Research Program

My major focus is on the physiology of ticks, emphasizing the role of the salivary glands in osmoregulation, the control of salivary fluid secretion, the endocrinological control of salivary gland development and degeneration and the control of vitellogenesis.

We work primarily with the tick, *Amblyomma hebraeum* (Acari: Ixodidae), one of the largest species that can be reared on rabbits in the laboratory.

1) Engorgement Factor in male Ixodid ticks

Female *A. hebraeum* require about 10 days to engorge. During the first 8-9 days, females increase in weight about 10-fold; during the next day or two, they increase in weight a *further* 10-fold. Thus, a slow phase of feeding is followed by a rapid phase; the transition between the two phases was named the *'critical weight'* (CW) by Harris & Kaufman (1984). Females forcibly removed from the host *below* the CW do not lay eggs, do not degenerate their SGs but do reattach to a host if given the opportunity. Females forcibly removed from the host *above* the CW do lay eggs, do degenerate their SGs within 4 days and do not reattach to a host if given the opportunity (Kaufman & Lomas; 1996). Recently, we have demonstrated that there is a slightly different CW for reattachment to the host, 20E-induced SG degeneration and egg development (Weiss & Kaufman, 2001).

A protein from the male gonad (named ‘male factor’ (MF) by Harris & Kaufman (1984), transferred to the female during copulation, is responsible for signalling the transition between the slow and rapid phases of engorgement. The evidence for this can be summarized as follows:

- Very few virgin females spontaneously feed beyond the CW, and none of these attain full engorgement (Harris & Kaufman, 1984; Lomas & Kaufman, 1992a).
- SGs of mated females above the CW degenerate within 4 days, whereas those of virgin females above the CW require 8 days to degenerate (Lomas & Kaufman, 1992a).
- Injecting an homogenate of male gonad into the haemocoel of virgin females above the CW, induces SG degeneration to occur within 4 (reduced from 8) days (Lomas & Kaufman, 1992a).
- MF acts by stimulating the synthesis/release of 20E once females exceed the CW (Lomas & Kaufman, 1992b).
- MF is produced in the testis/vas deferens (T/VD) but is not associated with the spermatozoa (Lomas & Kaufman, 1992b).

In the 1970s, Pappas & Oliver demonstrated that female *Dermacentor variabilis* do not feed to repletion when exposed to unfed males, or to those which had their genital apertures blocked. These experiments suggested the presence of an 'engorgement factor' (EF) contained in the spermatophore that promotes feeding to repletion; EF is not associated with the spermatozoa themselves. Because EF promotes feeding beyond the CW, and because MF is responsible for the behvioural and hormonal changes occurring at that time, we hypothesize that MF and EF are the same substance.

Using a differential cross-screening protocol of a cDNA library of *Amblyomma hebraeum*

male gonad, we isolated 35 clones which are up-regulated by feeding in the testis/vas deferens (Weiss et al., 2002). We have successfully produced recombinant (*rec*) proteins from 28 of these clones, and have identified *rec*AhEF from among them by use of an EF-bioassay. *Rec*AhEF comprises two peptides (*rec*AhEFα and *rec*AhEFβ); both peptides (but not either alone), when injected into the haemocoel of partially-fed virgin females, stimulate full engorgement (Weiss & Kaufman, 2004). Immunizing a rabbit against *rec*AhEF inhibited feeding and egg production in mated females by ~75%. We have proposed the name *voraxin* (Latin for gluttonous) for the native EFs of ixodid ticks (Weiss & Kaufman, 2004). Currently we are exploring the potential of producing an effective anti-tick vaccine based on voraxin.

Relevant publications:

Weiss, B.L. & Kaufman, W.R. (2004) Two feeding-induced proteins from the male gonad trigger engorgement of the female tick, *Amblyomma hebraeum*. *P.N.A.S.* **101:** 5874-5879.

Weiss, Brian L., Stepczynski, Jadwiga M., Wong Paul and Kaufman, W.R. (2002) Identification and characterization of genes differentially expressed in the testis/vas deferens of the fed male tick, *Amblyomma hebraeum*. *Insect Biochem and Molec. Biol.* **32:** 785-793.

Weiss, Brian & Kaufman W.R. (2001) The relationship between 'critical weight' and 20-hydroxyecdysone in the female ixodid tick, *Amblyomma hebraeum*. *J. Insect Physiol.,* **47:** 1261-1267.

Lomas, L.O. & W.R. Kaufman (1999) What is the meaning of 'critical weight' to female ixodid ticks?: A 'grand unification theory'! In: *Acarology IX*, Volume 2, Symposia (Eds. G.R. Needham, R. Mitchell, D.J. Horn, and W.C. Welbourn); The Ohio Biological Survey, Columbus Ohio, pp. 481-485.

