

## CUTANEOUS AND BLOOD LEUCOCYTE RESPONSE OF PIGEONS TO LARVAL ARGAS POLONICUS FEEDING

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**Abstract.** Dynamics of granular leucocyte response in peripheral blood of naive and sensitized pigeons infested with *Argas polonicus* larvae was only slightly dissimilar. In both cases a marked increase of heterophil and eosinophil counts and only a mild increase of basophil count were recorded at 6 hours post-infestation. In naive hosts the basophil count increased again at 96 hours post-infestation, but in sensitized hosts it did so as early as 48 hours post-tick attachment and was also accompanied by an increase of eosinophil levels 72 and 96 hours post-infestation. Cutaneous response of sensitized hosts at the tick feeding site was characterized by a large heterophil accumulation at 24 hours post-infestation, by an increased eosinophil count at 48 hours and basophil counts at 72 and 96 hours post-infestation. In primary hosts lesions were characterized by a mild increase of heterophil count later than 48 hours post-infestation and by slight eosinophil accumulations at 24 and 48 hours post-tick attachment, as well as basophil accumulation as late as 96 hours post-infestation. Cutaneous lesions of sensitized hosts were accompanied by apparent inflammatory changes which were mild and sometimes missing in primary hosts. Cytotoxic and degenerative alterations of basophils and other granulocytes were observed as well as vesiculation of secretory granules of basophils. Cutaneous response of sensitized hosts to larval *A. polonicus* feeding can be characterized as a cutaneous basophil hypersensitivity.

Acquired resistance to tick feeding was demonstrated and widely studied in mammalian hosts (rabbits, guinea pigs, murine rodents and cattle) after their infestation with ixodid ticks attached to host for several days. Until recently, however, no convincing proofs have been presented that short-term feeding of nymphs and adults or a few days' feeding of larvae of argasid ticks suffice to provoke host resistance (Brown 1985, Allen 1987). Despite the fact that Brossard et al. (1981) and Centurier et al. (1981) demonstrated the presence of antibodies to salivary gland antigen of *Ornithodoros moubata* in the serum of sensitized rabbits, they observed only a weak adverse effect on the tick biology. Neither the results of the pioneer work of Trager (1940) who studied the development of immune resistance in guinea pigs exposed to larval *Ornithodoros venezuelensis* feeding and in chickens exposed to larval, nymphal and adult *Argas persicus* feeding, proved any acquired immune resistance of these hosts to argasid ticks.

After repeated feeding of *Argas polonicus* larvae on pigeons we succeeded in demonstrating that pigeons sensitized by five consecutive infestations acquire the ability to reject 90-95 % of larvae (Dusbábek and Škárová-Špaková 1988) and we therefore decided to study acquired resistance of sensitized pigeons on cellular and tissue level. We were moreover inspired by the studies of McLaren et al. (1983b) and Brown et al. (1983b), drawing attention to some differences in terms of cellular composition and sequence of both the cutaneous and blood leucocyte involvement between hosts infested with ixodid and argasid ticks.

## MATERIALS AND METHODS

Pigeons supplied by breeders and urban feral pigeons captured beyond the area of distribution of the tick species studied were used as hosts. Prior to the experiment four pigeons were sensitized by five consecutive infestations with 100–200 larval ticks at 14 day intervals, four pigeons were used for primary infestation and two as uninfested control. Larval *Argas (Argas) polonicus* Siuda, Hoogstraal, Clifford et Wassef, 1979 ticks from colonies maintained at the Institute of Parasitology, Czechoslovak Academy of Sciences, originated from Czechoslovakia and Poland, were bred and fed as described previously (Dusbábek 1985, Dusbábek and Rosický 1976).

Leucocyte counts in the peripheral blood were made in samples collected from brachial vena in 100 large squares of Bürker chamber using differential staining with Brilliant Cresyl Blue after Procházka and Škrobák (1965). The per cent representation of individual blood elements was determined on blood smears stained with Giemsa and May-Grünwald solution and numerically expressed by conversion from total count of leucocytes in the peripheral blood.

Biopsy samples of the skin, locally anesthetized with Mesocain (Léćiva Praha), were excised together with the attached larval ticks at intervals 24, 48, 72 and 96 hours post-infestation. The site of excision was treated with gelatine spongeous hemostypticum Gelaspon (VEB Anker Werk Rudolfstadt, GDR). Samples for histological sections were fixed by Carnoy's and Helly's fixative, transferred to Paraplast and cut in series. Sections 5–8  $\mu\text{m}$  thick were stained with Wolbach-Giemsa solution, hematoxylin-eosin, trichrome and according to Van Gieson (Putt 1972). Semi-thin Durcupan sections 1  $\mu\text{m}$  thick were stained with May-Grünwald stain and additionally with Giemsa in a special procedure (Škárová-Špáková, in litt.).

Samples of ultra-thin sections were fixed with 4% glutaraldehyde in cacodylate buffer (pH 7.2) for two hours at 4°C or with 4% solution of paraformaldehyde in phosphate buffer (pH 7.2–7.4) after Sörensen, post-fixed with 1%  $\text{OsO}_4$ , transferred to Durcupan and cut on ultramicrotome LKB 8800 Ultratome III. The sections were contrasted for 45 minutes with 20% uranylacetate and Reynolds' solution of lead acetate, or for 20 min. with 0.02–0.04 g lead citrate in 20 ml  $\text{H}_2\text{O}$  and 0.2 ml 10 N NaOH and examined under transmission electron microscope Philips EM 420 and JEM 100B.

Our quantitative study of granular leucocytes in the host skin was performed under light microscope on histological sections in 25–50 square areas each measuring 0.01  $\text{mm}^2$  in close proximity to the tick hypostome at the dividing line between dermis and epidermis in pars papillaris and sub-papillaris.

The counts of basophil, eosinophil and heterophil granulocytes in cuticular lesion obtained at different time points were compared by means of one-way analysis of variance. The conclusive evidence of differences in time was tested by Duncan test, the differences in variance between primary and sensitized hosts by F-test. The comparison of mean counts of granulocytes in the peripheral blood was made by means of Student's t-test. The mathematical-statistical analysis was performed on Hewlett-Packard 9845 A computer.

