

Histologic Studies of Guinea Pig Skin: Different Stages of Allergic Reactivity to Flea Bites¹

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(Submitted for publication, 23 July 1963)

LARRIVEE, DENIS H., BENJAMINI, E., FEINGOLD, B. F., AND SHIMIZU MEIKYO. 1964. Histologic studies of guinea pig skin: different stages of allergic reactivity to flea bites. *Experimental Parasitology* 15, 491-502. A definite sequence of skin reactions occurs when guinea pigs are exposed to flea bites. The sequence is composed of stages that are observed at the bite sites and can be classified in terms of allergic phenomena. This study presents a sequential morphologic analysis of these allergic stages in terms of histopathologic events. When the different types of skin sensitivity in guinea pigs were defined both by macroscopic and microscopic characteristics, the following stages were noted:

- I. *Induction stage*, during which no abnormal macroscopic or microscopic changes occurred in the skin;
- II. *Stage of delayed skin reactions only*, characterized by intense mononuclear infiltration at the vicinity of the bite site some 24 hours following the bite;
- III. *Stage of immediate and delayed reactions*, immediate reactions appearing 20 minutes after the bite and characterized by an eosinophilic infiltration; the delayed reaction appearing within 24 hours, characterized by mononuclear infiltration;
- IV. *Stage of immediate reactivity only*, the reaction appearing 20 minutes after the bite and characterized by an infiltration of eosinophils at the bite site;
- V. *Stage of nonreactivity resulting from desensitization*, wherein no skin reactivity was observable and no cellular abnormalities occurred.

Benjamini *et al.* (1961) recently demonstrated a definite sequence of skin reactions occurring in guinea pigs as a consequence of repeated exposures to flea bites. Several stages of reactivity were determined by visual observations of the time of onset of skin responses following exposures. These stages were characterized as follows:

I—induction period (no reactivity), lasting

¹ This work was supported by research grant E-3966(02) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U.S. Public Health Service.

approximately 4 days following initial exposure;

II—stage of predominantly delayed skin reactions appearing 5 days after initial exposure and lasting about 4 days;

III—stage of delayed and immediate reactions, appearing 9 days after initial exposure and lasting approximately 48 days;

IV—stage of immediate reactivity, appearing after 57 days and lasting approximately 31 days;

V—stage of nonreactivity appearing in

about 88 days and lasting for at least 150 days.

The present studies were initiated to determine whether skin reactions noted visually during different stages of guinea pig reactivity to flea bites could be correlated with a sequence of morphologic and histopathologic responses having the classic cellular characteristics peculiar to skin responses during delayed and immediate phases of reactivity.

MATERIALS AND METHODS

Sensitization. Five animals were sensitized to flea bites by repeated exposures to bites of fleas (*Ctenocephalides felis felis*) according to the method described by Benjamini *et al.* (1960, 1961).

Histologic material. Twenty minutes and 24 hours after exposure to bites, biopsies of skin were taken from the bitten area on the abdomen. Each specimen was placed immediately in Bouin's solution and was fixed for 24 hours. It was then dehydrated and embedded in paraffin, except in the case of sample No. 2, which was fixed in Carnoy's solution for 3 hours and then processed as above. Paraffin serial sections, 8 μ thick, were obtained with a Spencer rotary microtome. The sections were deparaffinated and stained with hematoxylin-eosin. In some cases, Giemsa stain was used to demonstrate eosinophils, or Masson's trichrome stain was used to show collagen fibers.

The skin samples used in these studies, and

TABLE I
Summary of Skin Samples Used in These Studies and List of Figures Which Depict the Morphologic Aspects of Each Sample

Skin sample no.	Sensitivity stage	Days after initial exposure	Description of sample	Figures depicting sample
1	I (Induction period)	0	Unexposed (control)	1, 2
2		0	Nonsensitive animal approximately 20 min. after bite (flea's proboscis in the skin)	3
3		1	Nonsensitive animals 24 hours after bite	4
4	II (Predominantly delayed)	5-8	20 min. after bite	5
5		5-8	24 hours after bite	6-8
6	III (Delayed and immediate)	10-30	20 min. after bite	9, 10
7		10-30	24 hours after bite	11, 12
8	IV (Predominantly immediate)	62-73	20 min. after bite	13, 14
9		62-73	24 hours after bite	15
10	V (Nonreactivity)	100-150	20 min. after bite	16
11		100-150	24 hours after bite	17

the list of figures which depict the morphologic aspects of each sample, are shown in Table I.

