

The disappearance rate of skin-sensitizing antibody activity after intradermal administration

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The disappearance rate of skin-sensitizing antibody activity from the skin of normal volunteers was determined. Saline dilutions of serum from three patients sensitive to ragweed pollen were injected intradermally over varying periods of time and challenged with a ragweed extract. The loss of activity followed a logarithmic decay curve and was not significantly different for the three sera tested. The average half-time for loss of skin-sensitizing antibody activity of the three sera was 13 days (range: 11 to 15 days).

The studies of Loveless¹ demonstrated that skin-sensitizing antibody (SSA) activity persisted in the skin of nonallergic recipients for long periods of time (up to 12 weeks) after they were transfused with blood from allergic individuals. This work, together with the earlier observations of Coca and Grove,² led to the concept that SSA molecules specifically fix or bind to tissue. Recently, Fireman, Boesman, and Gitlin³ challenged this idea and claimed that SSA activity disappears from the tissue at a rate similar to other immunoglobulins. It was the latter report which prompted us to see if the observations of Loveless¹ could be verified, and if they could, to study the rate of disappearance of this activity. Prausnitz-Küstner (P-K) tests⁴ were performed by injecting dilutions of serum containing SSA intradermally over varying periods of time and challenging these sites with antigen. SSA activity was found to persist in tissue up to 12 weeks, and its disappearance followed a logarithmic decay curve with a half-time of 13 days (range: 11 to 15 days).

MATERIALS AND METHODS

Three sera (A, K, and M), each containing high titers of SSA to ragweed, were used in these studies. The average titers for A, K, and M were 1:450,

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1:640, and 1:825, respectively. The sera were obtained from 3 men who were healthy except for hay fever caused by ragweed pollen. All 3 had received hyposensitization treatment, but the sera used in these experiments were obtained 6 to 12 months after the last treatment. The sera were stored under sterile conditions without preservative at 4° C. and were never frozen.

P-K tests were performed by injecting intradermally with a 25 gauge needle 0.05 c.c. of saline dilutions of the sera, which had been prepared immediately prior to use. Injection sites were spaced approximately 5 cm. apart and were located at measured distances from body landmarks on the left side of the back of a nonallergic volunteer. The volunteers were healthy male prison inmates who had negative histories for allergies and negative intradermal skin tests to short ragweed extract.* Each dilution was injected in triplicate in a randomized pattern and coded so that the investigator did not know the strength of the dilutions until the results of the tests were recorded. The most frequently used serum dilutions were: undiluted, 1:5, 1:10, 1:25, 1:50, 1:75, 1:100, 1:150, 1:200, 1:400, 1:600, 1:800, 1:1,000, 1:1,200, 1:1,400, 1:1,600, and 1:1,800.

The locations of the injection sites were maintained by marking them on alternate days with gentian violet. After an interval of time had elapsed (7 to 86 days), another set of serum dilutions were similarly injected into the right side of the back of the volunteer. Two days after the last set of serum dilutions was injected all sites were challenged by injecting 0.02 c.c. of short ragweed extract intradermally with a 25 gauge needle.

Sites were considered positive if a wheal-and-erythema reaction occurred within 20 minutes and the mean diameter of the erythema (the sum of the longest diameter measured through the center of the wheal plus the diameter perpendicular to it divided by 2) was at least twice the mean diameter of the erythema at the site injected with ragweed extract alone. All of the reactions which were considered positive had a wheal, while none of the controls had wheals. A serum dilution was considered to give a positive reaction if at least 2 of the triplicate determinations were positive. A single positive reaction was an uncommon event. This procedure was designed to eliminate false positive reactions. Since the method used to determine which dilutions were positive applied equally to the titrations done at both time periods, it is unlikely that the rate of SSA disappearance would have changed significantly if a different method had been used.

In this study a total of 1,864 P-K tests were performed in 28 recipients. Several individuals were dropped from the study either because they developed positive reactions to the pollen extract, as Connell⁵ has reported, or because an end point was not reached.

RESULTS

Assays which use a stepwise dilution of the test sample give discontinuous measurements. They determine the most dilute sample which will give a posi-

*Aqueous extract containing 1,000 PNU per milliliter, Center Laboratories, Inc., Port Washington, N. Y.

tive reaction and the next highest dilution which is negative. These two dilutions represent limits; the true end point lies somewhere between these limits. In order to minimize the inaccuracy of this procedure, the dilution intervals for the SSA assay were kept as small as practical. In addition, since the true end point can never be reached no matter how many dilutions are made, the midpoint between the last positive and first negative dilution was arbitrarily used rather than either of the limits. It is the reciprocal of this midpoint dilution that is used for all the calculations.

