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Immediate, Arthus, and delayed-type skin
reactions in rhesus monkeys infected with
Schistosoma mansoni or mycobacteria

**Shirley E. Maddison, Ph.D., Martin D. Hicklin, M.D., and
Irving G. Kagan, Ph.D. Atlanta, Ga.**

Gross and histologic observations of immediate, Arthus, and delayed-type skin reactivities in rhesus monkeys infected with Schistosoma mansoni or mycobacteria are described. Edema without erythema occurred in the eyelid and on the chest of animals with immediate-type hypersensitivity. Eosinophils characterized the cell infiltrations. Arthus reactivity resulted in blanching of the skin test sites and in a polymorphonuclear neutrophilic infiltration. Erythema was stimulated by intrapalpebral testing of animals with delayed hypersensitivity, but in these animals the chest site or back again showed a blanched area of induration; a large mononuclear, epithelioid cell type of infiltration was characteristic of this reactivity.

In our studies on immediate-type hypersensitivity and cell-mediated immunity in the rhesus monkey (*Macaca mulatta*) we were unable, for a considerable period of time, to evaluate either the host response or the efficacy of antigens by means of skin tests. The gross reactions we observed could not be characterized according to reported skin reactivity in the monkey. Skin reactions in species other than the monkey have also deviated from the classical pictures observed in the guinea pig and man.

A comparison of the stimulation and subsequent demonstration of delayed hypersensitivity in the rat with those in the guinea pig and rabbit was made

From the Parasitology Branch and Pathology Section, Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, U. S. Department of Health, Education, and Welfare.

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Reprint requests to: Dr. Shirley E. Maddison, Parasitology Branch, Center for Disease Control, Atlanta, Ga. 30333.

by Flax and Waksman.¹ They showed that higher doses of skin test antigen are required to elicit delayed-type skin reactions in the rat, and that the frequent absence of erythema in the reaction sites correlated with the distribution of cutaneous venous blood vessels. The marked differences in the skin of various species of nonhuman primates observed by Montagna² relate to gross appearance, including hair and pigmentation, and glands; cutaneous hypersensitivity reactions were not discussed.

Gershon and Hencin³ studied delayed-type skin tests in the flank of mice and hamsters. The addition of normal peritoneal cells to the antigen injected intradermally was found to be essential for stimulation of a characteristic delayed hypersensitivity reaction. These authors suggest that some species, such as the mouse and hamster, in which delayed hypersensitivity skin reactions are poor, may be unable to recruit the nonimmune cells essential for the reaction to the site.

Immediate-type hypersensitivity in the rhesus monkey has been stimulated by injection of *Ascaris* antigen⁴⁻⁶ and by bacterial enzymes in laundry detergents.⁷ Some investigators⁴ administered Evans blue dye intravenously to demonstrate increased capillary permeability in skin test sites that were positive at 30 minutes; others^{6, 7} described wheal-and-flare reactions at 20 to 30 minutes. With neither observation was the cellular infiltration described.

Delayed-type hypersensitivity has been demonstrated in rhesus monkeys infected with mycobacteria by a number of investigators.⁸⁻¹² While erythema has been described by some, the site of skin testing has frequently not been mentioned. Rhesus monkeys sensitized to p-azobenzene-*ortho*-N-acetyl tyrosine⁸ developed delayed hypersensitivity evidenced by induration. Trinitrophenyl-keyhole limpet-hemocyanin⁹ stimulated precipitating antibodies and delayed hypersensitivity. Skin testing elicited an erythematous Arthus reaction at 5 hours, which histologically progressed to a delayed hypersensitivity response by 24 to 48 hours. This was manifested by a predominantly polymorphonuclear cell infiltration at 5 hours and a shift to a mononuclear infiltration at 24 and 48 hours.

Stucki¹³ injected various *Schistosoma mansoni* antigens into sites on the backs of rhesus monkeys and studied the reactions. Wheals were measured by imprint after 15 minutes. A description of the skin test sites was not included in this report.

