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BIOCHEMICAL STUDIES ON THE SALIVARY GLANDS AND HAEMOLYMPH OF *AMBLYOMMA HEBRAEUM*

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ABSTRACT

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The functional significance of some components of salivary glands and of their secretion and of haemolymph of *Amblyomma hebraeum* and other tick species is reviewed with respect to host responses at the attachment site, the survival of specific pathogens in the vector, the transmission of pathogens and immunological responses of the host to tick infestation.

INTRODUCTION

The importance of biochemical studies on the salivary glands and their secretion as well as on haemolymph of ticks is evident from several review articles (Sauer, 1977; Nelson, Bell, Clifford & Keirans, 1977; Binnington & Kemp, 1980; Kemp, Stone & Binnington, 1982; Binnington & Obenchain, 1982).

These studies have practical as well as analytical objectives. They include the gathering of information on immunity to tick infestation (Wakelin, 1984), the survival of pathogens in salivary glands and haemolymph (Pereira, Andrade, Ribeiro, 1981), the direct or indirect role of salivary glands in the transmission of *Cowdria ruminantium* (Bezuidenhout, 1981) and the composition of nutrient media for tick cell culture for the growth of pathogens (Rehacek & Brzostowski, 1969; Kurrti & Büscher, 1979). A knowledge of the composition of these tick body fluids could also be of assistance in the study of the metabolism and general biochemistry of ticks and the mechanism of salivation and host responses, especially at the attachment site of the tick (Kemp *et al.*, 1982).

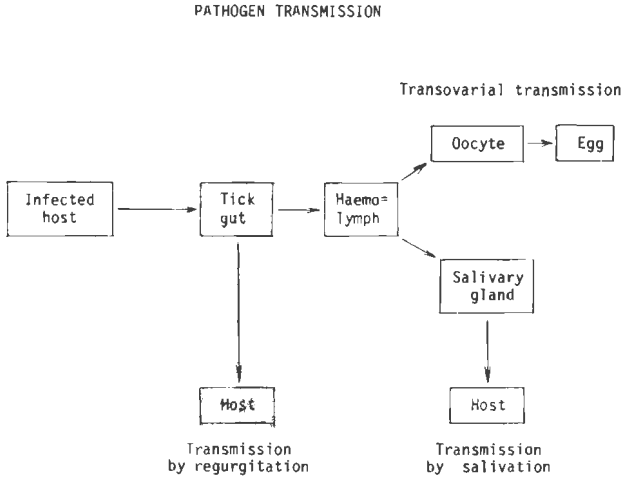

FIG. 1 Schematic presentation of possible involvement of salivary glands and haemolymph in pathogen transmission

The possible involvement of salivary glands and haemolymph in pathogen transmission as well as various ways by which tick feeding could cause deleterious effects to the host are schematically represented in Fig. 1 & 2, respectively.

COMPOSITION OF SALIVARY GLANDS AND THEIR SECRETIONS

A summary of some components present in tick salivary gland secretion together with the relevant references is presented in Table 1. It is evident that many of the components listed overlap in their activities. Thus, for example, antiproteases may function as anticoagulants, and several proteins may possess enzymatic or cytolytic activities or behave as toxins.

