

REGENERATIVE MODIFICATIONS IN THE EXTERNAL STRUCTURE OF HALLER'S ORGAN IN NYMPHS OF *IXODES RICINUS* L. (ACARI: IXODIDAE)

РЕГЕНЕРАТИВНЫЕ ИЗМЕНЕНИЯ В НАРУЖНОМ СТРОЕНИИ ОРГАНА ГАЛЛЕРА У НИМФ *IXODES RICINUS* L. (ACARI: IXODIDAE)

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ABSTRACT

The structural modifications in regenerated Haller's sensory organ in nymphs of *Ixodes (Ixodes) ricinus* from which forelegs have been amputated at larval stage, are similar with those in nymphs of another prostriate tick, *I. (Afrixodes) rubicundus*. In both cases the nymphal regenerates re-establish larval features through a reduction of some sensilla in two compartments of the organ (on the distal knoll and in the post-capsular area). This juvenilization of Haller's organ is explained as a result of peculiarities in regenerative processes under hormonal condition during larval-nymphal metamorphosis (high juvenile hormone content).

РЕЗЮМЕ

Нимфы *Ixodes (Ixodes) ricinus*, передние ноги которых были ампутированы на личиночной стадии, по регенеративным изменениям в строении сенсорных органов Галлера обнаруживают сходство с нимфами другого простриатного иксодового клеща *I. (Afrixodes) rubicundus*. В обоих случаях нимфальные регенераты восстанавливали личиночные признаки (в результате утраты некоторых сенсилл в дистальном и пост-капсулярном отделах этого органа). Предполагается, что такая juvenilization органа Галлера обусловлена особенностями регенеративных процессов при высоком содержании ювенильных гормонов, характерном для личиночно-нимфального метаморфоза иксодовых клещей.

INTRODUCTION

Data on the morphology of Haller's organ, the main and unique sensory organ of ixodid ticks, are widely used in studies of the taxonomy and phylogeny of this extremely important group of Acari [Balashov and Leonovich, 1978, 1981; Homsher and Sonenshine, 1979; Homsher et al., 1991; Baker, Jackson, 1996]. Some novel approach for solving these problems is represented by investiga-

tions of structural changes in the organ after its regeneration [Leonovich and Belozеров, 1992, 1993; Belozеров and Leonovich, 1995; Belozеров, Kok and Fourie, 1997]. This approach may give new perspectives in studies of ethology and, especially, of developmental biology of ticks also.

These changes, as was demonstrated in the initial study of Haller's organ regenerates in *Ixodes ricinus* L. adult ticks, are very regular being concerned the number and topography of its sensilla [Leonovich and Belozеров, 1992]. The further studies of nymphal and adult regenerates of Haller's organ in *Hyalomma asiaticum* [Belozеров and Leonovich, 1995] and *I. rubicundus* [Belozеров, Kok and Fourie, 1997] have shown that the regenerative modifications in Haller's organ are in strong dependence on the developmental stage when regeneration occurs. Adult regenerates reveal atavistic multiplication of sensory elements in most parts of Haller's organ having re-established its ancestral features, while nymphal regenerates re-establish typically larval features of the parent species through a reduction in the number of some sensilla. This dependence, as followed from the studies on *H. asiaticum* and *I. rubicundus*, is common to both metastriate and prostriate ixodids [Belozеров, Kok and Fourie, 1997].

The character of structural modifications in adult regenerates of Haller's organ is the same in both investigated species of prostriate ticks — *I. ricinus* [Leonovich and Belozеров, 1992] and *I. rubicundus* [Belozеров, Kok and Fourie, 1997]. Unfortunately, the information concerning such changes in nymphal regenerates for *I. ricinus* was absent. The presented paper makes this information to be available, and enlarges our knowledge about pathways of Haller's organ regeneration during the whole life cycle of *I. ricinus*.

MATERIALS AND METHODS

The larvae for this study were obtained from the laboratory culture of Moldavian *I. ricinus* ticks. The

unfed larvae (second generation) were maintained under a short-day photoperiod (LD 12:12) at 18°C, and at the age of 6 month they were fed on white mice. The engorged larvae being transferred to a long-day photoperiod (LD 20:04) and higher temperature (25°C) were treated by amputation of the left foreleg in 1–2 week after their drop-off.

The operation was undertaken before the beginning of apolysis in nymphs when they were in the state of active crawling. Forelegs were amputated at the level of the femur base, as in our previous studies on limb regeneration [Belozerov, 1993] and on regeneration of Haller's organ [Leonovich and Belozerov, 1992; Belozerov and Leonovich, 1995; Belozerov, Kok and Fourie, 1997]. In some larvae the operation was combined with amputation of distal part of palps. After moulting into nymphs the operated specimens (as well as non-operated control nymphs) were fixed and stored in 70% ethyl alcohol. Before examination by scanning electron microscopy (SEM 501B PHILIPS), tick specimens were dehydrated in an alcohol series. After air-drying and mounting on stubs, they were coated with gold in a sputter coater SC-500 EMScope (England). The images were recorded on photographic film (Kodak Academy 200). Haller's organ structure was examined in 20 operated nymphs (13 specimens after amputation of left foreleg only and 7 specimens after additional amputation of palpal tips) and in 7 control nymphs. Some previous data on external structure of larval Haller's organ are used.

