# Cuticular Plasticization Caused by Cuticular pH Descent, and Mitochondria-Rich Acidophilic Epidermal Cells in Adult Female Haemaphysalis longicornis (Acari: Ixodidae)

Authors: Okura, Nobuhiko, Mōri, Takayuki, Andō, Kōichi, and Shiraishi, Satoshi

Source: Zoological Science, 14(2) : 211-217

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.211

ZOOLOGICAL SCIENCE 14: 211–217 (1997)


# Cuticular Plasticization Caused by Cuticular pH Descent, and Mitochondria-Rich Acidophilic Epidermal Cells in Adult Female *Haemaphysalis longicornis* (Acari: Ixodidae)

Nobuhiko Okura[1]*, Takayuki Mōri[1], Kōichi Andō[2] and Satoshi Shiraishi[1]

[1]*Zoological Laboratory, Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812-81, Japan*
[2]*Biological Laboratory, Department of Regional Culture, Faculty of International Studies of Culture, Kyushu Sangyou University, Matsukadai, Higashi-ku, Fukuoka 813, Japan*

**ABSTRACT**—Measurements of pH in minced cuticle from the alloscutum of non-copulated and copulated female *Haemaphysalis longicornis* revealed a descent of cuticular pH after copulation. Sample cuticular loops were cut from the alloscutum of non-copulated females and immersed in buffers adjusted to pH 4, 5, 6, 7 or 8. Extensions of the cuticular loops subjected to a constant applied load were measured using a creep test. The cuticular loops at pH 7 and 8 showed small extensibilities. However, as the acidity of the cuticle increased, the sample loops displayed larger extensibilities. Extensibilities of sample loops from copulated females were significantly larger than those from non-copulated females. Based upon this difference of extensibility, the descent of cuticular pH after copulation is presumed to be about 0.5 pH units. These data suggest that the cuticular plasticization is caused by the cuticular acidification in the female tick. Moreover, novel acidophilic epidermal cells were situated around dermal glands. Their cytoplasm have an affinity for eosin, because of numerous mitochondria. A smooth endoplasmic reticulum like tubulovesicular system appeared in the apical cytoplasm after copulation. Such morphological features suggest that the acidophilic epidermal cells actively transport ions and provoke the pH descent of the cuticle after copulation.

## INTRODUCTION

In adult female ixodid ticks, expansion of the soft integument on the alloscutum during feeding is an important event, because this expansion enables the intake of a large blood meal for production of numerous eggs. The integumentary expansion consists of two phases; precopulatory "cuticle synthesis phase", and post-copulatory "rapid expansion phase" (Hackman and Filshie, 1982). During the former phase, epidermal cells become columnar and secrete cuticular materials resulting in an increase in endocuticular thickness. During the latter phase, the epidermal cells and the expansible endocuticle are flattened and nonexpansible epicuticular folds disappear, because of rapid feeding explosion.

It was not known if cuticular plasticization play a role in the integumentary expansion mentioned above. Recently, Okura *et al.* (1996) have revealed that cuticular plasticization induced by copulatory stimuli is necessary for initiation of rapid expansion of the soft integument in the adult female tick, *Haemaphysalis longicornis*. Thus, the importance of the cuticular plasticization is now recognized in ticks.

Cuticular plasticization has been thoroughly studied in the abdominal cuticle of the blood-sucking bug, *Rhodnius prolixus* (Bennet-Clark, 1962; Maddrell, 1966; Reynolds, 1974, 1975a, b). Reynolds (1975b) showed that the descent of the cuticular pH caused by some components of the epidermis brings about cuticular plasticization resulting in the expansion of the integument. Cuticular proteins of the alloscutum in the tick, *Boophilus microplus* are biochemically similar to those of the bug, *R. prolixus* (Hackman, 1975). Considering the similarities in the biochemical properties of the bug and the tick cuticles, Hackman (1975), and Hackman and Goldberg (1987) pointed out a possibility that a similar plasticization mechanism is operating in ticks. However, there is no direct evidence on cuticular plasticization caused by cuticular acidification in ticks.

In the present study, we demonstrate that cuticular plasticization depends on a decline in cuticular pH, and also describe novel acidophilic epidermal cells surrounding the dermal glands. These cells seem to acidify the cuticle by means of active transport of ions after copulation in the adult female tick, *H. longicornis*.

* Corresponding author: Tel. +81-92-642-2948; FAX. +81-92-642-2804.

## MATERIALS AND METHODS

*Animals*

Adult *Haemaphysalis longicornis* (bisexual race) used in the present study were collected with dragging from pastures at Kuju Highland, Oita Prefecture, Kyushu, Japan. Female and male ticks were separately fed on laboratory rabbits. Then, replete males were allowed to copulate with 5-day fed females. Copulated females rapidly engorged and detached from the rabbit within 48 hr after copulation. The female ticks in the various feeding stages, e.g. (1) partially fed and non-copulated (the 5th day in feeding), (2) just copulated (5-day fed and 5 hr after copulation), (3) copulated and rapidly feeding (24 hr after copulation or 6-day fed), and (4) engorged (40 hr after copulation) females, were used in the present experiments.

