

# Neonate blood IgE levels on filter paper as indicators of atopic disease

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Measurements of IgE levels in the blood of neonates were investigated using filter paper for blood collection in mass screening of congenital metabolic disorders. Time-resolved fluoroimmunoassay system for the measurement of filter paper blood IgE levels was also studied. In an analysis of the present results, IgE values of at least 0.015U/ml, the measurement limit, were considered as high. High IgE levels in filter paper blood were seen in 28 (7.2%) of the 389 cases. When the relation with serum IgE levels at 18 months of age was investigated in 134 of 389 subjects, high serum IgE levels were also found in about 86.7% of the subjects with high IgE levels in filter paper blood. In addition, when the relation between family history of atopic disease and presence of atopic disease in the first 18 months of age was investigated in 203 of the 389 subjects, about 90% of the subjects with a family history of atopic disease and high IgE levels in filter paper blood developed atopic disease. Since filter paper blood is routinely collected in Japan, IgE levels in this blood should be widely checked for the prediction of onset of atopic disease in infants.

*Key words:* atopic disease; filter paper blood; fluoroimmunoassay; IgE; neonate; prediction.

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## CLINICAL ASPECTS

In Japan, it is very difficult to obtain cord blood on a routine basis. In mass screening of neonatal diseases in Japan, the heels of neonates at 4 to 5 days of age are punctured and the obtained blood is dripped onto filter paper. Filter paper blood is collected from all neonates. Therefore, if it were possible to measure IgE values from filter paper blood, this would be highly useful as an indicator for the early prediction of the onset of atopic disease.

Attempts have been made to predict the onset of atopic disease in infants by measuring IgE levels in cord blood (3, 6, 7, 8, 10, 13, 15). Croner et al. (3) measured IgE levels in cord blood in 1,701 cases by Phadebas IgE PRIST, and then re-measured serum IgE levels between the ages of 18 months and 2 years. The results indicated that serum IgE levels in infants are already genetically coded at birth since cases with high IgE levels in cord blood showed similarly high

levels at 18 months to 2 years of age. Therefore, IgE levels in cord blood should be measured in mass screenings for early prediction of the onset of atopic disease in infants. Kimpen et al. (6, 7) reported similar results in cord blood measurements in 5,353 cases.

In Japan, it is very difficult to obtain cord blood on a routine basis. In the present study, IgE levels in the blood of neonates were measured by means of the filter paper for blood

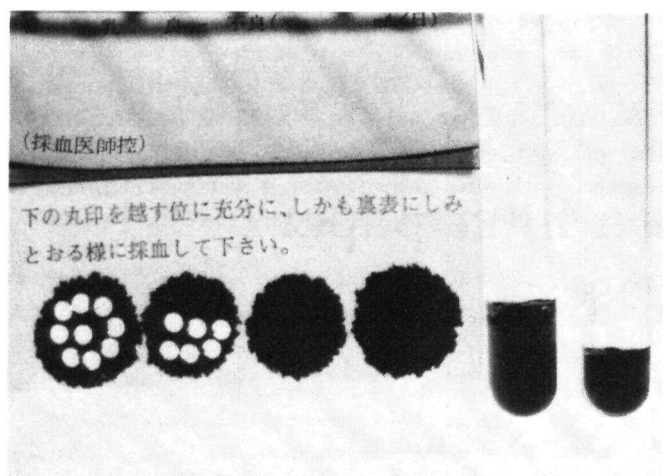


Fig. 1. Filter paper blood and 3-mm disks in assay buffer. Five or 10 3-mm disks were punched out from the filter paper with the blood of neonates at 5 days of age and suspended in 0.5 ml or 1.0 ml of assay buffer.

collection used in mass screening of congenital metabolic disorders. Time-resolved fluoroimmuno-metric assay system (4, 12) for the measurement of filter paper blood IgE levels was also studied.

