# THE HISTOLOGIC FEATURES OF THE HISTOPLASMIN SKIN TEST IN DOGS

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In 1941, van Pernis, Benson and Holinger [1] reported skin testing in histoplasmosis. Zarafonetis and Lindberg [2] described the preparation of an antigen from a culture filtrate and proposed its designation, histoplasmin. Extensive utilization of histoplasmin has established its usefulness in epidemiologic surveys. A histologic study of the cutaneous alterations in the histoplasmic skin test sites, however, has not as yet been reported. The authors have carried out routine histoplasmin testing in the course of investigating canine histoplasmosis.[3,4] The present report is concerned with the histologic features of these skin reactions.

## MATERIAL AND METHODS

Fifty-three dogs (34 male and 19 female) from Cincinnati and its vicinity were used. The mycologic and morphologic findings of the lesions in these animals have been reported elsewhere.[3,4]

For the histoplasmin skin test, 0.1 ml. of undiluted antigen was introduced intracutaneously on the left side of the shaved neck. Forty-eight hours later the reaction was read. In 6 of the 53 dogs, India ink was injected in the region of the test site in an attempt to mark the area for easier localization of the reaction.

The skin and subcutaneous tissue in the test site were excised after 48 hours and fixed in 10 per cent formalin. Paraffin sections were stained with hematoxylin and eosin and by the Grocott [5] and Gridley [6] stains.

## RESULTS

The results of the skin tests in the dogs are shown in Table I. Fourteen dogs (26.5 per cent) had positive reactions, and 39 (73.5 per cent) were negative. No remarkable sex difference was observed. Because of the relatively small number of cases, it is difficult to evaluate variations attributable to age; it appeared, however, that the intensity of positive reactions was less in older animals.

In general, sections at the positive reaction sites showed the following:

A vesicle, erosion or a shallow ulcer of the epidermis was observed in 8 of the 14 reactive lesions (Fig. 1). Moderate to abundant neutrophil infiltration was observed in and about these. In the other 6 specimens

This study was supported in part by Grant E-576 of the National Institutes of Health, United States Public Health Service.

Accepted for publication, January 15, 1962.

there were no remarkable changes in the epidermis. In the dermal papillary layer, hyperemia, edema, and a moderate cellular infiltration were observed (Fig. 1); the latter was composed chiefly of mononuclear cells with abundant basophilic cytoplasm and rounded nuclei with a fine reticular chromatin network. In addition, there were intermingled lymphocytes and neutrophils.

In the reticular layer of the dermis, especially about skin appendages, loosening of connective tissue fibers and an abundant mononuclear cell

TABLE I

EFFECTS (CLINICAL) OF HISTOPLASMIN SKIN TESTS IN STRAY DOGS *

| Age (yr.) | No. examined | Reaction negative (<5 mm.) | Reaction positive (>5 mm.) |
|---|---|---|---|
| 1– 5 | 6 | 4 (66.6%) | 2 (33.4%) |
| 6–10 | 24 | 18 (75.0%) | 6 (25.0%) |
| 11–15 | 22 | 16 (72.7%) | 6 (27.3%) |
| 16–20 | 1 | 1 (100.0%) | 0 |
| Total | 53 | 39 (73.5%) | 14 (26.5%) |

* Intracutaneous injection, 0.1 ml. undiluted histoplasmin; read at 48 hours.

infiltration were noted, often accompanied by a few neutrophils (Fig. 2). Perivenous and perineural cellular infiltrations and leuko-erythrostasis within veins and capillaries were also encountered (Fig. 3). In the subcutaneous tissue, the exudate was more diffuse and consisted chiefly of mononuclear cells, mainly located in perivascular spaces and usually extending into the musculo-fatty layer. In one instance, conspicuous lymph stasis was observed (Fig. 4). Neither necrosis, giant cell reaction nor arterial alterations were evident.

Thus the positive histoplasmin reaction was characterized by a mononuclear cell infiltration accompanied by a moderate number of neutrophils in the dermis and subcutaneous tissue, especially in relation to perivascular areas. The exudate in the rete was not as prominent as that in the reticular layer, and its greatest intensity occurred in the subcutaneous tissue. Hence the distribution of the lesions assumed a conical trapezoid configuration; the epidermis comprised the upper short margin of the trapezoid, and the base of the cone was situated in the subcutaneous tissue.

In most of the negative reactions there was a complete lack of cellular reaction. When an exudate appeared, it was more intense in the papillary than in the reticular layer; no infiltration appeared in the subcutaneous tissue. The configuration of the lesions in the negative reaction was therefore the reverse of that noted above; here the epidermis formed the base of the cone, and its apex extended into the dermis.

