

Article

Histopathologic studies on bite of a female American dog tick, *Dermacentor variabilis* (Acari: Ixodidae), in a man: Changes in skin

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Abstract

This study presents the histopathological changes of the skin indicating the hypostome with recurved denticles and the chelae of the chelicerae imbedded in the stratum corneum of the epidermis leading to cavitation in lower layers with extravasation of blood in the area of attachment by the tick mouthparts, shearing of skin tissue, liquifaction of cells, and other cellular changes in the area of the tick bite as indicated by several colored photos. No perivascular, lymphocytic, neutrophilic, eosinophilic, or granulomatous presence was seen in the skin indicating no evidence of acute or chronic inflammatory host reaction due to an early infestation of the host mostly localized to epidermis. No deep dermis or hypodermis was received attached with the tick and, thus, no tissue reaction was observed in these two deeper cutaneous layers. The study is of interest indicating removal of tick carefully from the host as soon as possible to avoid cellular-immunologic complex reaction and formation of granuloma or infection in the skin.

Key words: Tick bite, histopathologic changes, skin, USA.

Introduction

The American dog tick, also known as common wood tick, *Dermacentor variabilis* (Say, 1821) (Acari: Ixodidae), is widely distributed in North America and is especially common in the eastern and central United States (Terry & Williams 1980). It is usually found in woods and uncut grassy fields, parks and other areas of wild vegetation and is particularly numerous along paths used by animals (Williams 1977). Usually, it infests dogs but, occasionally, attaches to humans also. Its bite causes irritation and inflammation at the site of tick attachment. If the tick is not removed carefully from the host, the tissue may develop cellular-immunologic complex reactions leading to formation of granuloma or infection in the skin.

Several authors have reported histopathologic features of bites by the lone star tick, *Amblyomma americanum* Linnaeus, in humans (Fisher *et al.* 2006) and the three-host tick, *Hyalomma anatolicum anatolicum* (Koch), in rabbits (Gill & Walker 1985). Goldman *et al.* (1952) reported histopathologic studies on cutaneous reactions to the bites of various arthropods. Tavassoli *et al.* (2007) reported histopathologic changes from the bite of *Ornithodoros lahorensis* Neuman in a rat [species not specified] in Iran.

The second author (JWA) of this paper had indicated regular infestation of *D. variabilis* in men and women in West Virginia, USA, during spring and summer when animals were active after the winter. As no histopathologic studies were reported from human bites of *D. variabilis* in man in West Virginia, USA, and as the first author, other than being an acarologist, was also a pathology physician in Michigan, USA for years, had requested Dr. Amrine to send some ticks with attached human tissue for histopathologic study which he did. The results of that study are reported here. Presentation of histochemical changes using immunologic techniques were not done as this was beyond the scope of this preliminary study.

Materials & methods

The female of an adult tick of *D. variabilis* was noted to be present in left calf area of the leg of a friend of the second author (JWA), in Proctor, WV, USA in August 2009. The tick with attached tissue in 5% formalin was sent to the first author (VP) by the second author. Upon receipt, the first author made a few fine holes on the side of the idiosoma using a fine minuten needle to allow complete infiltration of the formalin for three days.

The tick with the tissue (Fig. 1), two weeks after collection, was taken to a pathology lab (Detroit Biomedical Laboratory, Farmington Hills, MI, USA) where it was processed as follows. The tick with attached tissue was removed from formalin, run through Tissue-Tek[®] VIP (Miles Scientific, now Sakura Finetek Inc., Torrance, CA, USA) automatic vacuum infiltration tissue dehydration processor overnight for better fixation and preparation. It was manually embedded in Paraffin wax, sectioned (ca. 2 μ m thickness) from dorsal to venter along the long axis of the body as instructed by the first author, including the capitulum, stained in Hematoxylin-Eosin stain, and mounted in Surgipath Acrytol (Surgipath Medical Industries, Inc., Richmond, IL, USA) mounting medium on 12, 3 x 1 inch glass slides for microscopic studies. The slides with the initial pathology report by the pathologist, Vincent Trent, M.D., were sent to the first author who studied the details, took photographs and prepared this report. The sections of the tick and skin tissue were examined using an American Optical trinocular microscope under 100-450x magnifications. Sections were photographed by the author at the same magnifications using a high definition Kodak EasyShare Z812 IS 8.1 mega pixels digital camera. The photos were enlarged and cropped for detail studies using PHOTOSHOP CS2. Thus, no scale for the photos is given. The female tick was identified by the second author.