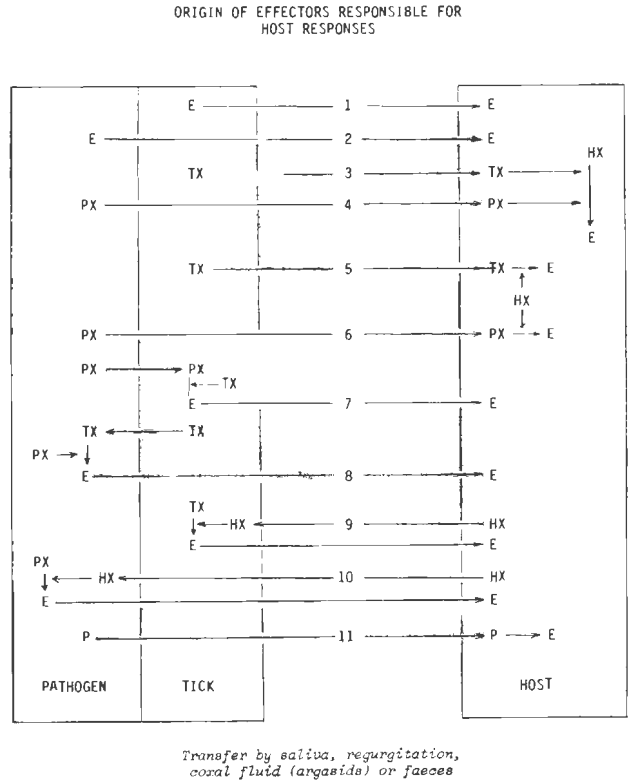

FIG. 2 Schematic presentation of possible ways by which tick feeding could cause effects in the host by effector subtances: the effector is a product of the tick tissue *per se* (1) or of a pathogen (2). The effector is formed in host tissue as the result of the action of a product from tick tissue (3) or from a pathogen (4) on a host component. A product of tick tissue (5) or from a pathogen (6) is converted to an effector by a host tissue component. A product of a pathogen is converted to an effector by tick tissue (7). A product of tick tissue is converted to an effector by a pathogen (8). Ingested host product triggers the tick (9) or pathogen (10) to produce an effector or causes conversion of products of tick tissue or of the pathogen to effectors. Pathogen transferred to host (11) in which effector is released or induced to form. PX, TX, HX: products of pathogen, tick and host respectively; E:effector; P:pathogen

Possible functions for some of the components in tick feeding and pathogen transfer have been suggested by several investigators. Thus it has been concluded that the antihaemostatic, anti-inflammatory and immunosuppressive properties of the saliva of *Ixodes dammini* probably facilitate the extended periods of feeding of this tick (Ribeiro, Makoul, Levine, Robinson & Spielman, 1985). The platelet anti-aggregation activity of the saliva

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TABLE 1 Summary of some components or activities described in tick salivary glands and their secretions

Component/activity	Tick species	References*
Enzymes		
Esterases (non-specific)	*Hyalomma anatolicum anatolicum*	1
	Boophilus microplus	2,3
cAMP phosphodiesterase	*Amblyomma americanum*	4,5
Hyaluronidase	*Amblyomma hebraeum*	6
	Ornithodoros savignyi	7
Acid phosphatase	*H.a. anatolicum*	1
	B. microplus	8
Acetylcholinesterase	*O. savignyi*	7
	B. microplus	8
Aminopeptidases	*H.a. anatolicum*	1
	B. microplus	8,9
Proteases	*O. savignyi*	10
Adenylate cyclase	*A. americanum*	5,11,12
cAMP dependent protein kinases	*A. americanum*	13,14
Carboxylic ester hydrolases	*B. microplus*	8,9
Monophenol monooxygenases	*B. microplus*	8,9
Triacylglycerol lipase	*B. microplus*	9
Polysaccharide splitting enzyme	*B. microplus*	3
Apyrase	*Ixodes dammini, A. hebraeum*	15,51
Kininase	*I. dammini*	15
Enzyme inhibitors		
Antiproteases	*B. microplus*	16
	O. savignyi	7
cAMP phosphodiesterase inhibitor	*A. americanum*	4
Anticoagulants	*I. dammini*	15
	Ixodes holocyclus	17
	I. ricinus	18
	O. savignyi	19
	Ornithodoros papillipes	20
	Ornithodoros moubata	21
	Argas persicus	22,23,24
Cement	*Hyalomma asiaticum*	25
	Ixodes ricinus	25
	B. microplus	26
	Dermacentor andersoni	27
	Haemaphysalis spinigera	28
Proteins (general)	*Rhipicephalus evertsi evertsi*	29
	A. americanum	30
	A. hebraeum	1
Toxins	*R. e. evertsi*	31,32
	I. holocyclus	33,34
	O. savignyi	7,10,35,36,37,49
Antigens/immunogens	*B. microplus*	2
	D. andersoni	38
	H.a. anatolicum	1
Haemolytic activity	*O. papillipes*	20
Bactericidal activity	*O. papillipes*	48
Amino acids	*A. hebraeum*	6
	O. savignyi	35
Ions	*Amblyomma maculatum*	39
	A. hebraeum	40
Other:		
Prostaglandins	*I. dammini*	15
	B. microplus	41
Bradykinin-like substance	*B. microplus*	42,43
Histamine/histamine-like substance or histamine releaser	*H. spinigera*	44
	Rhipicephalus sanguineus sanguineus	44
Histamine-blocker	*R.s. sanguineus*	44,45
Dopamine	*A. hebraeum*	46
cAMP	*A. hebraeum*	5,11,47
Noradrenaline	*A. hebraeum*	46
Pathogens	*A. hebraeum*	50,31
	Various other species	52