The histologic reactions to histoplasmin may be characterized as follows:

(a) Positive reaction: a conical trapezoid, the base placed deeply, the apex at the surface, with a moderate cellular infiltration, chiefly of mononuclear type.

(b) Questionable reaction: a cylindrical lesion with a small or moderate number of exudative cells.

(c) Negative reaction: a complete lack of inflammatory reaction or a minor degree of exudate with a reversed conelike distribution.

Table II indicates that a larger number of positive reactions were recognized histologically than could be detected "clinically." Five of the 39

TABLE II

HISTOLOGIC REACTION TO HISTOPLASMIN SKIN TEST

| Clinical impression of skin test | No. of cases | Negative reaction | Questionable reaction | Positive reaction |
|---|---|---|---|---|
| Negative (<5 mm.) | 39 | 34 | 0 | 5 |
| Positive (>5 mm.) | 14 | 1 | 2 | 11 |
| Total | 53 | 35 (66.0%) | 2 (3.8%) | 16 (30.2%) |

clinically negative reactions proved to be positive histologically. On the other hand, one of the 14 clinically positive cases proved to be histologically negative, and 2 were found to be questionable.

The cases in which India ink was introduced as an indicator adjacent to the histoplasmin injection provided somewhat confusing histologic patterns containing proliferating and actively phagocytic histiocytes.

Of interest is the fact that 2 of the 14 dogs with clinically positive reactions exhibited no pulmonary lesions of histoplasmosis at necropsy (Table III). It is also apparent that the intensity of the histoplasmin skin test did not parallel the titer of the complement fixation test.

## DISCUSSION

The relation of the histoplasmin skin test to histoplasmosis is analogous to that of the tuberculin test to tuberculosis.[7] The histologic features of the intracutaneous tuberculin reactions in human, bovine and experimental tuberculosis have been described.[8–11] The lesion here is characterized by an intense focal accumulation of mononuclear cells accompanied by a diffuse neutrophil infiltration. Hence the lesion is essentially similar to that induced by histoplasmin.

Feldman and Fitch[9] concluded that there was no correlation between the intensity of the histologic lesions and the appearance of the tuberculin test clinically. This was also the case with the histoplasmin test in

dogs. These authors [9,12] also remarked a predilection of the exudate for perivascular and perineural tissues, a giant cell reaction, thrombosis and obliterative endarteritis. Lesions of this nature were not observed in our dogs where the cellular infiltrations were especially prominent about skin

TABLE III

HISTOPLASMIN SKIN TEST IN DOGS; RELATION TO NECROPSY AND LABORATORY OBSERVATIONS

| Age (yr.) | Sex | Histoplasmin reaction | | Pulmonary histoplasmosis (necropsy) | Complement fixation test | | Cultural growth (*H. capsulatum* in pulmonary lesions) |
|---|---|---|---|---|---|---|---|
| | | Clinical (mm.) | Histologic | | A * | B † | |
| 12 | M | 20 | + | + | — | 1/16 | — |
| 10 | M | 20 | + | + | — | — | + |
| 7 | F | 15 | ? | + | 1/4 | 1/16 | + |
| 15 | M | 12 | + | + | 1/4 | 1/64 | + |
| 12 | F | 10 | + | + | 1/8 | 1/64 | — |
| 10 | M | 8 | + | + | — | 1/4 | + |
| 12 | M | 8 | ? | — | 1/4 | 1/32 | — |
| 5 | M | 7 | + | + | — | 1/4 | — |
| 8 | M | 7 | + | + | 1/4 | 1/32 | + |
| 12 | F | 6 | + | — | — | — | — |
| 12 | F | 6 | — | + | 1/4 | 1/16 | — |
| 6 | F | 5 | + | + | 1/4 | 1/32 | — |
| 4 | F | 5 | + | + | — | 1/32 | + |
| 8 | M | 5 | + | + | 1/8 | 1/16 | — |

* A = Antigen used was filtrate of the mycelial phase of *H. capsulatum* H 20 (Parke-Davis).

† B = Antigen used was whole yeast cells of our strain 5, *H. capsulatum*.

appendages known to be surrounded by a dense network of capillaries,[13] and about vessels.

Feldman and Fitch [12] found that the injection of tuberculin into the skin of nonsensitized calves failed to provoke demonstrable changes. The present study has shown that sections in most of the histoplasmin-negative areas exhibited a minor or even a moderate histologic response differing only quantitatively from that observed in the positive reaction.

The cutaneous erosions appearing in some of the positive lesions were probably due in part to the mechanical friction of the epidermal vesicle or degenerated epidermis by the dog's collar.

