

Changes in skin-sensitizing antibody titer after injections of aqueous pollen extract

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Titers of SSA decreased in 10 of 21 previously untreated patients with hay fever after treatment with injections of aqueous extract for 13 to 48 months. Quantity of antigen injected did not appear to influence the change in titer. Patients with the highest titers before treatment changed least after therapy. All patients with the lowest pretreatment titers showed significant declines in titer after treatment. Fifteen subjects did not receive injections of extract. Titers did not change in 12 and increased in 3 during a period of 2 to 3 years. Titers of SSA were determined in 2 other groups of patients. In one group, treated with aqueous extracts for many years, titers did not change during one additional year of therapy. The average of this group's first titers was low and suggested that previous therapy had caused a decrease in titer during early years of treatment. Forty other patients received repository injections of extract for 3 consecutive years. No difference existed between the average titers of pre- and posttreatment sera. Titers of 5 patients in this group increased significantly. Results suggest that aqueous injection therapy frequently causes a decrease in SSA, "hyposensitization." One hypothesis to explain hyposensitization is that injected antigen suppresses antibody synthesis. Hyposensitization may be analagous to partial immune tolerance.

Whether or not treatment with injections of antigens causes clinical improvement in allergic rhinitis has been disputed.¹⁻³ If treatment does cause improvement, the mechanism responsible for amelioration of symptoms is unknown.

We⁴ reported that a relationship existed between the patients' titers of skin-sensitizing antibody (SSA) and the severity of their symptoms of hay fever; the higher their titer, the greater their symptoms. Lichtenstein, Norman, and Connell⁵ found similar results in a smaller group of patients. In 369 clinic patients treated with injections of aqueous extract for periods varying from 0 to 20 years, we⁶ found that titers of SSA varied with the duration of treat-

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ment; the longer the term of therapy, the lower the titer. The results of these experiments ⁴⁻⁶ suggest that titers of SSA decrease during therapy and that the decreased titer of SSA is a factor in the amelioration of symptoms.

In our report⁶ relating the length of therapy to the decrease in titer, only one rather than serial titers, was determined for each patient. It is possible that this method of study distorted the results by unintentional selection (i.e., those patients who had no improvement in symptoms and possibly those with the highest titers of SSA were more prone to stop treatment in the early years). This factor may partially account for the finding that titers of SSA decreased with longer periods of treatment. On the other hand, many patients may have responded favorably to injection treatment with a decrease in titer accompanied by fewer symptoms and stopped treatment because of a favorable result.

MATERIALS AND METHODS

This study was designed to determine the effect of injections of aqueous ragweed pollen extract on titers of SSA. One group of previously untreated patients with ragweed hay fever was given injections of aqueous extract perennially. Titers of SSA were determined in 3 other groups of patients for comparison. All patients were from the Institute of Allergy, The Roosevelt Hospital.

Previously untreated patients treated with aqueous ragweed extract and followed with serial determinations of skin-sensitizing antibody

From 1961 through 1964, we obtained serum from 49 previously untreated patients before treatment with aqueous ragweed extract was begun. All had symptoms of allergic rhinitis during the ragweed pollen season and positive reactions to intracutaneous skin tests with aqueous ragweed pollen extract.

Twenty of these patients were eliminated because they stopped therapy after their first visit or during the next 6 months, most for unknown reasons. Eight others who received therapy for more than 6 months before stopping were eliminated because posttreatment sera were not obtained. In 21 patients one or more posttreatment sera were obtained: 10 in the first year, 14 after more than one year of treatment, 12 after more than 2 years, and 5 after more than 3 years.

Patients reported the severity of symptoms and amount of medication taken for relief of symptoms, daily, during the ragweed pollinating season, on a special card. We have previously reported the method of evaluation with the use of cards.⁴

In general, the dosage schedule followed for the administration of aqueous pollen extract was that described by Sherman.⁷ Injections were given weekly and the dose increased each week until the patient developed signs or symptoms of intolerance.⁷ The largest dose which could be given without ill effects was considered the maintenance dose and was administered every 3 to 4 weeks thereafter for the duration of the study.