RESULTS

Haller's organ and its sensilla in control ticks

The organ in *I. ricinus*, as well as in all ixodid ticks, is located on the dorsal surface of the tarsus of each foreleg and includes four distinct parts: a distal knoll, an anterior pit, a capsule and a post-capsular area (Fig. 1).

The set of sensillar elements in naive nymphs of *I. ricinus* includes two pairs of setae on the distal knoll (two porous, WP-SW*, and two smooth, NP-SW setae), two smooth, UP-DW bordering setae lateral to the knoll, six setae in the anterior pit (one porous, WP-SW; two grooved, WP-DW; two thin, NP-SW, and one conical, NP-DW), some porous capsular setae and six postcapsular setae (four grooved, WP-DW central setae and two smooth, UP-DW bordering setae). In regard to the topography of sensory setae (and to the shape of non-sensory parts), Haller's organ of the right foreleg is a mirror image of that on the left foreleg. Thus, sensillar set of Haller's organ in nymphs of *I. ricinus*

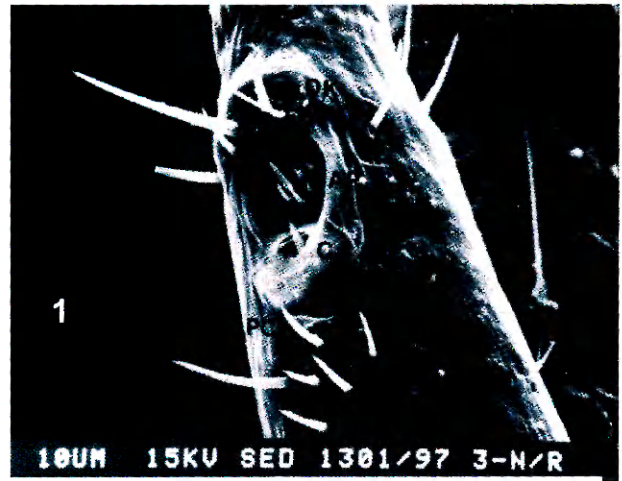


Fig. 1. Haller's organ in *Ixodes ricinus* non-operated nymph (right foreleg). AB — anterior bordering sensilla, AP — anterior pit, Ca — capsule, DK — distal knoll, PCA — post-capsular area. Bar = 10 μ m.

Fig. 2. Regenerated Haller's organ (left foreleg) in *I. ricinus* nymph with changed sensillar sets on the distal knoll and in the post-capsular area. For designations of compartments see the legend to Fig. 1. Bar = 10 μ m.

is completely similar to that in nymphs of *I. rubicundus*, a representative of another subgenus *Africoides* [Belozerov et al., 1997], but it differs from that of adult and larval ticks of both *I. ricinus* [Balashov and Leonovich, 1978] and *I. rubicundus* [Belozerov et al., 1997], inasmuch as the post-capsular group in adult Haller's organ includes four (in *I. ricinus*) and three (in *I. rubicundus*) UP-DW bordering setae, while larval organ includes only two WP-DW central and two UP-DW bordering setae. The distal and anterior-pit groups in larval organs consist also of reduced number of setae (one WP-SW porous and one NP-SW smooth setae on distal knoll, and one porous WP-SW, one grooved WP-DW, two thin NP-SW and one conical NP-DW setae in anterior pit).

Haller's organ and its sensilla in nymphal ticks after regeneration

Haller's organ on regenerated left foreleg shows significant changes in the number of sensilla and their topography, as well as in the shape of non-sensory compartments (Fig. 2). The character of these changes are peculiar in different parts of the organ.

*The abbreviated designations of Haller's organ sensillar elements used already for sense organs in ticks by Hess and Vlimant [1986], Leonovich and Belozerov [1993], Belozerov and Leonovich [1995] and Belozerov et al. [1997] are taken from a typology of insect sensilla by Altner [1977] with some modifications: wall-pore single-walled (WP-SW), no-pore single-walled (NP-SW), wall-pore double-walled (WP-DW), no-pore double-walled (NP-DW) and upper-pore double-walled (UP-DW).



Fig.3. The larval trait of regenerated Haller's organ in *I.ricinus* nymph. The sensillar set of the distal knoll is composed of one long porous and one short smooth sensillum, and that of the postcapsular area is composed of two bordering and two central sensilla. For designations of compartments see the legend to Fig.1. Bar = 10 μ m.