In our initial studies of *S. mansoni* infection in the rhesus monkey,¹⁴ erythema was not evident either at 15 minutes or at 24 to 72 hours when animals were skin-tested on the chest. At 15 minutes, a wheal at the antigen site that was greater than twice the size of the control was considered indicative of an immediate reaction. Although *S. mansoni* infection may stimulate delayed hypersensitivity in man,¹⁵ we were unable to obtain convincing gross evidence of this state in our infected animals. However, biopsies and histologic studies of skin test sites enabled us to confirm immediate and identify delayed type response in the rhesus monkey following injection on the chest or back. We later used this technique to assess the delayed hypersensitivity to *S. mansoni* or tuberculin conferred to normal recipient monkeys by injection of transfer

TABLE I. Gross and histologic observations of skin test reactions

Species	Site		Immediate 15 minutes	Arthus 5 hours	Delayed 72 hours
Rhesus	Eye	Gross	Edema and blanching	Edema and blanching	Slight edema and erythema
	Eye	Histologic	ND*	ND	ND
	Chest	Gross	Wheal and blanching	Wheal and blanching	Blanched area of induration
	Chest	Histologic	Eosinophils†	Neutrophils†	Large epithelioid cells†
	Back	Gross	Wheal and blanching	ND	Blanched area of induration
	Back	Histologic	Eosinophils	ND	Large epithelioid cells
Baboon	Chest	Gross	Wheal and flare	ND	ND
	Chest	Histologic	Eosinophils	ND	ND
Vervet	Chest	Gross	Wheal and blanching	ND	ND
	Chest	Histologic	Eosinophils		
Capuchin	Chest	Gross	Wheal and blanching	ND	ND
	Chest	Histologic	Eosinophils		

*ND = Not done.

†Characteristic cell of infiltration.

factor.¹⁶ Arthus reaction in the *S. mansoni* system was also studied histologically in the rhesus monkey. These observations and limited studies in three other species of nonhuman primates comprise the body of this report.

MATERIALS AND METHODS

Induction of immediate hypersensitivity

Sixteen rhesus monkeys were infected with *S. mansoni*; the duration of the respective infections varied from one to 3 years.¹⁴ Each animal was subsequently reexposed to cercariae from one to 3 times. In addition, 4 monkeys with single exposure to *S. mansoni* were skin-tested.

Assays for immediate hypersensitivity

The stimulation of reaginic antibody was demonstrated by skin testing, using the alcohol imprint method¹⁷ for recording results. The test was read after 15 minutes by circling the wheal with a ball-point pen and pressing a sheet of paper that had been slightly moistened with alcohol over the wheal to transfer the tracing. The areas of the wheals were measured from the paper tracings by means of a plastic grid. Immediate hypersensitivity was confirmed by passive cutaneous anaphylaxis (PCA) tests in normal rhesus recipients. For PCA testing,¹⁴ undiluted antisera were injected intradermally on the cheek, eyelid, and chest. Forty-eight hours later the animals were injected intravenously with a mixture of antigen and Evans blue dye. A blue zone developing within 30 minutes at the site of serum injection was considered positive.

Induction of delayed hypersensitivity

Twenty rhesus monkeys were infected with *Mycobacterium kansasii*, *Mycobacterium intracellulare*, *Mycobacterium scrofulaceum*, or *Mycobacterium bovis* (BCG), and were made available to us by Dr. K. D. Stottmeier and Mr. R. E. Beam of the Mycobacteriology Unit, Center for Disease Control. The animals were reinfected approximately 2 years after the first exposure to mycobacteria, and during the 2 year period they had been repeatedly skin-tested with old tuberculin. Transfer factor and skin test studies were initiated with this group



FIG. 1. Rhesus monkey infected with *S. mansoni*. Control saline solution injected into right eyelid and side of chest. Corresponding injections of *S. mansoni* antigen were given on left side. Photographed 15 minutes after injection. Note wheal and pseudopodia on left side of chest. Edema in left eyelid does not show up well. No erythema in eyelid or on chest.

of animals approximately one month after the reinfection with mycobacteria.

Specific delayed hypersensitivity to *S. mansoni* and tuberculin was conferred on 8 and 11 normal rhesus monkeys, respectively, by dialyzable or nondialyzable transfer factor prepared from cell lysates from monkeys infected with *S. mansoni* or mycobacteria or from humans with delayed hypersensitivity to purified protein derivative of tuberculin (PPD). The method of preparation of the transfer factors from peripheral blood leukocytes, the administration, and the assay for subsequent delayed hypersensitivity in the recipients have been detailed previously.¹⁶

Nonhuman primates studied other than the rhesus monkey

At the Southwest Foundation for Research and Education, San Antonio, Texas, through the kindness of Drs. R. E. Kuntz and B. J. Myers, immediate-type hypersensitivity was studied in 5 baboons (*Papio cynocephalus*), 3 capuchin monkeys (*Cebus apella*), and one vervet monkey (*Cercopithecus aethiops*). These animals were infected with various *Schistosoma* spp. and were skin-tested on the chest with *S. mansoni* antigen. The alcohol imprint method was used to record all reactions and, in addition, biopsies were performed on control and test sites of one baboon, one capuchin, and the vervet monkey.

