

THE EFFECT OF HYPOSENSITIZATION ON THE IN VITRO HISTAMINE RELEASE BY SPECIFIC ANTIGEN

Paul P. VanArsdel, Jr., M.D., Seattle, Wash., and Elliott Middleton, Jr., M.D., New York, N. Y.

HYPOSENSITIZATION of the allergic patient to specific antigen produces few changes which can be subjected to critical analysis and quantitative study. The appearance of blocking antibody and the rise in hemagglutinating antibody in the patient's serum during treatment provide us with some objective information, but the relationship of these antibodies to the physiologic effects associated with clinical improvement is unclear.^{1, 2}

It is now well known that incubation of specific antigen with whole blood from an allergic person induces release of histamine from the white cells into the plasma. This reaction can be studied quantitatively. Histamine is released in proportion to the antigen concentration up to a maximum of about 1 μ g protein nitrogen per 100 ml. of blood; higher concentrations, in general, release less histamine.³ Because of the physiologic implications of histamine release, it seemed appropriate to look for possible changes in this pattern of histamine release during the course of specific hyposensitization.

METHODS

Fourteen patients were studied. Thirteen had pollen sensitivity associated with clear-cut seasonal hay fever; most were sensitive to grass pollen, the remainder were sensitive to ragweed or English plantain. One other person, a laboratory technician, was sensitive to rat antigen clinically and by skin test. Prior to treatment of these individuals, and at intervals during treatment, blood was collected into silicone-treated centrifuge tubes, and aliquots were incubated for 30 minutes with concentrations of antigen varying from 0.1 to 40 μ g of protein nitrogen (PN) per 100 ml. of blood. Following incubation, the blood was centrifuged and the plasma assayed for histamine by the microchemical method of Lowry and his co-workers.⁴ More details of this procedure were reported earlier.³

From the Department of Medicine, University of Washington School of Medicine and College of Physicians and Surgeons, Columbia University.

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TABLE I. HISTAMINE RELEASED BEFORE AND DURING TREATMENT ($\mu\text{g/L}$ PLASMA)

PATIENT	ANTIGEN	TREATMENT		WHOLE BLOOD HISTAMINE ($\mu\text{g/L}$)	ANTIGEN CONCENTRATION ($\mu\text{g PN PER 100 ML. BLOOD}$)				
		DURATION	AMOUNT* ($\mu\text{g PN}$)		0.2	0.5	2.0	5.0	
Llo.	Ragweed	0	0	—	13†	28	49	31	—
		10 wk.	1.3	87	3	—	18	27	—
Dun.	Ragweed	0	0	91	0	3	17	45	—
		6 wk.	4.4	119	—	0	10 (8)	29 (22)	—
Fer.	Ragweed	0	0	—	7	20	38	39	—
		4 wk.	0.9	108	0	7	37	41	—
		12 wk.	13.6	72	0	8	35	28	—
Pal.	Ragweed	0	0	71	0	14	34	35	—
		28 wk.	40	—	0	3	12	15	—
					0.1	0.4	1.0	4.0	40
Wes.	Timothy	4 wk.	0.4	70	—	65	84	75	47
		16 wk.	8.4	71	—	0	1	3	3
		6 mo.	99	74	—	3	9 (8)	62	3
Pat.	Timothy	0	0	48	—	76	61	27 (23)	21 (18)
		14 wk.	32	57	2	8 (7)	22 (19)	35 (51)	39 (57)
		16 mo.	520	33	—	13 (19)	19 (28)	27	—
Tom.	Timothy	0	0	74	14	50	50	20 (16)	25 (20)
		8 wk.	3.6	91	—	37 (30)	16 (19)	32 (38)	14 (17)
		6 mo.	64	61	0	2 (2)	—	17 (20)	6 (7)
Wil.	Timothy	11 mo.	119	64	—	9 (10)	—	28	23
		0	0	121	1	22	43	22 (34)	4 (6)
		6 mo.	88	78	—	4 (6)	10 (15)	8 (10)	16 (20)
		19 mo.	208	97	—	4 (5)	4	43	30
Kum.	Timothy	0	0	36	41	49	51 (44)	32 (27)	21 (18)
		4 mo.	10.7	42	57 (49)	23	32	25	13
		14 mo.	92	35	14	61	65	57	30
Pla.	Timothy	0	0	50	45	7	32 (31)	46 (44)	35 (34)
		7 mo.	33	52	0	52	59	38	39
Mnl.	Timothy	0	0	46	31	9 (5)	13 (7)	21 (12)	34 (20)
		17 wk.	97	80	—	4 (3)	7 (5)	29 (22)	23 (17)
		4 mo.	220	62	—	—	10 (9)	23 (20)	13 (11)
		10 mo.	365	53	—	—	35 (24)	59 (40)	49 (33)
		16 mo.	550	68	—	24 (16)	59 (53)	55 (50)	57 (52)
		17 mo.	600	51	—	45 (41)	51	18	0
Ear.	Plantain	0	0	66	27	56	—	3 (5)	3 (5)
		9 wk.	6.7	40	—	5	8 (9)	7 (8)	0
		8 mo.	67	61	—	—	11	10	4
		15 mo.	137	68	—	—	83	59	39
Rad.	Plantain	0	0	101	70	97	90 (78)	59 (51)	23 (20)
		9 wk.	2.5	116	—	62 (54)	23 (32)	30 (42)	10 (14)
		5 mo.	44	72	3 (4)	14 (20)	37 (38)	48 (50)	29 (30)
		9 mo.	92	97	—	34 (35)	27	82	98
Han.	Rat	0	0	94	0	6	1 (2)	37 (70)	69 (130)
		4 mo.	32	50	0	0	13 (17)	44 (59)	54 (72)
		8 mo.	42	70	—	0	—	0	0
Van.	Timothy	0	0	113	—	0	—	0	2
	Control	4 wk.	350	118	—	—	—	0	—

