

Allergens of mammalian origin

IV. Evidence for common allergens in cat and dog serum

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IgE antibodies present in serum from 3 patients clinically sensitive to cat and dog were shown to combine with whole cat and dog sera linked to cellulose particles. Employing a modified radioallergosorbent technique (RAST), it was shown that cat and dog sera inhibited the binding of IgE antibodies to insolubilized cat serum and to insolubilized dog serum. These findings suggest that cat and dog sera have common allergens. Other mammalian sera were also tested and found to have little inhibitory activity. Cat and dog sera did not inhibit the binding of IgE antiragweed antibodies to an insolubilized ragweed fraction. Following separation of cat serum by gel filtration on Sephadex G-200 or electrophoresis in acrylamide gel, several fractions were found to inhibit the binding of IgE antibodies to insolubilized cat and dog sera. Whether the heterogeneity observed reflects the presence of single allergens with diverse size and charge properties or multiple allergens remains to be determined.

Patients who are clinically sensitive to cats or dogs can frequently be shown to exhibit immediate hypersensitivity reactions to a number of components derived from either of these animals. One or more of these components predominate in the pelt or skin of the animal,^{1, 2} while others predominate in the serum.^{3, 4} A previous report of skin test reactions to extracts of cat and dog skin indicated that the molecular size characteristics of cat and dog allergens were similar.² The following study provides direct evidence for common allergens in cat and dog sera and reports some of the molecular size and electrophoretic charge properties of the allergens in cat serum.

METHODS

Serum

Whole blood was obtained by cardiac puncture from three mongrel cats and dogs anesthetized with pentobarbital. After clotting of the blood, serum was separated, pooled, and stored at -20° C.

Gel filtration

Sephadex G-200 was equilibrated in 0.001 M Tris-buffered saline (0.15 M) at pH 8.0 as recommended by the manufacturer. Two-milliliter samples of serum were dialyzed against the

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same solution and applied to 2×100 cm columns at 4°C ; 4-ml fractions were collected. The optical density of various fractions was measured at 280 nm. Human γ -globulin (obtained from Schwarz Mann, Orangeberg, N. Y.) and cat albumin¹ were used as markers of known molecular size in calibrating these columns.

Polyacrylamide disc gel electrophoresis

Polyacrylamide disc gel electrophoresis was performed as previously described using tubes measuring 6×100 mm.¹ The gel contained 7% acrylamide plus 0.18% N,N'-methylenebisacrylamide. The sample, consisting of 2 μl of cat serum plus 50 μl of 40% sucrose, was applied to the top of the column and a current of 4 mA per tube was applied until the bromphenol blue indicator had migrated 50 mm into the gel. The gel columns were then removed from the glass tubes and stained with Coomassie blue. Fractions of the gel columns were prepared by embedding the columns in 1% agar immediately after electrophoresis and slicing the gel into 5-mm cylinders beginning at the bromphenol blue indicator. The cylinders were then homogenized and fractions were eluted with 1 ml of phosphate-buffered (0.01 M) saline (0.15 M), pH 7.5 (PBS), for 6 hr at 20°C and overnight at 5°C . A gel column that had been prepared under identical conditions was marked at 5-mm intervals with India ink and stained; this procedure permitted us to correlate the activity of eluates with specific bands of protein.

Radioallergosorbent test

Fifty microliters of cat or dog serum were coupled to 200 mg of cyanogen bromide-activated cellulose particles. The radioallergosorbent test was performed as described previously.¹ Fifty microliters of patient serum was diluted with an equal volume of assay buffer and added to plastic test tubes containing 0.5 mg of cat serum cellulose or dog serum cellulose. After rotation of the tubes for 12 hr at room temperature, the cellulose particles were washed three times with borate-buffered (0.005 M) saline (0.15 M), pH 8.0 (BBS), and approximately 10 ng of anti- ϵ antibody with an activity of 50,000 to 100,000 cpm, was added to each tube. The tubes were again rotated at room temperature for 12 hr and the cellulose particles washed 3 times with BBS. The radioactivity remaining bound to the cellulose was measured in a crystal scintillation spectrometer.