Kaufman, W.R. and Lomas, L. (1996) 'Male Factors' in ticks: their role in feeding and egg development. *Invert. Reprod. Devel.* **30:** 191-198.

Lomas, L.O. & W.R. Kaufman (1992a) An indirect mechanism by which a protein from the male gonad hastens salivary gland degeneration in the female ixodid tick, *Amblyomma hebraeum*. Arch. *Insect Biochem. Physiol*. **21**: 169-178.

Lomas, L.O., and Kaufman, W.R. (1992b) The influence of a factor from the male genital tract on salivary gland degeneration in the female ixodid tick, *Amblyomma hebraeum*. *J. Insect Physiol.* **38**: 595-601.

2) Development and degeneration of salivary glands during the feeding cycle

The functional state of the salivary glands depends on the degree of engorgement, there being a 60-fold increase in fluid secretory competence during the first 5-7 days of feeding. Our evidence suggests that a blood-borne factor triggers salivary gland development, although the identity of the factor is not known (Coons & Kaufman, 1988). However, within a few days following engorgement, the salivary glands atrophy under the influence of an ecdysteroid hormone - - probably 20-hydroxyecdysone (20E). The release of 20E occurs only after a critical degree of engorgement has been achieved (~10X the unfed weight; Weiss & Kaufman, 2001).

The timing of release of 20E is also influenced by a protein from the male gonad passed to the female during copulation. This is discussed above in project 1.

Since 1995 we have been publishing on the ecdysteroid receptor (EcR) in the salivary glands, elucidating the distribution, pharmacological and biochemical properties and ontogeny of these receptors (Mao et al., 1995; Mao & Kaufman, 1998, 1999). We are also beginning to study some of the post-receptor events mediating salivary gland degeneration.

Relevant publications:

Weiss, B. and Kaufman W.R. (2001) The relationship between 'critical weight' and 20-hydroxyecdysone in the female ixodid tick, *Amblyomma hebraeum*. *J. Insect Physiology,* **47:** 1261-1267.

Mao, H & Kaufman, W.R. (1999) Profile of the ecdysteroid hormone and its receptor in the salivary gland of the adult female tick, *Amblyomma hebraeum*. *Insect Biochemistry and Molecular Biology* **29**: 33-42.

Mao, H & Kaufman, W.R. (1998) DNA binding properties of the ecdysteroid receptor in the salivary gland of the female ixodid tick, *Amblyomma hebraeum*. Insect Biochemistry and Molecular Biology **28**: 947-957.Lomas, L.O., Gelman, D. and Kaufman, W.R. (1997) Ecdysteroid regulation of salivary gland degeneration in the ixodid tick, *Amblyomma hebraeum*: A reconciliation of *in vivo* and *in vitro* observations. *Gen. Comp. Endocrinol.* **109**: 200-211.

Charrois, G., Mao H. and Kaufman, W.R. (1996) Putative ecdysteroid antagonists and agonists: impact on salivary gland degeneration in the ixodid tick, *Amblyomma hebraeum*. *Pest. Biochem. Physiol.* **55**: 140-149.

Mao, H., McBlain, W.A. and Kaufman, W.R. (1995) Some properties of the ecdysteroid receptor in the salivary gland of the ixodid tick, *Amblyomma hebraeum. Gen. Comp. Endocrinol.* **99**: 340-348.

Coons, L.B. and W.R. Kaufman (1988) Evidence that developmental changes in type III acini in the tick, *Amblyomma hebraeum* Koch (Acari: Ixodidae) are initiated by a haemolymph-borne factor. *Exptl. Appl. Acarol.* **4**: 117-139.

3) Vitellogenesis in ixodid ticks

Although some progress has been made in understanding the endocrinology of egg development in the argasid family of ticks, we know very little about the process in the ixodid family. Ovary transplant experiments on *A. hebraeum* demonstrated that egg development is definitely under hormonal control (Lunke & Kaufman, 1993), and 20E triggers yolk-synthesis in vivo (Friesen & Kaufman, 2002). But 20E is not sufficient on its own to stimulate the whole process: yolk uptake following yolk synthesis. JH and JH-analogues are also ineffective in triggering yolk synthesis or uptake, either alone or in combination with 20E. It intrigues us that the tick ovary has so far shown itself to be refractory to treatments which have traditionally stimulated complete egg development in other arthropods. Our data strongly suggest that yolk uptake requires a separate signal from yolk synthesis (Friesen & Kaufman, 2004), and we intend to look for such a yolk-uptake factor in the haemolymph of engorged females.

Relevant publications:

Friesen, K. and W.R. Kaufman (2004). Effects of 20-Hydroxyecdysone and other Hormones on Egg Development, and Identification of a Vitellin-Binding Protein in the Ovary of the Tick, *Amblyomma hebraeum*. *J. Insect Physiol.* In Press.