## RESULTS

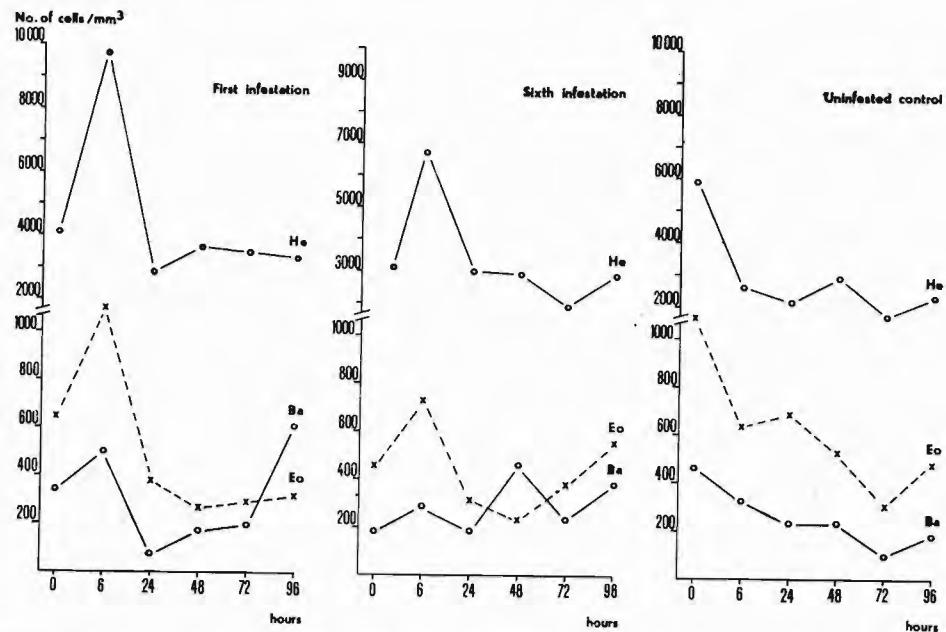
### A. BLOOD LEUCOCYTE RESPONSE

Leucocyte levels in the peripheral blood were variable in individual experimental pigeons and throughout the experiment ranged from 4,000 to 25,000 in control pigeons, from 4,000 to 34,000 in primary infested pigeons and from 6,000 to 21,000 in pigeons after sixth infestation. While in primary hosts a marked increase in the mean count of blood leucocytes appeared at 6 hours post-infestation, in sensitized pigeons this increase was not so marked (Table 1). Levels of leucocytes in the peripheral blood of control pigeons had a generally subsiding tendency in the course of the whole experiment. A similar situation became evident in the varying levels of heterophil and eosinophil granulocytes, where their levels increased at 6 hours of experiment twice as high as the initial levels in naive and sensitized hosts, while in control pigeons more than twice their decreased numbers were recorded.

Although the increase of these granulocyte levels was very well pronounced in both groups of experimental pigeons, it was statistically significant ( $P < 0.05$ ) due to considerable dispersion only in the case of eosinophils in primary hosts (Fig. 1).

**Table 1.** Total leucocyte count in 1  $\text{mm}^3$  of peripheral blood of pigeons during first and sixth infestation by *Argas polonicus* larvae and of uninfested control (Mean  $\pm$  S. E. M.)

Hours	Uninfested control	First infestation	Sixth infestation
0	20,000 $\pm$ 5,000	15,000 $\pm$ 3,524	13,000 $\pm$ 3,035
6	15,000 $\pm$ 1,250	26,000 $\pm$ 5,022	14,000 $\pm$ 2,552
24	9,000 $\pm$ 1,875	10,000 $\pm$ 1,990	10,000 $\pm$ 856
48	11,000 $\pm$ 1,250	12,000 $\pm$ 2,208	8,000 $\pm$ 825
72	6,000 $\pm$ 2,500	10,000 $\pm$ 4,116	7,000 $\pm$ 959
96	8,000 $\pm$ 625	8,000 $\pm$ 1,942	10,000 $\pm$ 2,022



**Fig. 1.** Variation of heterophil (He), basophil (Ba) and eosinophil (Eo) counts in the peripheral blood of pigeons during the first and sixth infestations with *Argas polonicus* larvae and of uninfested controls.

In sensitized pigeons there was another mild increase of the levels of eosinophil granulocytes at 72 and 96 hours post-infestation, which was not observed in primary hosts. In the dynamics of basophils there was a slight difference between the naive and sensitized pigeons. While in primary hosts, after a mild increase at 6 hours post-infestation, another marked increase of levels of these elements was observed as late as 96 hours post-infestation, in sensitized pigeons the increased basophil levels became evident as early as 48 hours post-infestation. In control uninfested pigeons this variation in numbers of granulocytes was not manifest and mean basophil counts had a generally subsiding tendency throughout the experiment (Fig. 1).

## B. CUTANEOUS LEUCOCYTE RESPONSE

Dynamics of the granulocyte occurrence in the skin at the feeding site of larval *A. polonicus* in primary and sensitized pigeons showed great differences in the course of experiment (Table 2). In primary infested pigeons the statistically significant increased levels of eosinophils ( $P < 0.01$ ) were found at 24 and 48 hours post-infestation, those of heterophils at 48–96 hours post-infestation and those of basophils as late as 96 hours post-infestation. In sensitized pigeons similar increased eosinophil levels became manifest at 24–48 hours post-infestation, but the levels of heterophils increased within the first 24 hours of the experiment and those of basophils soared at 72 and 96 hours post-infestation. The determined levels of polymorphonuclear granulocytes were significantly higher ( $P < 0.01$ ) in sensitized pigeons than in naive hosts. The cutaneous response in sensitized pigeons was consequently earlier and significantly higher than in primary infested hosts.

Table 2. Quantitation of cutaneous cellular response of pigeons to the first and sixth infestation by *Argas polonicus* larvae in  $0.01 \text{ mm}^2$  area around the site of tick attachment (Mean  $\pm$  S. E. M.)

Hours	Primary host		
	Basophils	Eosinophils	Heterophils
24	0.41 $\pm$ 0.09	4.05 $\pm$ 0.37	1.32 $\pm$ 0.20
48	0.08 $\pm$ 0.06	4.12 $\pm$ 0.61	6.12 $\pm$ 1.04
72	0.40 $\pm$ 0.15	1.92 $\pm$ 0.47	5.00 $\pm$ 1.13
96	1.00 $\pm$ 0.20	1.75 $\pm$ 0.27	4.35 $\pm$ 0.48
Sensitized host			
24	3.67 $\pm$ 0.39	2.83 $\pm$ 0.21	14.10 $\pm$ 1.24
48	0.53 $\pm$ 0.16	6.47 $\pm$ 0.83	1.70 $\pm$ 0.38
72	18.30 $\pm$ 1.69	0.90 $\pm$ 0.26	1.00 $\pm$ 0.36
96	20.08 $\pm$ 1.67	0.32 $\pm$ 0.13	1.20 $\pm$ 0.33