*Measurement of pH in minced cuticle from non-copulated and copulated females*

Soft integuments were removed from non-copulated (5-day fed), and copulated (5, 24 and 40 hr after copulation) female ticks. Cuticles were cleaned by scraping the epidermis and were quickly washed with physiological saline (0.8% NaCl). Fifty mg (wet weight) of cuticle was minced in the 100 μl of distilled water using razor blades. The pH of the mixture was measured using a pH meter (B-212, Horiba).

*Measurement of cuticular extensibility*

Following three kinds of measurements were performed with the creep test at 25°C; (1) extensibilities of the cuticle from non-copulated females under wide range of pH from 4 to 8 and (2) around physiological range of pH from 6 to 8, and (3) cuticular extensibilities in non-copulated and copulated females.

Sample loops of cuticle from females were transversely cut from the alloscutal body wall just posterior to the scutum (Fig. 1). The length and width of the cuticular loops were checked under a stereo microscope with an ocular micrometer, and then loops of the same size, 4 mm in length and 1 mm in width, were selected for the creep test. The epidermis sticking to the cuticle was carefully scraped using a small piece of filter paper. For the measurements of cuticular extensibilities under different pH conditions (for measurements 1 and 2), the loops from non-copulated females were respectively immersed in 0.01 M McIlvain buffer adjusted to pH 4, 5, 6, 7 or 8 for more than one hr, and were then measured with the creep test. The period of time for immersion of the loops into buffers was based on the preliminary experiment indicating that cuticular extensibilities became constant after one hour of the immersion. For the measurement of cuticular extensibilities in non-copulated (5-day fed) and copulated (5 hr after copulation) females (for measurement 3), the loops from these females without immersing in the buffer were immediately measured with the creep test. Although the non-copulated and the copulated females had almost the same body size, the cuticle was already plasticized in the copulated ticks (Okura *et al.*, 1996). Thus, we could obtain non-plasticized and plasticized cuticular loops having the same size.

In accordance with Reynolds (1975a), the behaviour of sample loops of cuticle subjected to a constant load was examined using an apparatus for the creep test (Fig. 2). The sample loops were placed in a chamber filled with paraffin oil, in order to prevent them from drying out and to avoid changes in the aqueous phase of the cuticle. The extension of a sample loop was detected by a differential transformer (TD-111S, Nihon Koden). The direct current output from the differential transformer was proportional to the cuticular extension, and was continuously monitored with a pen recorder (SP-H6P, Riken Denshi). The rate of creep of the cuticular loops after the imposition of the load was taken as a measure of the extensibility of the cuticle. For the measurement under wide range of pH (for measurement 1), the load in the creep test was only a 2.5 g weight for 5 min. The loops in pH 4 and 5 showed very high extensibilities and were easily cut by heavy load (more than 3 g weight) in the preliminary experiment. The

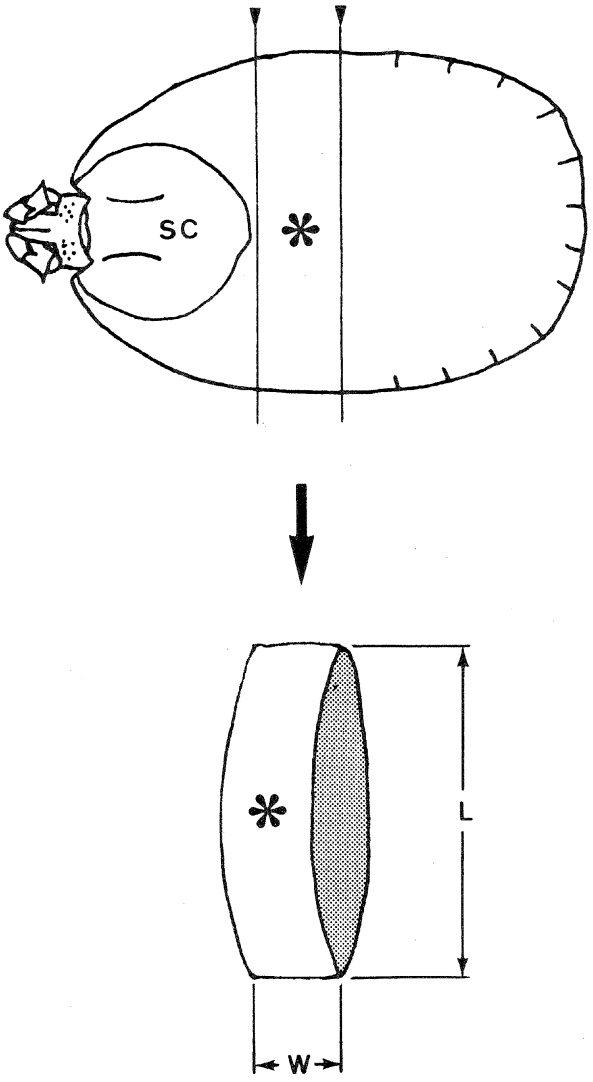


Fig. 1. A schematic diagram showing the preparation of a sample cuticular loop from a female tick. The loop is cut just posterior to the scutum (SC). L, length of the loop; W, width of the loop.

data around physiological pH were not precise in the present conditions. For the measurement around physiological range of pH and the measurement of the extensibilities in the non-copulated and copulated females (for measurements 2 and 3), the load in the creep test was a 25 g weight for 10 min.