Antigens

An extract of *S. mansoni* adult worms¹⁸ (0.04 mg. N per dose) and 0.005 mg. tuberculin PPD (Parke, Davis & Co., Detroit, Michigan)* in 0.1 ml. volumes were used as skin test antigens.

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the United States Department of Health, Education, and Welfare.

Skin testing and histologic examinations

Intradermal testing was done under Sernylan anesthesia (Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri). We could find no evidence of this drug affecting any of the three types of skin reactivity. Tests were done on the eyelid or on the chest or back. Dye was not used. A 5 mm diameter skin punch was used for biopsies of sites on the chest or back at 15 minutes or 72 hours after skin testing. Biopsies were not performed on intrapalpebral sites. In a few instances, sequential biopsies at 15 minutes and at 5 and 24 hours were performed on the back of each animal. All animals in the transfer factor study were skin-tested on the back. The biopsy tissues were immediately fixed in buffered formalin, and sections were stained with hematoxylin and eosin. Special observation periods to study evidence of irritation of skin test sites by scratching were not set up. None of the animals scratched the sites while the investigators were present for periods up to half an hour within the 72 hour period. However, during these periods the attention of the animals was essentially focused on the investigators.

Induction of Arthus reactivity

Arthus type reactivity was studied by gross observation and by biopsy of skin test sites at 5 hours after injection of rhesus monkeys infected with *S. mansoni*. In addition, 2 uninfected animals were injected intradermally with *S. mansoni* antigen/antibody complex, and biopsy of the injection sites was performed at intervals from 15 minutes to 72 hours. The complexes were prepared by mixing the *S. mansoni* antigen with a pool of infected monkey sera with high titers in complement fixation ($1/64$ to $1/256$) and bentonite flocculation ($1/80$ to $1/320$). The sera were collected from animals 6 to 7 weeks after infection with *S. mansoni*, at which time reaginic antibody is not stimulated.¹⁴ The antigen/antibody mixtures were allowed to react at 37° C. for one hour and then were placed at 4° C. overnight. Little visible precipitate was formed, and this was removed by centrifugation at 200 G for 15 minutes prior to injection.

RESULTS

The gross and histologic observations of reactions at the various skin test sites are summarized in Table I.

Immediate hypersensitivity

Reaginic antibody was demonstrable by skin tests and PCA tests in all animals following multiple exposure to *S. mansoni*. Intrapalpebral and chest skin test reactions at 15 minutes to *S. mansoni* antigen are shown in Fig. 1.* The animal's right eyelid and right side of the chest were injected with a control saline solution; corresponding injections of *S. mansoni* antigen were given on the left side. The marked edema of the left eyelid does not show clearly in the photograph. Erythema was noticeably absent. On the chest, the area of reactivity to the antigen was a blanched wheal with two well-defined pseudopodia. More frequently the immediate-type reaction was characterized by a wheal without pseudopodia. Reactivity was considered positive if the wheal with antigen was more than twice that of the control.¹⁴ There was no appreciable difference between reactions on the chest and back. Fig. 2 shows a typical cellular infiltration in a 15 minute reaction. Histologically, there are large numbers of leukocytes

*Cost precluded a color plate, but a limited number of Kodachrome slides of the gross appearance of immediate and delayed skin tests on the chest and eyelid, as well as of the histologic observations illustrated in the figures, are available to interested investigators on request to the authors.