An inhibition procedure was carried out as previously described¹; in this procedure, 100- μL samples of cat or dog serum were added to test tubes containing insolubilized cat or dog serum. Soluble and insoluble serum allergens were allowed to compete for binding sites on human IgE antibodies. Following the initial incubation, the cellulose particles were reacted with ^{125}I -labeled anti- ϵ antibody; the amount of radioactivity bound to the cellulose particles was compared to a control in which soluble serum allergen had not been added. The results were expressed as percent inhibition of IgE antibody uptake by insolubilized cat or dog serum. All tests were carried out in duplicate. The variation in results between duplicate tests was less than 5%.

Skin testing

Prick tests were performed as described previously using fractions prepared in 50% glycerol.¹ Informed consent was obtained prior to testing. The average diameter of the flare in millimeters was noted after 15 min. Reactions of less than 5 mm in diameter were disregarded. Each subject's maximum reaction was taken as 100%; the skin test reactions were expressed as a percent of the maximum reaction.

RESULTS

Binding of human IgE antibodies to insolubilized cat and dog serum

Serum was obtained from 16 patients known to have clinical sensitivity to cats and dogs as well as intense skin reactivity to cat and dog dander extracts. Each serum was then tested for IgE antibody binding to insolubilized cat and dog sera. IgE antibody binding was significantly different from normal control serum

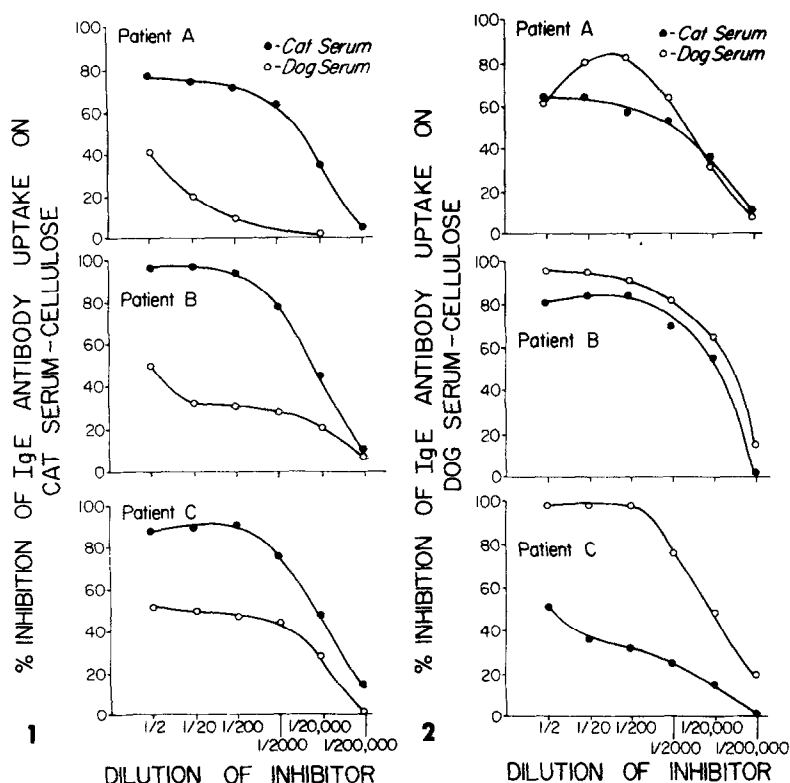


FIG. 1. Inhibition of binding of IgE antibodies to insolubilized cat serum. One hundred microliters of diluted cat and dog sera were added to 0.5 mg of insolubilized cat serum. The serum from Patients A, B, or C was then added to the system. With all three patients sera, cat serum was more effective than dog serum in inhibiting the binding of IgE antibodies to insolubilized cat serum. Dog serum had a lesser but significant inhibitory effect.

FIG. 2. Inhibition of binding of IgE antibodies to insolubilized dog serum. One hundred microliters of diluted cat and dog sera were added to 0.5 mg of insolubilized dog serum. The serum from Patients A, B, or C was then added to the system. With Sera A and B, cat serum was nearly as effective as dog serum in inhibiting the binding of IgE antibodies to insolubilized dog serum. With serum from Patient C, dog serum was a more effective inhibitor.

in three sera from Patients A, B, and C (Table I). All three sera contained IgE antibodies that bound to both insolubilized cat serum and to insolubilized dog serum. The binding with serum from Patients A and C was greater to insolubilized cat serum than to insolubilized dog serum. Sera from Patients A, B, and C did not bind significantly to a ragweed fraction linked to cellulose.