## C. HISTOPATHOLOGICAL EVALUATION OF CUTANEOUS LESIONS

The general histopathological picture of a cutaneous lesion at the tick feeding site was distinctly different in primary and sensitized hosts (Pl. I, Figs. 1 and 2). In primary infested hosts no inflammatory reaction and discernible necrosis at tick feeding sites were observed within 24 hours post-infestation. The cellular infiltrate was not defined and consisted mostly of erythrocytes and mononuclear cells, with a prominent level of heterophil granulocytes, presence of eosinophils and with occasional basophils (Pl. II, Fig. 1). In pars papillaris cori there were mast cells with hypertrophied cytoplasm releasing granules. After 48 hours post-infestation a weak inflammatory reaction began to appear. Mast cells penetrated the cellular infiltrate and in the vicinity to the tick's hypostome they degranulated. Degranulated mast cells were also observed in pars papillaris. Numerous extracellular eosinophilic granules were present in the vicinity to hypostome. At the next stage of the experiment, at 72 hours post-infestation, the inflammatory reaction was still weak and sometimes missing. Lymphocytes and erythrocytes in the infiltrate were prevalent, which fact was an evidence of an active phase of tick bloodsucking. As for granulocytes, heterophils predominated, while eosinophils and basophils were scarcely represented (Pl. II,

Fig. 2; Pl. III, Fig. 1). As late as 96 hours post-infestation symptoms of hyperemia and edematous swelling of skin were visible. The inflammatory reaction, however, was sometimes missing, in some cases it was prominent, with the necrotic lesion covered with a dense mixed inflammatory infiltrate, where lymphocytes were prevalent. Mast cells were present in the infiltrate. A disintegration of granulocytes and extracellular release of granules were recorded. Numerous mononuclear cells were present, while basophils and cells resembling plasmocytes were sporadic (Pl. III, Fig. 2).

Within the first 24 hours post-infestation hyperkeratosis was seen in sensitized hosts as well as a considerable activation and proliferation of the young connective tissue in subepidermal layers, inflammatory edema and abundant inflammatory infiltrates with local eosinophilia. In adjacent blood vessels there were symptoms of arteritis with obstruction of vascular lumens. The cell infiltrate contained basophils, heterophils and eosinophils, many extravasal erythrocytes and also present were macrophages and plasmocytes with secretory vacuoles (Pl. II, Fig. 3). Mast cells appeared in the cellular infiltrate adjacent to hypostome in degranulated form (Pl. VI, Fig. 1). Granular leucocytes adjacent to the inserted hypostome released granules extracellularly (Pl. II, Fig. 3). The onset of necrobiosis and tissue necrosis appeared at 48 hours post-infestation. The massive cellular infiltrate consisted of erythrocytes, mononuclear cells and of many disintegrating granulocytes, which, however, always remained intact in blood vessels and in their vicinity. In macrophages phagocytosis of free granules and cellular remainder was observed in cellular infiltrate. At the next stage of experiment, at 72 hours post- tick attachment the inflammatory reaction was very well defined and penetrated the skeletal muscles. The necrotic changes around the tick hypostome projected deep into corium. The massive cellular infiltrate consisted of mononuclear cells and granulocytes, and also discernible were the decreased numbers of erythrocytes, indicating a low bloodsucking activity of the ticks at this phase of feeding. Around the hypostome and debris of necrotic cells (Pl. II, Fig. 4) the cellular infiltrate was penetrated and surrounded by many free, mainly basophil granules (Pl. III, Fig. 4), followed up by a layer of disintegrating as well as intact granulocytes, primarily basophils. The presence of plasmocytes was also recorded. After 96 hours post-infestation the zone of necrotic tissue adjacent to the hypostome was penetrated by abundant nuclear detritus and at the periphery of the necrotic tissue there was a trace of a pyogenic rim. The inflammatory process penetrated in some places the skeletal muscles which became dystrophic and destroyed by changes characteristic of obscure swelling, lumpiness and Zenker's necrosis. Within the range of the inflammatory infiltrate many vascular changes were seen, characterized by traces of vasculitis, activation of the intima and hypertrophy of the medium. The cellular infiltrate contained mononuclear cells with a high level of disintegrating granulocytes, mainly basophils, and with a large number of free granules. Intact granulocytes occurred only in blood vessels and around them (Pl. III, Fig. 3). Mast cells continued to penetrate the infiltrate and degranulated.

## D. ELECTRON MICROSCOPY OBSERVATIONS

In primary hosts the most granular leucocytes remained unchanged 24 hours post-infestation. Some basophil granulocytes, however, were slightly damaged, their cytoplasm was vesiculated and lost its usual homogeneity (Pl. IV, Fig. 1). The cytoplasmic membrane was destroyed in them with subsequent cell disintegration and extracellular release of granules. A similar destruction could be also observed in eosinophil and heterophil polymorphonuclear cells (Pl. IV, Fig. 2). Macrophages with a lobular nucleus and cytoplasmic phagocytic processes and phagosomes were seen.

Degranulated mast cells with a destroyed cytoplasmic membrane (Pl. IV, Fig. 3) and fibrocytes were detected. Less discernible haloes were seen round some free granules. Within 48 hours post-infestation, in the cytoplasm of basophil leucocytes observed were granules surrounded by thin haloes and membrane fragments suppressing the cellular nucleus (Pl. IV, Fig. 4) and membranous vesicle-like formations which had apparently originated due to the release of granules. At 96 hours post-infestation most granulocytes showed distinctly degraded cytoplasm with vacant defined vesicles, apparently due to extracellular excretory degranulation (Pl. IV, Fig. 6).

In sensitized hosts only a minor number of granulocytes remained with little change within 24 hours post-infestation (Pl. IV, Fig. 5; Pl. V, Fig. 1). In the majority of basophil granulocytes a marked degeneration of cytoplasm was recognized with vesicles filled with a dense material and membrane-free granules or granules surrounded by membranous remains (Pl. V, Fig. 2). Some granules were surrounded with less discernible haloes (Pl. V, Fig. 3). Frequent was the disintegration of cytoplasm with the extracellular liberation of granules (Pl. V, Fig. 4). The elongated shape of some basophils was an evidence of their active tissue motion (Pl. V, Fig. 5). Eosinophil elements with granules with electrodense one-axis crystals and some heterophils were also subject to destruction of cytoplasm and cellular disintegration with the release of granules. Macrophages were activated with a very dense cytoplasm containing phagosomes and possessing cytoplasmic processes (Pl. V, Fig. 6). Mast cells with the preserved cytoplasmic membrane and cytoplasm penetrated by numerous vesicles, apparently due to secretory degranulation, were active (Pl. VI, Fig. 1). At 48 hours post-infestation the destruction of cytoplasm with extracellular release of granules and nuclear disintegration in basophils and eosinophils was very frequent (Pl. VI, Fig. 3). Many free granules had indistinguishable rims with the remainder of membranes. Prominent was an intensive formation of vesicles in the cytoplasm of mast cells and above all, the destruction of their cytoplasmic membrane (Pl. VI, Fig. 5). At 72 hours post-infestation vesicles were formed in the secretory granules of basophils, with a dense content (Pl. VI, Fig. 2). Heterophil granulocytes exhibited strongly vesiculated, damaged cytoplasm (Pl. VI, Fig. 4). An intensive vesiculation and disintegration of degranulated mast cells was seen, as well as phagocytosis of cellular remains (Pl. VI, Fig. 6). Abundant occurrence of free basophil, heterophil and eosinophil granules was typical.