*Calculation of presumptive cuticular pH in non-copulated and copulated females from cuticular extensibilities*

A regression equation of correlation between pH and extensibilities of buffered cuticle from non-copulated females was obtained from the measurement of the extensibilities around physiological pH using the least squares method. To presume pH of the cuticle in the non-copulated and copulated females, the cuticular extensibilities of these females were substituted into the equation.

*Light and electron microscopy*

Soft integuments just posterior to the scutum were removed from non-copulated (5-day fed), just copulated (5 hr after copulation), and copulated and rapidly feeding (24 hr after copulation) female ticks. The integuments were fixed with 2.5% glutaraldehyde for 5 hr and

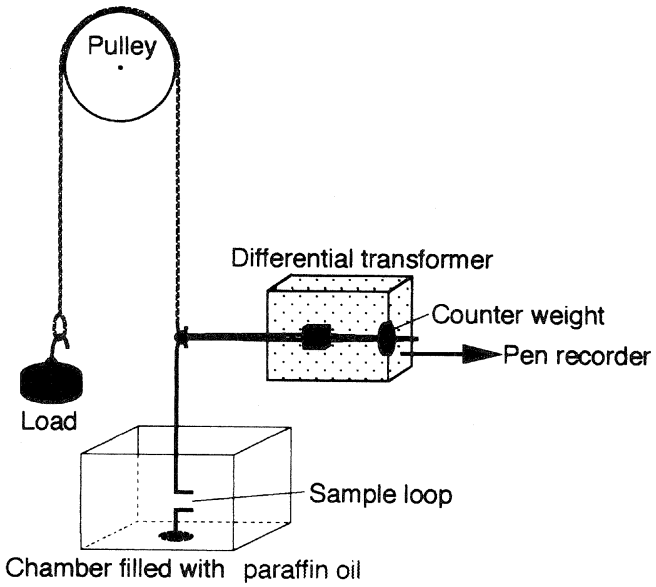


Fig. 2. Apparatus used for the creep test.

dehydrated in a graded ethanol series, and then embedded in a methacrylate resin (Technovit 7100®, Heraeus Kulzer GmbH). Semi-thin sections (≈ 2 μm) were cut on a microtome (JB-4, Sorvall) with a glass knife, and were doubly stained with haematoxylin and eosin.

The soft integuments from the females as mentioned above were doubly fixed with 2.5% glutaraldehyde for 5 hr and 1% $OsO_4$ for 2 hr. The tissues were dehydrated in a graded ethanol series, and embedded in epoxy resin. Thin sections (≈ 60 nm) were cut on a microtome (MT-6000, RMC) with a diamond knife, and doubly stained with uranyl acetate and lead citrate before examination in an electron microscope (H-600A, Hitachi) at 75 kV.

## RESULTS

### *pH of minced cuticle from non-copulated and copulated females*

Table 1 shows pH of minced cuticle from non-copulated (5-day fed), just copulated (5 hr after copulation), copulated and rapidly feeding (24 hr after copulation), and engorged (40 hr after copulation) female ticks. The pH after copulation were significantly lower than the pH before copulation, and seemed to be almost constant at least from 5 to 40 hr after copulation. The pH difference in the minced cuticle between non-copulated and copulated females was about 0.5 pH units.

### *Extensibilities of cuticle from non-copulated females under different pH conditions*

Figure 3 shows typical creep curves of the cuticle in wide range of pH from 4 to 8. A relationship between pH and the extensibility of cuticle is shown in Fig. 4. The cuticular loops immersed in pH 7 or 8 showed small extensibilities. In contrast, as the acidity of the cuticle increased, the sample loops displayed larger extensibilities. In addition, in the acidic buffers (pH 4 and 5), sample loops possessed an appearance of swelling.

The cuticular loops re-immersed into pH 7 from 4 exhibited

Table 1. pH of minced cuticle from soft integument in non-copulated (5-day fed virgin), copulated (5 hr after copulation), copulated and rapidly feeding (24 hr after copulation), and engorged (40 hr after copulation) female ticks

| Cuticle from | pH (mean ± S.D.) |
|---|---|
| non-copulated females (n = 5) | 6.92 ± 0.08 |
| copulated females (n = 3) | 6.47 ± 0.06* |
| copulated and rapidly feeding females (n = 3) | 6.43 ± 0.06* |
| engorged females (n = 3) | 6.47 ± 0.06* |

n, number of measurements.
*Significantly different from the pH in non-copulated ticks at $P < 0.05$ (Mann-Whitney U test).