Results

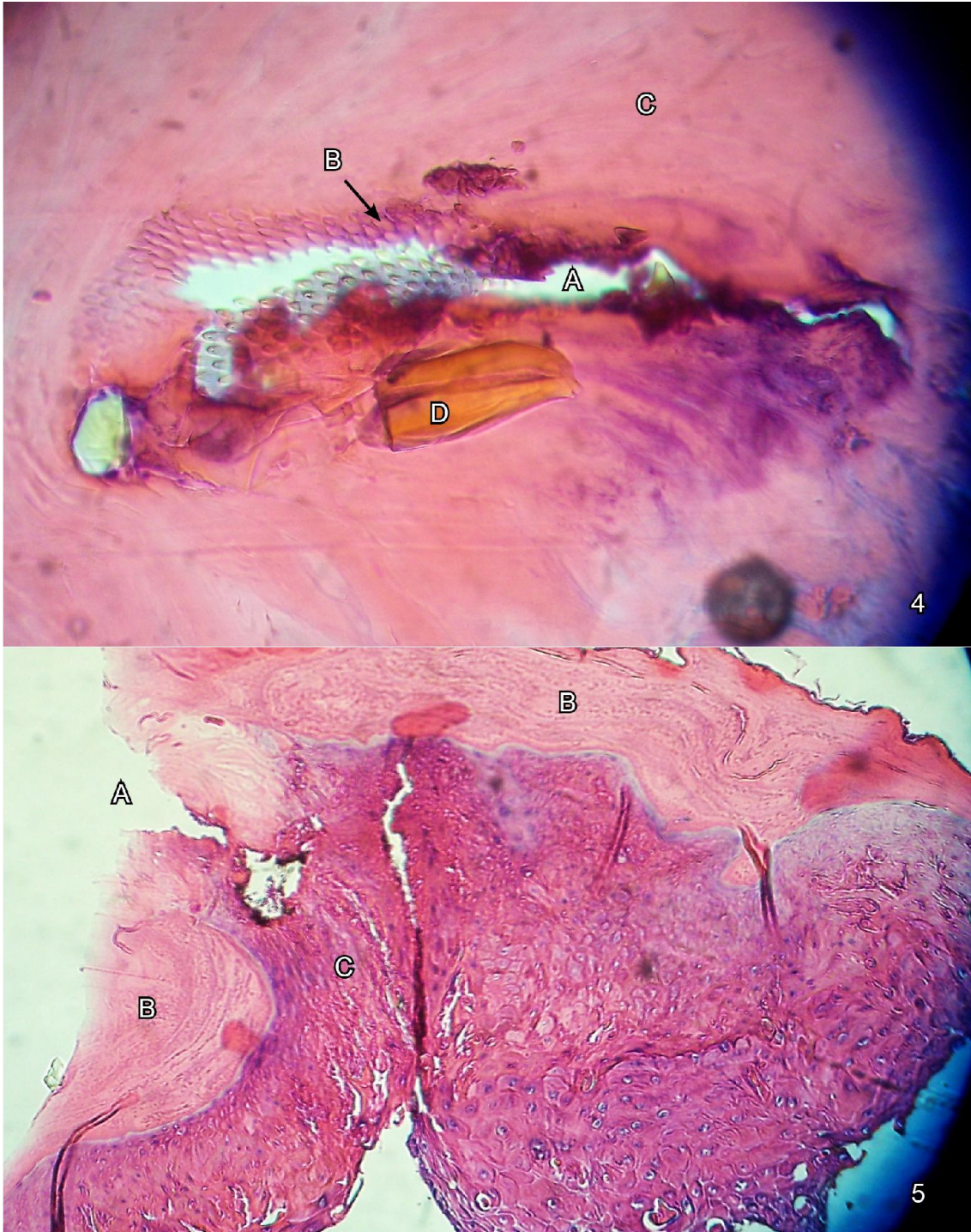
Attachment of tick with the host tissue (Fig. 1) - Figure 1 shows ventral part of the tick (A) and skin tissue (B) attached with the mouthparts of the tick before sectioning for histopathologic studies. Two lightly stained or hypokeratotic areas (D, E) just anterior and posterior to a deeply stained narrow band (C) are seen in the tissue. These are discussed later indicating holding of the tissue by the mouthparts and release of salivary enzymes in this area. First pair of legs I (F) located outside the skin tissue are also evident. Idiosoma of the female tick appears to be enlarged due to the blood meal (seen in several sections, unpublished).