The amount of antigen injected into any individual patient may be de-

scribed as the total of all antigen injected over the course of the study or as the maintenance dose. There is no evidence which suggests that one figure is better than the other. In fact, when antigen injections are administered perennially for several years, the maintenance dose for different patients is proportional to the total dose they received. We arbitrarily chose the maintenance dose for use in evaluating results.

One possible element of bias was eliminated in that a number of physicians in the clinic treated these patients and made all decisions pertaining to treatment. These physicians did not know that the patients were members of a study group.

Untreated patients

A placebo-treated group was not studied, since the length of the study was indefinite and this would have deprived patients of treatment we thought beneficial. Furthermore, the evaluation of a change in titer of SSA is an objective measure not believed to be affected by psychological stimuli. As a substitute for a group treated with placebo, we studied 15 subjects with hay fever who refused injection therapy. Their titers of SSA obtained originally were compared with their titers obtained 2 to 3 years later.

Patients on long-term therapy with aqueous ragweed extract

Determination of titers of SSA in 2 other groups of patients with hay fever are included to define changes in titer under different conditions or with a different method of treatment. In 23 patients treated with injections of aqueous ragweed extract perennially for more than 5 years, titers were determined in 2 successive years. During these 2 years the patients continued to receive maintenance doses of aqueous extract monthly. This group served to define short-term changes in titer in patients after many years of treatment.

Patients treated for 3 consecutive years with emulsions of aqueous ragweed extract

Forty other patients received treatment of water-in-oil emulsions of aqueous extract with one injection per year for 3 consecutive years. Some of these had never received injections of extract before. Others had been treated with aqueous extract in the past but had not received injections for one to 25 years before the first repository injection. To define changes in this group, titers of SSA before repository treatment were compared with the titers of sera taken one year after the last injection of emulsified extract. We used the titer taken one year after the last emulsion injection for comparison with pretreatment titers because it was known that such injections almost always cause a temporary increase in SSA in the 3 to 12 weeks following the injection.⁸ This increase in titer was usually followed by a decrease during the ensuing year.⁸

Since the primary purpose of this study was to determine effects of injections of aqueous ragweed extract on SSA in previously untreated patients, the other 3 groups described (no injection treatment, long-term aqueous

therapy, and water-in-oil emulsions) are included as control groups to establish the effect of other types of treatment on SSA.

Titers of skin-sensitizing antibody

Titers of SSA of sera were determined by passive transfer with the dilution technique previously described.⁶ We collected blood samples with sterile technique, removed the serum aseptically, and stored it in glass bottles with rubber caps, without preservative, at 3 to 5° C. Titers of all sera collected from one individual before and during the period of treatment were determined in the same passive transfer recipient at the same time. We verified the stability of the SSA in sera thus stored by determining the titer of sera shortly after they were obtained and at intervals during the next 4 years. No significant change in titer occurred in these sera during this period of storage. If titers of SSA had decreased during storage, sera obtained before treatment began and stored the longest should be affected most; this would tend to minimize the finding that treatment decreases titers.

So that titers in different patients could be compared, we determined the titers in recipients specifically selected on the basis of their skin reactions. All recipients gave essentially the same reaction to the same dilutions of the control serum.

RESULTS

Previously untreated patients who were then treated for more than one year with aqueous injections

Change in titer of SSA. Table I gives titers of SSA of the 21 patients who were treated for more than one year. Only changes greater than twofold are considered significant. In 10 patients the last posttreatment titer obtained was significantly less than their pretreatment titer. Two other patients had a twofold decrease in titer. Seven subjects were unchanged and 2 showed twofold increases.

Relationship of pretreatment titer to effect of treatment. Eleven patients' pretreatment titers were 1,000 or greater. After treatment, titers decreased in only 2, remaining the same or increasing in the other 9 patients.

Titers in 10 patients were less than 1,000 before therapy. All showed a decrease in titer of SSA, although in 2 the decrease was only twofold.

Maintenance dose of antigen related to change in titer of SSA (Table II). Results established no definite dose-response trend in the small number of patients studied. Three of the 7 patients who received a top dose of less than 1,000 protein nitrogen units (PNU) per injection did have a striking decrease in titer. In 5 of 7 patients who received more than 5,000 PNU per injection, titers decreased.