OBSERVATIONS

The following descriptions present the characteristics noted for each stage of reactivity and their differences from nonsensitized unbitten skin, shown in Figs. 1 and 2.

I. *Induction period.* This period begins immediately after the initial flea bite. During the bite the epipharynx of the flea penetrates the epidermis and then the dermis in its search for a capillary. The bite mechanism per se causes mild trauma in the skin which is characterized by the invasion of a few lymphocytes in the vicinity of the epipharynx (Fig. 3). In some instances evidence of epidermal

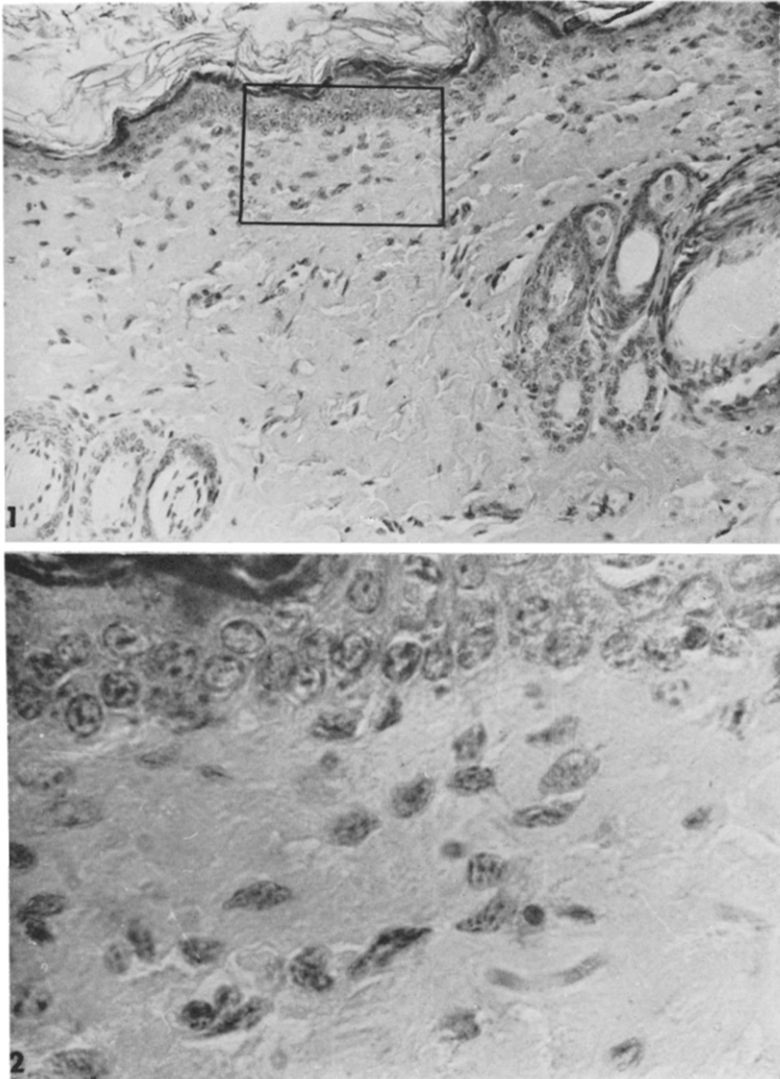


FIG. 1. Control guinea pig skin (nonsensitized, unbitten) showing epidermis, dermis, and hair follicles. Hematoxylin and eosin (H&E); $\times 125$.

FIG. 2. The framed area of Fig. 1, showing fibroblasts. $\times 500$.

cell fragmentation was noted. Twenty-four hours after the bite, this mild lymphocytic infiltrate, if still present, is greatly reduced (Fig. 4). Generally, 24 hours after the bite, evidence of trauma is not visible macroscopically. The lymphocytic infiltration, if present, does not exhibit the variety of components

seen during an allergic skin reaction (Figs. 6–8).