In every volunteer used in this experiment, one of the sets of serum dilutions was injected 2 days prior to challenge with ragweed extract. This was the final serum injection and henceforth will be referred to as the 2 day SSA titer. The SSA assays for all other time intervals were compared with the 2 day SSA titer, and loss of activity was expressed as a percentage of this titer.

A typical experimental protocol follows:

1. On Dec. 3, 1966, 8 dilutions of serum A were injected in triplicate in a random pattern on the left side of the volunteer's back.
2. On Jan. 7, 1967, 8 dilutions of serum A were similarly injected on the right side of the volunteer's back (2 day SSA titer).
3. On Jan. 9, 1967, all sites were challenged with ragweed antigen (Table I).

The disappearance rate of SSA activity of serum A tested in 20 individuals is shown in Fig. 1. Each point represents the midpoint between the last positive and first negative dilution as determined from a set of 24 to 48 injections (for the 46 and 86 day intervals a smaller number of tests was used). The decline in SSA activity seemed to approximate best a logarithmic decay curve. The mean of the slopes of all points from serum A is -1.15 (S. E., ± 0.08). This slope gives a half-time of 13 days. Positive reactions were observed in one

Table I. *Typical experimental protocol: Challenge with ragweed antigen*

<i>Dilutions injected</i>	<i>Mean diameter of each lesion (mm.)</i>		
Dec. 3, 1966			
1:5	35	31	30
1:10	32	26	30
1:25	6	16	20
1:50	<5	<5	<5
1:75	<5	<5	<5
1:100	<5	<5	<5
1:150	<5	<5	<5
1:200	<5	<5	<5
Ragweed control	5		
Jan. 7, 1967			
1:200	32	35	27
1:300	27	33	25
1:400	28	29	15
1:500	11	12	5
1:600	18	12	10
1:800	<5	<5	<5
1:1,000	<5	<5	<5
1:1,200	<5	<5	<5
Ragweed control	5		

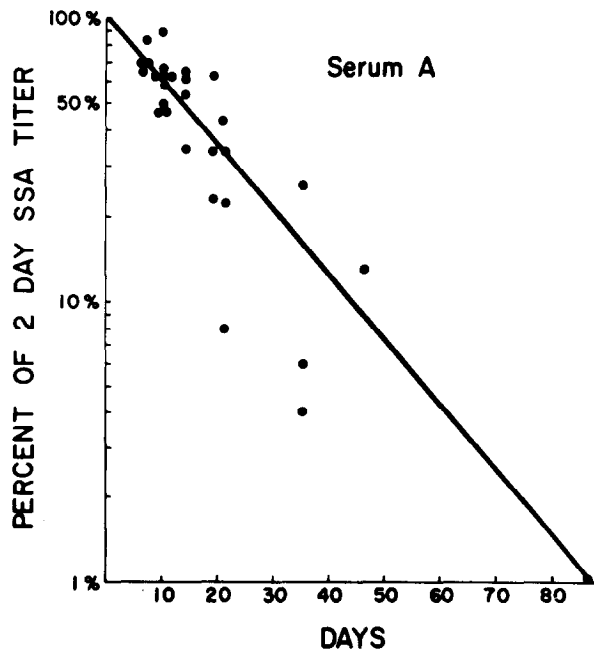


Fig. 1

Disappearance rate of SSA activity from *serum A*. The points are all related in time and level of SSA activity to the *2 day SSA titer*. Loss of activity was expressed as a percentage of this titer. The titer is the midpoint between the dilution of the last positive and first negative reaction.

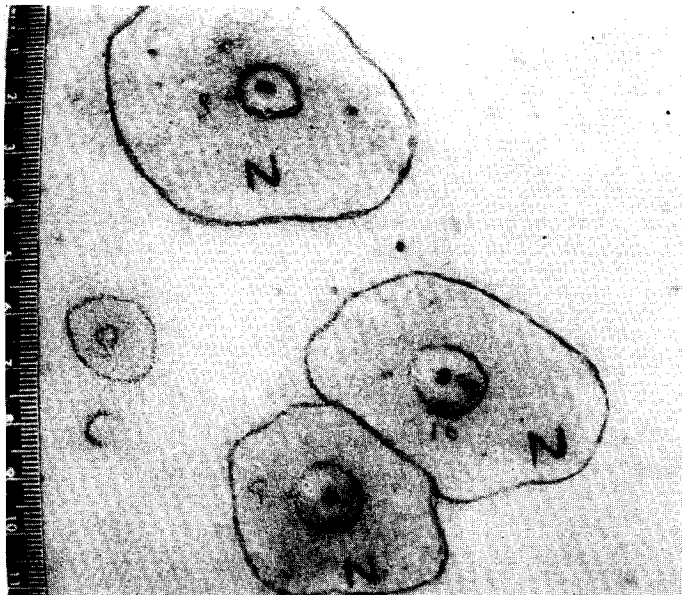


Fig. 2

The lesions marked *N* were injected with undiluted (neat) serum *A* 86 days earlier and challenged with ragweed extract 20 minutes before the photograph was taken. Lesion *C* received only ragweed extract.