Fig.4. The modified sensillar set of the anterior pit in regenerated Haller's organ in *I.ricinus* nymph. The set is composed of two porous and normal number of other sensilla. The modified sensillar sets on the distal knoll and in the postcapsular area are composed of three and four sensilla respectively. For designations of compartments see Fig.1. Bar = 10 μ m.

On the distal knoll these changes are characterized by reduction of sensillar setae (of smooth, NP-SW type in 100% cases, and of porous, WP-SW type in 40% cases). Hence, 60% of regenerates had three setae (two porous and one smooth), while 40% of regenerates had only two setae (one porous and one smooth). The retained sensilla in the latter case occupied the «larval» position on the right (inside) side of the distal knoll of the left regenerated foreleg (Fig.3). Some changes concerned also the bordering, UP-DW sensilla which normally (in naive nymphs) are situated symmetrically on both sides of the knoll, but in regenerates this symmetry is disturbed sometimes by a shift of left (outside) bordering sensillum in the proximal direction (to the level of the capsula or even of postcapsular sensilla). Such a shift occurred in 30% of regenerates.

Regenerative changes in the anterior pit were less pronounced. They concerned the duplication of porous sensilla in some regenerates only (Fig 4). This occurred in 30% of operated specimens also.

But the frequency of such a duplication after the combined amputation of left foreleg and palp's tip was much higher (in 4 from 7 regenerates) than after the amputation of left foreleg only (in 2 from 13 regenerates). In the distal knoll, on the contrary, the combined operation evoked an opposite, e.g weakening effect (a relationship of regenerates with 2 and 3 distal-knoll sensilla were 6:7 after ordinary amputation and only 2:5 after combined amputation).

Regeneration in the postcapsular area resulted practically always in a reduction of central, WP-DW sensilla. Their number decreased up to 3 (Fig.2) in 25%, and up to 2 (Fig. 3-4) in 70% of regenerates. The changes concerned 95% of regenerates as a whole. In most operated nymphs the sensillar set in the postcapsular area acquired the «larval» traits characterized by two central, WP-DW, and two bordering, UP-DW sensilla (Fig.3-4). An additional amputation of palpal tips had no effect on regenerative changes in this area.

Regenerative induction in nymphal Haller's organ of *Ixodes ricinus*

The phenomenon of regenerative induction described earlier in *Ixodes ricinus* adult ticks [Leonovich and Belozarov, 1992] and then in *I.rubicundus* nymphs and adult ticks [Belozarov et al., 1997] consists of specific structural changes in Haller's organ of the non-treated foreleg in response to amputation of opposite foreleg. Such changes were never detected in *Hyalomma asiaticum* [Belozarov and Leonovich, 1995]. It is supposed therefore that this phenomenon is peculiar to prostrate ticks only [Belozarov et al., 1997].

In nymphs of *I.ricinus* some inductive changes occurred in the distal knoll and in postcapsular area (but never in the anterior pit) of Haller's organ on the right foreleg. The changes were revealed in the loss of one smooth, NP-SW sensillum on the distal knoll (in two nymphal specimens which retained only three sensilla in this compartment), and in the loss of two central, WP-DW postcapsular sensilla (in two specimens also). In both cases the described changes were resulted from combined amputation of larval left foreleg and palps only. Though these inductive events in both cases were of the same low frequency (in 10% of operated specimens for each case), they were quite comparable in appearance with typical regenerative changes on the left foreleg of nymphs, where these changes appeared about in all (95-100%) treated specimens. The absence of inductive changes in the anterior pit can be explained, therefore, by low frequency of regenerative changes (in 30% of cases) occurred in this compartment of the left Haller's organ.

DISCUSSION

Structural changes in regenerated Haller's organ of *I.(Ixodes) ricinus* nymphs described above, are very similar to those in nymphs of *I.(Afrixodes) rubicundus* studied recently [Belozarov et al., 1997]. This is especially obvious from information of

Table.
Comparative data on changes in sensillar sets of Haller's organ in nymphs and adult ticks of *Ixodes ricinus* and *I. rubicundus*

Таблица.
Сравнительные данные по изменению набора сенсилл органа Галлера у нимф и взрослых клещей *Ixodes ricinus* и *I. rubicundus*

Compartment	Type of sensilla	<i>I. ricinus</i>		<i>I. rubicundus</i>	
		Nymphs	Adults	Nymphs	Adults
Distal knoll	WP-SW porous	--	++	--	++
	NP-SW smooth	--	--	--	--
	UP-DW bordering	!	!	!	++
Anterior pit	WP-SW porous	++	++	++	++
	WP-DW grooved	..	++	..	++
	NP-SW smooth
	NP-DW conical
Post-capsular area	WP-DW central	--	-+	--	-+
	UP-DW bordering	..	--	..	-+

Symbols: — reduction, ++ multiplication, .. retention of number, -+ increase of variability, ! proximal shift

both species of these prostrate ticks belonging to different subgenera of the same genus, the regenerative changes in nymphs are connected mainly with a reduction of some sensilla on the distal knoll and in the postcapsular area. It is essential that in both cases these events concern, as a rule, those sensilla which are absent in larval Haller's organ. And, therefore, the nymphal Haller's organs after regeneration re-establish features characteristic to the organ at the larval stage. The only case of sensillar multiplication in nymphs of both species is represented by porous sensilla in the anterior pit (but in limited number of cases).