*(1) Gill *et al.* (1986). (2) Willadsen & Williams (1976). (3) Geczy *et al.* (1971). (4) McMullen, Bantle, Essenberg & Sauer (1983). (5) Sauer & Essenberg (1984). (6) Neitz *et al.* (1978). (7) Neitz (1976). (8) Binnington (1978). (9) Schleger & Lincoln (1976). (10) Neitz *et al.* (1981). (11) Hume, Essenberg, McNew, Bantles & Sauer (1984). (12) Schramke, McNew, Schmidt, Essenberg & Sauer (1984). (13) McSwain, Essenberg & Sauer (1985). (14) Mane, Darville, Sauer & Essenberg (1985). (15) Ribeiro *et al.* (1985). (16) Willadsen & Riding (1980). (17) Ross (1926). (18) Foggie (1959). (19) Howell (unpublished data, 1969). (20) Pawlowsky & Chodukin (1929). (21) Hellman & Hawkins (1967). (22) Nuttall & Strickland (1908). (23) Cornwall & Patton (1914). (24) Chinery (1974). (25) Balashov (1972). (26) Moorhouse & Tatchell (1966). (27) Meredith & Kaufman (1973). (28) Chinery (1973). (29) Neitz & Gothe (1986). (30) McSwain *et al.* (1982). (31) Viljoen (1985). (32) Viljoen *et al.* (1986). (33) Kaire (1966). (34) Stone *et al.* (1979). (35) Howell, Neitz & Potgieter (1975). (36) Howell (1966). (37) Neitz *et al.* (1969). (38) Wikel, Graham & Allen (1978). (39) Guenther, Barker & Sauer (1980). (40) Neitz (unpublished data, 1979). (41) Dickinson, O'Hagan, Schotz, Binnington & Hegarty (1976). (42) Tatchell & Binnington (1973). (43) O'Hagan, Schotz, Binnington & Hegarty (1973). (44) Chinery (1981). (45) Chinery & Ayitey-Smith (1977). (46) Kaufman & Wong (1983). (47) Krolak, Ownby, Barker & Sauer (1983). (48) Podboronov *et al.* (1975). (49) Neitz, Bezuidenhout, Vermeulen, Potgieter & Howell (1983). (50) Bezuidenhout (1981). (51) Neitz (unpublished data, 1986). (52) Binnington & Kemp (1980).

is probably due to ADP degradation by apyrase and to the presence of prostaglandins (PG) of the E series. In addition, the formation of thrombin, which is involved in blood coagulation and platelet aggregation, is inhibited. Willadsen & Riding (1980) have isolated a proteolytic enzyme inhibitor which is presumably produced in the salivary glands of *Boophilus microplus*. This inhibitor affects blood coagulation since it prolongs activated partial thromboplastin time and prothrombin time. All these effects result in effective prevention of host haemostasis (Ribeiro *et al.*, 1985).

Salivary apyrase may furthermore prevent inflammatory responses stimulated by ATP, which include mast cell degranulation and aggregation of neutrophils (Ribeiro *et al.*, 1985). The effects of extracellular ATP on mast cell secretion, platelet aggregation and membrane permeability as well as its involvement in immunomodulation, vascular tone and neurotransmission have been reviewed by Gordon (1986). The removal of ATP by salivary gland apyrase may thus have complex effects in the host animal.