Years of treatment related to changes in titer of SSA (Table III). In 8 patients tested in the first year, titers of SSA were not significantly changed as compared with pretreatment levels. In the second year, 5 of 11 decreased significantly, in the third year 6 of 11, and in the fourth year 2 of 5 showed decreases. Unfortunately, we did not obtain each patient's serum during each

Table I. *Titers of SSA and doses of antigens administered to individual patients*

Case	Dose*	Months of treatment									
		0	2 to 6	7 to 12	13 to 18	19 to 24	25 to 30	31 to 36	37 to 42	43 to 48	
1	17,000	2,000§					2,000†				
2	8,500	2,000				5,000†					
3	400	2,000		2,000				2,000†			
4	1,500	1,000			2,000†						
5	1,200	1,000	1,000		1,000		1,000†				
6	750	1,000								1,000†	
7	900	1,000		1,000					1,000†		
8	1,000	1,000					1,000†				
9	200	1,000			1,000†						
10	10,000	1,000			100	100		50†			
11	6,500	1,000				100†					
12	2,000	800								400†	
13	8,000	800		800		800		100†			
14	300	500	500	750		100	100	50†			
15	10,000	500							100†		
16	500	500		500		100†					
17	2,500	500	500	500				50†			
18	5,000	500					100†				
19	600	300		300						10†	
20	20,000	300			50		50†				
21	1,500	200			500		100†				
Geometric average		780		700		360		190		210	
				710†		740†		710†		660†	

*Highest dose attained per injection (PNU).

†Last obtained titers of SSA.

‡Geometric average of pretreatment titers for the patients titrated during period listed.

§Titer of SSA.

Table II. *Dose of antigen compared with titer of SSA*

Dose*	Fold change in titer of SSA			
	Patients with decrease		Patients with no change	Patients with increase (2-fold)
	> 2-fold	2-fold		
< 1,000	3		4	
1,000-5,000	2	2	2	1
> 5,000	5		1	1

*Highest dose attained in PNU on last injection.

Table III. *Years of treatment compared with titer of SSA*

Years of treatment	Fold change in titer of SSA					
	Patients with decrease			Patients with no change	Patients with increase	
	10-fold or >	3- to 9-fold	1- to 2-fold		1- to 3-fold	4-fold or >
< 1 to 1				8		
> 1 to 2		2		3	3	
> 2 to 3	3	3	1	4		
> 3 to 4	1	1	1	2		

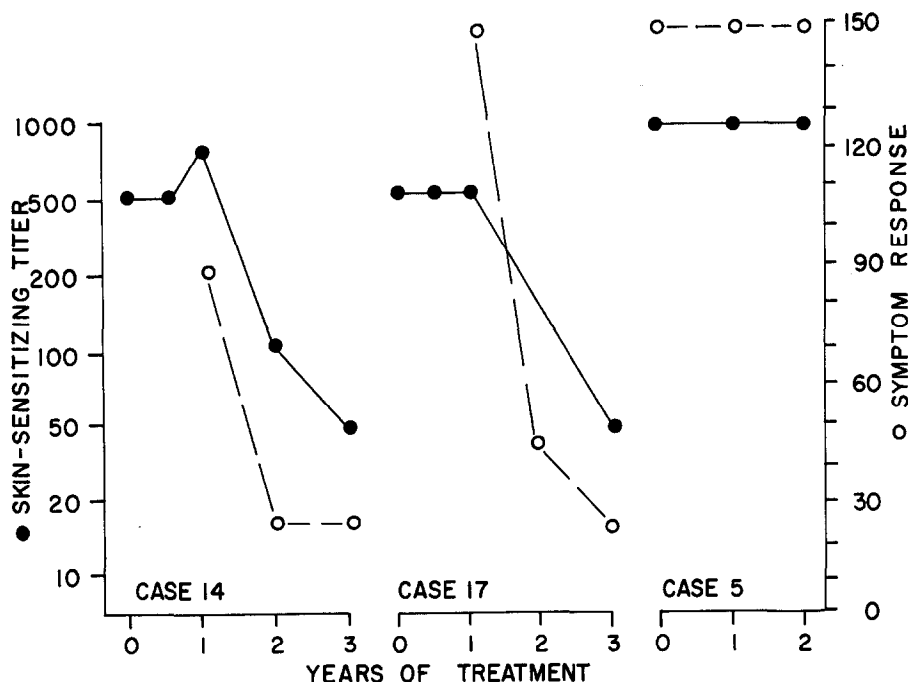


Fig. 1
Titer of SSA compared with symptom response in 3 cases.

year of therapy, so that the year in which titers decreased could not be exactly ascertained.