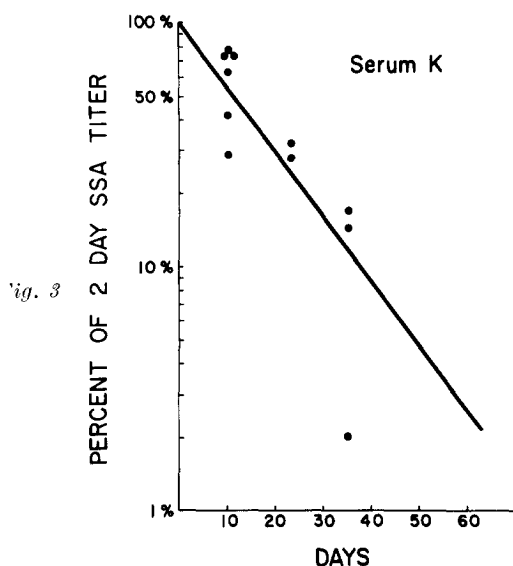


Fig. 3

Disappearance rate of SSA activity from *serum K*.

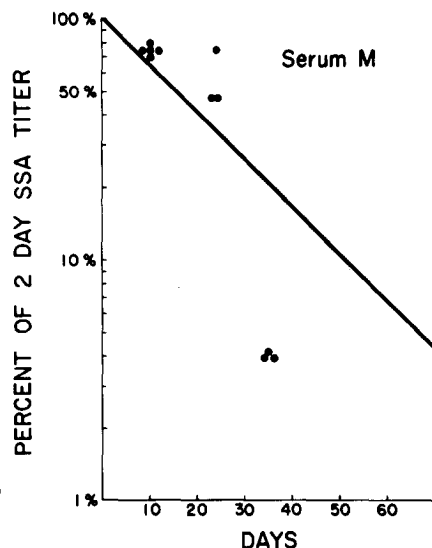


Fig. 4

Disappearance rate of SSA activity from *serum M*.

individual in sites which had been injected with whole serum 86 days before challenge (Fig. 2).

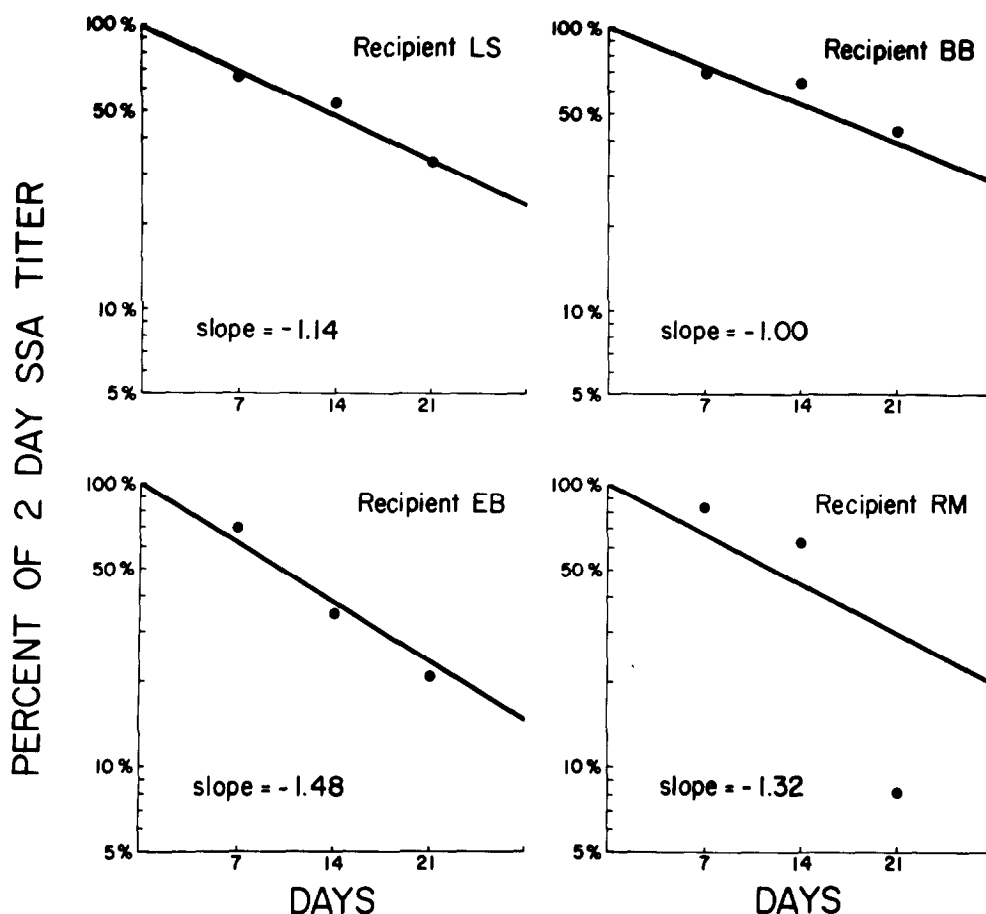
The mean slope of serum K, obtained in 11 individuals, was -1.34 (S. E., ± 0.23), giving a half-time of 11 days (Fig. 3). Serum M, also with 11 individuals, had a mean slope of -0.98 (S. E., ± 0.22) and a half-time of 15 days (Fig. 4). There was no significant difference in the slopes of these 3 sera by t-test.