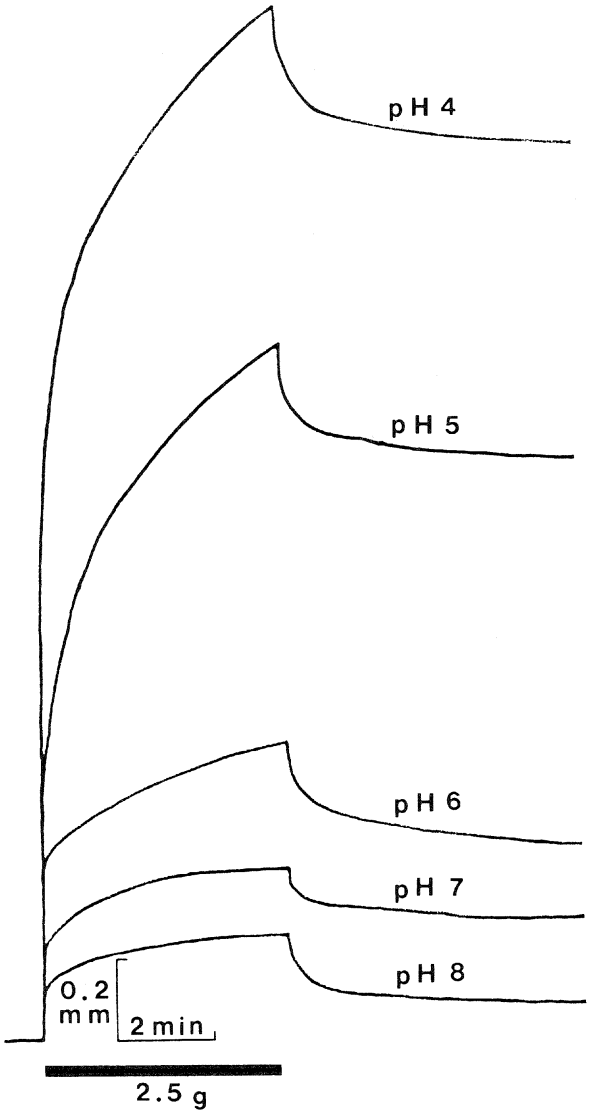


Fig. 3. Typical creep curves of the cuticular loops from non-copulated females after immersion into buffers (pH 4-8).

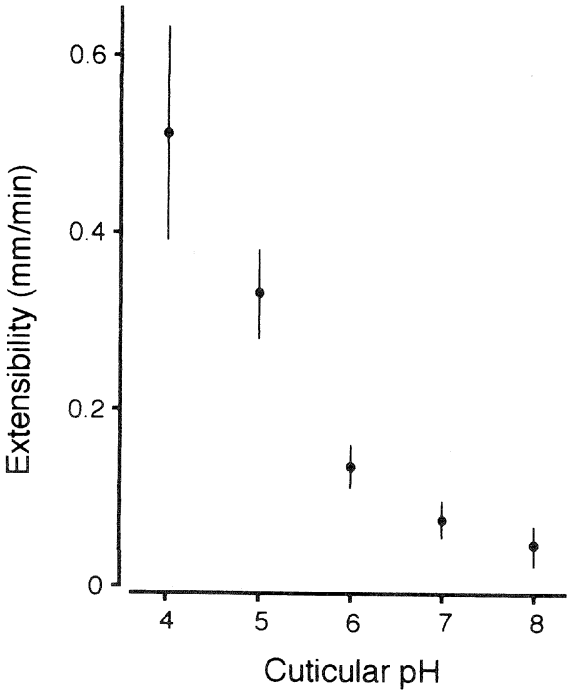


Fig. 4. The relationship between cuticular pH and extensibility of the cuticular loops from non-copulate females under a 2.5 g load for 5 min. Closed circle and vertical bar show mean and S.D. calculated from five measurements, respectively.

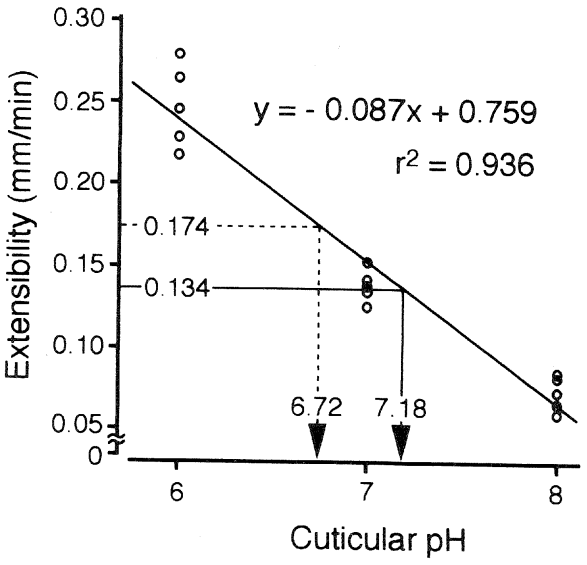


Fig. 5. The correlation between cuticular pH and extensibility of the cuticular loops from non-copulate females under a 25 g load for 10 min. Open circles indicate extensibilities of the loops in these pH conditions. Solid and broken arrows indicate presumptive cuticular pH of non-copulated and copulated ticks, respectively, on the basis of substitutions of cuticular extensibilities shown in Table 2 into the regression equation.

small extensibilities and no swelling. Therefore, these alterations of the cuticular properties caused by the pH change were reversible.

A precise correlation between pH from 6 to 8 and the extensibility of cuticle is shown in Fig. 5. There was a linear correlation between pH and the cuticular extensibility, and a regression equation was obtained using the least squares method.