In Cases 3, 5, 7, 10, 13, 14, 16, 19, 20, and 21, we collected a pretreatment serum and more than one serum during treatment (Table I). The time at which a change in titer occurred varied for different patients. Some did not change even after 4 years of treatment.

Table I compares the average pretreatment with the average posttreatment titers of the same patients for the individual years of treatment. The average titer in the first year of treatment was not significantly different from the pretreatment average. During the second year of treatment, titers decreased significantly. A further decrease occurred in the third year. There was no change during the fourth year.

Comparison of symptoms to change in titer of SSA (Fig. 1). Few data were available for such a comparison, since we included in this group only patients who had recorded the severity of hay fever symptoms, daily, and from whom sera had been drawn in 3 consecutive years. Shown in Fig. 1 are the only 3 patients who met these criteria. In Patients No. 14 and No. 17, high sensitizing titers after the first year of treatment were associated with more than average hay fever. During the second and third years of treatment, lower titers were associated with less severe symptoms. In Patient No. 5, the titer of SSA remained elevated and severe symptoms persisted during 3 years of treatment.

Changes in titers of SSA in control groups (Table IV)

No injection treatment. Fifteen subjects, observed for 2 to 3 years, did not receive injections of extract. The average of their first titers was only moderately high, since the sensitivity of these individuals varied from slight to marked. No significant change occurred in their average titer during 2 to 3 years of observation.

Variations between the initial and final titer were observed in different patients. Titers were unchanged in 7 patients; 2 had a twofold titer decrease, while 3 had a twofold increase. A significant increase occurred in 3 patients.

Patients receiving long-term aqueous therapy. We secured sera in 2 consecutive years from 23 patients treated continuously for the previous 5 to 20 years. The average of the first titer was only 81, a low figure which probably reflects the long period of previous therapy. The average titer in these same patients after one additional year of aqueous treatment was 78, not a significant change.

Only one of the 23 patients showed a decrease in titer of more than twofold, and none increased significantly.

Effect of repository therapy on titer of SSA. Forty patients received treatment with oil-in-water emulsions of aqueous ragweed extract for 3 consecutive years. Their average pretreatment titer was 220, but variations in titer of sera of individual patients indicated a wide range of sensitivity of persons in the group. This variation may have been caused by the fact that the patients had previously received differing amounts of aqueous therapy, varying from one to many years of treatment, while some had never been so treated.

We compared titers before the first repository injection with the titer of the last serum, obtained one year after the last repository injection, because of the following observations. Titers increased sharply in the first 3 weeks following emulsion injections.⁸ A slow decrease in titers followed the initial rise, began approximately 2 months after the injection, and continued for one year. In most cases the decrease at the end of a year equaled the initial rise.

The average titer of sera obtained one year after the third yearly repository

Table IV. *Change in titer of SSA in 4 groups of patients*

Treatment groups	Number of cases	Titer of SSA*		Fold change in titer of SSA						
		Pre-treatment	Post-treatment	Patients with decrease			Patients with no change	Patients with increase		
				10-fold or >	3- to 9-fold	1- to 2-fold		2-fold	4-fold	> 4-fold
Aqueous treated 1 to 4 years	21	780	260	5	5	2	7	2		
No treatment	15	200	250			2	7	3	3	
Long-term aqueous	23	81†	78		1	7	8	7		
Repository treated 3 years	40	220	270				35		3	2

*Geometric average.

†First titer obtained during treatment.

injection was slightly, but not significantly, elevated when compared with pretreatment titers. Titers of 35 patients were essentially unchanged one year after the last repository injection. However, in 5 patients titers were significantly increased.

DISCUSSION

While aqueous solutions of allergenic extracts have been used for over 50 years as treatment for allergic rhinitis, it is only recently that reports of controlled clinical studies^{1, 2} suggest that such injections are an effective form of treatment.

The immunological mechanism causing clinical improvement is not definitely established. Loveless⁹ believed improvement following injection therapy was caused by blocking antibody, produced as a result of the injections. Others^{4, 10, 11} agreed on the presence of blocking antibody after injections, but could not relate the titer of such antibody to the degree of amelioration of symptoms.