II. *Stage of delayed skin reactivity.* Macroscopically, this stage appears 5 days after initial exposure and lasts approximately 4 days. Biopsies taken 20 minutes after the bite (Fig. 5) reveal a situation very similar to

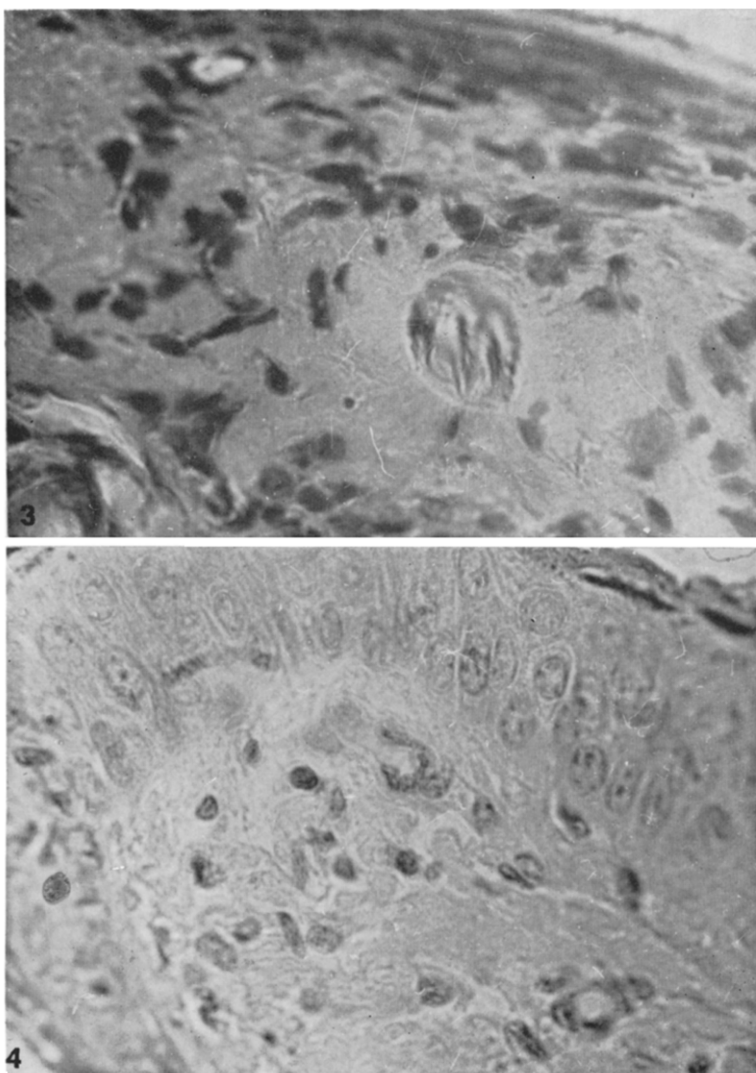


FIG. 3. Guinea pig skin during the induction period, 20 minutes after initial exposure to flea bites; shown are a few lymphocytes at the vicinity of the penetrating proboscis (cross section of proboscis seen off focus at the center of the slide). H&E; $\times 500$.

FIG. 4. Guinea pig skin during the induction period, 24 hours after flea bite. H&E; $\times 500$.

that seen in biopsies taken 20 minutes after the bite from nonsensitized animals (Fig. 3). This is in keeping with the fact that macroscopically, except for trauma, no immediate skin reactivity is observable at this stage.

Skin biopsies taken 24 hours after the challenging bite exhibit intense monocyctic and

lymphocytic infiltration of the dermis, accompanied by a conspicuous hyperplasia and mononuclear invasion of the epidermis (Figs. 6-8). This predominantly mononuclear response is characteristic of a delayed reaction whenever it occurs in the sequence of reactivity stages shown here. The number of

