

Allergy to Flea Bites. I. Experimental Induction of Flea-Bite Sensitivity in Guinea Pigs¹

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Sensitization of guinea pigs to flea bites, by exposure to the bites of *Pulex irritans*, *P. simulans* and *Ctenocephalides felis felis*, resulted in a delayed 12 to 24-hour reaction. The development of hypersensitivity and resulting lesions very closely duplicated the early phases of sensitivity in human beings subjected to intradermally injected foreign proteins or to the bites of insects such as mosquitoes or blackflies. The present results differed sharply from the reported results of experimental sensitization of rabbits to the bites of various mosquitoes.

Guinea pigs sensitized to one species of flea as a rule reacted to all flea species used in the experimental work reported here. This would seem to indicate the presence of a common antigenic factor present in *P. irritans*, *P. simulans*, and *C. f. felis*.

The reaction of the host to insect bites is due to the injection by the insect of various antigenic materials or specific toxic or venomous substances. When the injected material serves as an antigen it may induce either a protective or a non-protective immunologic response. Trager's work (1939a, 1939b, 1940) on tick immunity offers examples of the protective immunologic response. The non-protective immunologic response, which is tantamount to an allergic reaction, may in many instances cause not only serious discomfort to the host but even serious harm. The local allergic response to the bites of blood feeders such as fleas, mosquitoes, and bedbugs may range from a simple papule or wheal to various degrees of vesiculation or even bullous formation.

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In the more severe local reactions, necrosis of tissues with sloughing may occur at the site of the bite (DeMeillon, 1949; Atkins *et al.*, 1958). A review of the literature reveals no instance of a constitutional or anaphylactic reaction to the bite of hematophagous insects, but in the case of venomous insects such as bees, yellow-jackets, and hornets, many cases of constitutional or anaphylactic reactions have been reported. Reports of death following stings of insects are not rare in the literature.

Human subjects have been sensitized experimentally to sand flies by Theodor (1935) and to mosquito bites by Mellanby (1946). Sensitization of animals has been achieved by using the bites of mosquitoes (McKiel, 1955) and by the injection of mosquito extracts (Dubin *et al.*, 1948; McKiel, 1955).

The pattern of skin sensitization in the human follows a rather definite course. Individuals with a history of no previous exposure manifest, as the initial lesion, a 24-hour delayed inflammatory response which may persist in variable degree for 1 to 2 weeks. As exposure to the bites of the insect continues, the 24-hour delayed inflammatory lesion is supplanted by an immediate whealing reaction developing within 20 to 60 minutes after exposure and fading within

a matter of minutes or hours. Upon continued exposure, the whealing reaction diminishes in intensity, presumably until no skin responses occur following the insect bites (Mellanby, 1946). Animals which have been sensitized by either injection of antigen or exposure to insect bites develop an immediate 1-5 hour inflammatory or whealing reaction which may or may not be followed by a residual lesion persisting from 1-4 days (Dubin *et al.*, 1948; McKiel, 1955).

In no cases in the literature have experimentally sensitized animals exhibited the 24-hour delayed type of reaction without the accompanying immediate reactions which are so characteristic of experimental sensitization in humans. Our studies in flea bite allergy in the San Francisco Bay Area indicate that the delayed type of inflammatory lesion is the important response in humans. In view of this observation it was felt that a corollary study of experimental induction of sensitization to flea bites in laboratory animals would be instructive. No such studies have been reported. The procedure used was suggested in part by Professor A. S. West of Queen's University, Kingston, Ontario.

METHODS AND MATERIALS

Fleas

Fleas used were *Ctenocephalides felis felis* (Bouché), *Pulex irritans* (L), and *P. simulans* Baker; these were reared according to methods described elsewhere (Hudson and Prince, 1958a, 1958b). The fleas were collected 1-2 days after emergence from the pupal stadium; in the case of *C. felis* and *P. simulans*, fresh fleas were used each day, and in the case of *P. irritans*, 1-2 day-old fleas were used on the first day and retained for use on subsequent days during the period when bites were administered daily.

Animals

Male albino guinea pigs of 400 to 500 g were used in all work.

Procedure

The hair was removed from the ventral surface of all guinea pigs with hair clippers, followed by an electric shaver. The ventral surfaces were kept free of subsequent growing hair by daily shavings with the electric shaver. Fleas were applied to the shaved surfaces in gauze-covered pillbox cages. After a 15-minute exposure the cages were removed, the fleas were anesthetized with CO₂ or killed with hot water, and the number and sex of blooded (fed) fleas were recorded. Observations of the reactions to flea bites were made at 20 minutes, 1 hour, 6 hours, 1 day, 2 days and 3 days after initial exposure. In certain cases observations were taken on each subsequent day for a period of 10 days.