The regenerative changes in nymphs of these ticks being similar qualitatively, differ in quantitative parameters. The portion of nymphs with larval set of distal-knoll sensilla (one WP-SW and one NP-SW in larval position) in regenerated Haller's organs was essentially less in *I. ricinus* than in *I. rubicundus* (40 and 65% respectively). The difference was even more pronounced in regard to the proximal shift of UP-DW sensillum bordering the distal knoll (30% of regenerates in *I. ricinus* and 86% of regenerates in *I. rubicundus*), and to the duplication of porous, WP-SW sensillum in the anterior pit (30% of regenerates in *I. ricinus* and 65% of regenerates in *I. rubicundus*). But concern-

ing the larval traits in post-capsular area (by retaining two central, WP-DW, and two bordering, UP-DW sensilla only) the relationships between these species was opposite (70% of regenerates in *I. ricinus*, and only 46% of them in *I. rubicundus*).

It is of interest to note that the frequency of regenerative changes in *I. ricinus* nymphs was influenced by additional operative treatment (dissection of palpal tips), and this effect could be either weakened (in the distal knoll), or strengthened (in the anterior pit), or even absent (in the post-capsular area). Really, in the distal knoll the percent of regenerates with larval two sensilla was 46% after ordinary treatment and only 29% after combined treatment, whereas in the anterior pit the duplication of porous, WP-SW sensillum occurred in 15% of regenerates after ordinary treatment and in 57% of regenerates after combined treatment. The additional treatment didn't affect the regenerative response in the post-capsular area. The effect of combined treatment was especially strong in regard to the phenomenon of regenerative induction (an occurrence of some structural changes induced in non-treated right foreleg by amputation of left foreleg). The inductive changes concerning both distal-knoll and post-capsular sensilla were observed only after the combined operation (in two

from seven such nymphs). No such changes were observed in the anterior pit.

The data concerning both an effect of combined operative treatment and a regenerative induction in *I. ricinus* nymphs give especially convincing evidences for independent regenerative response in different compartments of Haller's organ. This confirms a conclusion [Leonovich, Belozero, 1992; Belozero et al., 1997] that the evolutionary changes in different parts of the organ have happened independently.

From the Table summarizing the data on regenerative changes in Haller's organ of *I. ricinus* nymphs (described in details in this paper) and of *I. ricinus* adult ticks [studied earlier by Leonovich and Belozero, 1992], we can see that these stages are principally different in this regard: the nymphal regenerates are characterized by a reduction of some distal-knoll and post-capsular sensilla with retaining of larval features (it is possible to designate these events as juvenilization of Haller's organ), while the adult regenerates reveal multiplication of sensory setae on the distal knoll and in the anterior pit with re-establishing of some atavistic traits in Haller's organ structure. Thus the pathways of regenerative development of this complex sensory organ are common in both studied species belonging to different subgenera of the same genus *Ixodes*. From the comparison of data given in the Table, we can see that the nymphal regenerates of *I. ricinus* and *I. rubicundus* are completely similar in their external structures, while adult regenerates of these ticks reveal only some minor differences concerning UP-DW bordering sensilla.

For a conclusion it is necessary to mention that the regularities of ontogenetic changes in regenerative processes (at successive life-cycle stages) are common not only for different representatives of genus *Ixodes*, but, as was shown recently [Belozero et al., 1997], for both main groups of ixodid ticks (prostriate and metastriate ticks) also. This similarity is connected, of course, with commonness of physiological mechanisms enabling regulation of developmental (particularly morphogenetic) processes in ixodid ticks. One of the probable peculiarities of these mechanisms was suggested in the aforementioned paper [Belozero et al., 1997] that the direction of developmental processes (both normal, and regenerative) is dependent on the hormonal situation (in particular on changes in the content of the juvenile hormones) at corresponding life-cycle stages of ticks. According to this suggestion, juvenile hormones can play a direct role in the elaboration of regenerates [in contrast to an opinion of Bulliere and Bulliere, 1985], and the development of juvenile features in ticks, as in insects [Wigglesworth, 1972], is determined by high content of juvenile hormones, while adult features

develop under condition of low content of these hormones.

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