Penetration of tick mouthparts within the host tissue (Figs. 1-4) - Section of attached tissue (Fig. 1B) shows mouthparts imbedded (Fig. 4B) and a cavity formed in

the tissue (Figs. 3K, 4A). Figure 2 shows ventral part of posterior hypostome with mentum (part of basis capituli) (B) and anterior hypostome (C) with posteriorly curved teeth (A).



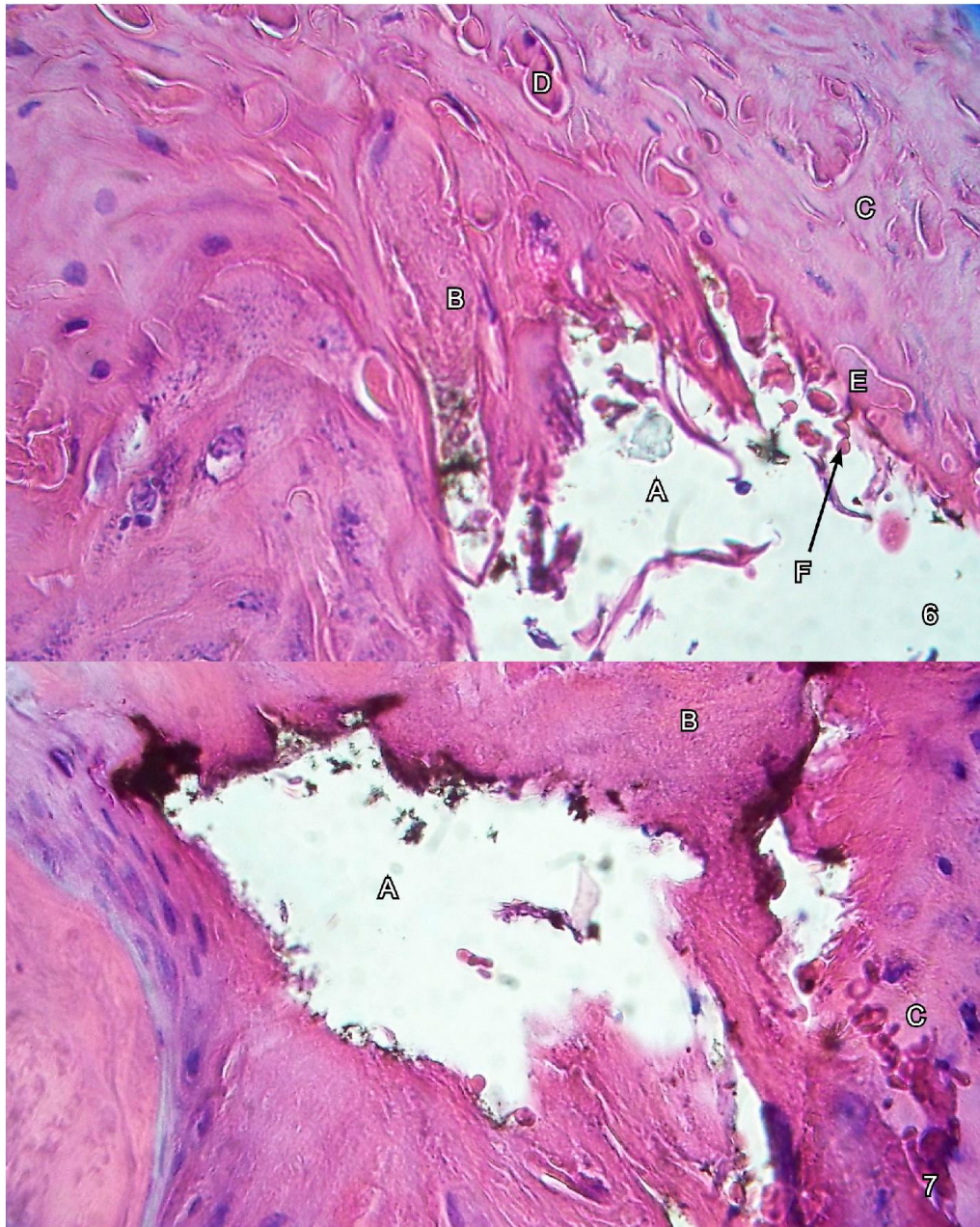
Figures 1–3. 1. Ventral and anterior part of the adult female tick (A), *Dermacentor variabilis*, with attached skin tissue (B) showing lightly stained hypokeratotic change (D, E) near a deeply stained tissue (C) after removal from formalin and before sectioning for histopathologic study. Pair of legs I (F) are evident just below the attached tissue (legs II-IV and female genital opening are also seen). Tick idiosoma is partly enlarged due to blood meal; 2. Ventral view of hypostome of tick with mentum (B) and anterior hypostome (C) with posteriorly curved teeth (A) (hypostome broken and folded over tip projecting posteriorly). Each recurved tooth appears to arise from a roughly rectangular “basal segment”; 3. Longitudinal section of anterior part of tick showing part of idiosoma with festoons (A), scutum (B), gnathosoma (C), basis capitulum (D), mentum (E), and hypostome with recurved tooth (F). Longitudinal section of dislodged skin tissue (G) showing epidermis with stratum corneum (H), stratum germinativum and stratum granulosum (I), and hypokeratotic stratum corneum (J) with cavity (K) that extends through to the cavity in the lower cellular layers of epidermis (L).