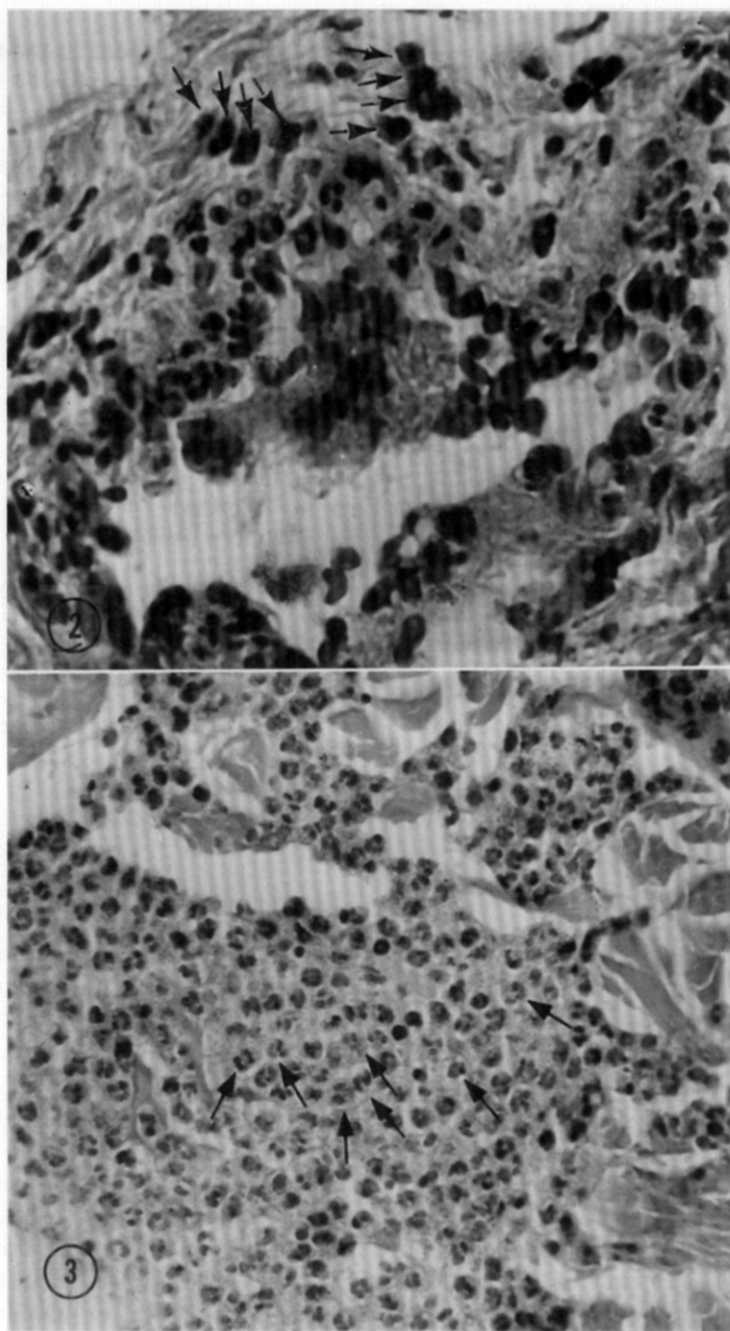


FIG. 2. Hematoxylin and eosin stain of skin biopsy from immediate-type reaction (15 minutes) to *S. mansoni* antigen. Arrows depict characteristic eosinophilic infiltration. (Original magnification, $\times 500$.)

FIG. 3. Hematoxylin and eosin stain of Arthus reaction 5 hours after injection of *S. mansoni* antigen/antibody complex into noninfected animal. Note the almost pure polymorphonuclear infiltration. Arrows point to some of these characteristic cells. (Original magnification, $\times 500$.)

that are almost entirely eosinophils adhering to endothelial surfaces and in perivascular foci in the dermis and subcutaneous tissue. The eosinophils are accompanied by occasional neutrophils and lymphocytes. Giemsa-stained preparations demonstrated that fewer tissue basophils were present in specific reactions than in control preparations.

Arthus reactivity

In two monkeys infected with *S. mansoni*, a blanched area was present at the chest skin test site at 5 hours. The intrapalpebral site at this time appeared edematous without erythema. These monkeys were re-skin-tested and the multiple sites biopsied at 15 minutes, 5 hours, and 24 hours. At 15 minutes a similar histologic picture to that described above was observed. The cell infiltration at 5 hours consisted of eosinophils with the addition of polymorphonuclear neutrophils. At 24 hours the eosinophils in the perivascular spaces were degenerating. Neutrophils were also still present, and, in addition, small and large mononuclear cells were observed. The presence of edema and neutrophils at 5 hours suggested Arthus type reactivity.