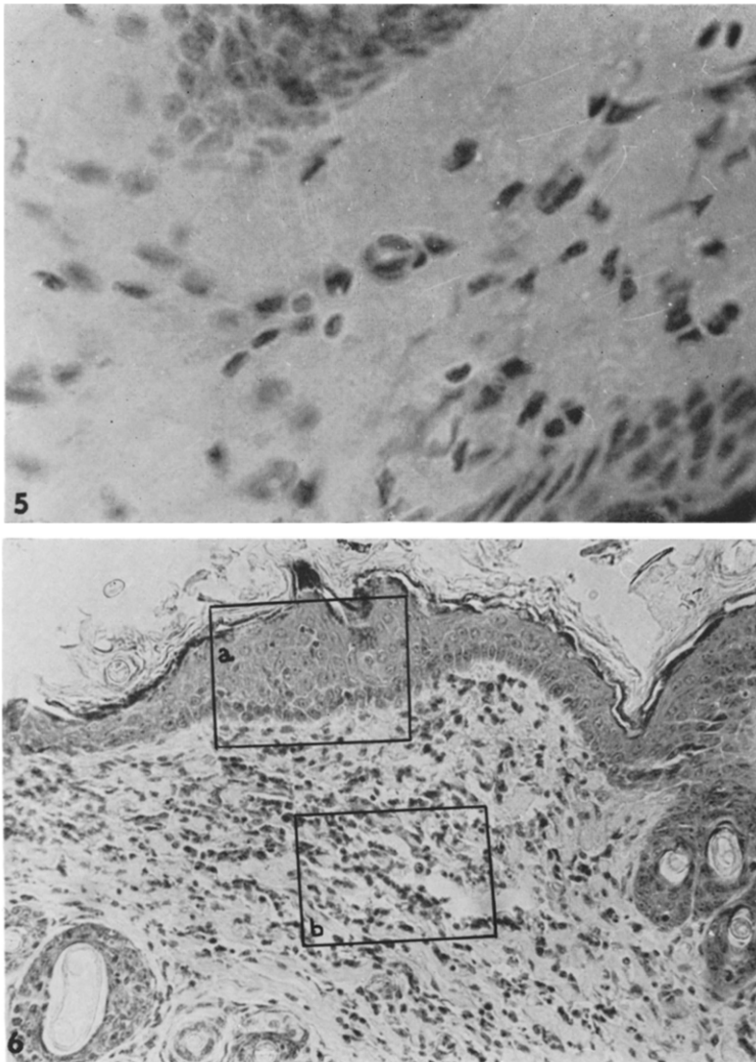


FIG. 5. Guinea pig skin during the stage of delayed reactivity, taken 20 minutes after the flea bite. H&E; $\times 500$.

FIG. 6. Guinea pig skin during the stage of delayed reactivity, taken 24 hours after flea bite, showing an intense generalized monocyctic infiltration and a few eosinophils, and accompanied by hyperplasia of the epidermis. H&E; $\times 125$.

eosinophils is always small, especially when compared with that present 20 minutes after challenge of animals showing immediate reactivity (Stages III and IV). These cellular characteristics associated with delayed skin reactivity in guinea pigs responding to flea bites agree with observations by Dienes and Mallory (1932) and Kaplan and Dienes (1958), who showed that delayed skin reac-

tions appearing on the 4th or 5th day after initial sensitization with protein antigens, were characterized by a predominance of mononuclear infiltration over polymorphonuclear cells.

III. *Stage of immediate and delayed skin reactions.* This stage begins approximately 9 days after the initial bite and lasts for approximately 48 days. During this stage there is

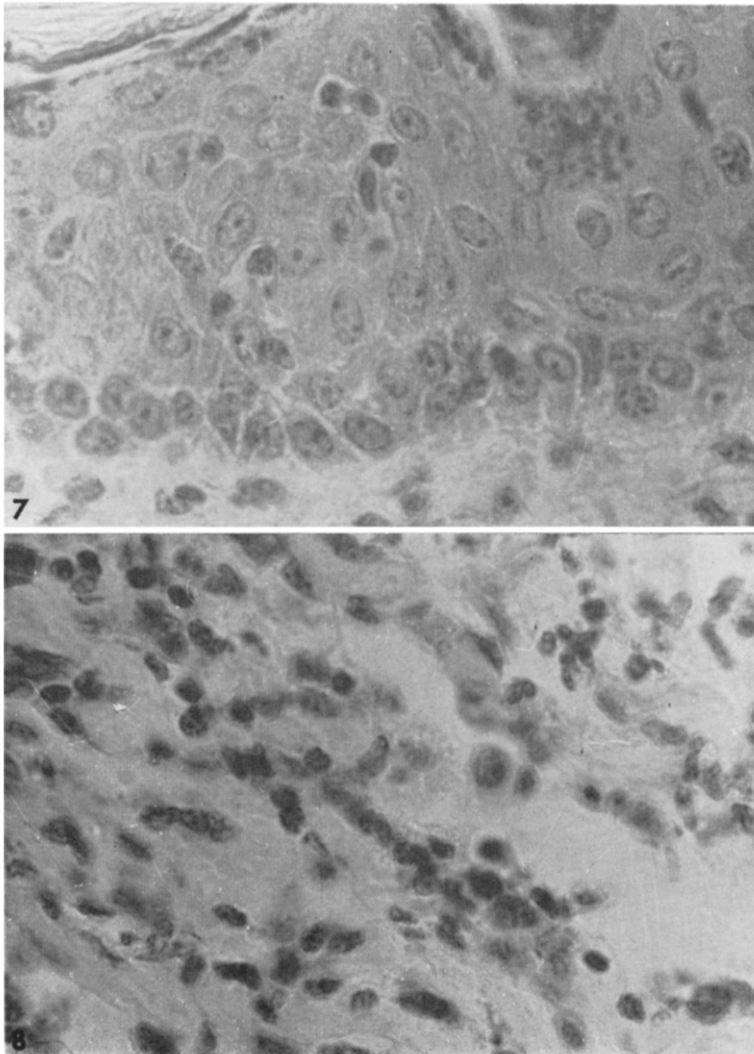


FIG. 7. The framed area (a) of Fig. 6, showing hyperplasia of the epidermis and its infiltration by mononuclear cells. $\times 500$.