Friesen, K. and W. Reuben Kaufman (2002). Quantification of vitellogenesis and its control by 20-hydroxyecdysone in the ixodid tick, *Amblyomma hebraeum. J. Insect Physiol*. **48:** 773-782.

Kaufman, W.R. (1999) Chelicerate Arthropods. In: Encyclopedia of Reproduction, (Ernst Knobil and Jimmy D. Neill, Eds) Academic Press, pp. 564-571.

Kaufman, W.R. (1997) ARTHROPODA-Chelicerata. Chapter 7 in: Reproductive Biology of Invertebrates (series editors: K.G. Adiyodi and Rita Adiyodi) Vol. 8 Part A, *Progress in Reproductive Endocrinology* (Ed. Terrance S. Adams), Oxford and IBH, New Delhi, India, and Wiley, NY, NY, pp. 211-245.

Mao, H., McBlain, W.A. and Kaufman, W.R. (1995) Some properties of the ecdysteroid receptor in the salivary gland of the ixodid tick, *Amblyomma hebraeum. Gen. Comp. Endocrinol.* **99**: 340-348.

Lunke, M.D. and W.R. Kaufman (1993) Hormonal control of ovarian development in the tick *Amblyomma hebraeum* Koch (Acari: Ixodidae). *Invert. Reprod. Devel.* **23**: 25-38.

4) The effect of the avermectins on ticks

The avermectins (AVMs) are very potent, broad spectrum antihelmintic drugs that are also effective against ticks. The AVMs potentiate the activity of GABA-pathways in mammals and invertebrates; this interaction with GABA may be responsible for antihelmintic activity, although several recent reports suggest instances in which GABA-pathways may not be involved. The mechanism of action against ticks is not known. We have demonstrated that the AVMs do not influence salivary gland activity, but they do inhibit vitellogenesis, the application of egg-wax, and oviposition. As with many other organisms that are sensitive to the AVMs, high concentrations of the drug induce a general paralysis. AVMs are likely to have several sites of action in ticks, and we are studying how they induce their numerous effects.

Relevant publications:

Friesen, K., Suri, R. and W. Reuben Kaufman (2003). Effects of the avermectin, MK-243, on ovary development and salivary gland degeneration in the ixodid tick, *Amblyomma hebraeum*. *Pest. Biochem. Physiol.* **76:** 82-90.

Lunke, M.D., and Kaufman, W.R. (1992) Effects of the avermectin analogue MK-243 on vitellogenesis and reproduction in the ixodid tick, *Amblyomma hebraeum*. *Exptl. Appl. Acarol.* **13**: 249-259.

Lomas, L.O., and Kaufman W.R. (1991) Ivermectin is not an agonist at a GABA-receptor in tick salivary glands. *Expt. Appl. Acarol.* **12**: 129-133.

Kaufman, W.R., S.G. Ungarian and A.E. Noga (1986) The effect of avermectins on feeding, salivary fluid secretion and fecundity in some ixodid ticks. *Exp. Appl. Acarol*. **2**: 1-18.

5) Pharmacological control of fluid secretion

Three receptors control fluid secretion: (a) catecholamines act via a dopamine (DA)-receptor. (b) Ergot alkaloids are agonists at a receptor distinct from the DA-receptor (Kaufman & Wong, 1983; Minion & Kaufman, MS in preparation). The natural ligand for the 'ergot receptor' is unknown. (c) A γ-aminobutyric acid (GABA)-receptor modulates the activity of the first. Although GABA has little intrinsic activity of its own, in the presence of DA, GABA increases fluid secretion by up to 100% or more two (Lindsay & Kaufman, 1986). This potentiation can be mimicked by butyrophenone drugs -- a surprising result, because butyrophenones are potent *antagonists* of some DA-receptors in the mammalian CNS (Kaufman & Wong, 1983).

Relevant publications:

W. Reuben Kaufman, B. Duff Sloley, Roger J. Tatchell, Geoff Zbitnew, Tom Dieffenbach and Jeff Goldberg (1999) Quantification and cellular localization of dopamine in the salivary gland of the ixodid tick, *Amblyomma hebraeum* and the effect of organ culture on dopamine content. *Exp. Appl. Acarol*. **23**: 251-265.

Kaufman, W.R. and Sloley, B.D. (1996). Catabolism of dopamine and 5-hydroxytryptamine by monoamine oxidase in the ixodid tick, *Amblyomma hebraeum*. *Insect Biochem. Molec. Biol*. **26**: 101-109.

Lucien, J., Reiffenstein, R., Zbitnew, G. and Kaufman, W.R. (1995) γ-aminobutyric acid (GABA) and other amino acids in tissues of the tick, *Amblyomma hebraeum* throughout the feeding and reproductive periods. *Exp. Appl. Acarol.* **19**: 617-631.