*Total amount of antigen given, in micrograms of protein nitrogen. For approximate amount of each injection, see text.
†Each figure gives the observed plasma histamine value less the control plasma value.

RESULTS

The amount of histamine released into plasma for each study is given in Table I. The antigen concentrations were somewhat different for the ragweed group (0.2, 0.5, 2.0 and 5.0 μg protein nitrogen per 100 ml. blood) than for the rest (0.1, 0.4, 1.0, 4.0). The plasma value was obtained by subtracting the control plasma concentration for each patient from the amount found after incubation with antigen. There was little variance in control plasma histamine levels; for the whole group, the mean plasma histamine was $7.2 \pm 2.7 \mu\text{g}$ per liter. By contrast, blood histamine levels in some patients varied widely. Thus it was

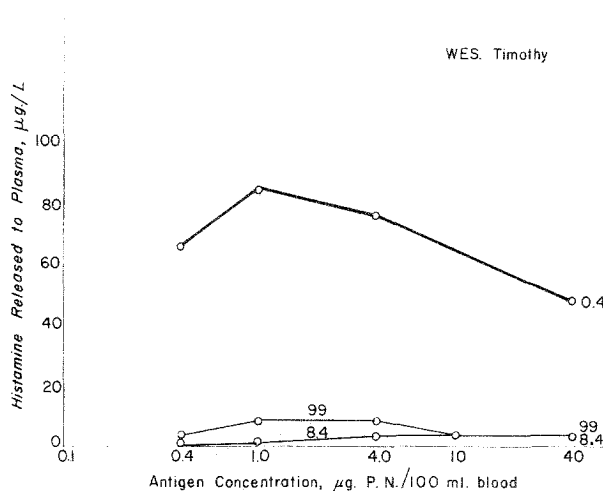


Fig. 1.—Histamine released to plasma in three studies on blood from patient *Wes*. The heavy line shows the results of the first study, which was done 4 weeks following onset of therapy. The numbers with each line give the total accumulated antigen dose in micrograms of protein nitrogen (PN).