*Extensibilities and presumptive pH of cuticle in non-copulated and copulated females*

Table 2 shows extensibilities of the cuticle from non-copulated and copulated females. The cuticular extensibility of copulated females was significantly larger than that of non-copulated females.

The extensibilities shown in Table 2 were substituted into the regression equation of correlation between pH and the extensibility of buffered cuticle from non-copulated females (Fig. 5). As a result of the substitution, cuticular pH of non-copulated and copulated females were assumed to be pH 7.18 and 6.72, respectively. Consequently, the descent of the cuticular pH after copulation on the basis of the equation was about 0.5 pH units.

Table 2. Extensibilities of sample cuticular loops from non-copulated (5-day fed virgin) or copulated (5 hr after copulation) female ticks under a 25 g load for 10 min. The extensibility is indicated by the rates of creep (mean ± S.D.).

| Cuticular loops from | Rate of creep (mm/min) |
|---|---|
| non-copulated females (n = 5) | 0.134 ± 0.010 |
| copulated females (n = 5) | 0.174 ± 0.018* |

n, number of analyzed loops.
*Significantly different from the rate in non-copulated ticks at $P < 0.05$ (Mann-Whitney U test).

*Morphological observations of the integuments*

Acidophilic epidermal cells were distinguishable in the epidermis at the end of the pre-copulatory cuticle synthesis phase (the 5th day in feeding) (Fig. 6). These cells surrounding the dermal gland had a cytoplasm which stained with eosin and a nucleus filled with chromatin (Fig. 6b). These cells and glands were only distributed in the stretchable soft integument of the alloscutum.

The acidophilic epidermal cells became larger in size after copulation, and reached a maximum size at the middle stage of post-copulatory rapid expansion phase (24 hr after copulation) (Fig. 7). The nucleus became larger in size and paler in color after copulation.

The cytoplasm of the acidophilic epidermal cells was filled with small mitochondria in both non-copulated and copulated females (Fig. 8). Smooth endoplasmic reticulum like the tubulovesicular system appeared in the apical cytoplasm 5 hr after copulation (Fig. 8 inset b).

These morphological changes occurring in the acidophilic

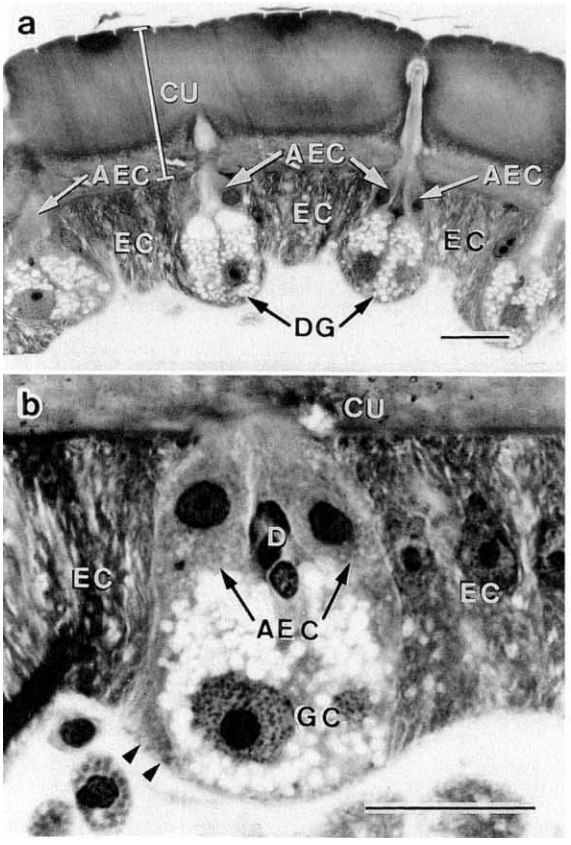


Fig. 6. Light micrographs showing integument of alloscutum in the 5-day fed non-copulated female *Haemaphysalis longicornis*. Scale bars indicate 30 μm. (**a**) A low power view. Beneath the cuticle (CU), epidermis consists of epidermal cells (EC) and dermal glands (DG). Note that acidophilic epidermal cells (AEC) having cytoplasm stained with eosin (lightly stained in the micrograph) are associated with the dermal gland. (**b**) A high power view. A dermal gland comprises glandular cells (GC) and a duct (D). Between the dermal gland and epidermal cells (EC), acidophilic epidermal cells (AEC) are situated. Basal part of the cell reaches the basement membrane of the epidermis (arrowheads).