## DISCUSSION

A great variability in the count of leucocyte elements in the peripheral blood of pigeons is common knowledge, as well as the variation in the occurrence of individual cell types (Schermer 1958, Lukas and Jamroz 1961). The total leucocyte count in 1 mm<sup>3</sup> of peripheral blood of healthy adult pigeons varies from 10,000 to 30,000 (average 15,000), the occurrence of heterophils from 0 to 66 %, of eosinophils and basophils from 0 to 5 % etc. From this aspect it is necessary to evaluate the absolute counts of individual blood elements in our experimental pigeons and to trace the relative changes in their numbers and the tendencies within experimental groups.

Our study of blood cell response in pigeons parasitized by larval *Argas polonicus* ticks demonstrated only a little difference in the kinetics of heterophil and eosinophil granulocytes in naive and sensitized hosts, in which a steep increase of their levels was noted within 6 hours post-infestation. However, in sensitized hosts there was another slight increase of eosinophil count at 72 and 96 hours post-infestation.

A significant difference was recorded in the kinetics of blood basophils where the slight increase in their levels at 6 hours post-infestation in sensitized hosts was followed by a prominent rise of their levels in the circulating blood at 48 hours post-infestation again, while in primary hosts it was as late as 96 hours post-infestation. Similar results were published by Brown et al. (1983b) and Johnston and Brown (1985) who demonstrated a significant increase of blood basophil levels in sensitized guinea pigs after larval and adult feeding of *Ornithodoros tartakovskyi* and *O. parkeri* as early as on the first or second day post-infestation, while in primary hosts they detected this increase on the second up to the fourth day post-infestation. Brown et al. (1983b) who also studied *Rhipicephalus appendiculatus* nymphs, detected an earlier basophilia (at day 3 post-infestation) in primary hosts, while in sensitized hosts the blood basophilia appeared at day 4 post-infestation. In sensitized hosts, on the other hand, they observed diphasic blood eosinophil response with the first peak at day 2 and 3 post-infestation. In primary hosts the second rise of eosinophil count in the peripheral blood up to day 4 of experiment was not recorded.

The kinetics of cutaneous leucocyte response, in comparison with the blood leucocyte response, was a little delayed and differed markedly in primary and sensitized hosts. An increase of heterophil levels in the tick feeding site was noted in sensitized hosts as early as 24 hours post-infestation, in primary hosts as late as 48 hours post-infestation. An increased number of eosinophil granulocytes was found in lesions of sensitized hosts at 48 hours, in primary hosts at 24–48 hours post-infestation. A dramatic rise of basophil leucocyte levels was observed in sensitized hosts at day 3 post-infestation, in primary hosts there was a slight increase in their levels as late as day 4 post-infestation. Similar kinetics of granular leucocytes in the skin, with a prominent rise of basophil levels as late as at days 2–5 post-infestation was recorded by Brossard and Fivaz (1982) and Brown et al. (1983a, 1984) during the larval feeding of *Ixodes ricinus* on sensitized rabbits and of *Rhipicephalus appendiculatus* and *Ixodes holocyclus* on sensitized guinea pigs. But Brown and Knapp (1981) recorded the basophil accumulation in the feeding site of larval *Amblyomma americanum* in sensitized guinea pigs much sooner, within 12 hours post-infestation. Likewise McLaren et al. (1983b) and Johnston and Brown (1985) reported an early cutaneous accumulation of basophils in sensitized hosts (24 hours post-infestation) while studying the feeding of nymphal and adult *Ornithodoros tartakovskyi* and *O. parkeri* in sensitized guinea pigs. In Gill's (1986) opinion variations among the different tick-host systems might suggest that the intensity of the basophil response is also dependent upon the species of the tick and host involved. However, different antigenic potency of the products of salivary glands of individual tick stages and different tick populations should be also taken into consideration here (Dusbábel and Škárová-Špáková 1988).

Allen (1973) characterized the response of sensitized guinea pigs to the *Dermacentor andersoni* feeding as the cutaneous basophil hypersensitivity reaction and Askenase and Worms (1979) demonstrated this type of hypersensitivity in the same host after feeding of argasid ticks. This type of delayed hypersensitivity reaction reminiscent of Jones-Mote reaction is apparently characteristic of reactions to the feeding of most hematophagous arthropods (Brown 1985). The results of our histological studies also indicate the occurrence of this type of hypersensitivity in pigeons sensitized by exposure to larval *Argas polonicus*.

Electron microscopy studies of the lesions in primary and sensitized hosts showed similar results as presented by McLaren et al. (1983b) in guinea pigs exposed to *Ornithodoros tartakovskyi* nymphs and adults. In our experiments especially notable was an early degranulation of mast cells at the initial phase of larval feeding which supports the conclusion of Kishimoto et al. (1986) that the granules of mast cells

may have a factor to gather basophils into cutaneous basophil hypersensitivity skin reaction site. Also prominent was the destruction of cellular membranes and cytoplasm of polymorphonuclear cells, primarily of basophil granulocytes, accompanied by the extracellular liberation of membrane-free granules or with less discernible haloes reminiscent of cytotoxic or degenerative alteration indicating the toxic effect of the products of salivary glands (toxins and enzymes) of argasid ticks. This toxic effect of the tick saliva on the host cells seems to be characteristic of argasid ticks (Argasidae) and likewise was observed in guinea pigs after their exposure to nymphs and adults of *Ornithodoros tartakovskyi*, but not to such an extent after their exposure to ixodid ticks, where the anaphylactic type of basophil degranulation predominated (McLaren et al. 1983a, b). At the next stage of our experiment, at 72 hours post-infestation, also observed was a prominent formation of dense vesicles inside basophil granules of granular leucocytes of the sensitized host, without any distinguishable halo, similar to those observed by Gill (1986 — Fig. 3B) in the skin of *Bos taurus* at a female *Hyalomma anatomicum anatomicum* feeding site, 24 hours after tertiary attachment. This vesiculation of granules resembles that occurred during the vesicular transport of eosinophil peroxidase by granulocytes from the extracellular fluid and its concentration within cytoplasmic granules, as described by Dvorak et al. (1985). While the destruction of granulocytes may be connected with the pathogenic effect of enzymes and toxins comprised in the tick saliva (McLaren et al. 1983b), the formation of cytoplasmic vesicles and release of membrane-free granules by granulocytes may depend on the interaction between the salivary gland antigen of the argasid ticks and specific homocytotropic antibody bound to the surface of basophils and mast cells (Gill 1986). The vesiculation of basophil granules of granulocytes may be connected with vesicular transport of tick enzymes and toxins through cytoplasm and its concentration within granules (cf. Dvorak et al. 1985).