Early workers¹²⁻¹⁴ examined titers of SSA after injections of extract and reported a variety of changes. However, they rarely followed their patient's titers for more than one year. Later, Sherman, Stull, and Cooke¹⁵ reported an increase in sensitizing-antibody titer after several months of injections, and a fall to pretreatment levels after one year of treatment. Titers decreased further in 3 of 7 patients observed for 4 years.

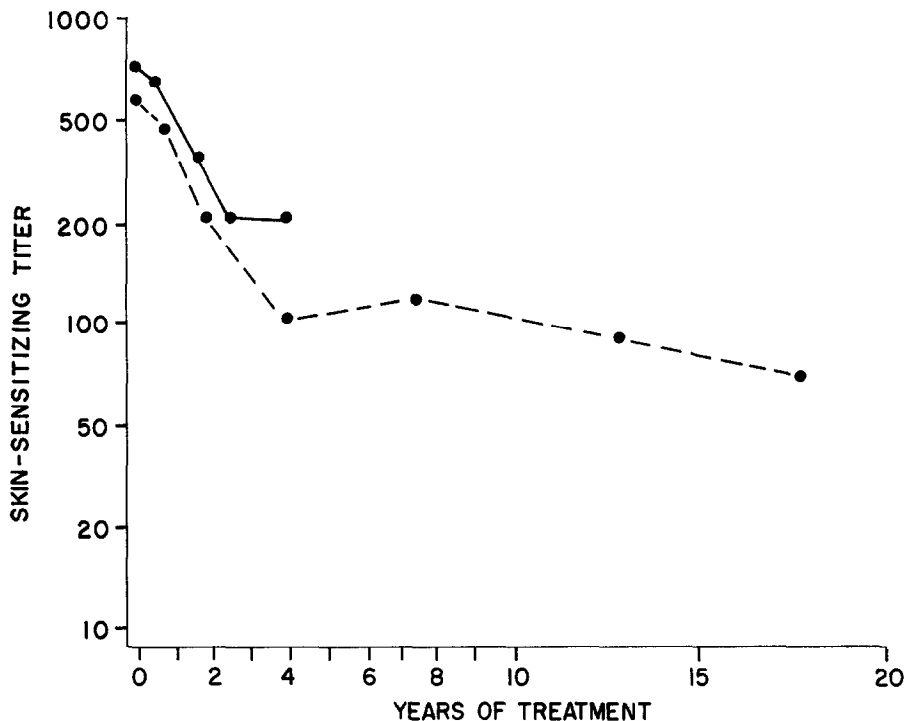
We⁶ reported an association between titers of SSA and the severity of hay fever symptoms. The finding of fewer symptoms associated with lower titers of SSA suggested that a decrease in SSA would prove clinically beneficial.

In the present study, the relationship between SSA and symptoms could be compared in only 3 patients. In these 3 patients, data collected for 3 consecutive years suggested a direct relationship between symptom response and antibody titer. Severe symptoms present in 2 patients with high titers improved in succeeding years when titers decreased. One patient simultaneously had severe symptoms and a high sensitizing titer over a 3 year span.

In the present study, titers of SSA determined in 8 patients before and during the first year of treatment with aqueous injections showed no significant decrease. A significant decrease in titer of SSA occurred in 10 of 21 patients after the first year and during the next 3 years of treatment. The occurrence of the decrease in titer varied in different patients. Titers of some patients decreased during the second year of therapy, while others declined in the third or fourth year of treatment.

Amelioration of symptoms in our own clinical experience and in the studies of Lowell and Franklin¹ and Norman, Winkenwerder, and Lichtenstein² took place most often during the second or third years of therapy, with little or no improvement during the first year. The time of the clinical improvement which they reported^{1, 2} coincides with the time at which our patients showed significant decreases in the titer of SSA. This suggests there may be a direct relationship between the two events. This finding is also compatible with our previous report⁴ relating titer of SSA to the severity of symptoms.

Almost all of the patients with the highest titers (greater than 1,000) failed

**Fig. 2**

Titer of SSA related to years of treatment. The solid line represents the average titer of SSA for the periods of treatment in this study. The dash line is the average titer of SSA related to years of treatment, plotted from data of a previous retrospective study.⁶

to respond to injections of extract. Titers declined during therapy in patients with lower pretreatment titers (less than 1,000).