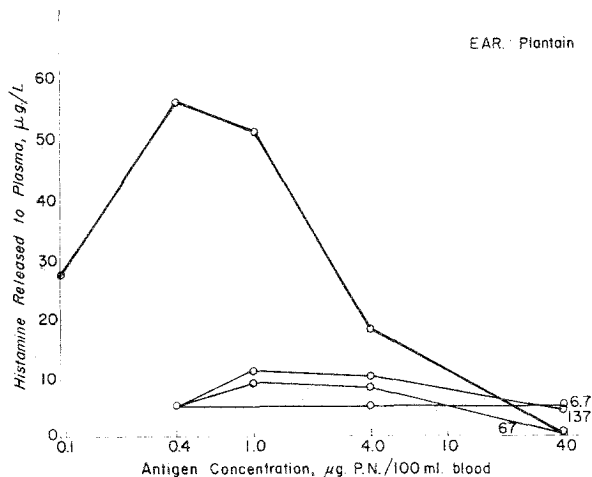


Fig. 2.—Histamine released to plasma in four studies on blood from patient *Ear*. The heavy line indicates the pre-treatment values. The numbers with each line give the total accumulated antigen dose in micrograms of PN.

necessary to relate the amount of histamine released to the amount of *available histamine* (whole blood histamine). Accordingly, many of the plasma values were corrected to correspond to the whole blood histamine of the pre-treatment studies. This was done by the following calculation. For each study: Histamine released (corrected) equalled

$$\text{Observed histamine released} \times \frac{\text{Pre-treatment whole blood histamine}}{\text{Whole blood histamine in that study}}$$

It can be seen that the effects of treatment varied considerably among the patients studied. Two patients, *Wes.* and *Ear.*, were unique, since histamine release was markedly inhibited early in treatment and the inhibition occurred at all antigen concentrations. Figs. 1 and 2 show these impressive changes.

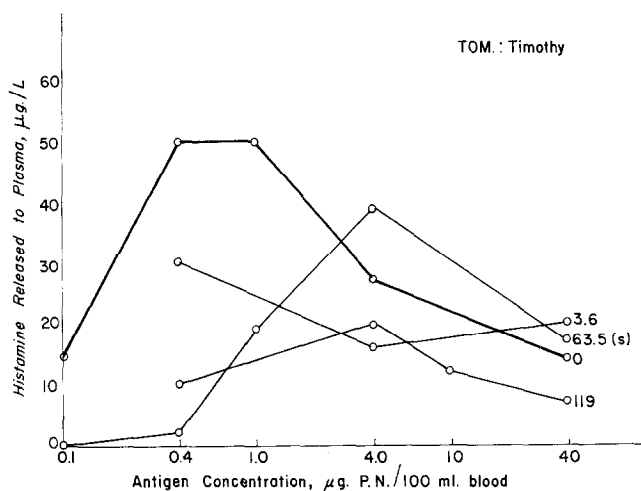


Fig. 3.—See legend for Fig. 2. (s) indicates that the study was done during the pollen season.

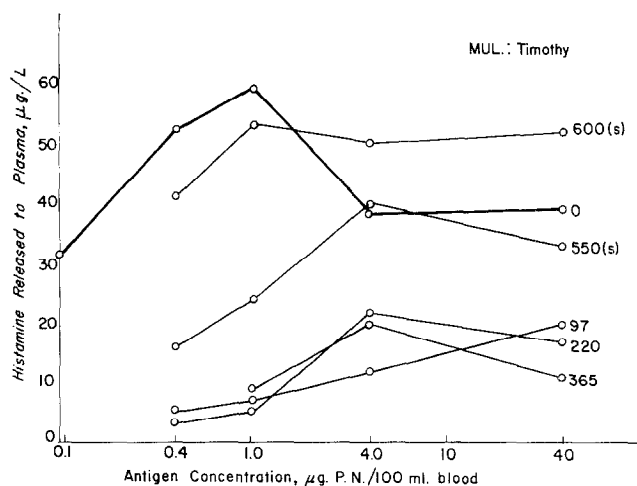


Fig. 4.—See legend for Fig. 2. (s) indicates that the study was done during the pollen season.

All others in this study had progressive suppression at lower antigen concentrations during their course of hyposensitization, but changes at antigen concentrations of $4 \mu\text{g}$ per 100 ml. and above were variable, and usually insignificant. Figs. 3 and 4 show our findings on the 2 timothy-sensitive individuals who were studied most extensively. Continuation of specific antigen therapy to a total of more than $100 \mu\text{g}$ protein nitrogen (10,000 PNU*) produced little additional change. Indeed, studies carried out during the pollen season (indicated by [s] in Figs. 3 and 4) showed considerable increase in histamine release; this was most impressive in studies on patient *Mul.* (Fig. 4) who had received maximum amounts of desensitization and remained clinically well during the season.