To define a pure Arthus reaction uncomplicated by preceding immediate and subsequent delayed-type reactivities, *S. mansoni* antigen/antibody complexes were injected intradermally into 2 noninfected animals and the skin reactivities examined sequentially. As early as 15 minutes capillaries showed some margination by neutrophils. At one hour the infiltration was essentially perivascular, but by 5 hours had become more diffuse and consisted essentially of neutrophils with only scanty eosinophils and lymphocytes (Fig. 3). Thereafter, collagen destruction and degenerating polymorphonuclear cells were observed.

Delayed hypersensitivity

Delayed-type reactivity was evidenced by erythema in the eyelid with slight edema. In marked contrast, chest sites had blanched areas of induration. Histologically the infiltration in the chest site or back was characterized by focal or diffuse infiltration of large epithelioid type mononuclear cells (Fig. 4), with a variable lesser component of polymorphonuclear neutrophils, eosinophils, and lymphocytes.

Monkeys infected with mycobacteria did not develop immediate hypersensitivity or Arthus reactivity to PPD. Seventy-two-hour reactions to PPD in this group of animals had the same characteristics as delayed reaction to *S. mansoni* in the animals infected with this parasite.

Occasionally, *S. mansoni* and/or PPD antigens elicited weak chronic inflammatory responses in nonsensitized animals. These were not evident grossly, but at 72 hours were characterized histologically by small lymphocytic cells and occasional eosinophils (Fig. 5). As intrapalpebral sites were not biopsied, it was not possible to determine if chronic inflammatory reactions ever occurred at this site.

There was no gross evidence of either immediate hypersensitivity or Arthus reactivity in rhesus monkey recipients of transfer factor. Because of the minimal gross appearance of the skin test sites in the transfer factor recipients and in

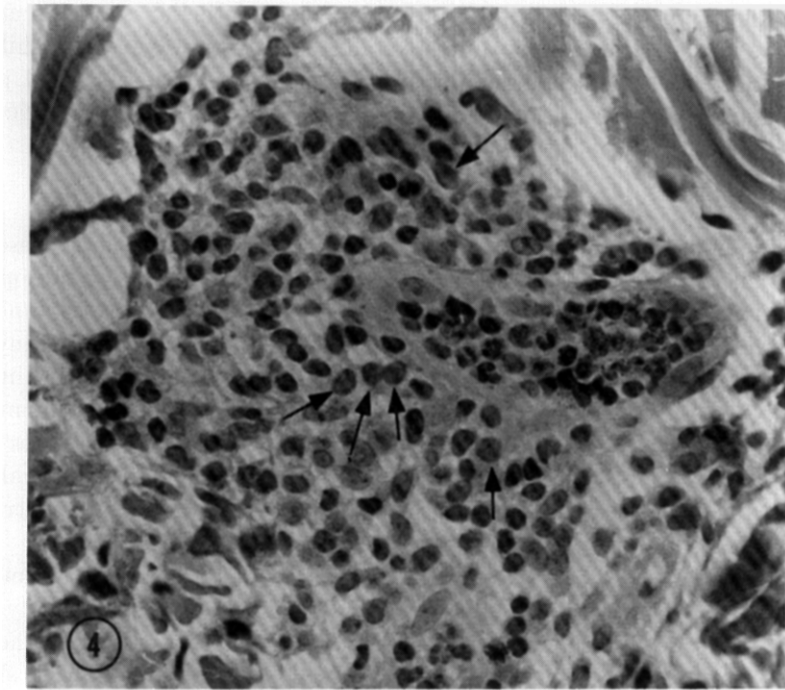


FIG. 4. Hematoxylin and eosin stain of skin biopsy of delayed-type reaction (72 hours) to *S. mansoni* antigen showing the large mononuclear cells that characterize the infiltration. Some of these cells are marked by arrows. (Original magnification, $\times 500$.)

monkeys studied at 20 weeks following single exposure to 1,000 cercariae of *S. mansoni*, the detection of a delayed hypersensitivity reaction in these animals was based on the histologic finding of large mononuclear cells predominating in the infiltration.

Findings in nonhuman primates other than the rhesus monkey

The one vervet and 3 capuchin monkeys tested on the chest for immediate-type sensitivity were all positive and reacted in a way similar to that of the rhesus monkey.