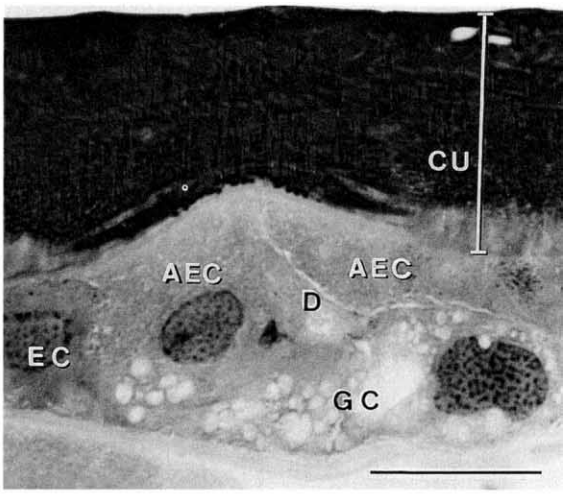


Fig. 7. A light micrograph showing integument of alloscutum in female *Haemaphysalis longicornis* 24 hr after copulation. Scale bar indicates 30 μm (the same magnification as Fig. 6b). Acidophilic epidermal cells (AEC) with a large nucleus become bigger. CU, cuticle; D, duct; EC, epidermal cell; GC, glandular cell.

epidermal cells are schematically summarized in Fig. 9.

## DISCUSSION

A precise measurement of cuticular pH is difficult. The present data of pH in minced cuticle are only rough estimations of exact cuticular pH, because the aqueous phase in cuticle is considerably diluted by distilled water. Other methods such as a direct measurement of cuticular pH using a $H^+$ specific microelectrode must be developed to measure the precise cuticular pH. However, the present data clearly shows the descent of cuticular pH after copulation.

In the bug, *Rhodnius prolixus*, abdominal cuticular pH descends below the isoelectric points of the cuticular proteins at the beginning of feeding, so non-covalent bondings between protein chains are replaced by bondings between proteins and water, finally the abdominal cuticle is plasticized because proteins are no longer bonded one to another (Reynolds, 1975b; Hackman and Goldberg, 1987). Cuticular proteins in the alloscutum from the adult female tick, *Boophilus microplus* are remarkably similar in biochemical properties, e.g. molecular weight and isoelectric points, to proteins in the abdominal cuticle of the bug, *R. prolixus* (Hackman, 1975; Hackman and Goldberg, 1987). Thus, these authors predicted a similar plasticization mechanisms is present in ticks. The cuticular plasticization caused by a drop in pH, shown in the present study, is the first experimental evidence for their prediction.

The cuticular pH decline after copulation presumed on the basis of the substitution of cuticular extensibilities into the regression equation between pH and extensibility was about 0.5 pH units (from pH 7.2 to 6.7). These declines of the cuticular pH seem to be in good agreement with the decline in the bug of an order of 0.5-1 pH units (Reynolds, 1975b).

Endocuticle of soft integument has abundant pore canals in ixodid ticks including *H. longicornis* (Hackman and Filshie, 1982; Okura *et al.*, 1997). These pore canals appear to contribute to diffusion of the pH changes within the endocuticle.

Novel acidophilic epidermal cells were firstly described in the present study. These cells were electron microscopically mitochondria-rich cells. The richness of mitochondria appears to give the cytoplasm an affinity for eosin in these cells as in oxyntic cells of mammalian gastric glands (Fawcett, 1986).

A kind of chloride cell, mitochondria-rich α cell in euryhaline fish (*Oreochromis niloticus*) gill has a well developed tubulovesicular system in the apical cytoplasm and high activity of ion transport, when the fish is reared in deionized water (Pisam *et al.*, 1995). In these cells, the tubulovesicular system participates in the increase of the surface area of the apical plasma membrane (Pisam *et al.*, 1995). These apical membranes of the cells contain $H^+$-ATPase (Brown *et al.*, 1987; Lin and Randall, 1991; Lacoste *et al.*, 1993) and give rise to $H^+$ extrusion and $Na^+$ uptake (Avella and Bornancin, 1989;

