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Histopathology of Lesion Produced by *Haemaphysalis longicornis* Neumann (Acarina:Ixodidae) with Reference to Acquired Immunity

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Introduction

The tick *Haemaphysalis longicornis* Neumann, 1901, representing an ecto-parasite of highly specialized bloodsucking arthropods has long been regarded as the proven vector of zoonoses or human diseases including tick-borne encephalitis and several types of tick fever caused by viruses or rickettsiae (Hoogstraal 1966)[7], Hoogstraal et al 1968[8], and L'vov 1978)[15]. It has also became recognized as the vector of important animal diseases, such as *Theileria sergenti* infection (Nikol'sky and Meshcheryakova 1964)[17] and *Babesia ovata* (sp. n.) infection (Minami and Ishihara 1980)[16], which cause immense economic loss in livestock industry in some countries including Korea.

An extensive review on the classiffication, identification, geographical distribution, epidemiological/epizootiological vector role, biological/genetical significance and population dynamics of the tick has already been made by the author (Kang 1980).[10,12] Meanwhile, the author (Kang 1980)[11] has also presented an intensive manuscript on the principal biological properties including an observation of the life cycle under controlled conditions with particular reference to the laboratory colonization for mass production and on the water balance mechanism in relation to the water vapor exchange kinetics in the tick.

Although it was repeatedly experienced in the colonization procedure that rabbits acquire immunity against the ticks after one infestation with them, no successful detailed work on the host-tick relation, i.e. an acquired immunity in the host has yet been reported except that by Dicker and Sutherst (1981)[5] on the control of the tick with Zebu cross Europan cattle.

Therefore, a series of experiments was designed and carried out to assess the acquired immunity against the tick *H. longicornis* in the experimental host rabbit. The ecological findings on the variation of feeding performance of the tick have been reported separately (Kang 1981),[13] and the histopathological findings on the lesion produced by the tick bites are described here in this report.

Materials and Methods

New techniques for the colonization of the tick *H. longicornis* in the laboratory using rabbit and temperature-humidity controlling chambers were developed by the author (Kang 1980).[10]

These enabled a quantitative experimental model of the tick feeding on the experimental host rabbit to be studied. The strain of the tick used in these experiments was the progeny of the bisexual diploid *H. longicornis* females originally collected from cattle in Weongseon District, Gangweon-do Province located in the central eastern part of the Korean Peninsula and colonized in the laboratory, the Zoology Department, the University of Edinburgh by means of techniques described earlier.

The rabbits used in the tick feeding were of the conventional breed, and were supplied by the Centre for Laboratory Animals, Bush Estate and reared at he Animal Rooms of the Zoology Department, in separate standard cages and fed on a standard diet (SG1, Oxoid) provided by the University. They were all males aged four to seven months and were inexperienced to any tick infestation.

A batch of unfed adult females with same number of males was applied to the ears of the rabbit previously uninfested with tick in the first instance. Then the following batch of unfed adult ticks exactly same strain to the tick used in the primary feeding, was applied again to the ears of the rabbit previously infested with the tick. The interval between the primary and the secondary infestations was one month.

In each feeding trial, the primary and the secondary infestations, the samples of a small portion of ear cut off were taken on the days of 2nd, 7th and 14th after the application of the ticks, respectively. The fresh materials including the skin and the skeletal muscle were fixed in 10% formalin-ethanol and then embedded in paraffin wax in the usual manner. The paraffin blocks were cut at 7 μm to expose the tissue and the sections were then stained either in Giemsa's stain or in hematoxyline-eosin. The specimens were examined macroscopically and microscopically using dissecting microscope and light microscope, respectively.

Results

The histopathological alterations in the skin infested by the ticks varied with the length of time after the tick had been infested and the degree of the acquired immunity in the host. In general, the lesion caused by tick bites showed a wound in the skin where the epithelium is broken and an acute or chronic inflammatory reaction.

Shortly after the bite, the signs of hemorrhagic and edematous inflammation appeared in the area just beneath the mouthpart of the tick (Fig. 1). It was noted that an infiltration of leukocytes, primarily neutrophils, was present soon but cell destruction was not present in the area (Figs. 1 and 2).

As the condition progress, necrosis occurred in the area, and the hemorrhagic edematous inflammation extended into underlying subcutaneous tissue showing the formation of cement material around the wound, the lysis of cartilages and the infiltration of eosinophils (Figs. 3 and 4).