FIG. 8. The framed area (b) of Fig. 6, showing an intense mononuclear infiltration of the dermis, accompanied by a few eosinophils. $\times 500$.

evidence of both monocytic and eosinophilic cellular infiltrations resulting from the challenging bite. At 20 minutes after the bite there is a prominent eosinophilic infiltration in the dermis (Figs. 9 and 10). This predominantly eosinophilic response generally is associated with skin reactivity of the immediate type and has been demonstrated by various workers (Campbell *et al.*, 1935; Dienes and Mallory, 1932; Gell, 1959; Kaplan and Dienes, 1958; Oda and Puck, 1961; Raffel, 1951; Samter, 1949; Weiser, 1957). Kaplan and Dienes (1958), working with responses elicited by protein antigens, characterized the immediate type of skin reactivity as edema and a massive infiltration of eosinophils occasionally accompanied by focal accumulations of mononuclear cells. The results presented in Figs. 9 and 10 generally conform with the

above description. However, a focal accumulation of mononuclear cells was not consistently apparent.

At 24 hours after the challenging bite, the characteristic cellular response associated with the immediate skin reaction has been replaced by cellular characteristics of delayed skin reactivity (Figs. 11 and 12). These skin sections show a predominantly monocytic infiltration composed mainly of lymphocytes and monocytes. In some cases mononuclear follicles and hyperplasia were noted. Eosinophilic infiltration, when present, is small compared with that seen in immediate reactions.

From Figs. 9–12 it appears that the stage of immediate and delayed responses is well defined and that a marked difference in cellular response occurs at 20 minutes or 24 hours after the bite.

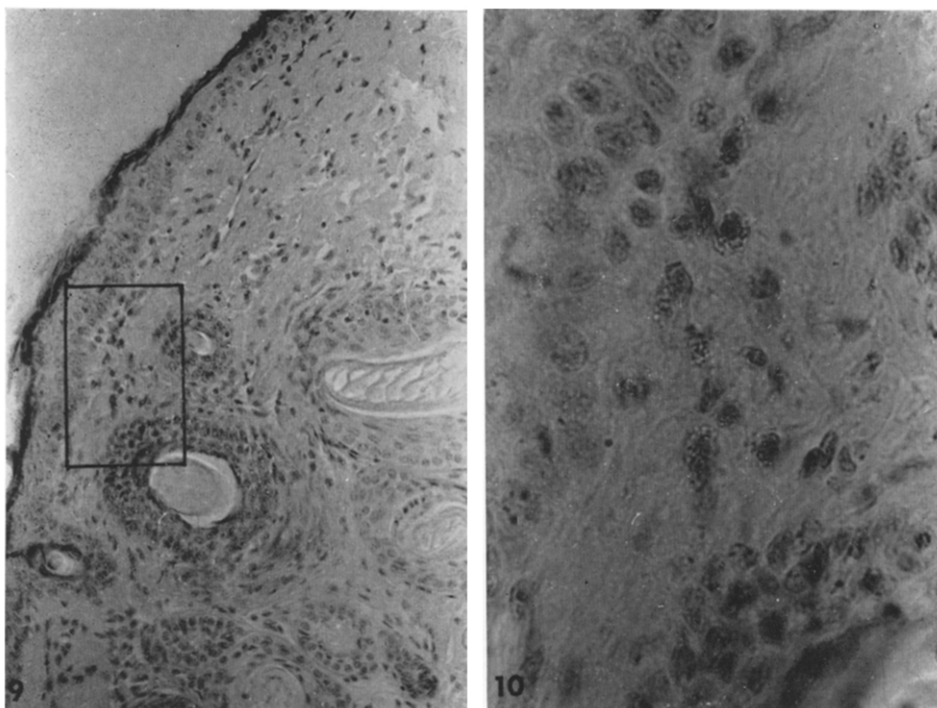


FIG. 9. Guinea pig skin during the immediate and delayed stage of reactivity, taken 20 minutes after flea bites; showing an eosinophilic infiltration and a lack of mononuclear infiltration. H&E; $\times 125$.