Two different sensitization schedules were utilized in these experiments. The first was an initial exposure of guinea pigs to bites of approximately 50, 100, or 200 fleas followed by exposure to the bites of 10 fleas after an interval of 10 days. The second was an exposure to the bites of 1-8 fleas each day for a period of 28-30 days. Delayed skin reactions were graded as follows:

Degree	Reaction (measured with micrometer caliper)
0	No observable lesion
1	0.0-0.9 mm induration
2	1.0-1.9 mm induration
3	2.0-2.9 mm induration
4	>3.0 mm induration

Following sensitization, all animals were checked for sensitivity to other species of fleas at from 30-35 days after initial bites.

RESULTS AND DISCUSSION

Tables I, II, and III show results of sensitization of guinea pigs by exposure to flea bites administered on a daily schedule. Under these conditions, in two groups the majority (6/8) and in one group all (8), animals were sensitive to flea bites in from 6-7 days. At the end of 35 days these sensitized animals reacted not only to the flea species used to achieve sensitivity, but also the majority (18/20) to all three flea species.

Tables IV, V, and VI show results achieved by sensitization of guinea pigs

TABLE I

Results of Sensitization of Guinea Pigs by Continuous Daily Exposure to the Bites of 1-8 Pulex simulans

Animal No.	Average No. fleas fed/day	Development of hypersensitivity (days)	Degree of sensitivity (days)				Reaction to flea bites at 35 days ^c		
			Max	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
1	1	7	2	1	1	0	1	1	1
1'	1	7	2	1 ^b	—	—	—	—	—
2	1.9	7	2	1	1	1	1	1	1
2'	1.9	7	2	1	0	—	1	1	1
3	3.8	7	1	1	0	—	1	1	0
3'	3.4	7	2	2	2	0	1	1	1
4	7.4	6	3	2	2	0	2	1	1
4'	7.4	6	3	1	0	—	1	1	1

^a 100% sensitized at end 30 days.^b Animal died at 28 days age.^c 100% cross reactivity at 35 days.

TABLE II

Results of Sensitization of Guinea Pigs by Continuous Daily Exposure to the Bite of 1-8 Ctenocephalides felis felis

Animal No.	Average No. fleas fed/day	Development of hypersensitivity (days)	Degree of Sensitivity (days)				Reaction to flea bites at 35 days ^b		
			Max	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
5	1	7	1	1	0	—	1	1	1
5'	1	—	0	0	—	—	0	0	0
6	1.9	7	1	0	—	—	0	0	0
6'	1.9	6	4	4	3	3	2	2	3
7	3.5	7	1	1	0	—	1	1	1
7'	3.7	7	2	2	1	0	1	1	1
8	6.5	8	1	1	0	—	1	0	1
8'	7.4	5	1	1	0	—	1	1	1

^a 75% sensitized at end 30 days.^b 100% cross reactivity.

after one exposure of up to 246 fleas followed by moderate exposures to 10 fleas at 10-day intervals. Under the conditions of mass exposure, 10/16 of the animals used showed a reaction to the mass bites at 5-6 days. At the end of 30-35 days 8/9 of those animals still reactive to flea bites showed cross reactivity to the other two species of fleas.

Sensitization to the bites of all three species of fleas followed the same general course of events. After the first bites there was no noticeable skin reaction for 24 hours.

At the end of from 5-7 days those animals developing sensitivity suddenly reacted to bites during a 12-hour period. During this 12-hour period all previous bites, whether given on consecutive days or on the first day only, suddenly flared and were characterized by reddened, indurated areas. These first reactions subsided within 2-7 days after they first appeared. Reactions to bites administered on days following the appearance of sensitivity all regularly appeared from 12-24 hours after administra-

TABLE III

Results of Sensitization of Guinea Pigs by Continuous Daily Exposure to the Bite of 1-8 Pulex irritans

Animal No.	Average No. fleas fed/day	Development of hypersensitivity (days)	Degree of sensitivity (days)				Reaction to flea bites at 35 days ^b		
			Max	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
15	1	8	2	2	1	0	1	1	1
15'	1	7	2	1	0	—	1	1	1
16	2	—	0	0	—	—	0	0	0
16'	2	7	1	1	1	0	1	1	1
17	4	7	2	1	1	0	1	1	1
17'	4	7	2	2	1	0	1	1	1
18	8	6	4	1	1	0	1	1	0
18'	8	7	1	0	—	—	0	0	0

^a 75% sensitized at end 30 days.^b 90-100% cross reactivity.