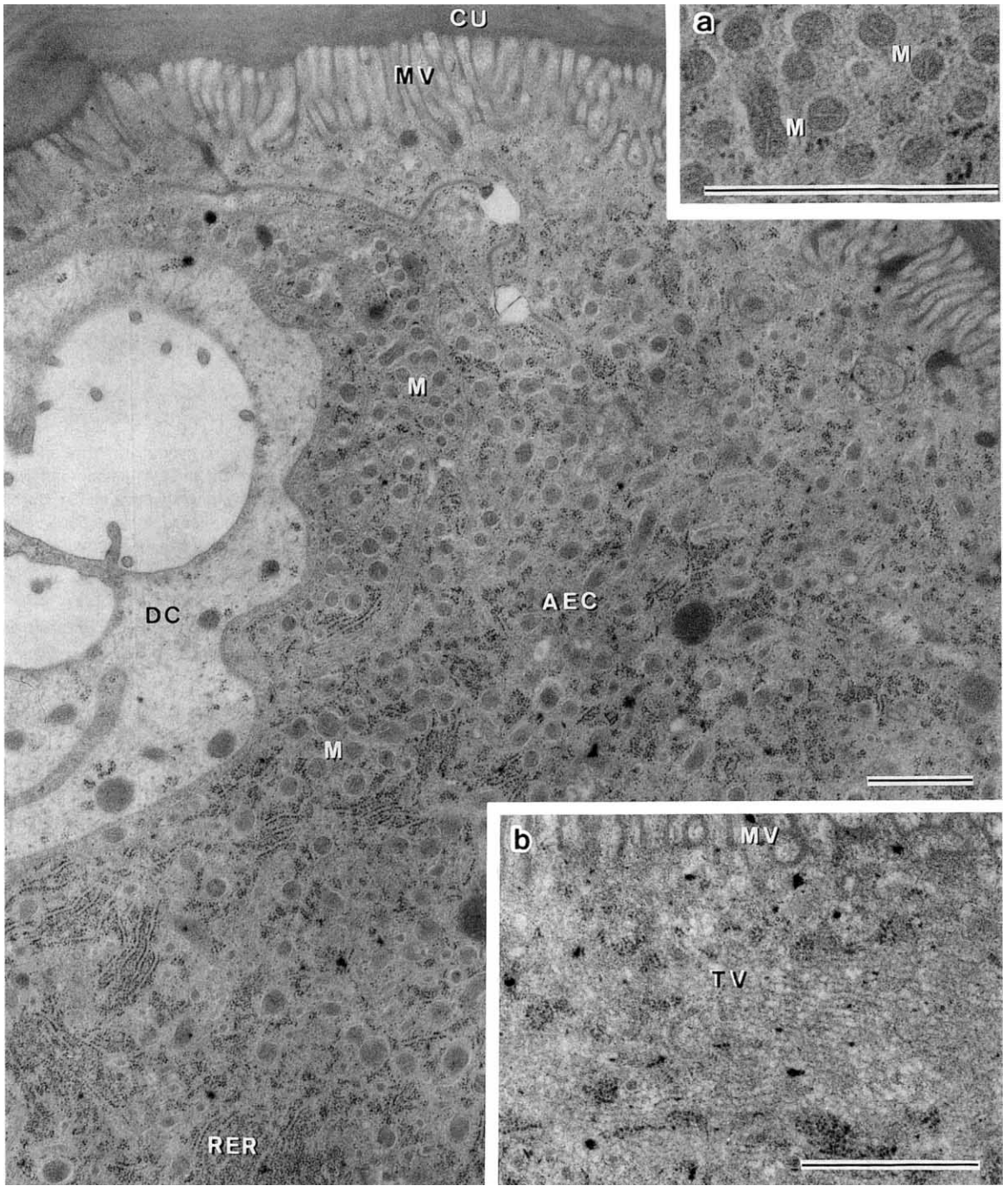


Fig. 8. Electron micrographs showing apical portion of the acidophilic epidermal cells. All scale bars indicate 1 μm. Acidophilic epidermal cells (AEC) in a 5-day fed virgin female, surrounding duct cells (DC) of the dermal gland, have apical microvilli (MV) which contact with the cuticle (CU). Cytoplasm of the AEC is filled with small mitochondria (M). Small mitochondria have typical cristae (inset **a**). Five hr after copulation, smooth surfaced endoplasmic reticulum like tubulovesicular system (TV) appear in the apical cytoplasm (inset **b**). RER, rough surfaced endoplasmic reticulum.

Ehrenfeld *et al.*, 1985; Lin and Randall, 1991). Morphological features of the acidophilic epidermal cells, the richness of mitochondria and the presence of tubulovesicular system, suggest that these cells have high activity in ion transport, presumably including $H^+$ extrusion from haemolymph into the cuticle, after copulation.

The cuticular plasticization occurring in the tick, *Haemaphysalis longicornis* was caused by the cuticular acidification, as in the bug, *R. prolixus.* On the other hand, cue stimuli for induction of the cuticular acidification are different in the tick and the bug; copulatory stimuli in the tick (Okura *et al.*, 1996), and bloodsucking stimuli in the bug