Moreover, characteristic is the prominent reduction in numbers of erythrocytes from the cellular infiltrate of sensitized hosts at 72 hours post-infestation, indicating a low activity of tick feeding at this stage of our experiment. This is in correlation with the reduction of the feeding time and with the decreasing weight of the engorged *Argas polonicus* larvae, with gradual host sensitization as demonstrated previously (Dusbábek and Škárová-Špáková 1988).

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#### ЛЕЙКОЦИТАРНАЯ РЕАКЦИЯ КОЖИ И КРОВИ ГОЛУБЕЙ НА КОРМЛЕНИЕ НА НИХ ЛИЧИНОК КЛЕЩА *ARGAS POLONICUS*

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**Резюме.** Динамика гранулоцитарных лейкоцитов в периферической крови необработанных и активизированных к чувствительности голубей, пораженных личинками клеща *Argas polonicus* не одинакова. В обоих случаях наблюдало значительное повышение числа гетерофилю и эозинофилов и лишь небольшое повышение числа базофилю через 6 часов после заклевания. У необработанных хозяев число базофилю повысилось опять через 96 часов после заклевания, но у активизированных к чувствительности голубей уже через 48 часов после заклевания и сопровождалось повышением числа эозинофилов через 72 и 96 часов после заклевания. Кожную реакцию активизиро-

ванных к чувствительности хозяев в месте укуса клеща характеризовали большое скопление гетерофилю через 24 часов после заклевания, повышенное число эозинофилов через 48 часов и базофилю через 72 и 96 часов после заклевания. У впервые пораженных хозяев отличительным признаком для повреждений являлось слабое повышение числа гетерофилю только через 48 часов после заклевания и небольшое скопление эозинофилов через 24 и 48 часов и базофилю лишь через 96 часов после заклевания. Повреждения кожи активизированных к чувствительности хозяев сопровождались выразительными воспалительными изменениями, которые были небольшие и иногда отсутствовали у впервые пораженных хозяев. Наблюдали цитотоксическое и дегенеративное изменения базофилю и других гранулоцитов и везикуляцию секреторных гранул базофилю гранулоцитов. Реакция кожи активизированных к чувствительности хозяев на кормление на них личинок *A. polonicus* имеет характер кожной базофилю сверхчувствительности.