Inhibition of binding by soluble cat and dog sera

Cat and dog sera were tested for their ability to inhibit the binding of human IgE antibodies to insolubilized cat serum. The addition of cat serum resulted in maximum inhibition of binding ranging from 78% to 97% depending upon the patient serum tested (Fig. 1). The displacement curves of all three patients were similar. Addition of dog serum also resulted in significant inhibition of binding

TABLE I. IgE antibody binding to insolubilized cat and dog sera

	Patient A	Patient B	Patient C	Control
IgE antibody binding to cat serum cellulose	1,535*	781	1,939	295
IgE antibody binding to dog serum cellulose	1,262	734	791	173

*Fifty microliters of patient serum was added to 0.5 mg of cat serum or dog serum cellulose. The IgE antibody binding to the absorbent was then measured as indicated in "Methods"; numbers represent counts per minute.

TABLE II. Inhibition of binding of IgE antibodies to insolubilized cat and dog sera by various other mammalian sera

Dilution of serum tested for inhibitory activity*	Percent inhibition of IgE antibody uptake on:					
	Cat serum cellulose			Dog serum cellulose		
	Patient A	Patient B	Patient C	Patient A	Patient B	Patient C
<i>Horse serum</i>						
1/2	—	20	32	12	25	16
1/20	2	9	19	7	13	9
1/200	—	8	—	—	—	—
<i>Rabbit serum</i>						
1/2	0	12	22	23	23	15
1/20	0	6	15	17	19	16
1/200	—	0	—	—	—	—
<i>Rat serum</i>						
1/2	13	31	26	13	24	37
1/20	3	14	13	8	23	1
1/200	—	10	—	—	—	—
<i>Mouse serum</i>						
1/2	8	33	17	14	40	12
1/20	4	20	12	6	26	10
1/200	—	12	—	—	—	—
<i>Guinea pig serum</i>						
1/2	9	38	21	22	38	39
1/20	12	26	10	9	17	8
1/200	—	13	—	—	—	—

*One hundred microliters of diluted serum were added to 0.5 mg of insolubilized cat or dog serum. The serum from Patient A, B, or C was then added to the system. Pooled test serum was obtained as described previously.⁵

of human IgE antibodies to insolubilized cat serum; maximum inhibition ranged from 40% to 53% (Fig. 1).

Cat and dog sera were also tested for their ability to inhibit binding of human IgE antibodies to insolubilized dog serum. The addition of dog serum resulted in maximum inhibition of binding ranging from 83% to 99% (Fig. 2). Addition of cat serum resulted in maximum inhibition of 51% to 84%. Cat and dog sera inhibited the binding of IgE antibodies present in serum from Patients A and B to a similar extent, while dog serum was considerably more effective than cat serum in inhibiting the binding of IgE antibodies present in serum from Patient C. These findings suggest that IgE antibodies in serum of Patients A and B are directed primarily toward components of cat serum, while the IgE antibodies of Patient C include some with specificity for unique components of dog serum.

The inhibitory effect of various other mammalian sera (horse, rabbit, rat, mouse, and guinea pig) was tested (Table II). Inhibition of IgE antibody bind-