## MATERIAL AND METHODS

Of 960 children born at Saiseikai Kawaguchi General Hospital, Saitama, during a 1-year period (1986), 389 were available for IgE determination in filter paper blood. As shown in Fig. 1, filter paper blood was collected from the heel by the usual method. Five 3-mm disks were punched out from the filter paper with the blood of 5-day-old neonates and suspended in 0.5 ml of assay buffer. The eluate obtained after 24 h was centrifuged at 3,000 rpm for 10 min and the supernatant obtained was frozen to  $-20^{\circ}\text{C}$  as test sample. IgE levels in the filter paper blood were measured by time-resolved fluoroimmuno-metric assay using DELFIA® total IgE (Pharmacia Diagnostics AB, Uppsala, Sweden).

The DELFIA total-IgE assay is a solid phase, two-site fluoroimmuno-metric assay in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the IgE molecule. Standards or samples containing IgE are first reacted with immobilized monoclonal antibodies directed

against a specific antigenic site on the  $\epsilon$ -chain. Europium-labelled antibodies directed against a different antigenic site on the  $\epsilon$ -chain of IgE are then reacted with the bound IgE. Enhancement solution dissociates europium ions from the antibody into solution where they form highly fluorescent chelates with components of the enhancement solution. The fluorescence in each well is then measured and is proportional to the concentration of IgE in the sample.

Briefly, 200  $\mu\text{l}$  of duplicates of diluted IgE standards 0.01, 0.05, 0.1, 0.5 and 1.0 U/ml using assay buffer and test specimens were pipetted into the bottom of each well, and the frame was incubated with slow shaking for 1 h at room temperature. After the incubation step, each strip was aspirated and washed with the plate-wash. Next, 200  $\mu\text{l}$  of diluted anti-IgE-europium tracer solution was added to each well after flushing it out a few times with the tracer solution, and the frame was incubated with slow shaking for 1 h at room temperature. After the second incubation step, each strip was aspirated and washed with plate-wash. Then, 200  $\mu\text{l}$  of enhancement solution directly from the reagent bottle was added to each well, and the frame with fluorescence was measured in the time-resolved fluorometer (Pharmacia Diagnostics AB, Uppsala, Sweden).

The possibility of predicting the onset of atopic disease was investigated in infants whose filter paper blood IgE levels had been measured. In 389 cases the IgE levels had been measured by the DELFIA system. Feeding method during the nursing period, presence of atopic disease in the first 18 months and serum IgE levels at 18 months were recorded in these subjects. 134 of 389 infants were examined clinically and serum IgE levels measured at 18 months of age. The children were selected at random without knowledge of IgE levels in filter paper blood. On 255 of 389 18-month-old infants, a questionnaire survey was performed concerning the presence of atopic disease and the feeding method during the nursing period. A possible history of atopic disease was obtained through a questionnaire filled in by the parents when their child was 18 months, covering family history of atopic disease, and the child's symptoms since

birth regarding skin, respiratory tract, eyes, nose, and gastro-intestinal tract. A family history of atopic disease was considered positive when atopic disease was confirmed in parents or siblings.

Serum IgE levels at 18 months were measured by Phadezyme IgE PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden) (2, 5). Serum IgE levels at 18 months were evaluated as high when the value was at least the mean value + 1 SD (30 units/ml) for a 1-year-old infant with no atopic disease in immediate family history. We have previously reported the normal range for serum IgE levels in Japanese non-atopic children (14).

## RESULTS

### *Measurement of IgE levels in filter paper blood*

Regarding the sensitivity of the DELFIA system, the fluorescence of 0.01 U/ml of standard IgE was almost the same as the fluorescence + 1SD of the blank when assay buffer fluorescence was taken as the blank. However, the

fluorescence of 0.015 U/ml was at least the blank fluorescence + 2SD, and values were sufficiently reliable at 0.015 U/ml or more. According to the present results, the test was calibrated for 0.01 U/ml of standard IgE, and an IgE concentration of 0.015 U/ml was chosen as the cut-off limit.