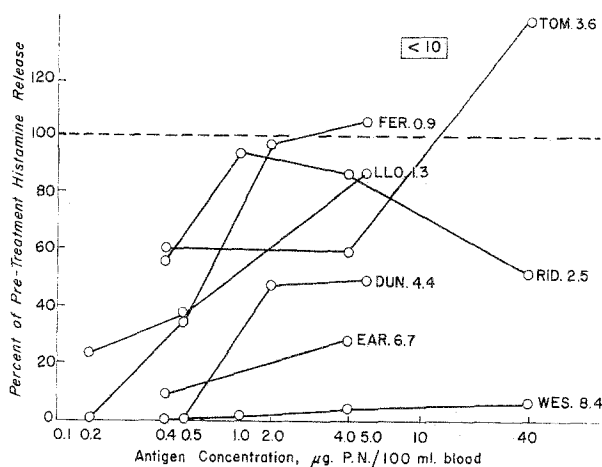


Fig. 5.—Seven early studies on patients during hyposensitization treatment (total antigen less than $10 \mu\text{g}$ PN). The data are given as the per cent of the pre-treatment histamine values at each antigen concentration. The numbers with each patient's name indicate the total antigen dose given.

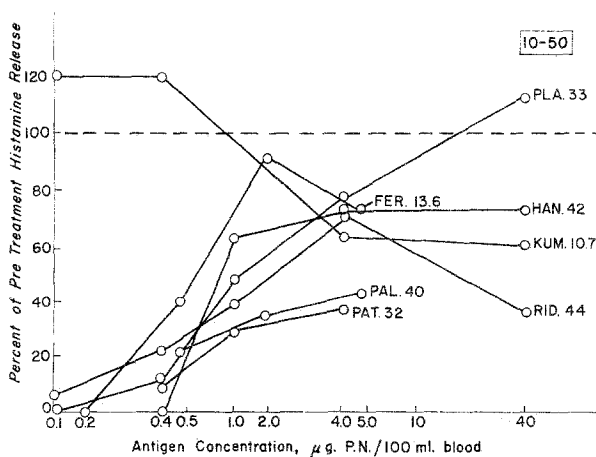


Fig. 6.—Same as Fig. 5, for 7 studies at total antigen doses from 10 to $50 \mu\text{g}$ PN. Only one of the two studies on patient *Han.* is charted.

*Protein nitrogen units.

Interpretation of our observations in toto was best achieved by considering the histamine released during various stages of hyposensitization in relation to pre-treatment histamine release. Figs. 5 through 8 summarize this information. The corrected values (those in parentheses in Table I) were used, and divided by the corresponding pre-treatment value for each antigen concentration. Fig. 5 shows that marked effects were produced during early hyposensitization at antigen concentrations up to $0.5 \mu\text{g}$ PN per 100 ml. blood in some subjects and at all concentrations for the exceptional patients *Ear.* and *Wes.* The total antigen dose (0.9 to $8.4 \mu\text{g}$ PN, or 90-840 PNU) represented a build-up to amounts ranging from 30 PNU to 200 PNU per injection. Fig. 6 (total amount, 10 to 50

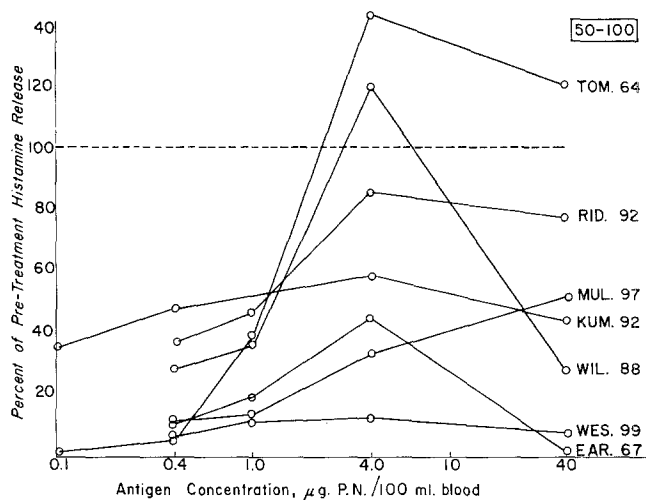


Fig. 7.—Same as Fig. 5, for 7 studies at total antigen doses from 50 to 100 μg PN. One study (on *Tom.*), which was done during the pollen season, is not included.