Further functions of prostaglandins during tick feeding may be to increase vascular permeability at the attachment site (Tatchell & Binnington, 1973) and vasodilation (Higgs, Vane, Hart, Potter & Wilson, 1976), resulting in increased blood flow to the tick. PGE_2 also inhibits mast cell degranulation and thereby serves to reduce the release of platelet-aggregating, oedema-promoting and vasoconstrictive factors (Ribeiro *et al.*, 1985).

It appears that tick saliva has inflammation promoting as well as inflammation reducing properties, and because inflammation both enhances and impairs feeding, ticks probably regulate host inflammatory processes selectively (Ribeiro *et al.*, 1985).

An entirely different function of salivary prostaglandins has been proposed by Oliver, Pound & Andrews (1984). These authors showed that haemocoelically injected salivary gland homogenates of male *Ornithodoros parkeri* stimulated 40 % of fed virgin females to oviposit. It thus seems likely that salivation by males immediately prior to spermatophore transfer not only serves to lubricate the males' mouthparts and to facilitate spermatophore transfer (Feldman-Muhsam, Borut & Saliternik-Givant, 1970), but also to stimulate ovum maturation through the introduction of prostaglandins, which are known to possess this stimulatory behaviour.

The immunosuppressive characteristics of *I. dammini* saliva may be due to PGE_2 present in the saliva (Ribeiro *et al.*, 1985). The proteolytic-enzyme inhibitor isolated by Willadsen & Riding (1980) blocks the action of complement and thus also exhibits immunosuppressive properties. The overall effect results in a delayed, reduced or abolished response of the host to the immunogens introduced by the tick (Ribeiro *et al.*, 1985).

The immunosuppressive characteristics of tick saliva may explain the observation made by Norval (1978) that rabbits and sheep are unable to acquire resistance to larvae and nymphae of *Amblyomma hebraeum*. It could also explain the fact that high concentrations of salivary gland antigens are not effective in the induction of resistance in guinea pigs. At high concentrations immunosuppresive components are present which are more readily eliminated by dilution (probably by denaturation) than are the immunogens (Wikel & Allen, 1982). This clearly indicates the need for the purification and characterization of salivary gland components.

Numerous immunogens have been shown to be present in salivary gland secretions (Gill, Boid & Ross, 1986), including the cement (Brown, Shapiro & Askenase, 1984). Using immunoblotting techniques Gill *et al.* (1986) showed that sera from hypersensitized rabbits reacted with 9 proteins in the saliva and 17 in the salivary gland extracts from female *Hyalomma anatolicum* fed for 96 h. One antigen exhibited acid phosphatase activity and another both non-specific esterase and aminopeptidase activity. In hosts which reject ticks, esterases are rapidly removed from the feeding site. This indicates an essential role for these enzymes during feeding (Gill *et al.*, 1986). Geczy, Naughton, Cleg & Hewetson (1971) have mentioned the possibility that esterases may increase vascular permeability by hydrolyzing cholesterol esters present in the membranes of certain cells.

According to Brown *et al.* (1984), esterases and aminopeptidases are likely to be involved in facilitating insertion of the tick's mouthparts into the host's integument as well as in effecting a beneficial feeding milieu by destroying the cellular and tissue integrity. Proteases and hyaluronidase present in *Ornithodoros savignyi* (Neitz, Bezuidenhout & Potgieter, 1981) and *A. hebraeum* (Neitz, Howell, Potgieter & Bezuidenhout, 1978) probably have similar functions.

Calcium, cAMP, dopamine, dopamine-sensitive adenylate cyclase, cAMP phosphodiesterase, phosphodiesterase inhibitors and phosphorylated proteins present in tick saliva are involved in the direct and indirect nervous control of salivary gland secretion (Sauer & Essenberg, 1984). Amongst other functions, certain ions in these secretions are involved in the reversed flow of fluid in the salivary gland ducts, i.e. in the uptake of vapour from unsaturated atmospheres (McMullen, Sauer & Burton, 1976).