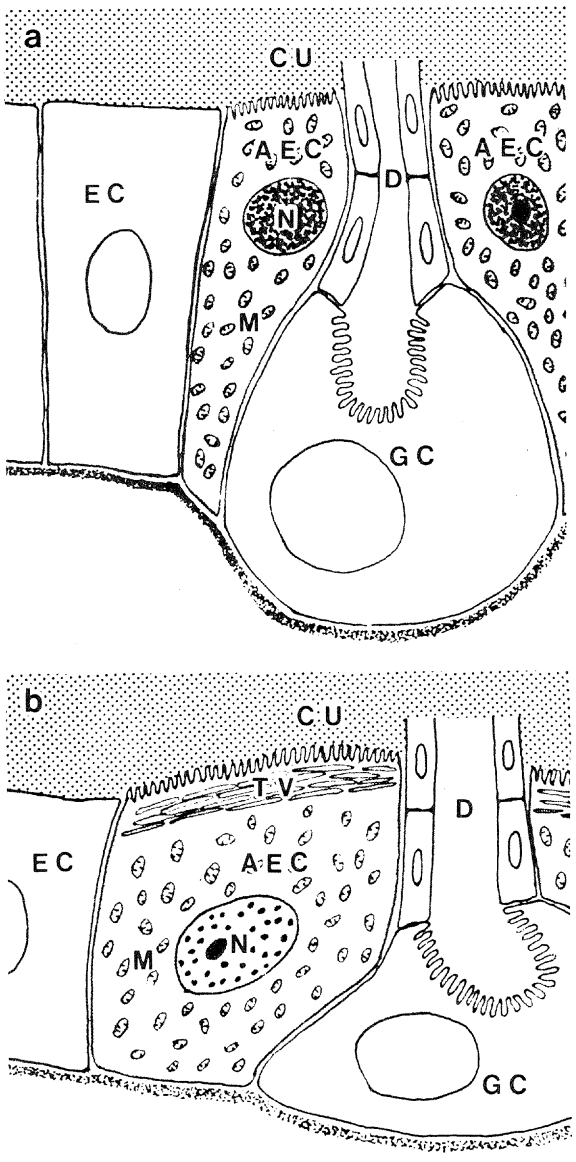


Fig. 9. Schematic diagrams showing morphological changes in the acidophilic epidermal cells (AEC). The AEC situates around the dermal gland composed of glandular cells (GC) and a duct (D). (**a**) The AEC, in the non-copulated (5-day fed) tick, has a small dense nucleus (N) and numerous mitochondria (M) in the cytoplasm. (**b**) The AEC, in the copulated (24 hr after copulation) tick, becomes larger in size. The nucleus (N) becomes thinner in staining density and smooth endoplasmic reticulum like tubulovesicular system (TV) appearing several hours after copulation is seen in the apical cytoplasm. CU, cuticle; EC, epidermal cell.

(Bennet-Clark, 1962; Maddrell, 1966). Therefore, it is possible that control mechanisms for the induction of cuticular plasticization are also different in the tick and the bug. Although the control mechanisms in the bug are considerably known (Maddrell, 1966; Reynolds, 1974, 1975b), almost nothing has been determined in ticks. These mechanisms in ticks must be elucidated in the future.

In conclusion, the present data in the tick, *H. longicornis* suggests that the acidophilic epidermal cells cause a decrease in the cuticular pH on the alloscutum and this cuticular acidification results in the cuticular plasticization.

## ACKNOWLEDGMENTS

We would like to thank the staff of Kuju Agricultural Research Center, Kyushu University for their kind help in collecting the ticks, and Professor N. Fujihara, Kyushu University for loan of the differential transformer and the pen recorder. We also thank Dr. D. Taylor, University of Tsukuba for critically reading the manuscript.

## REFERENCES

Avella M, Bornancin M (1989) A new analysis of ammonia and sodium transport through the gills of the fresh water rainbow trout (*Salmo gairdneri*). J Exp Biol 142: 155–175

Bennet-Clark HC (1962) Active control of the mechanical properties of insect endocuticle. J Insect Physiol 8: 627–633

Brown D, Gluck S, Hartwig J (1987) Structure of the novel membrane-coating material in proton-secreting epithelial cells and identification as an $H^+$ ATPase. J Cell Biol 105: 1637–1648

Ehrenfeld J, Garcia-Romeu F, Harvey BJ (1985) Electrogenic active proton pump in *Rana esculenata* skin and its role in sodium ion transport. J Physiol 359: 331–355

Fawcett DW (1986) A Textbook of Histology. (eleventh edition) Saunders, Philadelphia

Hackman RH (1975) Expanding abdominal cuticle in the bug *Rhodnius* and the tick *Boophilus*. J Insect Physiol 21: 1613–1623

Hackman RH, Filshie BK (1982) The tick cuticle. In "Physiology of Ticks" Ed by FD Obenchain and R Galun, Pergamon Press, Oxford, pp 1–42

Hackman RH, Goldberg M (1987) Comparative study of some expanding arthropod cuticles: the relation between composition, structure and function. J Insect Physiol 33: 39–50

Lacoste I, Brochiero E, Ehrenfeld J (1993) Control of $Na^+$ and $H^+$ transports by exocytosis: endocytosis phenomena in a tight epithelium. J Membrane Biol 134: 197–212

Lin H, Randall D (1991) Evidence for the presence of an electrogenic proton pump on the trout gill epithelium. J Exp Biol 161: 119–134

Maddrell SHP (1966) Nervous control of the mechanical properties of the abdominal wall at feeding in *Rhodnius*. J Exp Biol 44: 59–68

Okura N, Kitaura H, Mōri T, Shiraishi S (1996) Cuticular plasticization induced by copulatory stimuli in female *Haemaphysalis longicornis* (Acari: Ixodidae). J Med Entomol 33: 702–705

Okura N, Koga K, Mōri T, Shiraishi S (1997) Morphological changes in soft integument during feeding of adult female *Haemaphysalis longicornis* (Acari: Ixodidae). J Acarol Soc Japan 6 (1): in press

Pisam M, Le Moal C, Auperin B, Prunet P, Rambourg A (1995) Apical structures of "mitochondria-rich" $\alpha$ and $\beta$ cells in euryhaline fish gill: their behaviour in various living conditions. Anat Record 241: 13–24

Reynolds SE (1974) Pharmacological induction of plasticization in the abdominal cuticle of *Rhodnius*. J Exp Biol 61: 705–718

Reynolds SE (1975a) The mechanical properties of the abdominal cuticle of *Rhodnius* larvae. J Exp Biol 62: 69–80

Reynolds SE (1975b) The mechanism of plasticization of the abdominal cuticle in *Rhodnius*. J Exp Biol 62: 81–98

(Received November 11, 1996 / Accepted January 14, 1997)