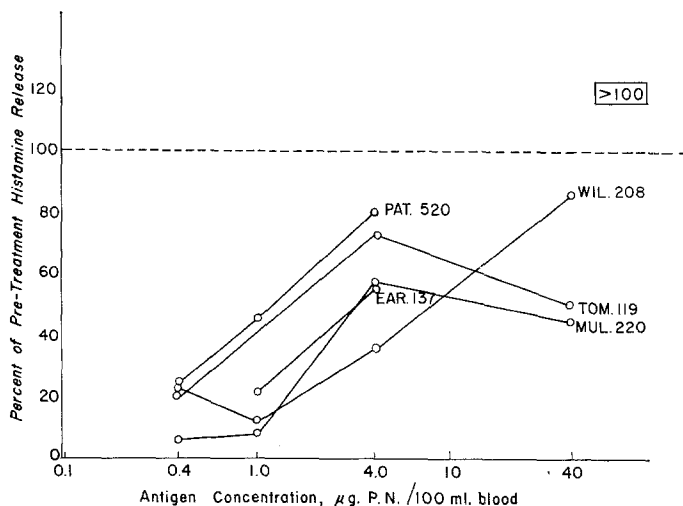


Fig. 8.—Same as Fig. 5, for 5 studies at total antigen doses over 100 μg PN. Two other studies, on patient *Mul.*, were done during the pollen season and are not included.

μg PN) shows general suppression of histamine release up to an antigen concentration of $0.5 \mu\text{g}$ PN per 100 ml. blood. This table presents those studies in which the amount of antigen for each injection varied from 250 to 600 PNU. Figs. 7 and 8 include all studies done when full maintenance dosage (1,000 to 5,000 PNU) was reached. A comparison of Fig. 7 with Fig. 8 will indicate how little was achieved relative to histamine release by prolonged maintenance hyposensitization.

The data from Figs. 5 through 8 were summarized by observing the frequency with which the four levels of hyposensitization suppressed histamine release to less than half the pre-treatment values. This information is charted

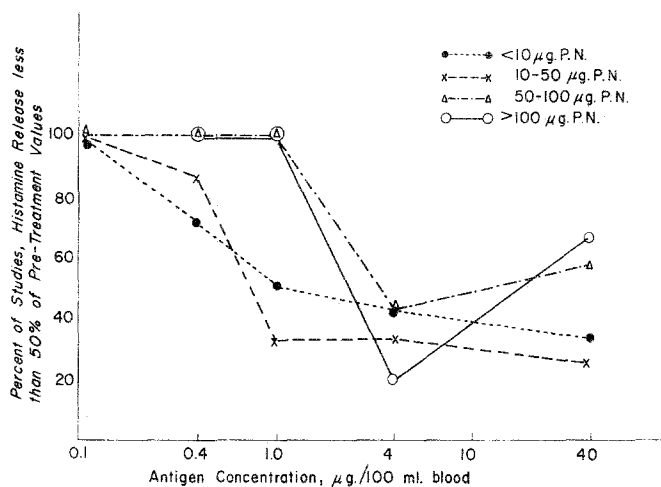


Fig. 9.—The four lines correspond to the four groups of studies presented in Figs. 5-8. The data are presented here as the percentage of studies at each antigen concentration with histamine release less than half the pre-treatment level. For simplicity, data for antigen concentrations relatively close together (e.g., 0.1 and $0.2 \mu\text{g}$ PN) were pooled and charted at the lower concentration.

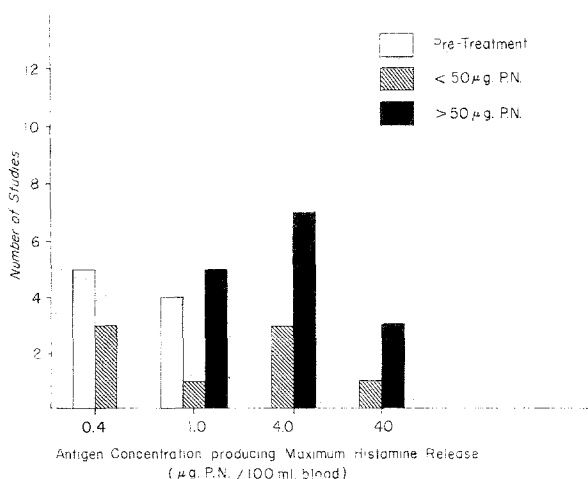


Fig. 10.—The shift in antigen concentration which occurred in 9 patients is charted separately for studies done early in treatment (total antigen dose less than $50 \mu\text{g}$ PN) and those done later (total antigen dose greater than $50 \mu\text{g}$ PN).

