

# Interdigital skin test for evaluation of delayed hypersensitivity and cutaneous basophil hypersensitivity in young chickens

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## SUMMARY

A skin test to assess T-cell mediated delayed hypersensitivity (DH) and cutaneous basophil hypersensitivity (CBH) was evaluated in the interdigital skin of young chickens. Three-day-old chickens were sensitized with *Mycobacterium tuberculosis*, and the DH reaction was elicited in the interdigital skin in 10-, 17-, 24-, and 31-day-old chickens by intradermal injection of tuberculin. Cutaneous basophil hypersensitivity was elicited in the interdigital skin of 10- and 14-day-old chickens by a single intradermal injection of phytohemagglutinin-P (200  $\mu$ g). The effect of immunosuppression on the results of interdigital skin test for DH and for CBH was evaluated in chickens that were treated with dexamethasone daily for 4 days before testing.

The DH reaction, as indicated by a significant ( $P < 0.01$ ) increase in the mean interdigital skin thickness, was detectable in 10-day-old chickens and was consistently evident in 17-, 24-, and 31-day-old chickens. The DH response in the interdigital skin of 24-day-old chickens was comparable with that elicited in the standard wattle test. The CBH reaction, as indicated by a significant increase ( $P < 0.005$ ) in skin thickness, was evident in the interdigital skin of 10- and 14-day-old chickens. Treatment with dexamethasone significantly decreased ( $P < 0.01$ ) the DH and CBH reactions. Results of the study indicated that the interdigital skin test may be used to evaluate normal and suppressed cell-mediated DH and CBH reactions in chickens as young as 10 and 14 days old.

Cell-mediated immunity may be evaluated in chickens by induction of T-cell-mediated delayed hypersensitivity (DH)<sup>1-6</sup> and cutaneous basophil hypersensitivity (CBH)<sup>7-12</sup> reactions. Skin testing is especially useful as a simple in vivo screening to evaluate DH and CBH reactions in chickens and for detection of deficient T-cell-mediated response.<sup>1-6,8-12</sup> Because most feathered skin of chickens is too thin for adequate intradermal injections,<sup>9</sup> the wattle is the standard site for skin testing; however, in young chickens < 2 or 3 weeks old, the wattle is undeveloped and intradermal injection and measurement of response

are difficult.<sup>13</sup> A simple skin test to assess cell-mediated immunocompetency in chickens < 3 weeks old would be especially useful. The purpose of the study reported here was to evaluate the interdigital skin as a skin test site for eliciting DH and CBH reactions in young chickens. Further, we evaluated the effect of immunosuppression on both types of T-cell-mediated reactions in the interdigital skin.

## Materials and Methods

**Chickens**—One-hundred forty-four 1-day-old male broiler chickens (Hubbard  $\times$  Hubbard) were obtained from a commercial hatchery, placed in electrically heated starter batteries<sup>a</sup> under fluorescent lighting, and provided feed and water ad libitum. The feed consisted of a commercial unmedicated corn-soybean meal-based diet that contained or exceeded levels of critical nutrients recommended by the National Research Council.<sup>14</sup> At 21 days of age, chickens were transferred to growing batteries,<sup>a</sup> were allotted at random into 3 groups of 48 chickens each, and identified by wing band number.

**Interdigital skin test for DH**—Each chicken was sensitized to *Mycobacterium tuberculosis* (H37R<sub>a</sub>) at 3 days of age by SC injection over the pectoral muscle with Freund complete adjuvant<sup>b</sup> containing 0.2 mg (group 1), 0.5 mg (group 2), or 1.0 mg (group 3) of the organism. Twelve chickens from each group were selected at random on days 7, 14, 21, and 28 after sensitization and injected intradermally between the third and fourth digits of the right foot with 0.05 ml of purified-protein derivative<sup>c</sup> (PPD) diluted 1:1 with physiologic saline solution (PSS). Preliminary studies (data not shown) indicated that tuberculin PPD produced from strains of *M bovis*<sup>c</sup> or strains of *M tuberculosis*<sup>d</sup> elicited equally intense DH reactions in chickens sensitized with *M tuberculosis* in Freund complete adjuvant. Tuberculin PPD bovis was, therefore, used during the present study. The skin between the third and fourth digits of the left foot of each chicken was injected with 0.05 ml of PSS (control).

Skin thickness was measured before injection, and 24 and 48 hours after injection by use of a constant-tension

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<sup>b</sup> Sigma Chemical Co, St Louis, Mo.

<sup>c</sup> USDA, Animal and Plant Health Inspection Service, Ames, Iowa.