In order to evaluate the possibility that the level of SSA activity in the serum might influence the rate of disappearance, the averages of the 2 day SSA titers for the 3 sera were compared with the slopes. Sera A, K, and M had mean 2 day SSA titers of 1:450, 1:640, and 1:825, respectively. There is no correlation with the corresponding slopes: -1.15 , -1.34 , and -0.98 .

To evaluate the variability of the disappearance rate of SSA due to differences in tissue characteristics of the recipients, the disappearance rates of one serum (serum A) were observed in 4 individuals. The slopes for these 4 recipients were -1.00 , -1.14 , -1.32 , and -1.48 , and the corresponding half-times were 15.5, 13.5, 12, and 10 days (Fig. 5). The procedure was limited by the number of test sites that could be used in one individual. The slopes were not statistically different but small differences could easily have been obscured by the variability of the test.

DISCUSSION

Some of the factors responsible for the variability of the P-K test include: (1) day-to-day changes in the skin reactivity of an individual; (2) regional differences in skin reactivity; (3) loss of SSA activity in the allergic serum

**Fig. 5**

Comparison of SSA activity disappearance rate in four volunteers injected with serum A.

with aging, freezing, and thawing; (4) variation in the response of individuals to P-K passive transfer; and (5) the difficulty of locating injection sites after long intervals of time. In the present study some of these factors were eliminated or minimized by: (1) challenging all the injection sites in an individual on the same day; (2) injecting the serum dilutions in a randomized pattern in triplicate to avoid error due to reaction variability in skin sites; (3) use of serum that had never been frozen and dilutions that were freshly prepared prior to injection; (4) placing each injection site a measured distance from skin landmarks and marking each site with gentian violet on alternate days; and (5) measuring the reactions without knowing the dilution strength.

Another difficulty that had to be resolved was the method of handling the SSA titer data. As described earlier, we arbitrarily chose to use the reciprocal of the dilution which was midway between the last positive and first negative dilutions. This choice was largely academic since the curves and half-time values were very similar when the reciprocal of the last positive dilution was used for our calculations (half-times of 11, 11, and 16 days for sera A, K, and

M, respectively); however, our approach seems more logical than the use of either of the limits.

In comparing our results with those of Fireman and colleagues,³ striking differences are apparent. They reported a half-time of 4.2 to 4.9 days and observed no positive reactions after 7 weeks. We have found a half-time of 13 days (11 to 15 days) and one reaction was positive 12 weeks after injection. The SSA titers for Fireman's sera were all 1:1,000, while those used in this experiment had lower titers. The results of these two experiments are so divergent that if a curve which showed a half-time of 4.9 days was superimposed on our graphs (Figs. 1, 3, and 4), all of the points for sera A, K, and M would fall above the curve.

Differences in experimental design might explain the dissimilar results. Fireman and colleagues tested each serum dilution only once in each of 3 individuals. In an assay system which is notoriously variable we think this is insufficient. The dilutions were injected in a nonrandom fashion, which we believe could lead to systematic error due to regional differences in skin reactivity. Another danger in using nonrandom injections is that an interpretation bias might result from an awareness of the nature of the previous injection. Finally, Fireman does not comment on the means of storing the sera. It is possible that some methods of preserving or handling the serum, especially freezing, might alter the disappearance rate.

Fireman argues that there is no evidence for irreversible binding of SSA to tissue, and with this we agree, but our study strongly suggests a great affinity and slow release of SSA from tissue. Our data show considerable difference between the disappearance rate of SSA and that reported for the other immunoglobulins³ and albumin.^{3, 6} Although there are many factors, including the size and shape of the molecule, that might influence the loss of SSA activity, tissue binding due to complementary structures on the antibody and the cells remains a distinct possibility. The rapid progress being made in the isolation and characterization of γ E-globulin will probably provide the opportunity for more definitive studies.

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