TABLE IV

Results of Sensitization of Guinea Pigs by Exposure to Bites of Pulex simulans Followed by Periodic Exposure to Bites of Additional Fleas of the Same Species at 10 Day Intervals for 30 Days

Animal No.	No. fleas fed	No. fleas in booster exposures	Reaction to mass bites		Degree of sensitivity (days)					Reaction to flea bites 30-35 days ^b		
			Days	Degree	10	20	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
9	49	10	6	1	1	0	0	—	—	0	0	0
9'	55	10	6	1	2	2	1	1	0	1	1	1
10	104	10	6	1	1	1	1	0	—	1	1	1
10'	104	10	—	0	3	2	1	1	1	1	1	1
11	246	10	6	1	0	0	0	—	—	0	0	0
11'	193	10	5	1	3	1	1	0	—	1	1	1

^a 66% sensitized at end 30 days.^b 100% cross reactivity.

TABLE V

Results of Sensitization of Guinea Pigs by Exposure to Bites of Ctenocephalides felis felis Followed by Periodic Exposure to Bites of Additional Fleas of the Same Species at 10 Day Intervals for 30 Days

Animal No.	No. fleas fed	No. fleas in booster exposures	Reaction to mass bites		Degree of sensitivity (days)					Reaction to flea bites 30-35 days ^b		
			Days	Degree	10	20	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
12	49	10	—	0	0	0	0	—	—	0	0	0
12'	47	10	—	0	1	0	1	0	0	1	1	1
13	102	10	—	0	1	0	0	—	—	0	0	0
13'	93	10	6	1	1	1	0	—	—	0	0	0
14	196	10	5	1	1	1	1	0	—	0	0	1
14'	170	10	—	0	0	0	0	—	—	0	0	0

^a 33% sensitized at end 30 days.^b 50% cross reactivity.

TABLE VI

Results of Sensitization of Guinea Pigs by Exposure to Bites of Pulex irritans Followed by Periodic Exposure to Bites of Additional Fleas of the Same Species at 10 Day Intervals for 30 Days

Animal No.	No. fleas fed	No. fleas in booster exposures	Reaction to mass bites		Degree of sensitivity (days)					Reaction to flea bites 30-35 days ^b		
			Days	Degree	10	20	30 ^a	60	180	<i>P. simulans</i>	<i>P. irritans</i>	<i>C. f. felis</i>
19	107	10	—	—	—	—	—	—	—	—	—	—
19'	91	10	5	1	1	1	1	1	0	1	1	1
20	216	10	6	1	1	1	1	1	0	1	1	1
20'	205	10	5	1	1	1	1	1	0	1	1	1

^a 75% sensitized at end 30 days.

^b 100% cross reactivity.

tion of the bite, reached a maximum in from 24-48 hours, and subsided in from 2-7 days.

Although the lesions had the typical morphology and chronology of tuberculin-type reactions they did not exhibit the permanence of tuberculin sensitivity. The reactions to bites reached a maximum in from 7-14 days and then declined to very low levels. One hundred eighty days from the time of the first bite no noticeable reactions occurred in most of the animals. The three animals (No. 2, Table I; No. 6', Table II; No. 10', Table IV) which remained reactive at the end of 180 days were those showing more severe reactions all during the course of experimental sensitization.

DISCUSSION

A number of published reports are of interest in relation to the results presented here. Theodor (1935) reported that persons exposed to bites of sand flies, *Phlebotomus* sp., developed a skin condition known as "Harara." When he administered bites to individuals from a previously unexposed population, an indurated "papule" developed in from 4-34 (avg. 8-14) days after administration of the first bite. Following this long delayed appearance of the first bite reaction, all subsequent responses to bites took from 2-4 days to appear. After 2-4 months of continued exposure this delayed, indurated-type of reaction was sometimes superseded by immediate 20-30 min. wheal-type reactions.

It has also been shown that persons with no previous exposure to the bites of mosquitoes, *Aedes aegypti*, will develop hypersensitive skin reactions upon periodic administration of bites (Mellanby, 1946). The following were the sequence of reactions observed: first, there was a short period in which no reaction was exhibited; following this initial induction period, the subjects exhibited a delayed, inflammatory-type reaction for a number of months; upon continued exposure, this delayed inflammatory reaction was gradually supplanted by an immediate whealing reaction which in turn faded as the exposure continued. In some cases the course of reactions took as long as a year or more for completion.

It thus appears that in man, at least, an allergic type of sensitization to biting insects goes through a definite sequence of characteristic reactions. These are an induction period in which the original bite sites remain quiescent, followed by a period in which sensitivity has developed and in which the original bite site may or may not "flare up" causing indurated, papular-like lesions, and then a period in which new bites are followed by indurated 24-hour delayed reactions, which in turn are followed by a period in which immediate 20-minute reactions begin to appear and delayed reactions subside; and finally, a period exclusively of immediate reactions, followed by a state of "immunity" to bites. These are

essentially the same conclusions arrived at by A. Hudson *et al.* (1958).