<sup>d</sup> Statens Seruminstitut, Copenhagen, Denmark.



dial micrometer.<sup>o</sup> The intensity of the DH reaction was calculated by 2 methods: (1) Increased skin thickness = (postinjection skin thickness, right foot) - (preinjection skin thickness, right foot); and (2) DH = (PPD response, right foot) - (PSS response, left foot).

The interdigital skin test was conducted on 12 nonsensitized chickens at 7, 14, 21, and 28 days of age. The chickens served as controls to evaluate the response to PPD injection in chickens non sensitized to *M tuberculosis*.

**Comparison of interdigital skin and the wattle tests**—Delayed hypersensitivity reactions elicited in the interdigital skin and in the wattle were compared in chickens sensitized with of *M tuberculosis* (0.2 mg in Freund complete adjuvant<sup>b</sup>) at 3 days of age. Twenty-one days after sensitization, the interdigital skin of the right foot and the right wattle of each chicken were injected with 0.05 ml of PPD diluted 1:1 with PSS. The left foot and left wattle were similarly injected with 0.05 ml of PSS. The DH reaction in the interdigital skin and in the wattle were measured 24 and 48 hours after injection as previously described.

**Effect of dexamethasone on the DH reaction in interdigital skin**—The effect of treatment with dexamethasone on the DH reaction elicited in the interdigital skin was determined in chickens sensitized with *M tuberculosis* (0.5 mg in Freund complete adjuvant) at 3 days of age. The sensitized chickens were injected SC with 4 mg of dexamethasone<sup>f</sup>/kg of body weight daily for 4 days prior to skin testing on days 14, 21, and 28 after sensitization. Twelve chickens were skin tested at each time point, and the DH reaction was measured as previously described.

**Interdigital skin test for CBH**—Ten-day-old and 14-day-old chickens were injected intradermally between the third and fourth digits of the right foot with 200 µg of phytohemagglutinin-P (PHA-P)<sup>g</sup> in 0.10 ml of PSS. The left foot was similarly injected with 0.10 ml of PSS. Twelve chickens were skin tested in each age group, and reactions were evaluated 12 and 24 hours after injection by measurement of skin thickness.

**Effect of dexamethasone on the CBH reaction in interdigital skin**—The effect of treatment with dexamethasone on the interdigital skin reaction elicited by PHA-P was determined in chickens that were injected SC with 4 mg of dexamethasone<sup>f</sup>/kg of body weight daily for 4 days prior to skin testing at 10 days and 14 days of age. Twelve dexamethasone-treated chickens were tested in each age group, and the reaction was measured and compared with that of nontreated groups of chickens of the same age.

**Histologic lesions**—Tissue specimens were collected from the interdigital test sites 24 hours after PPD skin testing on day 21 and 12 hours after skin testing with PHA-P in 14-day-old chickens. Specimens were fixed in 10% formalin, sectioned at 5 µm, and stained with hematoxylin and eosin for microscopic examination.

**Statistical analyses**—Data were analyzed by the Student *t* test for significant differences among groups.

<sup>o</sup> Mitutoyo Co, Tokyo, Japan.

<sup>f</sup> Azium, Schering Corp, Kenilworth, NJ.

<sup>g</sup> Wellcome Diagnostics, Dartford, England.

## Results

**Interdigital skin test for DH**—The skin reaction was maximal 24 hours after PPD injection and remained constant or decreased at 48 hours after injection. Therefore, skin measurements were determined 24 hours after PPD injection.

A significant ( $P < 0.01$ ) increase in the mean postinjection skin thickness compared with preinjection thickness was elicited by PPD on day 7 after sensitization with 0.2 mg of *M tuberculosis* (Table 1). The mean postinjection skin thickness increased significantly ( $P < 0.01$ ) in each of the groups of chickens during skin tests conducted on days 14, 21, and 28 after sensitization (Table 1). The interdigital skin reaction expressed as increase in skin thickness (Table 1), or expressed as the difference between the PPD and the PSS elicited responses (Table 2) were similar numerically.

