two of the loci (99D and 75C) encode proteins homologous with domains in the Xenopus transcription factor IIIA[3,4]; one locus (25D) encodes a protein which shares a homology domain with the fos oncogene[4]. A number of the blastoderm-specific genes are thus potential DNA-binding proteins. We are characterizing the localization of expression of these genes in the early embryo, using in situ hybridization to the RNA in whole embryos, and antibodies against synthetic peptides predicted by the protein sequence. By screening over small deficiencies, and using P-factor mediated transformation rescue, we are generating mutants in these blastoderm-specific genes and characterizing their phenotypes.

[1]Roark et al., Devel. Biol. 109:476
[2]Vincent et al., J. Mol. Biol., in press.
[3]Baldarelli et al., in preparation.
[4]Boyer et al., in preparation.

K107 GENETIC CONTROL OF BODY SEGMENTATION IN DROSOPHILA, E. B. Lewis, California Institute of Technology, Pasadena CA 91125.

A 16-mm color film will be presented to show selected homeotic mutant phenotypes of the bithorax (BX-C) and Antennapedia (ANTP-C) gene complexes in Drosophila. Examples include living flies with: 4 wings (homozygous for three mutants of the BX-C); 8 legs (hemizygous for another mutant of the BX-C); proboscises transformed to tarsi (a double mutant combining the proboscipedia and aristapedia mutants); and antennae transformed to legs (an Antennapedia mutant combined with Polycomb, a mutant that trans-regulates both ANTP-C and BX-C. Animation techniques are used to illustrate a model for the genetic control of the thoracic and abdominal segmentation pattern as seen in the late embryonic and first instar larval stages; specific BX-C gene functions are shown as being expressed in a sequential fashion starting with the third thoracic segment and proceeding posteriorly; remarkably, the order of the genes in the chromosome parallels the order in which they become expressed along the body axis. [The assistance of Sung Min Park in the preparation and filming of the animated sequences is gratefully acknowledged]. (Work supported in part by USPHS grant HD06331).

K108 THE DEVELOPMENT AND RISK ASSESSMENT OF A GENETICALLY ENGINEERED MICROBIAL INSECTICIDE, Robert J. Kaufman, Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, MO 63198

Conceptually, genetic engineering promises to make great contributions to environmentally acceptable and cost effective pest control. A model microbial insecticide was developed to test the validity of this concept and to identify key technical hurdles which need to be solved to make genetically engineered microbial pesticides technically, economically and environmentally viable. To develop such a model system, the gene coding for the active endotoxin of Bacillus thuringiensis kurstaki was cloned into isolates of corn root colonizing P. fluorescens. Criteria for assessing the risk of releasing genetically engineered microbes into the environment were developed and data was generated supporting the conclusion that a genetically engineered microbial pesticide can be safety released into the environment. These include lack of pathogenicity, low capacity for genetic exchange and limited environmental persistence of the microbial insecticide candidate described in this paper.