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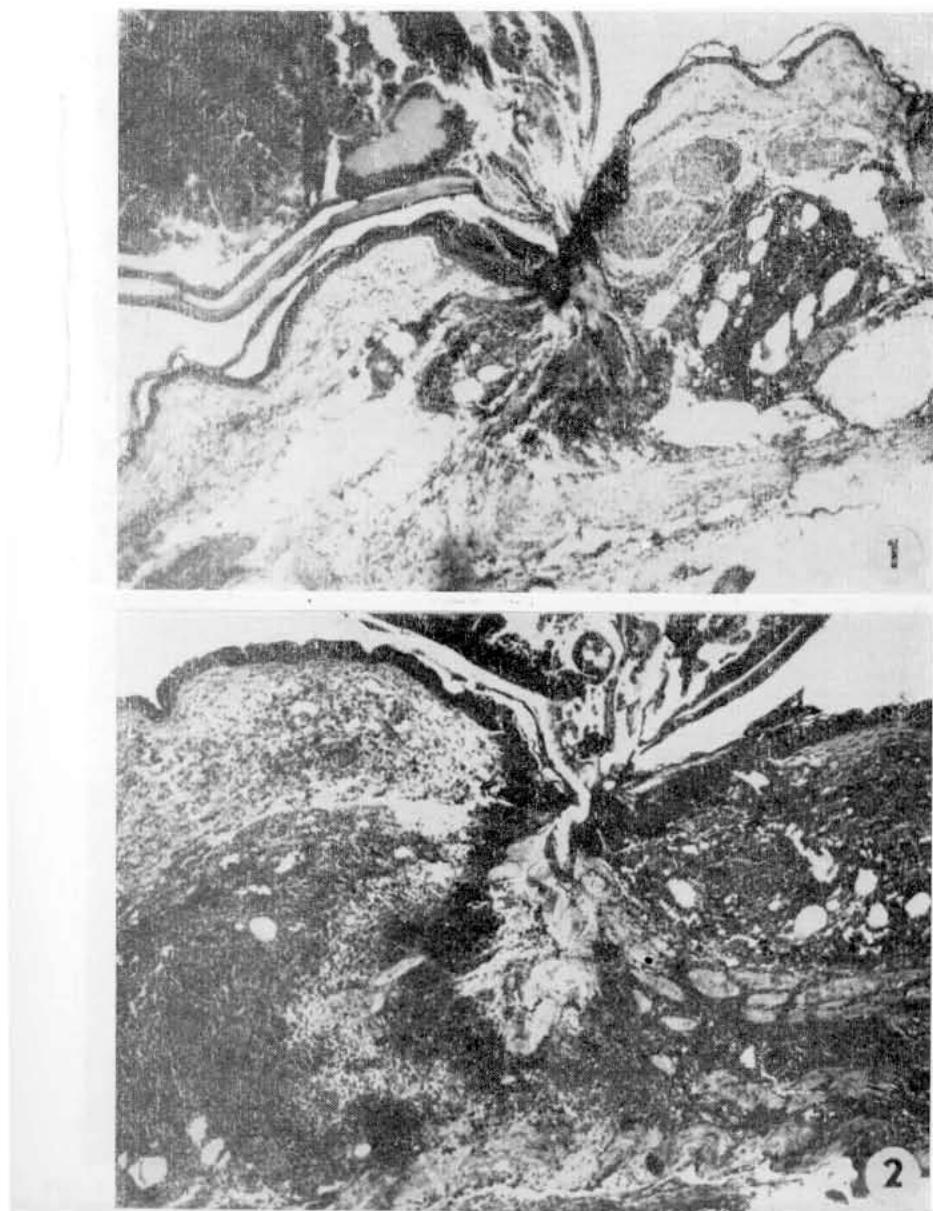
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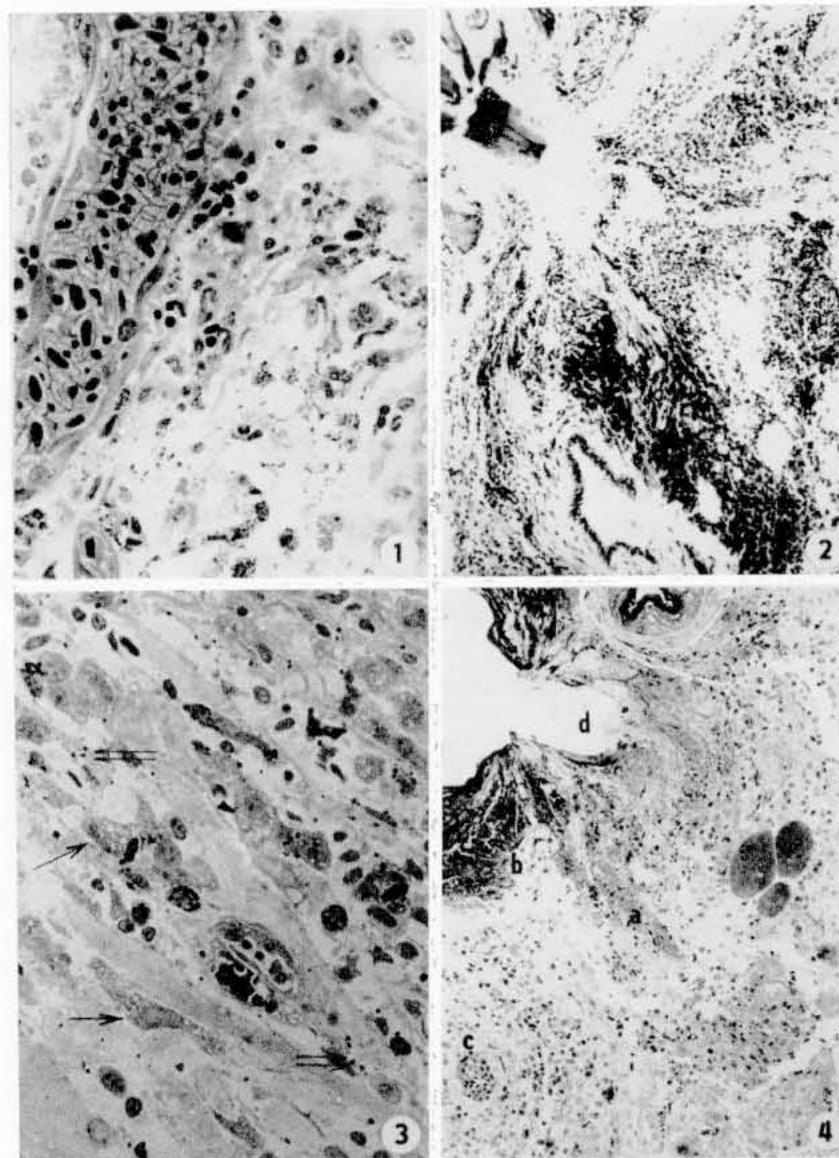
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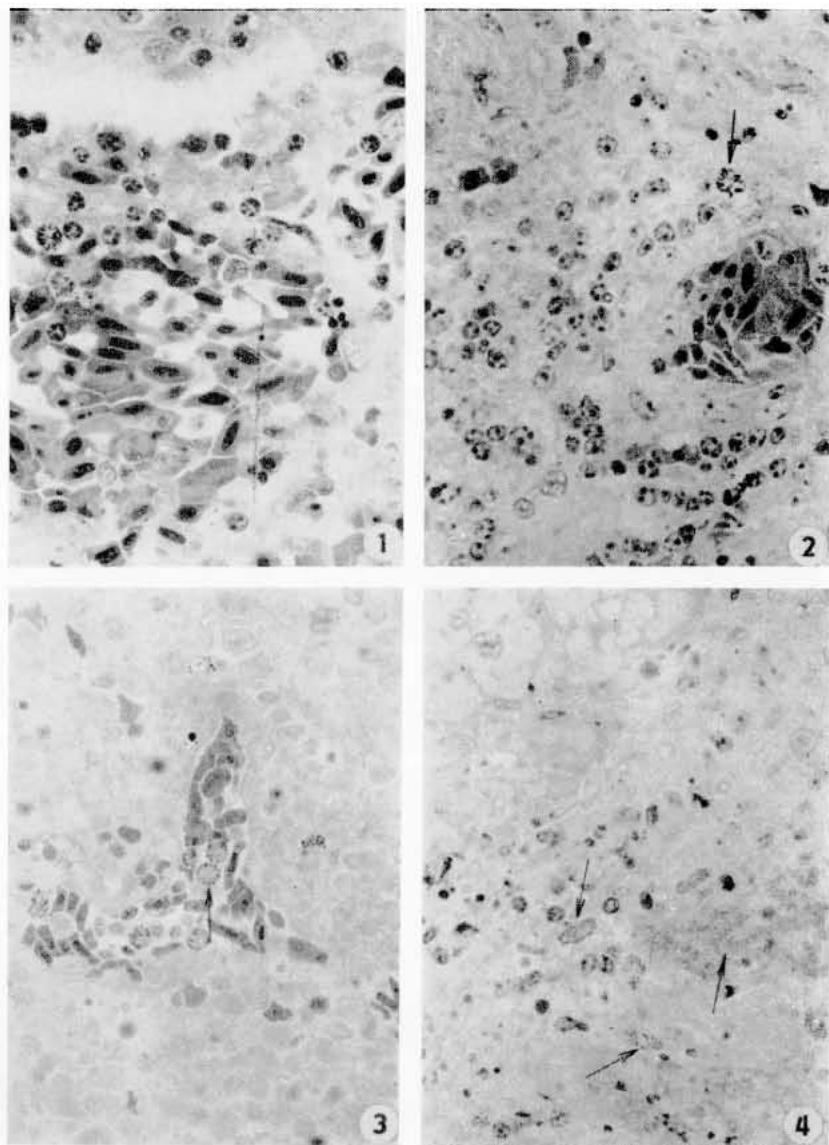
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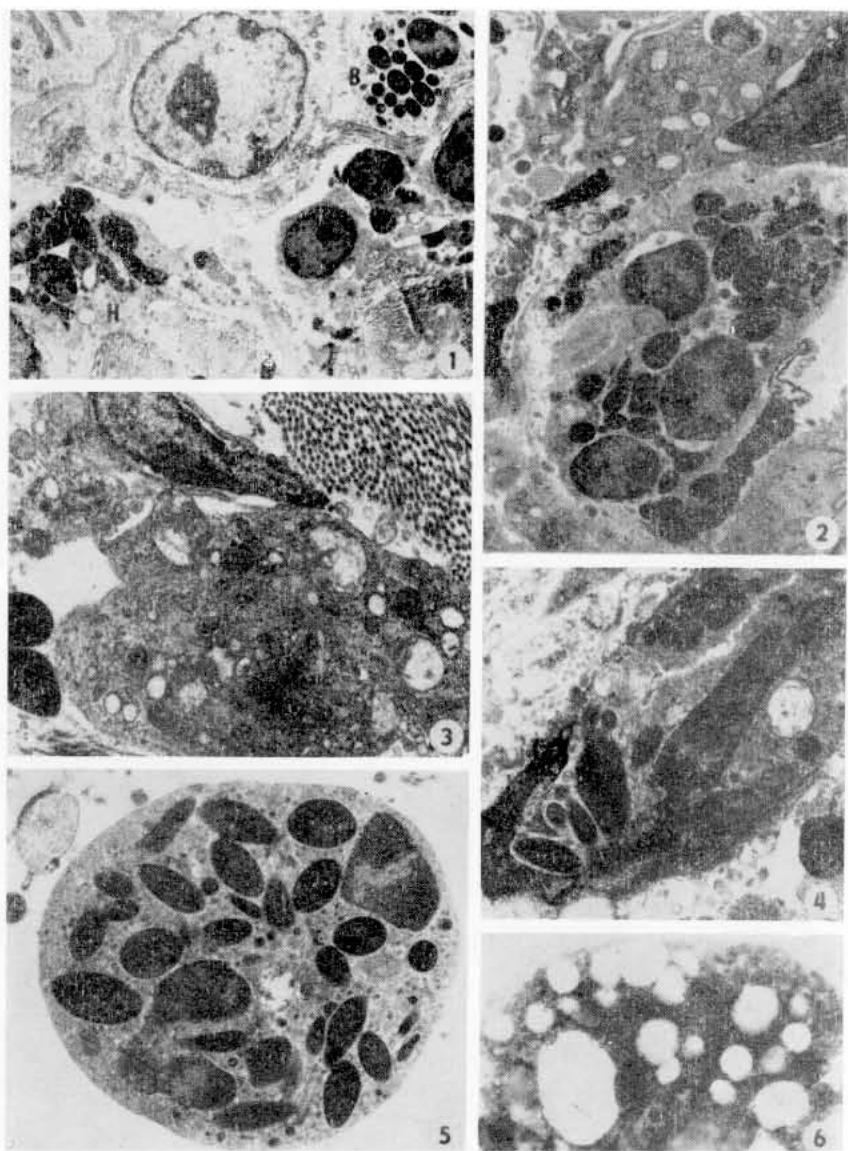
Figs. 1—2. General situation seen in histological section of pigeon skin at the feeding site of *Argas polonicus* larvae 96 hours post-infestation. Extensive infiltrate and marked necrosis in sensitized host are visible. Fig. 1. Primary host (Trichrome,  $\times 85$ ). Fig. 2. Sensitized host (Hematoxylin-eosin,  $\times 100$ ).