Similar sequences of skin reactivity have been reported to develop from the periodic intradermal administration of foreign proteins such as rabbit serum (Jones and Mote, 1934; Mote and Jones, 1936), and guinea pig serum (Simon and Rackeman, 1934). To explain the long delayed response to the initial injection, Simon and Rackeman advanced the theory that a small quantity of antigenic material remains fixed in the tissues at the site of the original injection. Sensitization develops uniformly and simultaneously throughout the entire skin area of the body. When it reaches a certain level, there still remains in the injected area a concentration of antigen sufficient to cause the inflammatory response. Subsequent injections cause reactions in 24 hours, and continued exposure results in the appearance of immediate skin whealing of the "atopic" type.

An investigation in Australia of allergic dermatitis (Queensland Itch) in the horse definitely implicated the sandfly, *Culicoides robertsi* (Riek, 1954, 1955). Evidence was obtained in this case of the presence in susceptible animals of a thermolabile, skin-sensitizing antibody which was capable of passive serum transfer. The papule observed following the bites of *C. robertsi* in susceptible horses persisted for about 72 hours. By 96 hours the accompanying edema was largely absorbed, and at the end of 7 days the crusts of serous exudate and epidermal cells over the original papules were lost. Other allergic conditions in animals, attributed to insect bites, are "summer eczema" in dogs (Kral and Novak, 1953) and a generalized allergic condition in cattle due to bites of *Simulium* sp. (Burghardt *et al.*, 1951).

Little work appears to have been done in the experimental hypersensitization of animals to the bites of arthropods other than ticks and various arachnids. Dubin and co-workers (1948), in attempts to protect rabbits against mosquitoes by vaccination with mosquito antigen, succeeded only in sensitizing the animals in contrast to the normal non-sensitive controls. In response

to bites the sensitized rabbits showed large, indurated, reddened papules at the bite sites. These reactions began in about 30 minutes and reached a maximum size in 5 hours. The authors reported that attempts at desensitization and passive serum transfer were unsuccessful.

McKiel (1955), in studies on mosquito bite reactions in experimental animals, reported success in developing sensitivity by several exposures to bites or by injections of mosquito extracts. He reported that the reaction of sensitized animals was quite different from that seen in sensitive human beings. The wheal was slower both in making its appearance and in disappearing. The wheal in rabbit, guinea pig, or rat began to appear in from 10–15 minutes and reached a maximum in one hour. McKiel indicated that the wheal may disappear entirely within eight hours or assume a papular appearance and persist for as long as two to three days. He also stated that delayed reactions, distinct from the immediate reaction, were never observed in animals in response to mosquito bites. Subsequently, other members of the same group, together with McKiel (A. Hudson *et al.*, 1958) concluded that the degree of sensitivity to heterologous species may be determined by the time during the sensitization period at which the test is made, specificity being higher during the early stages of sensitization.

In the experiments reported here, the data show a rather high degree of cross-reactivity in guinea pigs resulting from the bites of three species of fleas. This suggests that a non-specific allergenic factor may be common to all three flea species. On the other hand, this may be due to the tests having been performed on the 35th day, a date which would be late in the course of sensitization and which, according to the thesis of A. Hudson *et al.* (1958), would result in a high degree of cross reactivity.

The reports of Riek (1954), Dubin *et al.* (1948), McKiel (1955), and A. Hudson *et al.* (1958) would seem to indicate that the development of hypersensitivity to insect bites in animals is characterized by the appearance of immediate (1–4 hour) reactivity with or without accompanying re-

sidual effects which may last from 3-7 days. This contrasts sharply with the reports of Theodor (1935), Mellanby (1946), Simon and Rackeman (1934), and Mote and Jones (1936) who suggest that in man the course of sensitization to insect bites, or to intradermal injection of foreign protein, is characterized by an initial stage of delayed 24-hour reactions unaccompanied by immediate (20-minute to 1 hour) whealing, the latter appearing only after relatively longer periods of exposure.

The work with guinea pigs reported here indicates that the reaction to flea bites in these animals more closely approximated the initial stages of sensitivity in human beings. The development of sensitivity, and the reactions to initial bites, confirmed almost exactly the theoretical postulate of Simon and Rackeman (1934) with reference to the early phase of foreign protein sensitization. The apparent lack of immediate 1-4 hour reactions would seem to indicate that the response in guinea pigs may not go through the complete series of sensitization phenomena as reported by Mellanby (1946) and by Simon and Rackeman (1934) for man, and also suggests that the sensitization of guinea pigs to flea bites may be somewhat different from that shown in rabbits in response to mosquito bites.

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