Fig. 2 shows the standard curve prepared by measuring 5 times on different days. A clear standard curve was obtained at concentrations of 0.01 U/ml to 1 U/ml, and the reproducibility was good, coefficients of variation (CV) being 2.2% (0.1 U/ml) to 17.5% (0.01 U/ml). There were no lot-differences with regard to the standard curve (data was not shown). Two filter paper blood eluates were measured 5 times on different days. Table 1 shows the mean values  $\pm$  SD and CV for both cases; good reproducibility was obtained, CV being 5.2 and 8.2%, respectively.

When the correlation between IgE levels in serum and filter paper blood eluate was investigated in 25 cases of simultaneous serum and filter paper blood sampling, there was excellent positive correlation ( $r = 0.997$ ) as shown in Fig. 3 ( $P < 0.01$ ).

Simultaneous reproducibility and comparison between filter paper blood eluate and serum IgE levels were investigated in three samples (K, Y and O) showing high IgE levels in pair sampling of serum and filter paper blood. Filter paper blood eluate was measured eight times and serum six times for the three samples. Table 2 shows the mean values  $\pm$  SD and CV for each case. Good reproducibility was obtained for

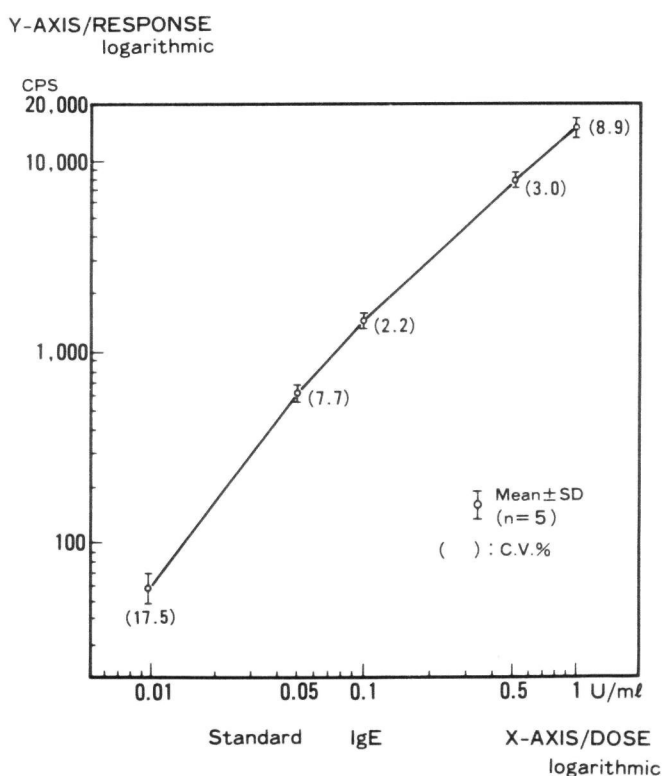


Fig. 2. Standard curve for DELFIA assay of IgE.  
CPS: count per second.

Table 1

Reproducibility by measuring five times on different days in filter paper blood eluate IgE levels

|               | Filter paper blood |                    |
|---------------|--------------------|--------------------|
|               | Sample A           | Sample B           |
| 1             | 0.060 U/ml         | 0.036 U/ml         |
| 2             | 0.063              | 0.041              |
| 3             | 0.066              | 0.037              |
| 4             | 0.058              | 0.035              |
| 5             | 0.064              | 0.042              |
| Mean $\pm$ SD | 0.062 $\pm$ 0.0032 | 0.038 $\pm$ 0.0031 |
| CV            | 5.2%               | 8.2%               |