The interdigital skin reaction first detectable at 7 or 14 days after sensitization was more marked during skin tests conducted on days 21 and 28 as indicated by a progressive increase in skin thickness (Tables 1, 2, 3). The maximal increase in skin thickness elicited during skin testing developed in the chickens sensitized with 0.2 mg of *M tuberculosis*, compared with chickens sensitized with

Table 1—Interdigital skin thickness elicited by purified protein derivative (PPD) in chickens sensitized with *Mycobacterium tuberculosis* at 3 days of age

Dose (mg)	Days after sensitization	Age (c)	Preinjection thickness (mm)	Postinjection thickness (mm)	Increase in thickness (mm)*
Group 1 (0.2)	7	10	1.07 ± 0.05	1.19 ± 0.13†	0.12
		14	1.34 ± 0.14	1.57 ± 0.15†	0.23
		21	1.49 ± 0.15	1.86 ± 0.21†	0.37
		28	1.52 ± 0.13	1.94 ± 0.30†	0.42
Group 2 (0.5)	7	10	1.05 ± 0.07	1.09 ± 0.11	0.04
		14	1.24 ± 0.12	1.45 ± 0.13†	0.21
		21	1.44 ± 0.12	1.69 ± 0.17†	0.25
		28	1.60 ± 0.15	1.88 ± 0.31†	0.28
Group 3 (1.0)	7	10	1.10 ± 0.09	1.11 ± 0.08	0.01
		14	1.24 ± 0.11	1.44 ± 0.12†	0.20
		21	1.39 ± 0.10	1.74 ± 0.12†	0.35
		28	1.55 ± 0.13	1.86 ± 0.26†	0.31

\* Increase = (postinjection skin thickness) - (preinjection skin thickness).  
† Significantly ( $P < 0.01$ ) different from preinjection thickness.  
Data are expressed as mean ± SD of 12 chickens.

Table 2—Interdigital skin reaction elicited by PPD in chickens sensitized with *M tuberculosis* at 3 days of age

Dose (mg)	Days after sensitization	Age (d)	PSS reaction left foot (mm)	PPD reaction right foot (mm)	Interdigital skin reaction (mm)*
Group 1 (0.2)	7	10	1.09 ± 0.07	1.19 ± 0.13†	0.10
		14	1.32 ± 0.16	1.57 ± 0.15†	0.25
		21	1.45 ± 0.12	1.86 ± 0.21†	0.41
		28	1.48 ± 0.15	1.94 ± 0.30†	0.46
Group 2 (0.5)	7	10	1.09 ± 0.11	1.10 ± 0.11	0.01
		14	1.28 ± 0.10	1.45 ± 0.13†	0.18
		21	1.49 ± 0.14	1.69 ± 0.17†	0.20
		28	1.58 ± 0.11	1.88 ± 0.31†	0.30
Group 3 (1.0)	7	10	1.10 ± 0.06	1.10 ± 0.09	0.00
		14	1.26 ± 0.06	1.44 ± 0.12†	0.18
		21	1.45 ± 0.08	1.74 ± 0.12†	0.29
		28	1.48 ± 0.15	1.86 ± 0.26†	0.38

\* Interdigital skin reaction = (PPD reaction) - (PSS reaction). † Significantly ( $P < 0.025$ ) different from PSS reaction. ‡ Significantly ( $P < 0.01$ ) different from PSS reaction.  
PSS = physiologic saline solution.  
Data are expressed as mean ± SD of 12 chickens.



Table 3—Effect of sensitization with 0.2, 0.5, or 1.0 mg of *M tuberculosis* on the interdigital skin reactions elicited by PPD

Days after sensitization	Interdigital skin reaction (mm)*		
	0.2 mg	0.5 mg	1.0 mg
7	0.10 ± 0.13†	0.01 ± 0.02	0.02 ± 0.03
14	0.25 ± 0.14	0.18 ± 0.07	0.18 ± 0.09
21	0.41 ± 0.24‡	0.20 ± 0.09	0.29 ± 0.08
28	0.47 ± 0.30	0.30 ± 0.30	0.38 ± 0.17

\* Interdigital skin reaction = (PPD reaction, right foot) - (PSS reaction, left foot). † Significantly ( $P < 0.05$ ) different from other groups on day 7. ‡ Significantly ( $P < 0.01$ ) different from 0.5 mg group on day 21.  
Data are expressed as mean ± SD of 12 chickens.

Table 4—Effect of dexamethasone on the interdigital skin reaction elicited by PPD in chickens sensitized with *M tuberculosis*

Days after sensitization	Increased skin thickness (mm)*		DH reaction (mm)†	
	Non-treated	Dexamethasone	Non-treated	Dexamethasone
14	0.21 ± 0.18	0.19 ± 0.12	0.18 ± 0.07	0.13 ± 0.09
21	0.25 ± 0.17	0.09 ± 0.13‡	0.20 ± 0.09	0.03 ± 0.04‡
28	0.28 ± 0.27	0.08 ± 0.13‡	0.30 ± 0.30	0.12 ± 0.14§

\* Increased skin thickness = (postinjection thickness) - (preinjection thickness). † Delayed hypersensitivity reaction = (PPD reaction) - (PSS reaction). ‡ Significantly ( $P < 0.01$ ) different from nontreated. § Significantly ( $P < 0.05$ ) different from nontreated.  
Data are expressed as mean ± SD of 12 chickens.