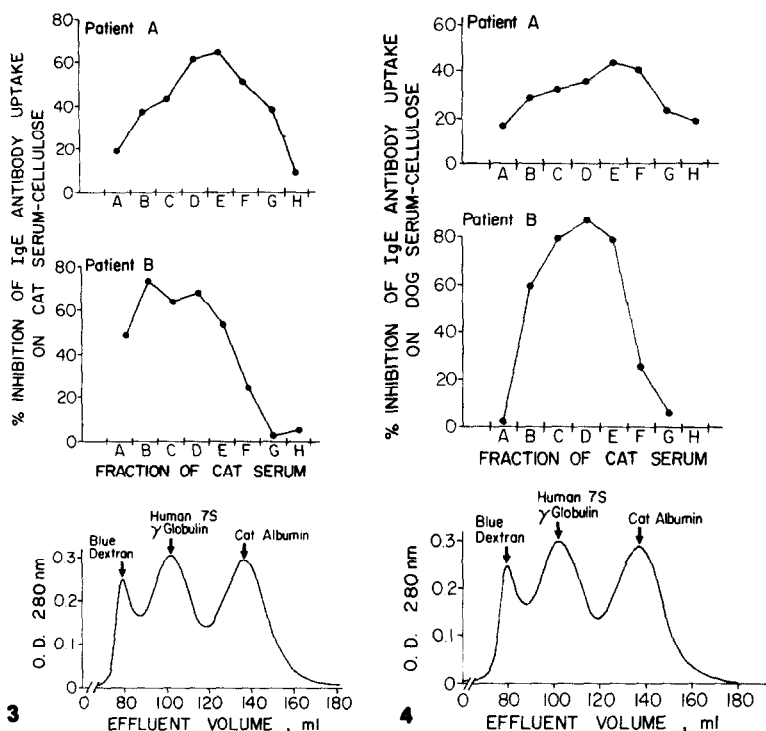


FIG. 3. Gel filtration of cat serum on Sephadex G-200. The eluate was pooled as shown; 100 μ L of a 1:200 dilution of each fraction was tested for its ability to inhibit the binding of IgE antibodies to insolubilized cat serum. With serum from Patient A, maximum inhibition occurred with fractions D, E, and F (upper panel); with serum from Patient B, maximum inhibition occurred with fractions B, C, and D (middle panel).

FIG. 4. Gel filtration of cat serum on Sephadex G-200. Fractions obtained as in Fig. 3 were used to inhibit the binding of IgE antibodies to insolubilized dog serum. With serum from Patient A, maximum inhibition occurred with fractions D, E, and F (upper panel); with serum from Patient B, maximum inhibition occurred with fractions C, D, and E (middle panel).

ing to insolubilized cat and dog sera by a 1:2 dilution of the various test sera ranged from 8% to 40%. Further dilution of these sera almost always resulted in a reduction of the inhibitory effect. While degrees of inhibition above 15% are probably significant, it is apparent that cat and dog sera are more potent inhibitors than these other mammalian sera.

The possibility that mammalian sera might nonspecifically inhibit IgE antibody binding to insolubilized allergen was tested in a second assay system. In this system, serum from a patient allergic to ragweed was combined with ragweed fraction D⁶ linked to cellulose, followed by the addition of ¹²⁵I-labeled anti- ϵ antibody. Uptake of radioactivity was six times greater than that obtained with a normal serum control. The addition of 100 μ L of a 1:2 dilution of cat, dog, horse, rabbit, rat, mouse, or guinea pig serum to the test system reduced the binding of IgE antibodies to insolubilized allergen by no more than 8%.

Inhibition of binding by fractions of cat serum separated by gel filtration

Preliminary studies indicated that extensive ultrafiltration (PM 30 filters, Amicon Corp., Lexington, Mass.) of cat and dog sera to eliminate proteins with a molecular weight of less than 30,000 daltons did not reduce the inhibitory effect of these sera on IgE antibody binding to insolubilized cat and dog sera.

In order to further define the molecular size characteristics of the inhibitory substances, cat serum was subjected to gel filtration on Sephadex G-200 (Fig. 3, lower panel). Three protein peaks were obtained: the first contained proteins of molecular size greater than 200,000 daltons, the second contained proteins with the approximate molecular size of γ -globulin (160,000 daltons), and the third contained albumin (68,000 daltons). The eluates were combined into 8 fractions; a 1:200 dilution of each fraction was tested for its ability to inhibit the binding of IgE antibodies to insolubilized cat serum. Two patterns of inhibition were seen. With serum from Patient A, maximum inhibition occurred with fractions D, E, and F (Fig. 3; upper panel); with serum from Patient B, maximum inhibition occurred with fractions B, C, and D (middle panel). The pattern of inhibition observed with serum from Patient C was similar to that seen with serum from patient A.

The fractions of cat serum were tested for their ability to inhibit the binding of IgE antibodies to insolubilized dog serum (Fig. 4). With serum from Patient A (and C, not shown), maximum inhibition occurred with fractions D, E, and F; with serum from patient B, maximum inhibition occurred with fractions C, D, and E. The inhibition experiments were repeated three times; the same pattern of inhibition was observed each time.