FIG. 10. The framed area of Fig. 9, showing the eosinophilic infiltration. $\times 500$.

IV. *Stage of immediate skin reactivity.* This stage appears about 50 days after the initial bite and lasts approximately 1 month. In general, this stage is characterized by an immediate (20-minute) eosinophilic response and a mild delayed (24 hours) mononuclear response (Figs. 13–15). With continued challenges, both responses diminish and the animal enters the stage of nonreactivity. As the animal approaches the stage of desensitiza-

tion, the decline in polymorphonuclear response occurs at a much higher rate than the decline in mononuclear response.

V. *Stage of nonreactivity.* This stage is probably achieved as a result of repeated exposures to bites. Biopsies taken 20 minutes and 24 hours after bites reveal negligible or no cellular responses (Figs. 16 and 17) and show a histologic similarity to control samples obtained from nonsensitized animals at com-

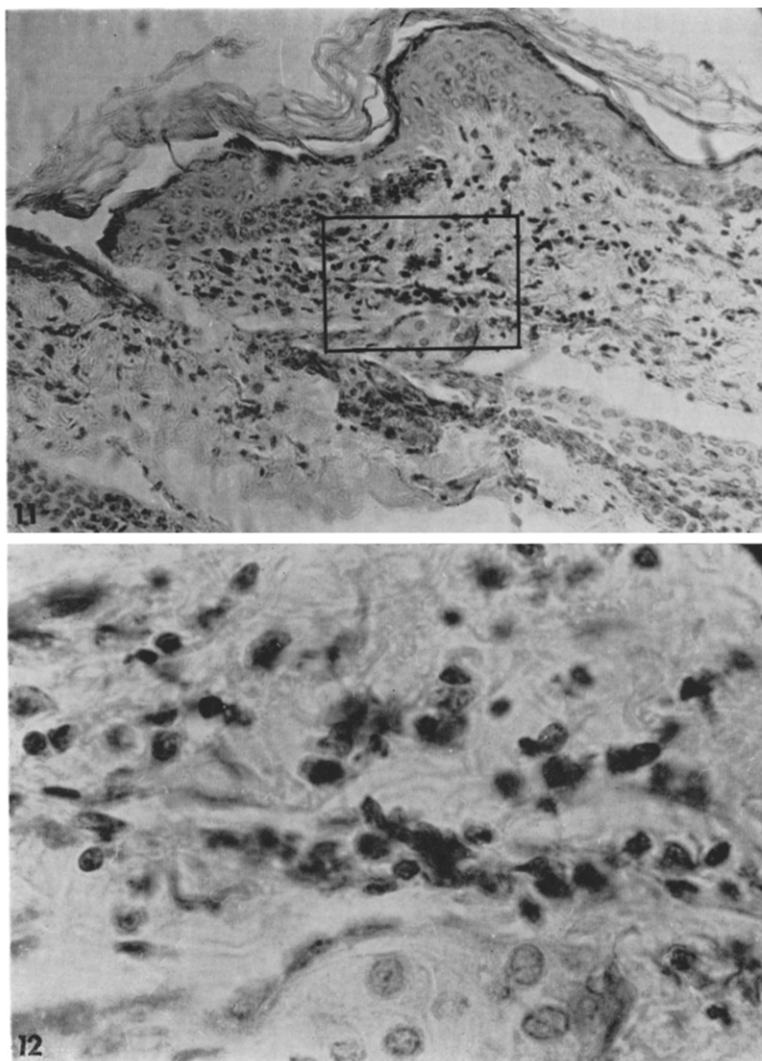


FIG. 11. Guinea pig skin during the stage of immediate and delayed reactivity, taken 24 hours after the flea bite; showing a mononuclear infiltration. H&E; $\times 125$.

FIG. 12. The framed area of Fig. 11, showing the predominant mononuclear response. $\times 500$.

parable time intervals after exposure to bites (Figs. 1-4).