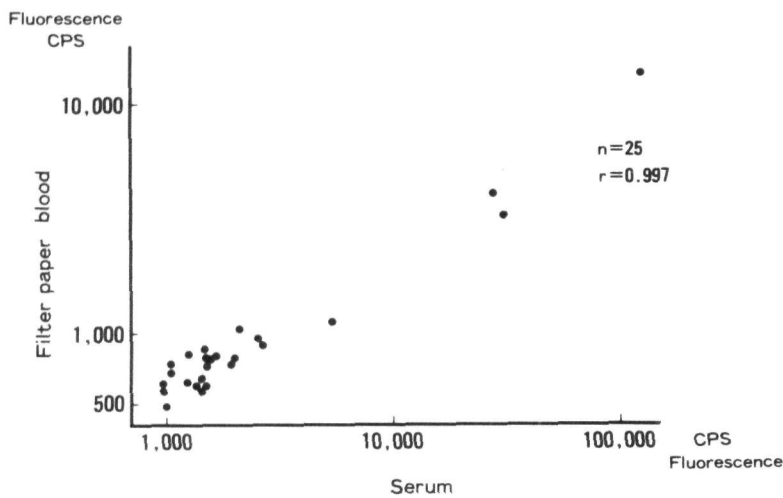


Fig. 3. Correlation between IgE levels in serum and filter paper blood eluate. CPS: count per second.

both the filter paper blood eluate and serum. The IgE values for filter paper blood eluate and serum were then compared for the three samples. As shown in Table 3, the ratio of the filter paper blood eluate IgE value to the serum IgE value was an average of  $0.014\pm0.002$ , i.e., 1 U/ml of serum IgE was equivalent to approx. 0.014 U/ml of IgE in filter paper blood eluate.

*Relation between filter paper blood IgE levels and atopic disease in infants*

Examinations were performed and serum IgE levels were measured at 18 months in 134 of the 389 subjects.

As shown in Fig. 4, 28 (7.2%) of the 389 subjects showed filter paper blood IgE levels of at least 0.015 U/ml, the measurement limit. This IgE level corresponds to about 1 U/ml in neonatal serum. Examinations and serum IgE measurements were performed at 18 months in 15 of these 28 cases. Table 4 shows family history, presence of atopic diseases in the first

18 months and serum IgE levels at 18 months in children with high filter paper blood IgE values ( $\geq 0.015$  U/ml).

A family history of atopic disease was confirmed in all 15 cases with high IgE values in filter paper blood but in only 66 (55.5%) of the 119 with low filter paper blood IgE values; there was a significant difference between the two ( $P<0.01$ ).

High serum IgE levels at 18 months were seen in 13 (86.7%) of the 15 cases with high filter paper blood IgE levels and in 37 (31.1%) of 119 cases with low values; there was also a significant difference between the two ( $P<0.01$ ).

Sixty-nine replied to the questionnaire on atopic diseases at 18 months. Family history of atopic disease and presence of atopic disease in the first 18 months were analysed with respect to filter paper blood IgE levels in 203 subjects, including the replies to the questionnaire survey. The feeding method in the nursing period was clear in 191 cases. No differences in feeding

Table 2  
Simultaneous reproducibility and comparison between IgE levels in filter paper blood eluate and serum

|                | Filter paper blood |             |             | Serum*    |           |            |
|----------------|--------------------|-------------|-------------|-----------|-----------|------------|
|                | Sample K           | Sample Y    | Sample O    | Sample K  | Sample Y  | Sample O   |
| Mean±SD (U/ml) | 0.883±0.05         | 0.263±0.014 | 0.249±0.013 | 74.05±2.5 | 16.15±0.7 | 16.25±0.75 |
| CV (%)         | 5.7                | 5.3         | 5.2         | 3.4       | 4.3       | 4.6        |
| n              | 8                  | 8           | 8           | 6         | 6         | 6          |

\*Serum was used at a dilution of 1:50 with assay buffer.

Table 3

| Ratio of filter paper blood eluate IgE to serum IgE level |             |
|-----------------------------------------------------------|-------------|
| Sample K                                                  | 0.012       |
| Sample Y                                                  | 0.016       |
| Sample O                                                  | 0.015       |
| Mean±SD                                                   | 0.014±0.002 |

methods were found between the high and low IgE level groups.

Fig. 5 shows the results of the relation between filter paper blood IgE levels, family history of atopic disease and presence of atopic disease in the first 18 months in 203 cases. Among the 20 cases with an atopic family history and high IgE levels, atopic disease developed in 90 % (18 of 20). In cases with an atopic family history but low IgE levels, atopic disease developed in 53 (50 %) of the 106 cases. However, in the 77 cases with low IgE values and no atopic family history it developed in only 19 (24.7 %). There were significant differences among these three groups ( $P < 0.01$ ).