Inhibition of binding by fractions of cat serum separated by electrophoresis in acrylamide gel

Cat serum was subjected to electrophoresis in 7% acrylamide gel (Fig. 5). A stained acrylamide gel is illustrated in the lower portion of the figure; numerous protein bands were visualized that had mobilities ranging from that of albumin (fraction 3) to that of γ -globulin (fraction 11). Twelve fractions were obtained from an identical acrylamide gel by slicing the gel and eluting with PBS. These cat serum fractions were tested for their ability to inhibit binding of IgE antibodies to insolubilized cat serum. Three peaks of inhibitory activity were seen that corresponded to stained protein with albumin, intermediate and γ -globulin mobility. The serum from Patient A was used in this experiment. With serum from Patient B, a similar pattern of inhibition was seen except that the fraction of γ -globulin mobility had a greater inhibitory effect (46%). With serum from Patient C, the pattern of inhibition was similar to that of Patient A.

The same fractions of cat serum were tested for their ability to displace IgE antibody from insolubilized dog serum (Fig. 6). Two peaks of inhibitory activity were seen that corresponded to stained proteins with albumin and intermediate mobility. Identical patterns of inhibition were seen with serum from Patients B and C. These findings suggest that components of cat serum which inhibit binding to insolubilized dog serum appear to be somewhat less heterogeneous than the components involved in inhibiting the binding to cat serum.

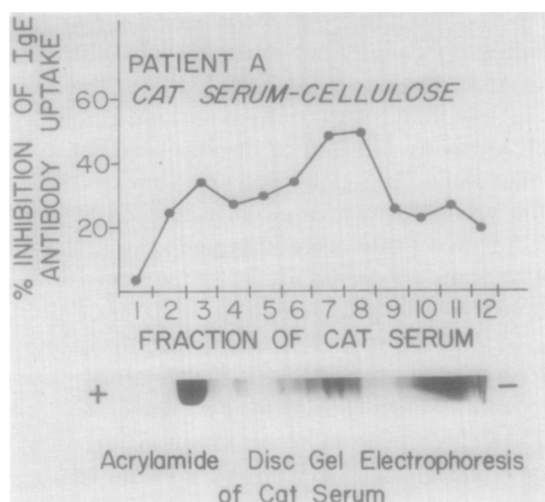


FIG. 5. Polyacrylamide disc gel electrophoresis of cat serum. Following electrophoresis, gel slices were obtained as shown and proteins contained therein were eluted with 1 ml of saline. Eluates were tested for their ability to inhibit the binding of IgE antibodies to insolubilized cat serum. Inhibitory activity was localized to areas of the gel that had albumin (fraction 3), intermediate (fractions 7 and 8), and γ -globulin mobility (fraction 11).

Skin test reactivity of fractions of cat serum prepared by gel filtration

Cat serum was separated into 8 fractions by gel filtration on Sephadex G-200 (Fig. 7); the elution profile obtained was similar to that seen in Fig. 3. Prick tests with undiluted pooled eluate were performed in 5 subjects who were allergic to cat serum. Average prick test reactions are indicated in Fig. 7. Fractions that produced the largest prick test reactions contained proteins with a molecular size between that of the albumin and γ -globulin markers. One of the subjects who was skin-tested with cat serum fractions was also skin-tested with fractions obtained by Sephadex G-200 gel filtration of dog serum (not shown). The fractions of dog serum that produced the largest prick test reactions also contained proteins with a molecular size between that of the albumin and γ -globulin markers.

DISCUSSION

This study utilized the radioallergosorbent assay to test the ability of cat and dog sera to inhibit the binding of human IgE antibodies to insolubilized cat serum and to insolubilized dog serum. The finding that cat serum inhibited binding to insolubilized dog serum and that dog serum inhibited binding to insolubilized cat serum strongly suggested the presence of common allergens in cat and dog sera. Cat serum was a particularly effective inhibitor of binding to insolubilized cat serum as well as to insolubilized dog serum.