Antiproteases in the salivary secretion of *O. savignyi* may function as inhibitors of microbial proteases, thereby preventing the multiplication of some species of invading organisms (Board & Fuller, 1974). Inhibition of animal proteases is most probably only incidental since they are similar in structure and function to some microbial proteinases (Davis, Zahnley & Donovan, 1969). The bactericidal property of the saliva from *Ornithodoros papillipes* (Podboronov, Stephanochenock-Rudnik & Grokhovskaya, 1975) may be due to the presence of such inhibitors. These inhibitors could well be responsible for specific pathogen associations in ticks and act as primitive humoral defence agents which do not depend on recognition of immunogens (Lackie, 1980).

In the salivary secretion of *Rhipicephalus sanguineus sanguineus*, either histamine, a compound closely related to histamine or a releaser of such a substance as well as an agent which antagonizes and potentiates the action of histamine and acetylcholine on guinea pig ileum have been found (Chinery & Ayitey-Smith, 1977; Chinery, 1981). These authors have suggested that small amounts of the histamine-blocking agent is released during the initial feeding phase and larger amounts during the final period of rapid engorgement. In this way the beneficial and detrimental effects of histamine in the host can be regulated during the entire feeding phase. The potentiating effect on the action of acetylcholine could lead to motor paralysis. This has been observed in several host species as a consequence of feeding of various ixodid and argasid tick species (Gothe, Kunze & Hoogstraal, 1979). The functional significance of tick paralysis toxins has been discussed by Gothe (1984).

The findings on tick salivary gland composition are of particular interest concerning the role of salivary gland secretions in pathogen transfer as well as the survival of pathogens in the host and vector. They may also have a bearing on practical considerations in the preparations and administration of vaccines containing live pathogens. For example, the heartwater vaccine, prepared from ground-up infected *A. hebraeum* nymphae, is only

effective when administered intravenously (Bezuidenhout, 1981). Subcutaneous inoculations with infected tick suspensions to which hyaluronidase, histamine, and Freud's complete adjuvant have been added are ineffective (Bezuidenhout, 1981). Some of these negative results can probably be related to components in the salivary gland secretions and hence in whole tick homogenates. Thus the presence of proteases, and histamine antagonists could nullify the activity of hyaluronidase and histamine respectively.

It should be stressed that a comparison of the biochemical compositions of salivary glands or their secretions obtained from the various tick species is difficult since different methods for the collection of material have been used. The material has also been obtained from ticks in various developmental stages and feeding phases and numerous techniques for the fractionation and detection of the components have been used. Barker, Burris, Sauer & Hair (1973) have compared infra-red heat, pilocarpine injections and electrical stimulation as methods for inducing salivation in ticks. They found differences in the responses of the ticks with respect to the volume and the nature of the components in the secretion. It is also to be expected that the composition of salivary glands will differ, both quantitatively and qualitatively, from that of salivary secretions. The methods employed for the preparation of salivary gland extracts (e.g. homogenization or sonification; time taken; temperature and pH and ionic strength of the extractant buffer) could also cause variations in the composition of the extracts. Furthermore, it is evident from studies on the morphological changes as well as changes in the protein content of salivary glands during feeding (Binnington & Stone, 1981; McSwain, Essenberg & Sauer, 1982; Gill *et al.*, 1986; Neitz & Gothe, 1986) that the feeding phase should be noted when making comparisons. Hajjar (1971) has also stressed the fact that, during the developmental cycle of ticks, physiological changes influence the biochemical composition of body fluids. In addition, detection methods for enzyme activities have included histochemical and spectrophotometric techniques utilizing whole tissue, crude extracts or purified fractions with natural or synthetic substrates. Obviously, these approaches differ with respect to sensititivy and specificity.

COMPOSITION OF HAEMOLYMPH

The haemolymph serves as a transport system for hormones, nutrients, intermediates of metabolism and specific pathogens to various internal organs of the tick. Haemolymph thus affects the biochemical characteristics of the entire tick organism as well as its vector potential (Dolp, 1970; Hefnawy, 1972). The transport function of the haemolymph is greatly enhanced by the absence of an epithelial lining to the haemocoel. Thus tissues of the internal organs such as the salivary gland alveoli, ovarian oocytes, fat body, malpighian tubules and epithelium of the midgut are only separated from the surrounding haemolymph by thin basement membranes (Binnington & Obenchain, 1982). The interrelationships of haemolymph with the internal organs and factors which may alter its composition (adapted from Mullins, 1985) are shown in Fig. 3.