Although more patients who attained higher doses of antigen per injection responded with a significant decrease in titer, the small number of cases studied precludes a conclusion that high doses are more beneficial than lower doses. Furthermore, since serial studies of antibodies in individual subjects were not sufficiently complete, it is possible that in some patients treated with high doses and in whom SSA decreased, the titer may have dropped before the high dose was attained. Indeed, in these patients, tolerance of high doses may have occurred because of decreased sensitizing titers. In this regard, we⁶ previously reported that tolerance to antigen is related to SSA and blocking antibody. Antigen doses were found to be higher in patients with lower titers of SSA; when blocking titers were elevated, large doses of antigen could be injected regardless of the titer of SSA. Nevertheless, it does seem significant that of 7 patients in this study who attained a maximal dose of less than 1,000 PNU per injection (considered low dose), titers diminished in 3. These findings suggest that the dose of antigen which caused a decrease in titer is not nearly so important as still unrecognized variables. One of these variables may be heredity, as suggested by the experiments of Fink and Quinn.¹⁶ In different groups of inbred animals which

they studied, antibody production related more to the animals' genetic background than to the quantity of antigen injected.¹⁶

The possibility exists that titers of SSA may change markedly from year to year due to causes other than injections of aqueous extract and that the changes we observed may be due to these causes. Comparison of the findings in the various groups described make this unlikely. Patients who were not being treated with injections of aqueous extract showed insignificant changes in titer when followed for 2 to 3 years, with the exception of 3 patients whose sera showed marked increases. Patients who had received aqueous therapy for more than 5 years did not show a significant change in the titer of sensitizing antibody when studied in 2 consecutive years. Since the average titer of the first sera drawn in this group was quite low, it is likely that they had already experienced a decrease in titer during earlier years of therapy and were in a relatively stable state at the time of this study. In 35 cases treated for 3 years with emulsified aqueous extract, titer of SSA one year after the last injection of emulsion was the same as that prior to treatment. Five other patients treated for 3 years with emulsions showed a significant elevation in posttreatment titers.

The evidence presented suggests that, in a significant number of patients, aqueous injection therapy causes a decrease in the titer of SSA. Although all patients did not show a decrease, it might be anticipated that this would occur in some additional subjects with continued treatment. Results of this study and our previous report¹⁷ suggest that a small number of patients may be refractory or even aggravated by treatment.

The decrease in titer of SSA, demonstrated in this study and suggested in a previous report⁶ (Fig. 2), represents a decrease in sensitivity, or hyposensitization. Several mechanisms could explain the decrease in titer. First, the quantity of antibody could remain the same, but its quality may be altered by treatment rendering it less active by passive transfer. Since SSA has not been isolated, this hypothesis cannot be tested. Second, injected antigen could neutralize SSA, but this is unlikely as a cause of the decrease in titer for several reasons. The amount of antigen required for neutralization is far in excess of that which could be safely injected at any one time. Furthermore, the production of antibody between injections of antigen could undoubtedly be in excess of that neutralized by the last injection. As injections of antigen are continued, blocking antibody will also be synthesized. Blocking antibody, which has a greater affinity for antigen than SSA, would then have to be neutralized before any additional SSA is neutralized. One of the most likely explanations for decreased titers of SSA with treatment is that the quantity of antibody is reduced because of decreased production. The decrease in synthesis of antibody may be analagous to "immune tolerance" produced in animals by antigen injections, although in adult hay fever patients treated with injections of antigen, complete cessation of synthesis of SSA specific for the antigen injected is rare. In this sense the result is a "partial immune tolerance." The mechanism causing immune tolerance is unknown.

Besides hyposensitization, injection therapy also stimulates the synthesis of an IgG class of antibodies, the blocking antibody. Thus, the patient is hypo-

sensitized and immunized simultaneously by injection therapy. We^{6, 17} have, in previous reports, presented evidence suggesting that blocking antibody protects the patient by decreasing the likelihood of systemic reactions when antigen is injected. However, we⁴ and others^{10, 11} have been unable to show that blocking antibody is a significant factor in preventing or decreasing nasal symptoms when the pollen is inhaled.

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