Healing did not take place in a short period of time as the mouthpart remains in the wound. Allergic dermatitis was noticed at the site of tick feeding in those animals that had been sensitized previousely to the tick. The focal reaction in the skin where the ticks were reapplied was a serous, hydropic, suppurative, or necrotic inflammations (Fig. 5).

As the condition progress, necrotic inflammation became more extended and the eosinophils became more numerous than they did in the primary tick infestation (Fig. 6).

When the tick had fallen off from the host, even though not fully engorged, the area became surrounded with leukocytes and connective tissue and an ulcerative inflammtion developed (Fig. 7).

Discussion

All arthropod bites have the similar basic lesion which consists of a wound in the skin and an inflammatory reaction. Extensive edema may appear in the area shortly after the ectoparasite bite, particularly if a toxin or proteolytic substance is injected into the host skin. Leukocytes appear in the area and in sensitized animals particularly eosinophils appear more abundantly.

Hooker et al (1912)[9] reported that the bites of adult ticks generally produce edematous inflammatory lesions and Wolbach (1919)[27] described that the histology of the adult tick bite. Later, it was shown by Trager (1939)[21,22] that one infestation of guinea pigs with larvae of the tick *Dermacentor variabilis* induced an acquired immunity which was solid enough to prevent larvae

from engorging and to reduce the amount of blood taken by nymphs and adults. It was confirmed by Allen (1973)[1] in the experiments on *D. andersoni* larvae. He detected large numbers of basophils in skin reactions of resistant guinea pigs.

Bagnall (1978)[4] demonstrated a similar phenomenon of cutaneous immunity to the tick *Ixodes holocyclus* in the experimental host guinea pig, however, he could find no basophil response in the rabbit and man although an acquired immunity to the tick was similarly demonstrated in the hosts. Skin-sensitizing antibody was detected by passive cutaneous anaphylaxis from the serum of guinea pigs exposed to a total of more than four infestations of larvae and the main activity was found in a heat-stable IgG-1 fraction. Precipiting antibody was also detected in the serum from multiple infested animals.

It has long been assumed that the lesions of tick bites result from extra-oral digestion through the action of cytolysins in ticks' salivary secretions. However, Tatchell and Moorhouse (1968[19] and 1969[20]) have suggested that on the base of a study of the development of the lesion caused by the tick *Boophilus microplus* specific vascular damage results from the saliva of the tick while tissue damage is caused by the host response and that much of the tissue destruction associated with tick feeding could be of host origin. On the other hand, Francis and Little (1964)[6] have already reported the resistance of droughmaster cattle to tick infestation and babesiosi and it was of host origin. More recently, Kemp (1978)[14] has suggested that part of the process of *B. microplus* salivation was the forcible ejection of saliva into the host by the action of the prepharyngeal valve.

Arthur (1970)[3] has described the histopathology of the lesion produced by the tick *Ixodes ricinus* and hypothesized that collagen could be polymerized as well as merely aggregating around the trophi of the tick. Later, Whitwell (1978)[26] has presented a histopathological findings on the bite of *Ixodes trianguliceps* and reported that the tick does not secret cement but stimulates host collagn to coagulate densely and in an organized fashion around its trophi.

Allen and Wikel (1978)[2] have suggested that tick resistance in guinea pigs is an immunological phenomenon which is acquired and demonstrable on challenge infestation of a different skin site one week following the primary infestation and that occurrence of high concentrations of basophil granulocytes at the reaction site in the skin of resistant animals might imply that something akin to cutaneous basophil hypersensitivity is at play. Wikel and Allen (1976[23,24] and 1978[25]) have also mentioned that guinea pig resistance to *Dermacentor andersoni* larvae is mediated by a strong cell-mediated component and a humoral component which involves the utilization of complement or complement-derived products for its expression.

Saito et al (1960)[18] have tried to make a series of observations on the changes of the host tissue caused by the infestation of several species of female ticks comprising *Ixodes persulcatus*, *I. japonensis*, *Haemaphysalis flava* and *H. dönitzi*, and reported that the main changes were characterized by producing hemorrhage, leukocyte infiltration, abscess formation and necrosis; and that the changes by *I. persulcatus* were most remarkable while those by *H. flava* were slightest compaired with those by all other ticks. However, they did not try to make a view in relation to acquired immunity in the host to the tick infestation.