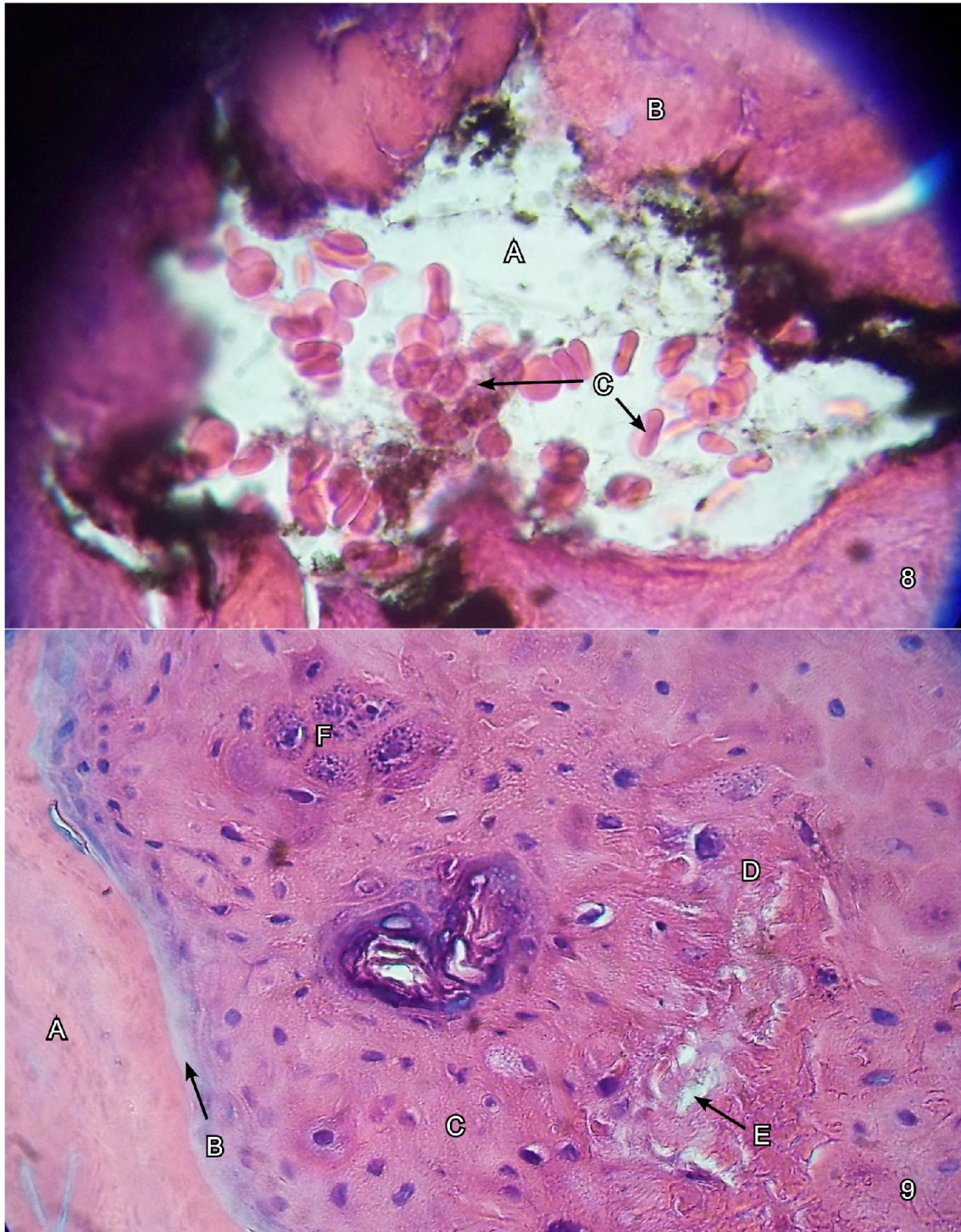
Figures 4–5. 4. Section of skin showing cavitation (A) where tick hypostome with recurved teeth was imbedded - microdentation of shafts of chelicera (B) in the stratum corneum (C). Parts of broken apical chelae (D) are also evident; 5. Deep section of skin showing cavitation (A) going deep in epidermis passing through hypokeratotic and degenerative stratum corneum (B) and stratum germinativum and stratum granulosum showing cellular changes (C).