DISCUSSION

Descriptions of histologic changes as part of a response syndrome to bites of hematophagous arthropods have been the subject of various reports (Allen, 1948; Arian and Fox, 1955; Goldman *et al.*, 1952; Gordon and Crewe, 1948; Lunsford, 1949; Riek, 1954,

1958; Rockwell and Johnson, 1952). Allen (1948) described pseudoepitheliomatous hyperplasia in human skin, characterized by cells in mitosis, disruption of the basement membrane, and accompanied by vesiculation and massing of eosinophilic leucocytes in the epidermis. The dermis contained a dense infiltration of mature eosinophils, plasma cells, and histiocytes with lymphocytic follicles. Goldman *et al.* (1952) demonstrated a se-

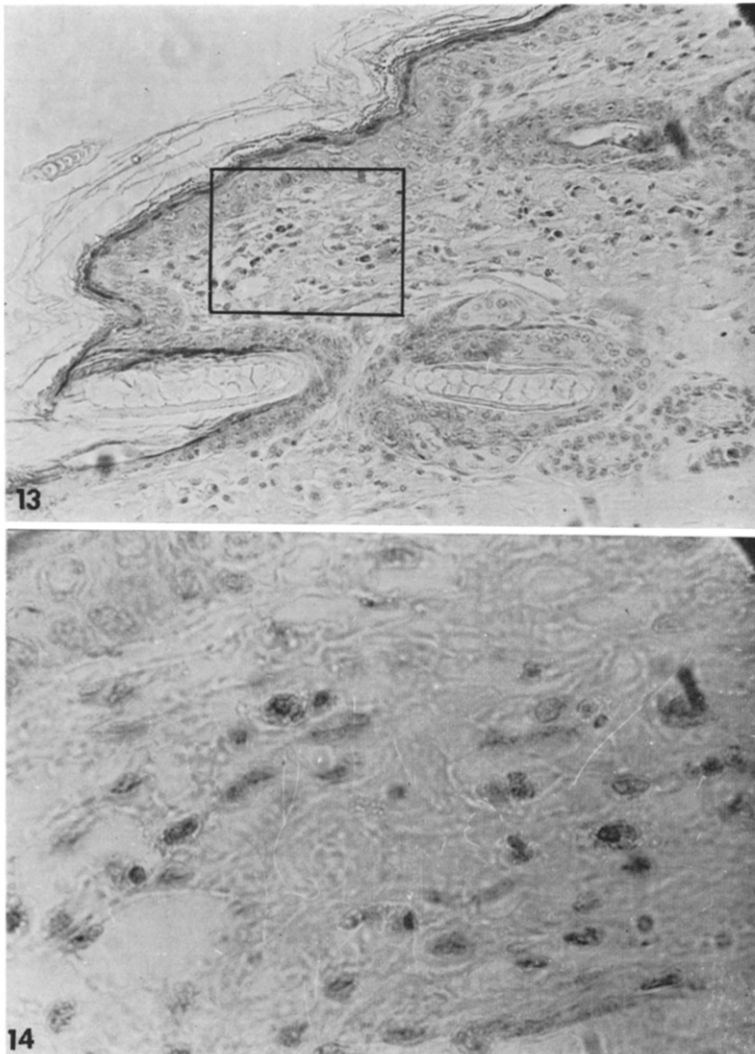


FIG. 13. Guinea pig skin at the stage of immediate reactivity, taken 20 minutes after flea bite; showing eosinophilic infiltration at the bite site. H&E; $\times 125$.

FIG. 14. The framed area of Fig. 13, showing predominant eosinophilic infiltration. $\times 500$.

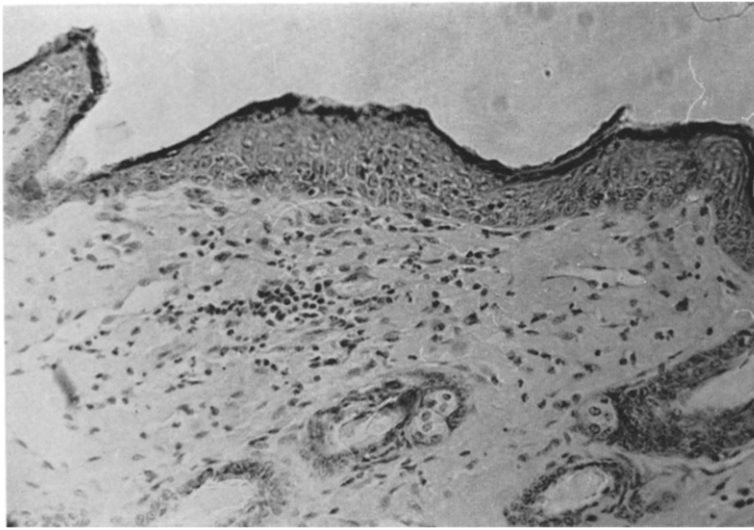


FIG. 15. Guinea pig skin at the stage of immediate reactivity, taken 24 hours after the flea bite; showing persistence of a mild, localized monocytic infiltration and slight hyperplasia. H&E; $\times 125$.

quence of histopathologic events following the bite of arthropods (particularly mosquitoes) on a reactive patient. Biopsies were taken at different time intervals after the bite. At 30 minutes the skin section showed edema with eosinophilic and lymphocytic infiltration. At 24 hours after the bite, there was a decrease in edema and an increase of lymphocytes and eosinophils. At 48 hours a mild edema and an increase of lymphocytes and histiocytes occurred. In subsequent days there was a marked decrease of cellular infiltration.