Kang (1981)[13] has presented the ecological findings on the variation of feeding performance of *H. longicornis* females with particular reference to acquired immunity in the host and concluded that the feeding performance of the ticks parasitized on the host previously infested with the ticks was remarkably reduced and that it was not true economy to use a rabbit more than twice for the colonization of the tick as some immunological response may occur.

Conclusions and Summary

In order to observe the histopathological changes on the bite lesion produced by the tick *Haemaphysalis longicornis* Neumann, 1901, with parti-

cular reference to the acquired immunity in the host, a series of experiments was designed and carried out. The findings observed are summarized as follows:

1. *H. longicornis* produced the bite wounds penetrating the epidermis producing a flat cone of cemet in the vicinity of the mouthparts which spreads superficially and invades the lesion extent.

2. The major histopathological changes of the lesion produced by the tick were characterized by a hemorrhagic and edematous inflammatory reaction, leukocyte infiltration and some necrotic signs.

3. The neutrophils appeared more numerous in the lesion taken at earlier stage of tick infestation and from the rabbit previousely not sensitized by the tick, whereas, the eosinophils appeared more numerous in the lesion at the later stage of tick infestation or from the rabbit previousely sensitized by the tick.

4. As the mouthparts of the tick remain in the wound, healing did not take place in a short period of time. Necrotic inflammatory reaction became more extended as the condition progress and especially in the sensitized host.

5. Ticks fed for seven to thirteen days both in the primary and the secondary feeding trials, however, the mean feeding period in the secondary feeding trials was somewhat delayed as compared with that in the primary feeding trials.

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Legends for Figures

Fig. 1. Section through the bite of *H. longicornis* adult female on the ear of rabbit previousely uninfested with ticks. (On the 2nd day, 40X, Giemsa).
The arrow indicates that the epithelium is lacking at the point of attachment. There are some signs of hemorrhagic inflammation(H), edema(Oed) and leukocyte infiltration(N; neutrophils).

Fig. 2. Section through the bite on the 7th day after the tick infestation in the primary feeding trial. (40X, Giemsa).
The signs are similar to those in Fig. 1., but the histopathological reactions are somewhat developed.

Fig. 3. Section through the bite on the 14th day after the tick infestation in the primary feeding trial. (100X, Giemsa).
Cement materials(Cm) around the mouthpart, broken keratin layer(K), epithelium(Ep), lyzed cartilages(C), edema(Oed) and leukocyte infiltration(E; eosinophils) are shown.

Fig. 4. Section through the bite on the 14th day after the tick infestation in the primary feeding trial. (430X, Hematoxyline-eosin).
Extensive edema(Oed), leukocyte replacement from neutrophil infiltration(N) to eosinophil infiltration(E), necrotic inflammation(Nc) are noticed.

Fig. 5. Section through the bite of *H. longicornis* adult female on the ear of rabbit previousely infested with ticks. (On the 2nd day after the tick infestation in the secondary feeding trial, 40X, Hematoxyline-eosin).

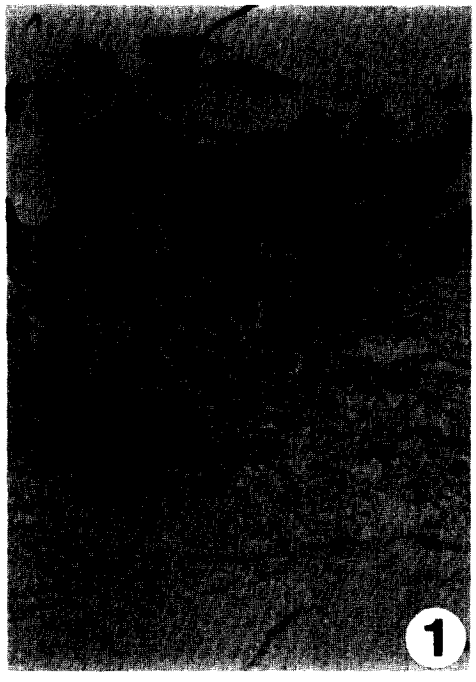
It is characteristic in the presence of the thickened epithelium(Ep) but broken around the mouthparts, keratin layer(K), necrosis(Nc), leukocyte infiltration(E; eosinophils), cement materials(Cm) and dilated capillaries(C).