It is evident that each tooth arises from a roughly rectangular basal segment. In Fig. 3, anterior part of tick located on left is seen in midlateral view (with gnathosoma) and

dislodged (during sectioning) host tissue on right, near the tick mouthparts. Section of the tick shows idiosoma with festoons (A), scutum (B), gnathosoma (C), basis capitulum (base of gnathosoma) (D), mentum (E), and hypostome with recurved teeth (F). The dislodged host tissue (G) shows epidermis with non-cellular stratum corneum (H), cellular layers stratum germinativum and stratum granulosum (I), hypokeratotic areas of stratum corneum (J), and cavity (K) formed in the stratum corneum which extends to lower cellular layers of epidermis (L). Dermis and hypodermis are not present.



Figures 6–7. 6. Section of skin with cavitation (A) in lower epidermis (C) where tick was attached for blood meal showing shearing, fragmentation, and liquification of tissue (B). Sections of several intact blood vessels (D), broken blood vessels (E) around the cavity, and released red blood cells (F) in the cavity are seen; 7. Section of skin showing severe liquification of stratum corneum (B) around cavitation (A). Extravasation of erythrocytes in the tissue can be seen (C).



Figures 8–9. 8. Section of skin showing numerous red blood cells (C) in lumen of the cavity (A). Severe liquifaction and degradation of stratum corneum (B) around cavitation is also seen; 9. Section of skin showing section of hypokeratinized non-nucleated stratum corneum (A) and nucleated cellular layers of stratum germinativum (B) and stratum granulosum (C). Glossy appeared degenerating cells (D) around lumen of the cavity (E) and cells with hyperactive nuclear and cytoplasmic changes are also evident (F).

Cavitation of host tissue near the tick mouthparts (Figs. 3–8) - Figure 3K shows

cavitation in upper layer and going down in lower layer of the epidermis. Figure 4 of the deep section shows tissue with cavity (A) and hypostome with recurved teeth (B) imbedded in the stratum corneum (c). Parts of chela of chelicera (D) are also evident.