The composition of tick haemolymph is summarized in Tables 2, 3 and 4.

The possible functional significance of some haemolymph constituents is reviewed below. Connat, Diehl, Gfeller & Morici (1985) have correlated ecdysteroids present in the haemolymph with cuticular changes during feeding, vitellogenesis and oviposition in *A. hebraeum*.

INTERRELATIONSHIPS OF HAEMOLYMPH WITH INTERNAL ORGANS AND FACTORS WHICH MAY ALTER ITS COMPOSITION

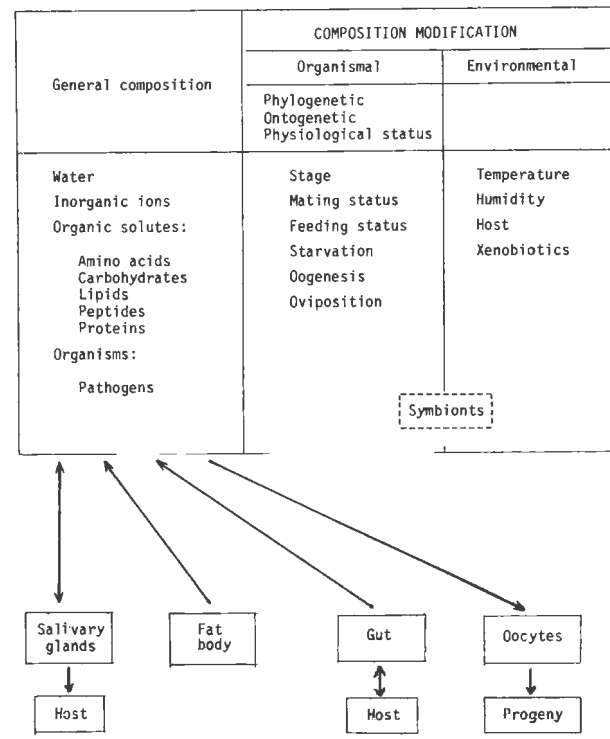

FIG. 3 Schematic presentation of interrelationships of haemolymph with internal organs and factors which may alter haemolymph composition

They conclude that these steroids are probably not involved in the synthesis of procuticular material and that large quantities of ecdysteroids are synthesized during the gonotrophic cycle and that these, consisting mainly of ecdysone and 20-hydroxyecdysone, are incorporated in eggs in the free form. They also postulate that circulating ecdysteroids might be similar to the 'tick salivary gland degeneration factor' (TSGDF) (Harris & Kaufman, 1984) which is involved in the degeneration of tick salivary glands in replete females. Harris & Kaufman (1985) have also shown that infusion of 20-hydroxyecdysone *in vivo* induces salivary gland degeneration.

Antibodies naturally synthesized by the host against tick saliva (Bowessidjaou, Brossard & Aeschlimann, 1977) probably cross the midgut epithelium of ticks and pass via the haemolymph to the internal organs where they disturb physiological mechanisms (Brossard & Rais, 1984). The passage of host antibodies across the digestive tract of ticks has also been demonstrated by Ackerman, Clare, McGill & Sonenshine (1981) and Fujisaki, Kamio & Kitaoka (1984).

The presence of pathogens in haemolymph most certainly has a bearing on pathogen transmission to the vertebrate host via the salivary glands as well as on transovarial transmission. Burgdorfer (1970) has exploited this fact by developing a hemolymph test for the detection of rickettsiae in ticks.