Fig. 6. Section through the bite on the 7th day after the tick infestation in the secondary feeding trial. (100X, Hematoxyline-eosin).

The signs are similar to those in Fig. 5., but the histopathological reactions are much developed.

Fig. 7. Section through the bite on the 14th day after the tick infestation in the secondary feeding trials. (100X, Hematoxyline-eosin).

The signs of cement formation(Cm), keratin layer(K) thickening, epithelium(Ep) regeneration, abscess(Ab) formation, leukocyte infiltration(E; eosinophils), capillary(C) dilatation and cartilages(C) are noticed, however, healing has already been taking place.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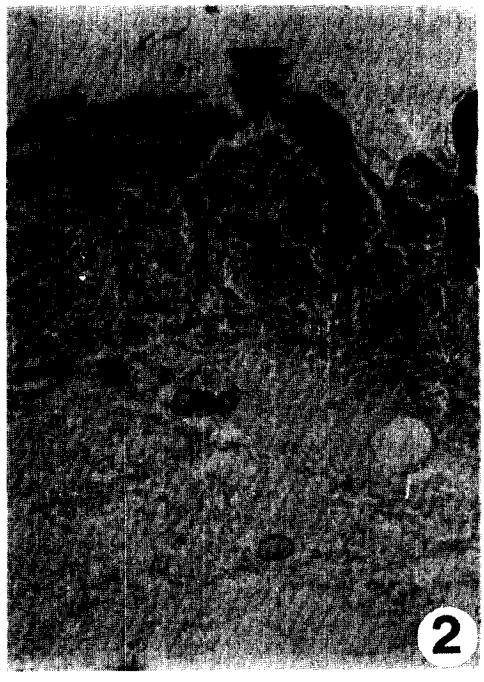
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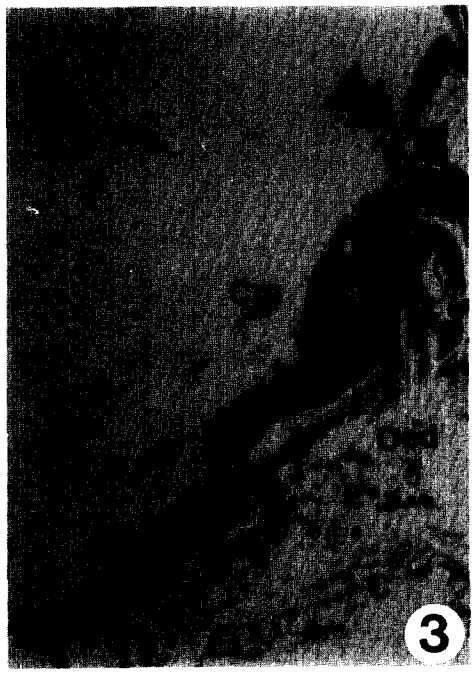
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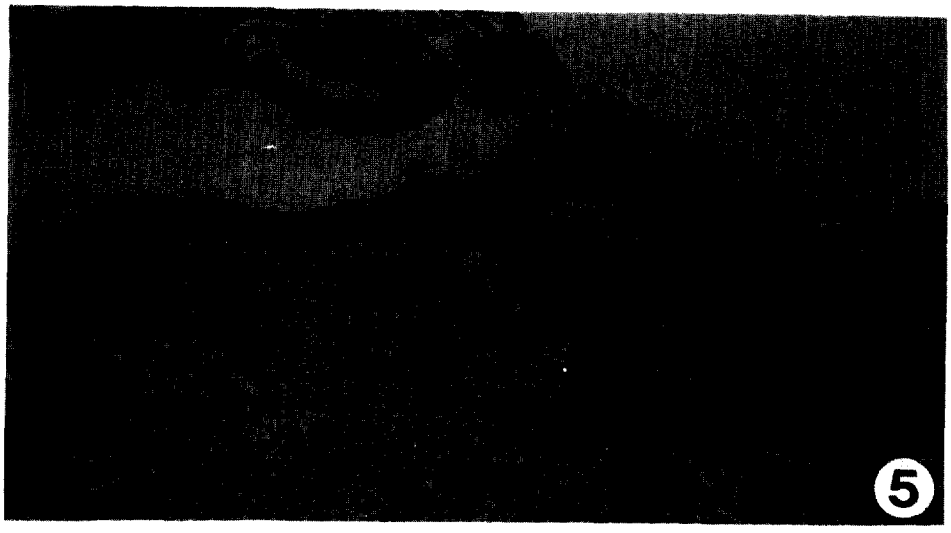
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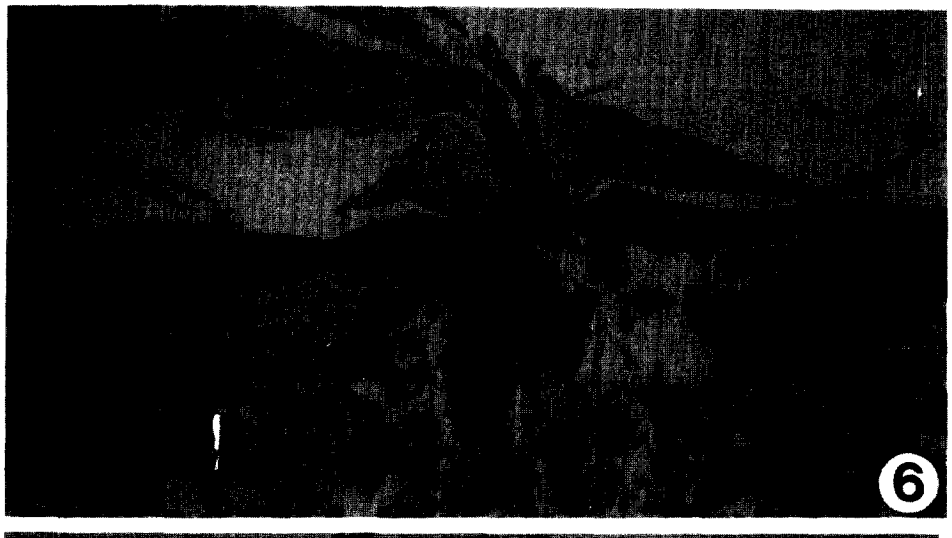
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實驗用 家兎에 있어서의 *Haemaphysalis longicornis* 진드기 咬傷에 대한 獲得免疫에 關聯된 組織病理學的 觀察