Lindsay, P.J. and W.R. Kaufman (1986) Potentiation of salivary fluid secretion in ixodid ticks: a new receptor system for γ-aminobutyric acid. *Can. J. Physiol. Pharmacol.* **64**: 1119-1126.

Kaufman, W.R. and D.L.-P. Wong (1983) Evidence for multiple receptors mediating fluid secretion in salivary glands of ticks. *Eur. J. Pharmacol.* **87**: 43-52.

Kaufman, W. (1979) Control of salivary fluid secretion in ixodid ticks. pp. 357-363. In: *Recent Advances in Acarology* (J.G. Rodriguez, Ed.) Vol. I Academic Press, New York.

Kaufman, W. (1978) Actions of some transmitters and their antagonists on salivary secretion in a tick. *Am. J. Physiol.* **235**: R76-R81.

6) Pathogen transmission by ticks

Since 1990, I have collaborated regularly with Prof. Patricia Nuttall at the NERC Institute of Virology and Environmental Microbiology, Oxford England. The overall theme of our project is to explore how pathogens exploit the physiology of the tick to gain access to the host. The model we work with is Thogoto virus and its tick vectors (*Amblyomma variegatum* and *Rhipicephalus appendiculatus*) in laboratory hosts (primarily the guinea pig). We have provided definitive data that virus is transmitted via saliva actively (i.e., by naturally infected ticks) and passively (i.e., following injection of virus into the haemocoel) (Kaufman & Nuttall, 1996). The

virus also inhibits salivary fluid secretion in partially fed (but not engorged) ticks; the intracellular mechanism of this inhibition remains unknown (Kaufman, Bowman & Nuttall, 2001).

We have also demonstrated that the tick does not simply act like a "needle and syringe": acquiring virus from one host and transmitting it to another. Rather, the salivary glands participate actively via a protein ('saliva activated transmission (SAT)-factor') that markedly facilitates the movement of virus among ticks and through the host (Jones et al., 1992). SAT-factor may have its effect on the host's immune system, although the physiological function of the protein to the tick remains obscure.

Relevant publications:

Kaufman, W.R., Bowman, A.S. AND Nuttall P.A. (2001) Salivary fluid secretion in the ixodid tick *Rhipicephalus appendiculatus* is inhibited by Thogoto virus infection. *Exptl. Appl. Acarol.* **25:** 661-674.

Wang, H., W. R. Kaufman, W. W. Cui *and* P. A. Nuttall (2001) Molecular individuality and adaptation of the tick *Rhipicephalus appendiculatus* (Ixodidae) in changed feeding environments. *Med. Vet. Entomol.* **15**: 1-9.

Kaufman, W.R. & Nuttall P. (2000) Secretion of Thogoto virus by *in vitro* salivary glands of *Rhipicephalus appendiculatus*. (*Proceedings of 3rd International Conference on Ticks and Tick-borne Pathogens : Into the 21st Century*. Eds: M. Kazimirova, M. Labuda & P.A. Nuttall, Institute of Zoology, Slovak Academy of Sciences.

Wang, H., Kaufman, W.R., Nuttall, P. (1999) Molecular individuality: polymorphism of salivary gland proteins in three species of ixodid ticks. *Exp. Appl. Acarol*. **23**: 969-975.

Kaufman, W.R. and P.A. Nuttall (1999), Secretion of Thogoto virus by salivary glands of *Amblyomma variegatum*. In: *Acarology IX*, Volume 2, Symposia (Eds. G.R. Needham, R. Mitchell, D.J. Horn, and W.C. Welbourn); The Ohio Biological Survey, Columbus Ohio, pp. 427-431.

Kaufman, W.R and Nuttall, P. (1996) *Amblyomma variegatum* (Acari: Ixodidae): Mechanism and control of arbovirus secretion in tick saliva. *Exptl. Parasitol.* **82**: 316-323.

Nuttall, P.A., L.D. Jones, M. Labuda and W.R. Kaufman (1994) Adaptations of arboviruses to ticks. *J. Med. Entomol*. **31**: 1-9

Dharampaul, S, W.R. Kaufman and M. Belosevic (1993) Differential recognition of saliva antigens from the ixodid tick *Amblyomma hebraeum* by sera from infested and immunized rabbits. *J. Med. Entomol.* **30**: 262-266.

Nuttall, P.A., L.D. Jones, M. Labuda and W.R. Kaufman (1992) Interactions between arboviruses and their tick vectors. Invited Symposium paper, First International Conference on Tick-Borne Pathogens at the Host-Vector Interface: An Agenda for Research, St. Paul Minnesota, 15-18 September, 1992: 37-41.

Jones, L.D, W.R. Kaufman, and P.A. Nuttall. (1992) Feeding site modification by tick saliva resulting in enhanced virus transmission. *Experientia* **48**: 779-782.

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