DISCUSSION

IgE screening was performed using the Phadebas IgE PRIST technique on the cord blood of

Table 4

Family history, presence of atopic disease in the first 18 months and serum IgE levels at 18 months in cases with high filter paper blood IgE values

| No. | Case | Sex | Family history of atopic disease | Atopic disease | IgE levels at 18 months (U/ml) |
|-----|------|-----|----------------------------------|----------------|--------------------------------|
| 1   | Y.Y. | M   | +                                | +              | 83                             |
| 2   | K.M. | M   | +                                | +              | 340                            |
| 3   | I.K. | M   | +                                | +              | 79                             |
| 4   | H.K. | M   | +                                | +              | 60                             |
| 5   | A.A. | F   | +                                | +              | 61                             |
| 6   | K.S. | M   | +                                | +              | 790                            |
| 7   | M.G. | M   | +                                | +              | 150                            |
| 8   | S.M. | M   | +                                | +              | 400                            |
| 9   | N.Y. | F   | +                                | +              | 58                             |
| 10  | T.O. | M   | +                                | +              | 31                             |
| 11  | Y.S. | M   | +                                | +              | 200                            |
| 12  | K.A. | F   | +                                | +              | 30                             |
| 13  | M.O. | F   | +                                | +              | 30                             |
| 14  | Y.H. | F   | +                                | +              | 20                             |
| 15  | H.T. | M   | +                                | -              | 8.5                            |

1,701 newborn infants according to Croner et al. (3). They reported that atopic disease developed in 73 % of infants with high IgE concentrations ( $> 1.3$  kU/l) in cord blood and a family history of atopic disease, but in only 3 % of infants with low IgE and no family history, during the first 18 months of life. In addition, a high IgE concentration in cord blood was more

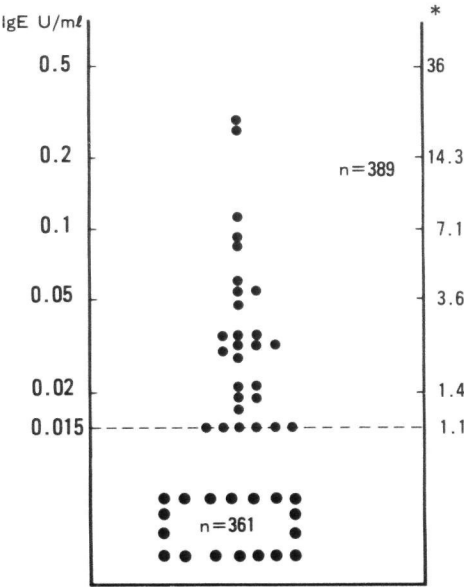


Fig. 4. IgE levels in filter paper blood of 389 subjects. \*This line is neonatal serum IgE levels (U/ml) converted from filter paper blood IgE levels.

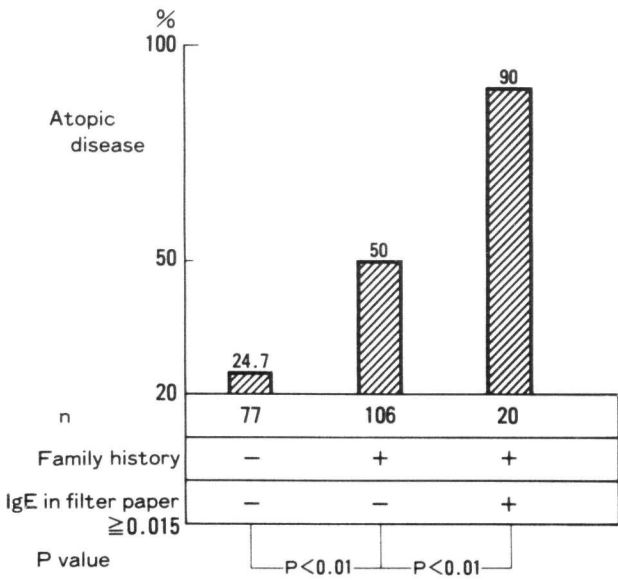


Fig. 5. Relation among filter paper blood IgE levels, family history of atopic disease and presence of atopic disease in the first 18 months in 203 cases.