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抄 錄

진드기의 吸血에 對하여 經驗이 없는 家兎를 實驗宿主로하여 *Haemaphysalis longicornis* 진드기를 附着 吸血시켰을 때 形成된 咬傷에 對한 組織病理學的 變化를 觀察하고, 진드기의 吸血에 經驗이 있는 感作된 家兎에 1個月 間隔으로 第二次 吸血까지 試圖하여 家兎體內의 獲得免疫의 形成과 그에 따른 組織病理學的 變化를 比較觀察하여 다음과 같은 結論을 얻었다.

1. *H. longicornis* 진드기는 口器를 使用하여 家兎 耳部位의 皮膚를 穿刺하므로서 咬傷을 일으키며, 흔히 진드기의 口器週邊에는 진드기의 安全한 附着을 企圖하는 扁平圓錐狀의 시멘트 物質이 形成되었다.

2. 진드기 咬傷에 對한 主要한 組織病理學的 變化는 局所部位의 出血性 炎症性 反應, 廣範圍한 浮腫, 白血球 浸潤, 그리고 細胞組織의 壞死로 特徵지워졌다.

3. 진드기 寄生의 初期에 形成된 病變 또는 진드기 吸血에 對하여 經驗이 없는 家兎의 病變에서는 好中球를 흔히 볼 수 있었으며, 진드기 寄生末期 또는 진드기 吸血에 對하여 經驗이 있는 感作된 家兎의 病變에서는 好酸球가 많이 觀察되었다.

4. 진드기의 口器가 創傷內에 殘存하는 한, 治癒은 短時日內에 이루어 지지 않았으며, 症狀이 進行됨에 따라 壞死性 炎症性 反應은 더욱 擴大 되었는 데, 이러한 所見은 感作되어 있는 宿主에서 더욱 確實히 觀察되었다.

5. 最初(一次) 吸血 試圖後 感作된 家兎에 二次的으로 附加吸血을 試圖하였을 때 볼수 있는 진드기의 吸血成就度의 低下現象은 宿主體內의 咬傷病變에서 일어나는 組織病理學的 所見과 獲得免疫에 깊이 關聯되어 있는 것으로 思料된다.