Benjamini *et al.* (1961) have demonstrated a definite sequence of events in skin reactivity of guinea pigs responding to repeated exposures to flea bites. This suggests that histologic responses to the arthropod's bite may be a function of a particular stage of skin reactivity in the host. Furthermore, the tissue changes probably will conform with histopathologic responses characteristic of immediate and delayed allergic manifestations. The present study corroborates the existence of a definite sequence in stages of skin reactivity in guinea pigs when exposed to flea bites. The study also shows that each stage of this se-

quence is accompanied by distinct and specifically characteristic histologic phenomena.

The present findings suggest a specific relationship between the delayed skin reaction and mononuclear skin infiltration, and between the immediate reaction and eosinophilia. The results further suggest that cellular and morphologic changes are induced, either directly or indirectly, by an antigen-antibody interaction, since such changes appear only in sensitized animals.

ACKNOWLEDGMENT

The authors wish to express their thanks to members of the Communicable Disease Center, San Francisco Field Station, U. S. Public Health Service, for the cooperation and generous use of laboratory facilities.

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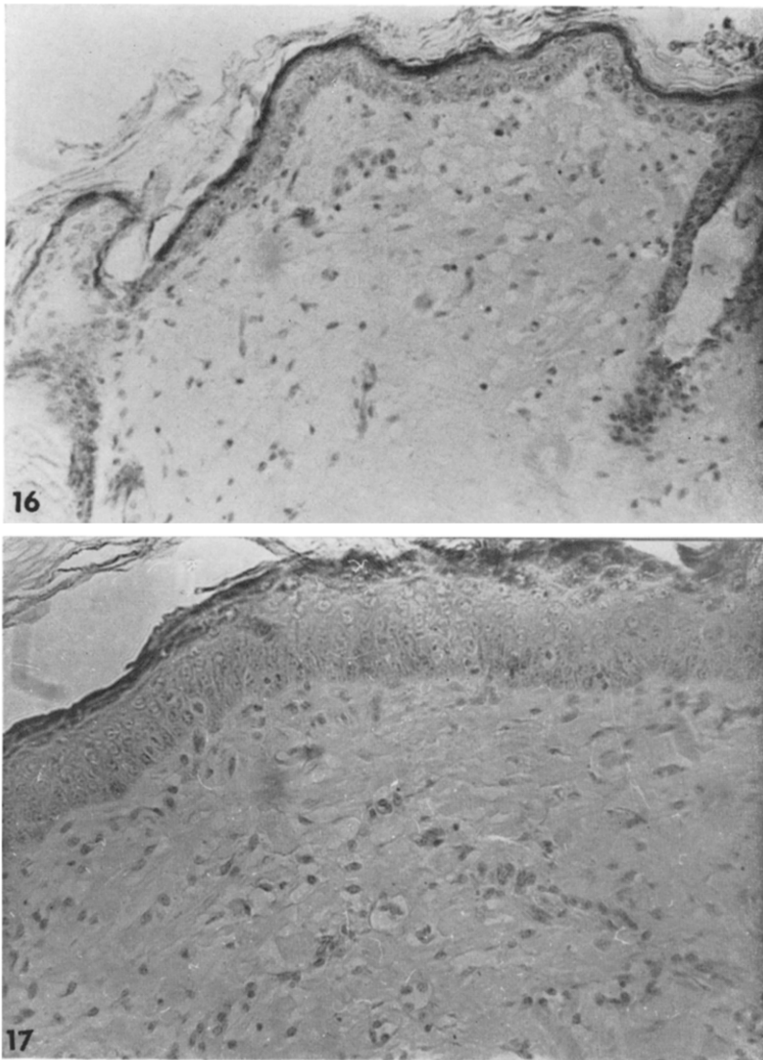


FIG. 16. Guinea pig skin at the stage of nonreactivity, taken 20 minutes after the flea bite. Note the absence of abnormal cellular infiltration. H&E; $\times 125$.

FIG. 17. Guinea pig skin at the stage of nonreactivity, taken 24 hours after the flea bite. Note the complete disappearance of cellular response to the bite. H&E; $\times 125$.

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