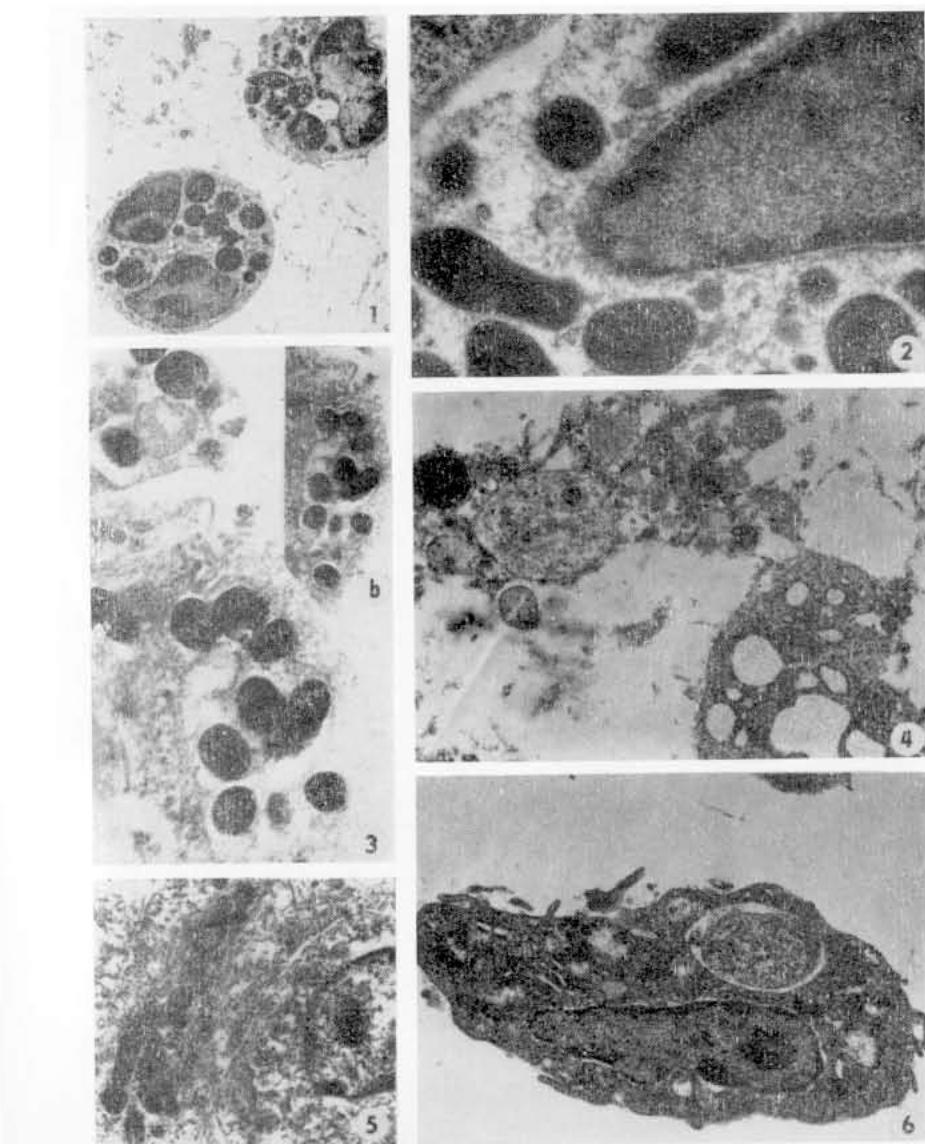
Figs. 1—4. Pigeon skin section at the feeding site of *Argas polonicus* larvae. Fig. 1. Primary host 24 hours post-infestation. Blood vessel with erythrocytes, cellular infiltrate with dominant heterophils ( $\times 630$ ). Fig. 2. Primary host 72 hours post-infestation. Cellular infiltrate, discernible tick hypostome ( $\times 140$ ). Fig. 3. Sensitized host 24 hours post-infestation. Plasmocyte-like cells with secretory vesicles (arrow) and extracellular granules (double arrow) ( $\times 750$ ). Fig. 4. Sensitized host 72 hours post-infestation. Destroyed fatty tissue (a), necrosis (b), blood vessel with erythrocytes (c), impression of tick hypostome (d) ( $\times 180$ ). Figs. 1, 3 and 4 semi-thin sections, May—Grünwald, Giemsa—Romanowski, Fig. 2 histological section, Wohlbach—Giems.