0.5 mg or 1.0 mg of *M tuberculosis* (Table 3). The intradermal injection of PPD in chickens not sensitized with *M tuberculosis* failed to elicit an interdigital skin reaction.

**Comparison of interdigital and wattle skin tests**—A significant ( $P < 0.05$ ) increase in skin thickness developed in the right wattle and the interdigital skin of the right foot. The mean increase in wattle thickness ( $0.52 \pm 0.31$ ) was not significantly different from the increase in interdigital skin thickness ( $0.37 \pm 0.29$ ). Likewise there was no significant difference between the wattle ( $0.36 \pm 0.14$ ) and the interdigital skin ( $0.41 \pm 0.24$ ) reactions when expressed as the difference in their respective PPD and PSS elicited responses.

**Effect of dexamethasone on the DH reaction**—Treatment with dexamethasone had no apparent effect on the skin-test response on day 14 after sensitization (Table 4). The interdigital skin reaction in the dexamethasone treated chickens was significantly ( $P < 0.01$ ) decreased as compared with nontreated chickens during skin tests conducted on days 21 and 28 after sensitization.

**Interdigital skin test for CBH**—The skin reaction was maximal at 12 hours after injection and remained constant or decreased at 24 hours. A significant ( $P < 0.005$ ) difference in the mean skin thickness was detected in the PHA-P injection sites, compared with the PSS-injected control sites in 10-day-old ( $1.63 \pm 0.20$  vs  $0.97 \pm 0.10$ ) and 14-day-old ( $1.78 \pm 0.29$  vs  $1.10 \pm 0.09$ ) chickens.

**Effect of dexamethasone on the CBH reaction**—The interdigital skin reaction elicited by PHA-P was significantly ( $P < 0.01$ ) less in the dexamethasone treated group than that detected in the nontreated group in 10-day-old ( $0.49 \pm 0.15$  vs  $0.66 \pm 0.17$ ) and 14-day-old ( $0.46 \pm 0.14$  vs  $0.68 \pm 0.23$ ) chickens.

**Histologic lesions**—The interdigital skin reaction evident 24 hours after intradermal injection of PPD was characterized by edema and the infiltration of the dermis with numerous mononuclear cells and fewer numbers of heterophils. The population of mononuclear cells was frequently more numerous in the perivascular tissue.

The inflammatory response elicited in the interdigital skin 12 hours after injection with PHA-P was characterized predominately by edema and the diffuse infiltration of the dermis with heterophils and fewer numbers of small mononuclear cells. Nearly all the mononuclear cells were located adjacent to capillaries and venules.

## Discussion

Delayed hypersensitivity reactions have been induced in chickens sensitized to tuberculin,<sup>1,3,5,15</sup> diphtheria toxoid,<sup>2,4</sup> *Salmonella* sp,<sup>3</sup> *Eimeria tenella*,<sup>13</sup> bovine serum albumin,<sup>5</sup> and human globulin.<sup>5,6,16</sup> The wattle skin test is commonly used to evaluate in vivo DH reactions in chickens and is considered to be a valid method of assessing T-cell-mediated immunity.<sup>1-6</sup> Because of small size, the wattle is not a suitable site for skin testing chickens < 2 or 3 weeks old. Data from our study indicated that chickens can be successfully sensitized with *M tuberculosis* at 3 days of age and the DH reaction elicited in the interdigital skin by the intradermal injection of PPD as early as 7 or 14 days later in chickens 10 and 17 days of age. The DH response was readily detectable in the interdigital skin by micrometer measurement of skin thickness in 10- and 17-day-old chickens. The thickened interdigital skin accompanied by erythema was visible in 24- and 31-day-old chickens that were skin tested 3 and 4 weeks after sensitization. As was previously reported,<sup>5</sup> and as developed during the present study, sensitization of young chickens with increasing doses of *Mycobacterium* did not enhance the DH reaction and apparently diminished reactivity (Table 3).

Comparison of the DH reactions elicited in the wattle and in the interdigital skin on day 21 after sensitization (24-day-old chickens) indicated that the response was similar in both test sites. In previous studies, PPD-elicited increases in wattle thickness of > 0.3 mm were considered as a positive DH reaction.<sup>5</sup> The increase in interdigital skin thickness, calculated by either of the 2 methods used during the study, exceeded 0.3 mm and was not significantly different from the increase that developed in wattle thickness. In our opinion, intradermal injections were more easily performed in the interdigital skin than in the wattle. In addition, skin thickness was more accurately determined by caliper measurement in the interdigital skin than in the wattle.