## SUMMARY

The histologic features of histoplasmin skin reactions have been reported. The pattern of these is similar to that observed in the tuberculin reaction. Exudative cells were chiefly of mononuclear type accompanied by neutrophils. The lesions had characteristic trapezoidal configurations, with the base in the deeper tissues and the apex at the cutaneous surface.

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*[Illustrations follow]*

## Legends for Figures

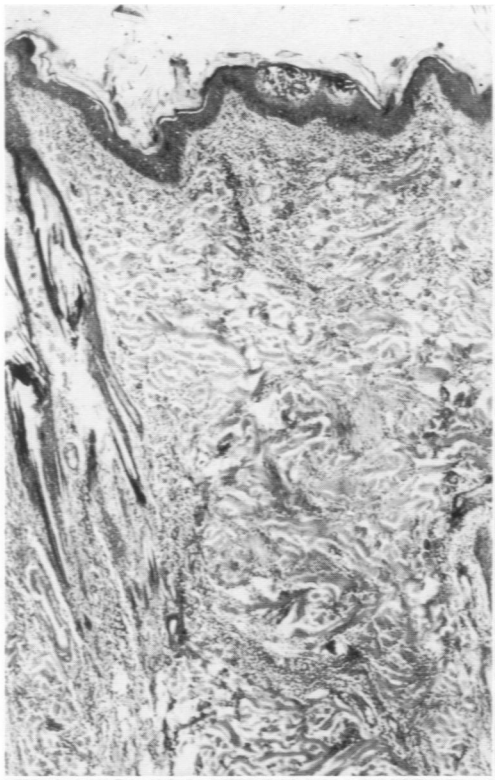
Photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Positive reaction to histoplasmin. An intra-epithelial vesicle is accompanied by a cellular infiltration in the dermis and subcutaneous tissue. × 36.

Fig. 2. A slight mononuclear cell exudate has a perifollicular location. × 150.

Fig. 3. A mononuclear cell infiltration appears in the dermis, especially in a perivenous region. There is no arterial involvement. × 50.

Fig. 4. There is marked dilatation of lymphatics and lymph stasis in the subcutaneous tissue. The cellular exudate is quite marked. × 150.



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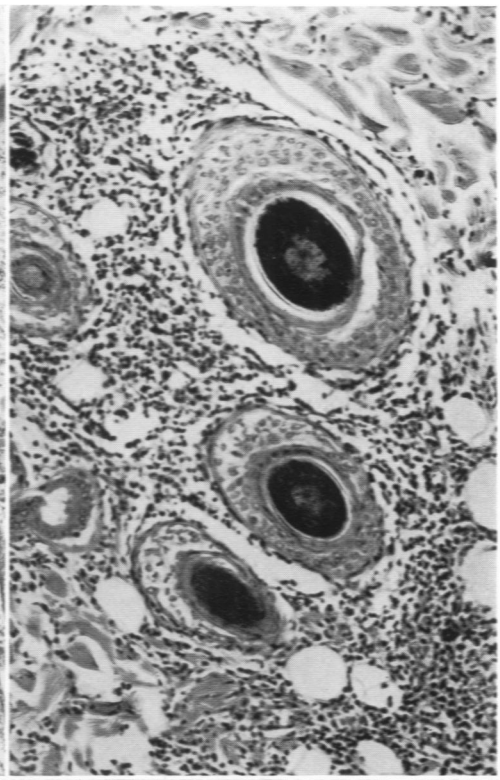

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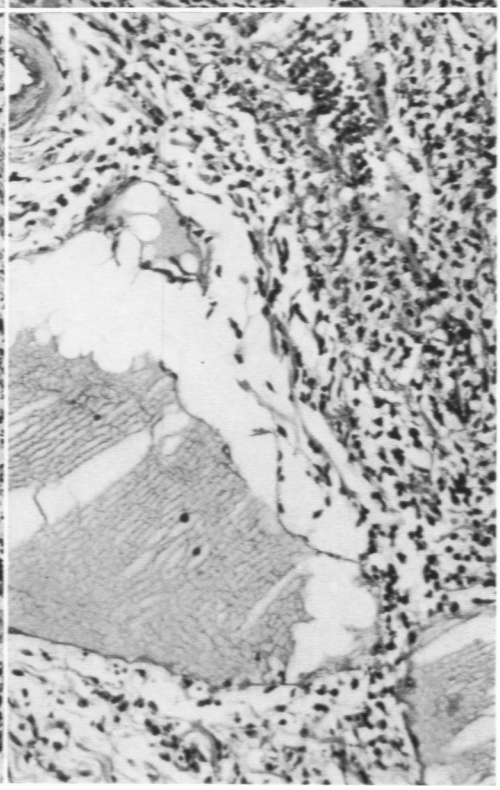

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