Erythema was present in varying intensities in the chest reactions of the 4 positive baboons at 15 minutes.

DISCUSSION

In the rhesus monkey, repeated exposure to *S. mansoni* stimulated immediate-type hypersensitivity demonstrable by skin testing with an extract of *S. mansoni*. The allergic nature of the cutaneous reaction was confirmed by histologic characterization of the cell infiltration and by PCA tests. We have thus been able to use skin tests and resulting wheal area to semiquantitatively monitor reagenic antibody response in our infected monkeys. In addition, skin tests have been used to assay a purified *S. mansoni* reagenic antigen (unpublished

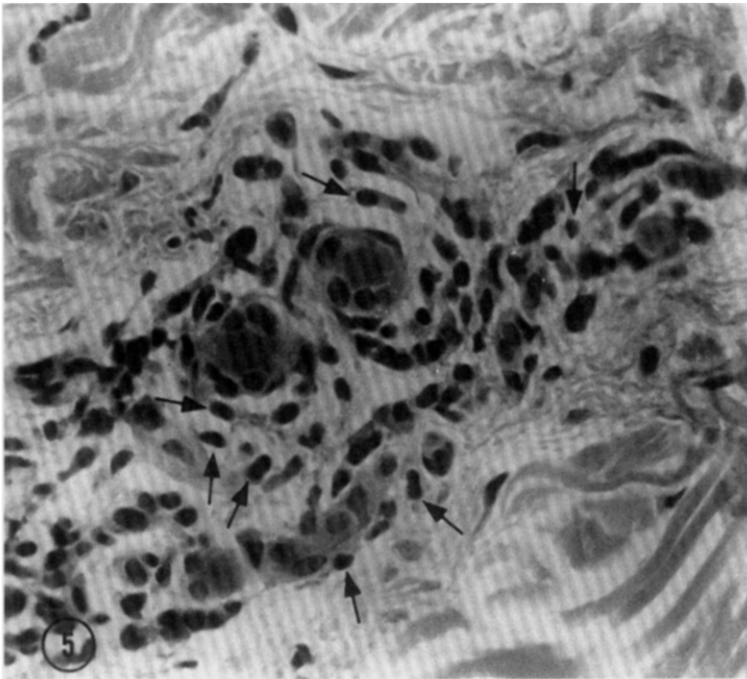


FIG. 5. Hematoxylin and eosin stain of nonspecific chronic inflammatory reaction occasionally elicited by *S. mansoni* antigen or PPD in nonsensitized animals. The infiltration consists essentially of small lymphocytes (see arrows). The larger mononuclear cells are for the most part hyperplastic endothelial cells. (Original magnification, $\times 500$.)

data). The blanching we observed in the area of the wheal, however, differs from the erythematous response reported by Malley and associates.^{6, 7}

Erythema was not observed in Arthus type reactions at 5 hours in either intrapalpebral or chest sites. An awareness of possible Arthus reactivity is important in studies of hypersensitivity as the accumulated polymorphonuclear neutrophils from the 5 hour reaction may still be present in appreciable numbers at 24 hours and thus modify the histologic pattern of a subsequent delayed-type reaction. Similarly, eosinophils from an immediate-type reaction persist well beyond the duration of edema. Therefore, in order to differentiate between these early responses and a delayed skin reaction, we routinely performed our biopsies for the latter at 72 hours. The differences between the erythema of the eyelid and the blanching of the chest or back at 72 hours in our monkeys infected with *S. mansoni* or mycobacteria are dramatic. While erythema has been reported in delayed hypersensitivity reactions by other investigators, the site of skin testing was frequently not mentioned. We confirmed the delayed hypersensitivity characteristic of our chest reactions by means of biopsy and cell identification. As biopsies were not performed on intrapalpebral sites, we do not have data on chronic inflammatory reactions in these sites, and, therefore, can do no more than speculate on the effect this type of reaction may have on erythema.

Our experience with species of nonhuman primates other than the rhesus

monkey is limited, but it is noteworthy that the baboons showed a wheal-and-flare type reactivity at 15 minutes. It would be of interest to determine whether this type of reactivity in nonhuman primates is related to the formation of the vascular bed and/or pigmentation of the skin.

Since an increasing number of investigators are using nonhuman primates in experimental models of allergy and delayed hypersensitivity, a study of the skin reactions in the various species would help resolve some of the problems that exist in interpreting results.

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