The inflammatory response elicited in the interdigital skin of chickens sensitized to PPD was microscopically the same as that reported to develop in the wattle.<sup>4,11</sup> Results indicated that both sites are equally suitable for eliciting the DH reaction.

Cutaneous basophil hypersensitivity elicited by a single intradermal injection of PHA is reported to be thymus-dependent<sup>7-12</sup> and has been used to evaluate T-cell-mediated immune response in chickens.<sup>8-12</sup> The CBH response differs from classic DH in that the reaction is elicited by a single injection of mitogen and is characterized by the infiltration and participation of basophils.<sup>9,11,17-20</sup> De-



granulating basophils may release vasoactive amines that initiate vascular changes resulting in an edematous reaction characteristic of an early inflammatory response.<sup>20</sup> The CBH response elicited by PHA-P during the present study was characterized by edema and the infiltration of heterophils and mononuclear cells identical to that reported in the wattle of older chickens.<sup>9,10,17-20</sup> Subsequently, even though T lymphocytes are apparently an essential element in the CBH response,<sup>7-12</sup> the skin reaction is indicative of acute inflammation.

Intradermal injection of 50 to 250 µg of PHA has been used to elicit positive CBH reactions in the wattle of chickens.<sup>8-10,12,21-23</sup> The 200-µg PHA-P dose used in the study reported here was, therefore, comparable with doses used to elicit the wattle response. An increase in wattle thickness of 0.34 mm to 0.94 mm was reported in 2-week-old chickens after a single injection of various dilutions of PHA-P.<sup>12</sup> Increases in wattle thickness ≥ 0.5 mm were reported in chickens ≥ 6 weeks of age.<sup>8,10</sup> The CBH reaction elicited in the interdigital skin of 10- and 14-day-old chickens by injection of PHA-P during the study was readily detectable by gross examination. The increase in interdigital skin thickness of 0.66 mm to 0.69 mm was comparable with that reported in the wattles of 2-week-old chickens<sup>12</sup> and in chickens ≥ 6 weeks of age.<sup>8,10</sup> Because the CBH reaction is elicited by a single injection of mitogen without prior sensitization, the PHA-P skin test is a rapid method of assessing T-cell-immune response in vivo.<sup>8</sup> The data from this study suggest that T-cell-mediated CBH reaction may be evaluated by the interdigital skin test in chickens as early as 10 days of age.

Treatment with dexamethasone immediately prior to skin testing significantly ( $P < 0.01$ ) decreased the PPD elicited DH reaction and the PHA-P-elicited CBH reaction in the interdigital skin of chickens. The anti-inflammatory immunosuppressive effects of dexamethasone on cell-mediated immunity have been studied extensively in human beings and animals.<sup>24-26</sup> Several studies have examined the effects of dexamethasone on adrenocortical activity in chickens<sup>27,h</sup> however, the effects of adrenocortical hormones and corticosteroids on avian cell-mediated immune responses are not as well understood as in other animal species.<sup>16,22,23,28,29</sup> Corticosterone has been reported to prolong homograft survival in chickens<sup>29</sup> and treatment with adrenocorticotropin prior to the wattle skin test was reported to suppress DH reactions to human γ-globulin<sup>16</sup> and CBH reactions elicited by PHA-P.<sup>22</sup> Because the effects of dexamethasone on avian immune response is not well documented, the dosage used in this study was selected to be higher than the reported pharmacologic dosage of 2 to 3 mg/kg.<sup>27,h</sup> The decreased DH reactions in dexamethasone-treated chickens suggested that the drug has a suppressive effect on avian T-cell-mediated immune responses similar to that reported in other animals. The suppressed DH reactions were readily detectable by the interdigital skin test in 24- and 31-day-old chickens. Because the CBH reaction is partly an inflammatory response, the suppressive effect of dexamethasone on the CBH response in the 10- and 14-day-old chickens may have resulted from impaired T-lymphocyte activity and/or the anti-inflammatory effect of the drug.

The results of the study indicated that the interdigital

skin is a suitable test site for the evaluation of DH and CBH reactions in chickens < 3 weeks old. Seemingly, the interdigital skin test may provide a simple rapid method of evaluating T-cell responsiveness in vivo in young chickens and for detecting deficient T-cell activity in immunosuppressed chickens.

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