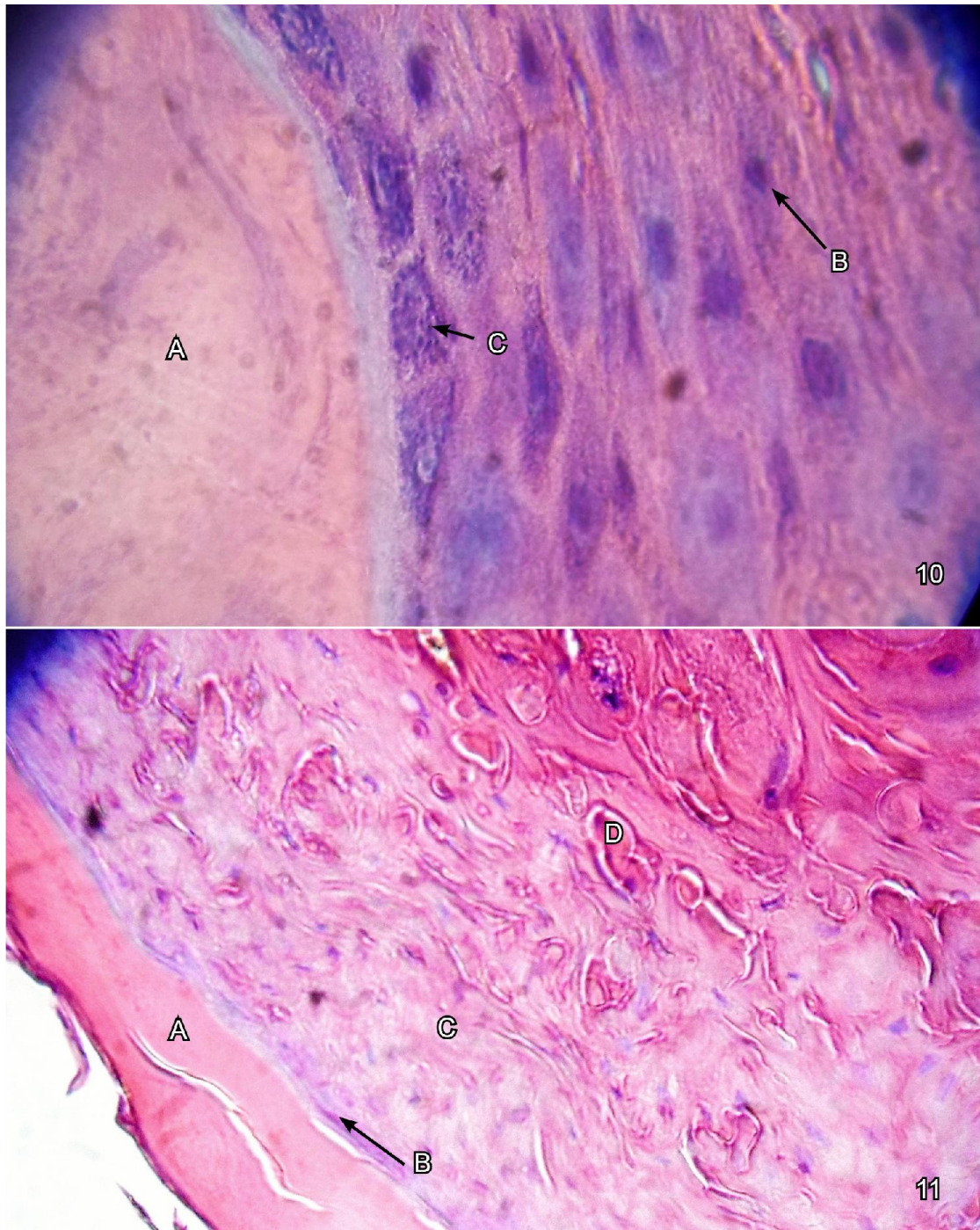
Deep section of tissue in Fig. 5 shows cavitation (A) going deep in the epidermis passing through stratum corneum (B), stratum germinativum and stratum granulosum (C). Hypokeratotic and degenerative stratum corneum is clearly evident around the cavity. Cellular changes in the epidermal cells are also evident (C). It should be noted that the tissue that was attached to the tick was irregular, and mouthparts were imbedded inside the tissue, there was no way to know where exactly to section the tissue to contain the mouthparts. Thus, the series of sections presented may not show continuous cavitation starting from stratum corneum and entering to lower layers of the tissue. No inflammatory cells are present. However, hypokeratotic area is present where enzymes from salivary glands may have been released by tick to soften the host tissue for penetration of mouthparts in it.

Shearing and liquifaction of host tissue near the tick mouthparts (Fig. 6) - From Fig. 6, it is evident that there is shearing of tissue and liquifaction of cells (B) around the cavity (A). Several, oval to round sections of intact blood vessels (C, D) in lower epidermis without release of blood in nearby tissue are seen on top and to right. But, sections of blood vessels around cavity (A) show some broken blood vessels (E) and released red blood cells (F).

Extravasation of blood in the host tissue (Figs. 7–9) - Section in Fig. 7 shows severe liquifaction of the cells (B) near the cavity (A). On right lower side of this section, red blood cells (C) are seen in the tissue. Figure 8 shows numerous red blood cells (C) in lumen of the cavity. Severely degraded keratin (B) around the cavity is also evident. Another section (Fig. 9) shows stratum corneum (A), stratum germinativum (B), and stratum granulosum (C), and gloss-like cellular appearance of the cells (D) around the cavity (E). Hyperactive nuclear and cytoplasmic changes in the cells are also evident (F).

Host tissue reaction after penetration of mouthparts by the tick (Figs. 4–11) - It is evident from Figs. 4–11 that there was no acute or chronic infiltration of lymphocytes, neutrophils, eosinophils, formation of granulomas, or vasculitis from bite of the tick in the present study. However, there was hyperactive nuclear and cytoplasmic changes in the cells as evident in Fig. 10 (C), right of stratum corneum (A), as can be compared with normal cells (B). Various other changes in the tissue have been mentioned before. Figure 11 shows sections of many blood vessels (D), right of stratum corneum (A), stratum germinativum (B), and stratum granulosum (C). Even here, no inflammatory changes or vasculitis are evident.