Dolph & Hamdy (1971) investigated haemolymph and coxal fluid proteins in an attempt to describe biochemically the internal milieu of ticks with the purpose of understanding interrelationships between the vector, the host and the pathogen as well as to assist with the establishment of tick tissue cultures. Changes in the haemoprotein concentrations in haemolymph have been studied by Tatchell (1971). He concluded that these proteins are

TABLE 2 Summary of some components or activities described in tick haemolymph

Component/activity	Tick species	References*
Enzymes		
Esterases; acid phosphatases, alkaline phosphatases; leucine aminopeptidase	*B. microplus*	1
Proteins (general)	*A. hebraeum*	2
	R. sanguineus	3
	Hyalomma dromedarii	4
	Hyalomma anatolicum excavatum	4
	Argas (Persicargas) persicus	4
	Argas (Persicargas) arboreus	4
Haemoproteins		
Haemoglycoproteins	*B. microplus*	1
Lipoglycohaemoproteins (lipovitellins)	*D. andersoni*	5
Host antibodies	*I. ricinus*	6
	Haemaphysalis longicornis	7
	O. moubata	7
	Dermacentor variabilis	8
Host haemoglobin digestion products		
Haematin	*O. moubata*	9
	B. microplus	10
Methaemalbumin	*I. ricinus*	9
Lipids		
Ecdysteroids	*A. hebraeum*	11
Phospolipids	Several species	12,13,14,15
Free sterols	Several species	14,15
Free fatty acids	Several species	14,15
Triglycerides	Several species	14,15
Sterol esters	Several species	14,15
Cholesterol	Several species	21
Carbohydrates		
Glucose	*D. andersoni*	16,17
	B. microplus	18
	Argas langenoplastis	18
Trehalose	*D. andersoni*	17
Glycerol	*D. andersoni*	16
Inositol	*D. andersoni*	16
	B. microplus	18
	A. langenoplastis	18
Mannose	*D. andersoni*	16
Amino acids	*A. (P.) persicus*	19
	A. (P.) arboreus	20
	(Additional data in Table 3 and 4)	
Ions	See Table 4	
Pathogens (in haemocytes)	*A. hebraeum*	22
	D. andersoni	23
	Several species	24

*(1) Tatchell (1971). (2) Neitz *et al.* (1978). (3) Araman (1979). (4) Dolp & Hamdy (1971). (5) Boctor & Kamel (1976). (6) Brossard & Rais (1984). (7) Fujisaki, Kamio & Kitaoka (1984). (8) Ackerman *et al.* (1981). (9) Wigglesworth (1942). (10) O'Hagan (1974). (11) Connat *et al.* (1985). (12) Hussein & Kamal (1977). (13) Kamal & Kamel (1977). (14) Hajjar (1972). (15) Maroun (1972). (16) Levenbook, Boctor & Fales (1980). (17) Barker & Lehner (1976). (18) Rehacek & Brzostowski (1969). (19) Boctor & Araman (1971). (20) Boctor (1972). (21) Maroun & Kamal (1976). (22) Du Plessis (1984). (23) Kocan, Oberst, Ewing, Hair & Barron (1983). (24) Burgdorfer (1970).

taken up selectively by the developing oocytes. However, he could not demonstrate an immunological similarity between haemoproteins in female *B. microplus* haemolymph and egg homogenates. Boctor & Kamel (1976) showed that 2 lipovitellins of *Dermacentor andersoni* eggs were immunologically identical. They did not cross-react with host haemoglobin.

There are indications that haemolymph proteins may be transported to the salivary glands in *B. microplus* (Binnington & Kemp, 1980). If this proves to be true for ticks in general, it could raise doubts as to the origin of tick toxins which are at present presumed to be products of the salivary glands (Neitz, Howell & Potgieter, 1969; Stone, Doube, Binnington & Goodger, 1979; Viljoen, Bezuidenhout, Oberem, Vermeulen, Visser, Gothe & Neitz, 1986).

Compared with insect haemolymph (Chen, 1985), tick haemolymph contains much lower concentrations of free amino acids. It appears that the osmotic pressure of tick haemolymph is maintained principally by the high sodium and chloride concentrations.

The presence of ornithine and citrulline in the haemolymph of *A. hebraeum* may indicate the operation of the urea cycle or more likely implicate the synthesis of arginine which in turn could be utilized for arginine phosphate synthesis, the phosphagen involved in ATP production in invertebrate muscle (Bender, 1975). Correlations between free amino acids in haemolymph and metabolism in ticks should become evident in the future.