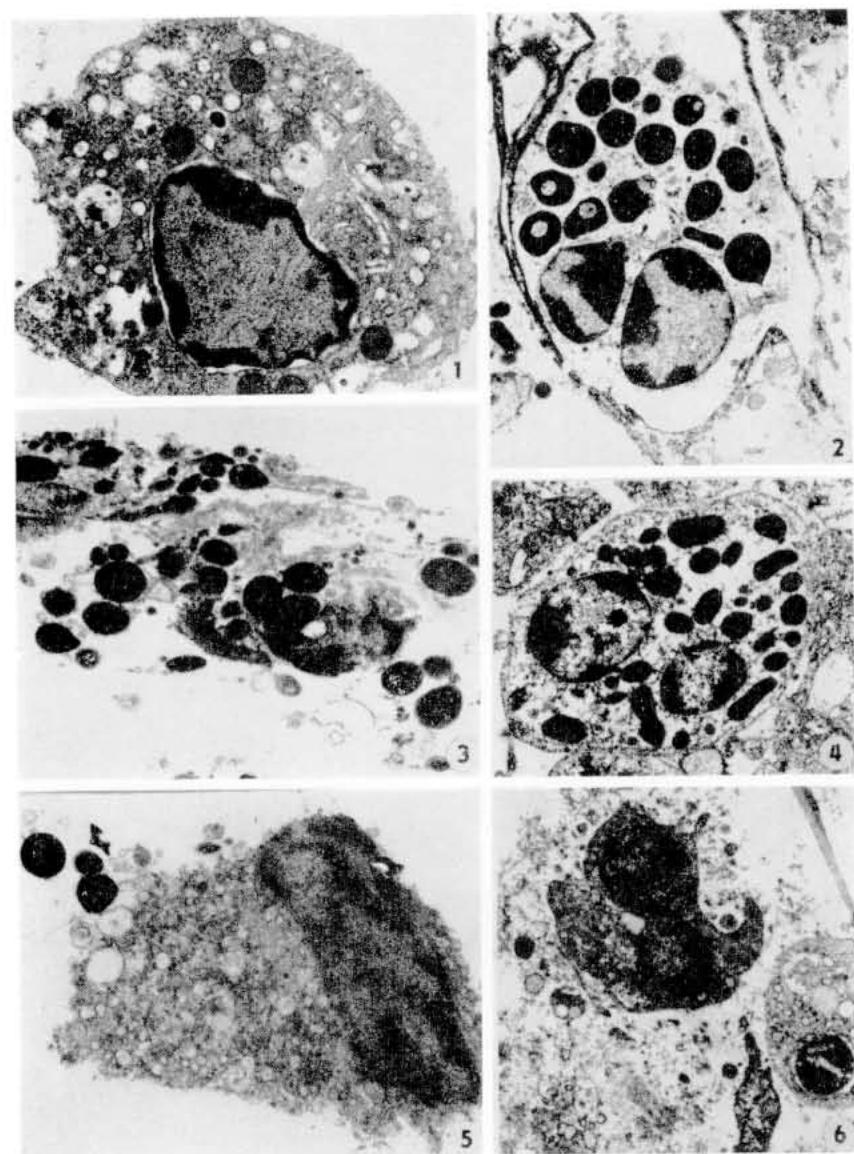
Figs. 1—4. Cellular infiltrate in pigeon skin at the feeding site of *Argas polonicus* larvae. **Fig. 1.** Primary host 72 hours post-infestation. Infiltrate consisting mostly of erythrocytes and heterophil leucocytes ( $\times 700$ ). **Fig. 2.** Primary host 96 hours post-infestation. Blood vessel with erythrocytes and basophil granulocytes (arrow) ( $\times 725$ ). **Fig. 3.** Sensitized host 96 hours post-infestation. Intact granulocytes in vascular lumen and its surroundings. Basophils (arrow) and heterophils ( $\times 600$ ). **Fig. 4.** Sensitized host 96 hours post-infestation. Disintegrating infiltrate cells, numerous free basophil granules (arrow), basophils and heterophils (arrow) ( $\times 780$ ). (Semi-thin sections, May—Grünwald, Giemsa—Romanowski).



Figs. 1—4. Pigeon skin at the feeding site of *Argas polonicus* larvae. **Fig. 1.** Primary host 24 hours post-infestation. Basophil (B) and heterophil granulocytes (H), other infiltrate cells and collagenic fibres ( $\times 1200$ ). **Fig. 2.** Primary host 24 hours post-infestation. Destruction of cytoplasmic membrane of heterophil granulocyte ( $\times 7500$ ). **Fig. 3.** Primary host 24 hours post-infestation. Degranulation of mast cell with numerous ribosomes, disintegrated cellular membrane and mitochondria ( $\times 12000$ ). **Fig. 4.** Primary host 48 hours post-infestation. Basophil granulocyte with nucleus extruded into the cell periphery, granules with thin haloes projecting into nucleus ( $\times 12000$ ). **Fig. 5.** Sensitized host 24 hours post-infestation. Intact heterophil granulocyte ( $\times 10000$ ). **Fig. 6.** Primary host 96 hours post-infestation. Total destruction of cytoplasmic membrane and cytoplasm of granular leucocyte with vesicles left by released granules ( $\times 7500$ ). (Ultra-thin TEM sections.)



Figs. 1—6. Skin of a sensitized pigeon at the feeding site of *Argas polonicus* larvae 24 hours post-infestation. **Fig. 1.** Unchanged basophil and heterophil granulocyte ( $\times 4300$ ). **Fig. 2.** Degenerative changes in the cytoplasm of basophilic leucocyte, destroyed nuclear membrane, some granules without any compact rim ( $\times 42500$ ). **Fig. 3.** Some basophil granules encompassed by indistinct haloes ( $\times 24000$ ), b — reduced cut-out ( $\times 11000$ ). **Fig. 4.** Disruption of cytoplasm of the infiltrate cells is accompanied by extracellular release of granules ( $\times 3900$ ). **Fig. 5.** Elongated shape of basophil granulocyte indicates active cell motion ( $\times 8500$ ). **Fig. 6.** Active form of macrophage with a very dense cytoplasm and peripheral microvillous processes ( $\times 850$ ). (Ultra-thin TEM sections.)



Figs. 1—6. Skin of a sensitized pigeon at the feeding site of *Argas polonicus* larvae. **Fig. 1.** Active mast cell with intact cytoplasmic membrane and cytoplasm proliferated by granules and vesicles with electron-dense material, 24 hours post-infestation ( $\times 11300$ ). **Fig. 2.** Inside the granules of a basophilic leucocyte appear vesicles containing dense material, 72 hours post-infestation ( $\times 13000$ ). **Fig. 3.** Disintegrating granulocyte, releasing granules, 48 hours post-infestation ( $\times 9000$ ). **Fig. 4.** Heterophil granulocyte with strongly vesiculated and disrupted cytoplasm, 48 hours post-infestation, ( $\times 8000$ ). **Fig. 5.** Mast cell with disintegrated cytoplasmic membrane and vesiculated cytoplasm, 48 hours post-infestation ( $\times 8000$ ). **Fig. 6.** Phagocytosis of cellular remains by a macrophage, 72 hours post-infestation ( $\times 4800$ ). (Ultra-thin TEM sections.)