As indicated above, in all of the histologic sections presented in this paper, none indicated acute or chronic inflammatory changes having lymphocytes, neutrophils, eosinophils, or granulomas. The pathology report indicated: "Sections of keratotic skin with small parts of arthropod, epidermal changes with reactive acanthosis, intercellular edema, parakeratosis, some necrotic keratinocytes, cavitary space, extravasation of red blood cells, and without significant epidermal inflammatory reaction" due to reactive changes of the tick bite. Thus, this tick infestation of the human host is considered an early infestation, possibly of less than 48 hours duration, by the authors. Additional studies of known duration of infestation may be essential to understand the complete pathology of the human host tissue from the bite of *D. variabilis*.



Figures 10–11. 10. Section of skin showing hypokeratinized stratum corneum (A), normal squamous cells (B), and cells with hyperactive nuclear and cytoplasmic changes (C); 11. Section of skin showing stratum corneum (A), stratum germinativum (B), stratum granulosum (C), and sections of many blood vessels without showing vasculitis or any acute or chronic inflammatory cells (Lymphocytes, neutrophils, eosinophils, or granulomas). No hemorrhage in this part of tissue is evident.

Discussion

The sequence from anchoring of tick to suitable host for its feeding, growth and survival leading to formation of clinical dermatologic lesions in the host, is a complex

phenomenon and beyond the scope of this paper. In general, it may depend on stage of species of tick, type of host, season of infestation, duration of feeding, size of mouthparts, type of tick secretions, changes in secretion during feeding, previous exposure to the tick, and allergic reactions of the host (Krinsky 1983). However, the main histopathologic changes in the skin include: (1) cutaneous necrosis, (2) vasculitis, and (3) inflammatory cellular reactions.

Anchoring of tick mouth parts in skin of host - After a site for attachment to host and feeding has been selected, the tick begins to lacerate the epidermal layers of the skin using horizontal action of its chelicerae with the sharp chelae. These are used for cutting, ripping and tearing of the skin. At this time, the hypostome with recurved teeth plunges inside the skin and anchoring of the tick with the host tissue takes place. Once mouthparts anchor in the host tissue, interaction between the host's tissue and the antihemostatic, anti-inflammatory, and immunomodulatory chemical modulators contained in the saliva are released by the tick. While tick is firmly attached, blood flows into the wound site, the tick starts feeding, and the buccal canal becomes a common duct for the intake of host tissue fluids while releasing the saliva (Kemp *et al.* 1982).

In the present study, cavitation in the host tissue was present where tick was attached. Shearing and irregular fragmentation of tissue were also present. Broken cheliceral chelae imbedded in the cuticle along with hypostome and recurved teeth in the feeding cavity were evident also.

Cement cone - Castelli *et al.* (2008) reported presence of a cement cone anchoring the mouthparts to the skin. No such cement cone was found in the present study.

Extravasation of blood cells - Castelli *et al.* (2008) reported presence of some severed blood vessels in the dermis allowing copious blood extravasation. The cavitation in the skin at the bite site of the present study revealed extravasation of fresh, unhemolyzed, blood response. Ticks are known to release specific proteins from salivary gland inhibiting hemostasis in host skin for easy transport of blood meal in the pharynx and stomach (Brossard & Wikel 2004).

Cuticular changes - Castelli *et al.* (2008) reported presence of loose multilayered endothelial proliferation, with plump endothelia, permeated by erythrocytes. In some cases, they reported less characteristic cellular changes even though fragments of mouthparts and vascular changes were present. In the present study, degeneration and necrosis of keratin around the bite site was seen. It is possible that the tick secreted some chemical from its salivary gland to soften the cuticle for easy penetration of chelicerae and the hypostome in the less hypokeratotic area.

Dermal changes - Castelli *et al.* (2008) reported spongiform appearance of the superficial dermis. These changes in epidermis and upper dermis are seen in the present study but no deep dermis or hypodermis are present in the slides studied. Thus, host response of dermal or hypodermal tissue could not be evaluated in the present study.