Conclusions and Perspectives

A knowledge of the composition of salivary gland secretions and haemolymph has led to a better understanding of numerous aspects of tick physiology and biochemistry as well as the effects of tick feeding on the host. Practical applications of this knowledge should emerge in the future. Undoubtedly, analysis by means of modern separation techniques, such as HPLC and FPLC, will reveal additional constituents and provide more in-

TABLE 3 Amino acid analysis of haemolymph of female *Amblyomma hebraeum* (A.h.), *Boophilus microplus* (B.m.), *Argas lagenoplastis* (A.l.) and *Argas (Persicargas) arboreus* (A.P.a.) (values in mg/ℓ)

Amino acid	A.h.[1]	B.m.[2]	A.l.[3]*	A.P.a.[4]
Lysine	48	44	16	110
Histidine	36	44	10	114
Arginine	6	8	1	Trace
Aspartic acid	0	23	11	53
Methionine	2	7	7	Trace
Threonine	72	44	18	53
Serine	39	67	16	69
Glutamic acid	75	63	14	580
Proline	75	38	9	Trace
Glycine	45	42	20	63
Alanine	154	71	20	40
Cysteine	nr	nr	nr	44
Valine	67	164	27	249
Isoleucine	2	13	4	5
Leucine	30	50	15	5
Tyrosine	5	33	8	Trace
Phenylalanine	12	43	8	Trace
Citrulline	81	nr	nr	nr
Ornitine	35	nr	nr	nr
α-amino butyric acid	51	nr	nr	nr
γ-amino butyric acid	6	nr	nr	nr
3-methylhistidine	5	nr	nr	nr
Total	688#	754	204	1 475

[1] Semi-engorged; Neitz *et al.* (1978)

[2] Semi-engorged; Rehacek & Brzostowski (1969)

[3] Semi-engorged; Rehacek & Brzostowski (1969)

[4] Engorged; Boctor (1972)

nr Not reported

\# Total excludes the last 5 listed amino acids

* Haemolymph collected 2–3 months after the last blood meal

formation on the internal biochemical milieu of ticks on which the survival of specific pathogens and the vector potential of ticks depend. The latter is closely associated with immunity in ticks. Therefore, an analysis of tick haemolymph for the presence of phenoloxidases, lectins, lysozyme and cecropins which are involved in humoral immunity in insects (Gotz & Boman, 1985), could prove to be valuable in describing vector-pathogen interrelationships.

From the data supplied in this review, the lack of knowledge on the chemical characteristics of many components, especially with respect to the proteins is evident. The main obstacle in this regard resides in the very small quantities of material available for analyses. This may be circumvented by applying recombinant DNA technology.

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TABLE 4 Ions and total amino acids in female tick haemolymph

Tick species	Feeding phase	Ionic concentration (mEq/ℓ)								Amino acids mM/ℓ†	References*
		Na	K	Cl	Ca	Mg	Mn	Fe	Cu		
A. hebraeum	Semi-engorged	157	43	—	4	1	0	0,4	0,1	6	1
B. microplus	Engorged	136	15	118	8	4	—	—	—	6	2
H. dromedarii	Engorged	186	13	104	—	—	—	—	—	22	3
H. anatolicum excavatum	Engorged	186	8	79	—	—	—	—	—	35	3
D. andersoni	Engorged	170	7	125	—	—	—	—	—	—	4
A. persicus	Engorged	193	7	140	—	—	—	—	—	—	5
A. arboreus	Engorged	195	7	126	—	—	—	—	—	7	5,6
A. lagenoplastis	Semi-engorged	—	—	—	—	—	—	—	—	2#	7

*References:

1. Neitz *et al.* (1978), Neitz (unpublished data, 1979)
2. Tatchell (1969)
3. Araman (1972)
4. Kaufman & Phillips (1973)
5. Araman & Said (1972)
6. Boctor (1972)
7. Rehacek & Brzostowski (1969)

† Assuming average molecular mass of amino acid is 120

\# Haemolymph collected 2–3 months after last blood meal

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