Considerable heterogeneity in pattern of inhibition and molecular size and charge properties of the allergens was observed. It is not known whether the latter heterogeneity reflects the occurrence of a limited number of allergens occurring as multiple size or charge moieties, or whether a large number of aller-

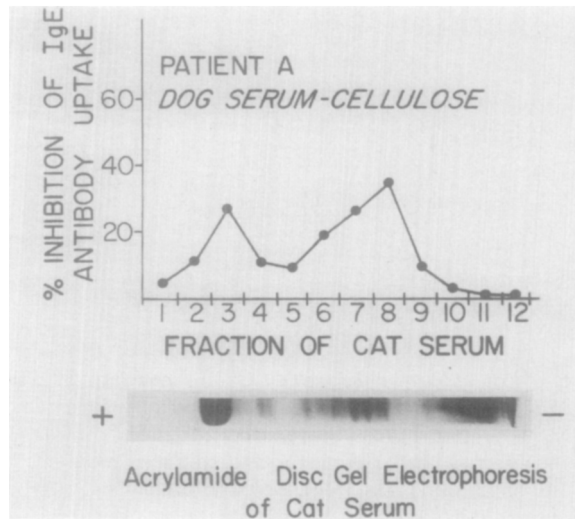


FIG. 6. Polyacrylamide disc gel electrophoresis of cat serum. Fractions obtained as in Fig. 5 were tested for their ability to inhibit binding of IgE antibodies to insolubilized dog serum. Inhibitory activity was localized to areas of the gel that had albumin (fraction 3) and intermediate mobility (fractions 7 and 8).

gens are involved. The pattern of inhibition (Fig. 2) suggests that one of the three patients, who were all clinically sensitive to cats and dogs, did form IgE antibodies to unique allergens in dog serum. Further characterization of the serum allergens will be required in order to determine the number of antigen-antibody systems involved.

The measurement of allergenic activity by an *in vitro* test as described in this report may not be equivalent to an *in vivo* procedure such as skin testing. Some investigators have suggested that allergens with a small number of determinants per molecule may be less reactive *in vivo* than *in vitro*.⁷ It is also possible that certain allergenic components may combine poorly with cellulose particles. Cat serum was therefore separated into eight fractions by gel filtration on Sephadex G-200 (Fig. 7). Allergenic activity as determined by skin testing was recovered in fractions containing proteins with a molecular weight between that of the albumin and γ -globulin markers. These studies indicate that the molecular size characteristics of at least some of the allergens in cat serum are similar whether measured by an *in vitro* or an *in vivo* method. In addition, one patient reacted by skin test to allergenic determinants in cat and dog sera that had a similar molecular weight. A more direct *in vivo* demonstration of common allergens in cat and dog sera might be achieved by the use of a passive transfer cross-neutralization test.⁸

The allergens in cat serum are distinct from the most active component present in cat pelts. The major allergen in cat pelt (cat allergen 1) has recently been isolated and found to have a molecular size between 30,000 and 60,000 daltons and a pre-albumin mobility on electrophoresis in 7% acrylamide gel.¹

The clinical significance of the various allergens that a cat or dog produces re-

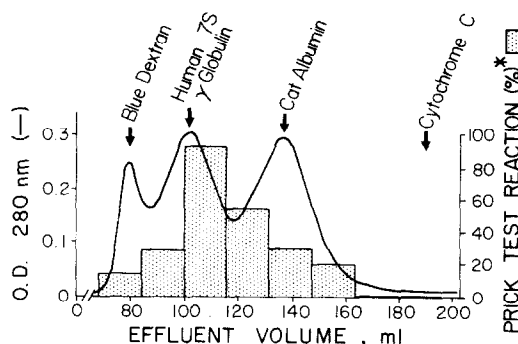


FIG. 7. Gel filtration of cat serum on Sephadex G-200 (see Fig. 3). Prick tests were performed with undiluted pooled eluate in 5 patients clinically sensitive to cats. Allergic activity was largely recovered from pooled fractions that contained proteins with a molecular size between that of the γ -globulin and albumin markers. *, The diameter of the flare of each patient's maximum reaction was taken as 100%; other skin tests reactions were expressed as a percent of the maximum reaction. Average values are indicated.

quires further study. It must be shown that these allergens are present in sufficient concentration during natural exposure to result in manifestations of disease. Patients may become sensitized by, and respond with the production of IgE antibody to, a dose of allergen that is below the dose required to produce significant clinical symptoms.

Other investigators have shown that skin test reactions to animal dander extracts correlate with one another.⁹ This study provides evidence that common allergens are present in serum derived from two species of mammals.

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