Cellular infiltrates - Amosova (1994) reported histopathologic changes at the site of attachment to the host skin by larvae of ixodid tick, *Ixodes ricinus* (Linnaeus, 1758), showing intensive neutrophil migration and presence of erythrocytes and active fibroblasts in the feeding site. Tavassoli *et al.* (2007) reported edema and hyperemia of capillaries in their experimental rats when adult tick, *O. lahorensis* Neuman, were fed for two days. They found vascular damages with endothelial swelling and fibrinoid necrosis. Perivasculitis was present in some vessels with infiltration of lymphocytes and eosinophils. In addition, foci of necrosis in the epithelium with remnant of infiltration of polymorphonuclear cells with severe hemorrhages were also evident. On the other hand,

mostly lymphocytes were found in the early infestation but when ticks had fed for two weeks on the rat, severe cellular infiltration, loosely arranged newly formed fibrous tissue and active inflammatory process with predominant lymphocytes and macrophages were present. They also found eosinophils along with numerous lymphocytes. Castelli *et al.* (2008) reported presence of mild neutrophilic and eosinophilic infiltration of skin in local reactions to tick bites in human beings.

In the present study, significant cellular changes causing perivasculitis, lymphocytic, and polymorphonuclear infiltration were absent. The absence of these changes is considered due to short duration of attachment of skin (less than 48 hours) by the tick giving no consequential or delayed cellular histopathologic response by the host.

Immunologic changes - No such study was done in the present study and considered beyond the scope of this paper. However, various immunologic response studies about tick bites are reported (Alarcon-Chaldez *et al.* 2006).

Granuloma formation & other symptoms in host - Castelli *et al.* (2008) reported presence of erythema chronicum migrans-like lesions, foreign body granulomas – sometimes containing remnants of the mouthparts – with cutaneous lymphoid hyperplasia, either of the T-cell or the B-cell type, and tick bite alopecia in the host. Some excellent histopathologic photos of tick bites with granulomatous and heavy cellular reactions are reported (www.som.tulane.edu/classware/pathology/Z_medical_pathology/inflamdermato/S12). However, the present study, in spite of presence of hypostome and broken chelicerae in the skin, did not indicate these changes indicating short duration of tick attachment to the host, possibly 24–48 hours, as mentioned before.

Conclusions

The sequence from anchoring of tick to a suitable host for its feeding, growth and survival leading to formation of clinical dermatologic lesions in the host, is a complex phenomenon. These and various immunologic studies, lead to identification of the tick antigens, development of suitable vaccines, and prevention of infestation or infection by early removal of tick from the host. The American dog tick, *D. variabilis*, is widely distributed in North America biting mostly dogs but, occasionally, human as well. It causes inflammatory dermal changes at the attachment site including formation of a nodule or granuloma at the bite site if the tick and its mouthparts are not removed from the host as soon as possible.

The present study of human tissue indicated hyper reactive changes in the skin at the bite site, liquifaction or necrosis of the tissue, and extravasation of blood. It did not show acute or chronic inflammatory host response indicating short duration (less than 48 hours, confirmed by Dr. Kenneth Rastall, see acknowledgement) of infestation of tick on the host. The study is of interest indicating removal of tick carefully from the host as soon as possible (within 24–48 hours) to avoid cellular-immunologic complex reaction and formation of granuloma or infection in the skin.

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مطالعات بافت آسیب‌شناسی گزش کنه ماده سگ امریکایی، *Dermacentor variabilis* (Acari: Ixodidae) در انسان: تغییرات در پوست

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چکیده

مطالعه حاضر تغییرات بافت آسیب‌شناسی پوست را با هیپوستوم دارای دندان‌های برگشته و انگشت متحرک کلیسرها که در کورنئوم بافت اپیدرم نشان می‌دهد که منجر به ایجاد حفره در لایه‌های پایینی همراه با نشت خون در ناحیه اتصال قطعات دهانی کنه، شکافتن بافت پوست، آب آوردن سلول، و دیگر تغییرات سلولی در ناحیه گزش کنه می‌شود همچنان‌که با چندین عکس رنگی نشان داده شده است. هیچ اثری پیرامون رگی، لمفوسیتی، نوتروفیلی، ائوزینوفیلی یا گرانولوماتوزی در پوست دیده نشد که نشان‌دهنده واکنش التهابی حاد یا مزمن میزبان به واسطه آلودگی اولیه میزبان تمرکز یافته در اپیدرم باشد. پوست رویی یا زیری عمیقی چسبیده به کنه جدا نشد و بنابراین واکنش بافتی در این دو لایه عمیق‌تر پوست دیده نشد. این مطالعه از این نظر جالب است که جداسازی دقیق و هر چه سریع‌تر کنه برای پرهیز از واکنش ترکیبی سلولی-ایمنی و تشکیل گرانولوما یا عفونت پوست را نشان می‌دهد.

واژگان کلیدی: گزش کنه، تغییرات بافت آسیب‌شناسی، پوست، ایالات متحده امریکا.

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