in Fig. 9. The build-up in hyposensitization resulted in a significant inhibition of histamine release at antigen concentrations up to at least 1 μg PN per 100 ml. blood. The least effects were seen at an antigen concentration of 4 μg PN per 100 ml. blood; suppression was observed more frequently, but with no consistent pattern, at the top antigen concentration of 40 μg PN per 100 ml. blood.

Another, more subtle, effect was observed in the data from the 9 timothy- and plantain-sensitive patients, who were studied more completely than the rest. This effect was a progressive rise during treatment of that antigen concentration producing the *greatest* amount of histamine release. Fig. 10 shows this shift graphically. The optimum antigen concentrations for histamine release before treatment were 0.4 or 1.0 μg PN per 100 ml. blood, similar to those reported earlier.³ Following hyposensitization, the modal concentration for optimum release was 4 μg PN per 100 ml. blood.

DISCUSSION

The mechanism by which hyposensitization is achieved has been attributed to neutralization of antigen by blocking antibody, but the correlation of blocking antibody titer with clinical improvement has not been impressive.¹ Our subjects have all experienced worth-while clinical relief, but we are unable to quantitate the clinical effects adequately for correlation with the changes in histamine release.

It was most interesting to observe that the patients *Ear.* and *Wes.* experienced complete clinical remission, since histamine release in their blood was so clearly suppressed during hyposensitization at all antigen concentrations. We have not studied them further, but passive transfer and symptom-provocation tests with specific antigen would be of great interest. It is possible that the mechanism of remission in these individuals is unique, with true desensitization occurring through destruction of the antibody-forming "clones." If this were true, such patients should have no recurrence of symptoms and perhaps no increase in in vitro histamine release after therapy is stopped.

The changes seen in the studies on the other patients are consistent with the appearance of a blocking antibody, increasing in titer only during an increase in the amount of antigen injected. Fig. 9 can be interpreted to indicate that upward of 1 μg PN (100 PNU) of antigen per 100 ml. blood is neutralized at full desensitization. The shift in the concentration producing maximum histamine release during treatment (Fig. 10) is also consistent with in vitro antigen neutralization of concentrations somewhat less than 4 μg PN per 100 ml. blood. Observations at several intermediate antigen concentrations would have allowed more significant conclusions from these data. The interesting, but variable, decreases in histamine release at the highest antigen concentration are not explained adequately by the neutralizing effect of blocking antibody, and may indicate that changes in cellular responsiveness to antigen also occur during specific antigen treatment.

The possibility of a cellular effect is intriguing. Certainly the changes observed during treatment of patients *Ear.* and *Wes.* cannot be explained by the mere presence of blocking antibody. It is now known that antigen will release

histamine from washed leukocytes collected from sensitive persons.⁵ If antigenic histamine release from these washed cells is also suppressed during specific treatment, it will no longer be necessary to attribute the clinical effects of hyposensitization entirely to the production of blocking antibody.

SUMMARY

Whole blood from 14 untreated allergic patients was incubated with various concentrations of specific antigen and the amount of histamine released into the plasma was measured chemically. During the course of specific hyposensitization of these patients, a total of 30 similar studies were carried out, and the pattern of histamine release was compared with the pre-treatment data for each patient.

Studies on 2 patients showed that treatment was associated with a suppression of histamine release which was practically complete at all antigen concentrations. In the remainder, suppression of histamine release occurred only at low antigen concentrations, and was more effective as treatment progressed. However, surprisingly little change occurred at an antigen concentration of 4 μ g protein nitrogen per 100 ml. blood, even though more suppression was often seen at the highest concentration (40 μ g PN per 100 ml. blood).

Some of our observations are explained by the production of serum blocking antibody during treatment, but other changes are more consistent with an alteration in cellular reactivity during hyposensitization.

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