often associated with a high IgE level and a positive radioallergosorbent test between the ages of 18 and 24 months than a low initial IgE level, indicating that in man as well as in animals high and low IgE responders are already genetically coded at birth. Croner et al. proposed that IgE screening in cord blood is recommended if there is obvious atopy in both parents or if severe atopic disease is present in a sibling or in one parent. Kimpen et al. (6) analysed 5,353 cord samples for IgE by means of an enzyme linked immunosorbent assay. They reported that descriptive statistics of the distribution curve of cord blood IgE concentrations showed that most normal values were well below the cut-off value of 1.0 IU/ml, which was the commonly cited cut-off point, and that most abnormal values were in the neighbourhood of 1.0 IU/ml. Therefore, they recommended lowering the cut-off value for cord blood IgE. These reports suggest that cord blood IgE appears to be the single best indicator of atopic disease.

However, it is not possible to collect cord blood on a routine basis in Japan. Usually, pediatricians only attend births when there is a problem, such as a premature birth, and obstetricians never collect cord blood. In mass screening of neonatal diseases in Japan (11), the heels of 4-5-day-old neonates are punctured and the blood dripped onto filter paper. It is possible to detect phenylketonuria or hypothyroidism by measuring phenylketone or TSH values in this blood. As filter paper blood is collected from all neonates in Japan, it would be highly useful if the IgE levels in this blood could be used as indicators for the early prediction of the onset of atopic disease.

It was reported from experiments using tracers that the amount of serum on the 3 mm disk was one part in 60 or 70 of the amount of serum obtained from whole blood in venous puncture, and this ratio was constant between 40% and 60% in hematocrit (9). Accordingly, it must be possible to measure extremely small amounts of IgE when measuring the IgE levels in filter paper blood. The DELFIA system was developed to measure IgE levels in an even lower concentration range than that measured

by Phadezym IgE PRIST. The authors were able to measure standard IgE diluted down to 0.01 U/ml with the DELFIA system. Originally, samples of 25 µl were measured by diluting to 225 µl with assay buffer, but the authors used 200 µl samples obtained by eluting five 3 mm disks of filter paper in 0.5 ml of assay buffer. With this method, values as low as 0.015 U/ml of IgE were considered reliable. When the assay buffer was taken as the blank, the fluorescence in a number of samples was almost always the same or less than that of the blank, and IgE levels in filter paper blood eluate of at least 0.015 U/ml and lower could be evaluated. Therefore, in an analysis of the present results, IgE values of at least 0.015 U/ml, the measurement limit, were considered high. When 0.015 U/ml of IgE in filter paper blood was converted to the serum value in pair sampling of filter paper blood and serum, the result was approx. 1.0 U/ml. This value was in agreement with the results of experiments using tracers mentioned previously.

When filter paper blood IgE levels were measured by the DELFIA system and values of at least 0.015 U/ml were taken as high, high IgE levels in filter paper blood were seen in 28 (7.2%) of the 389 cases. When the relation between atopic family history and serum IgE levels at 18 months was investigated in 134 of 389 subjects, it was revealed that all cases with high IgE levels at birth had an atopic family history and that 86.7% of these had high IgE serum levels at 18 months. When the relation between atopic family history and presence of atopic disease in the first 18 months was investigated in 203 of the 389 subjects, about 90% of the subjects with a familial history and high IgE levels in filter paper blood developed atopic disease.

Since IgE levels in this study were measured in filter paper blood of neonates (4 to 5 days of age) and not in cord blood, high IgE levels in the filter paper blood indicate the results of sensitization in the fetus and/or very early IgE production in neonates. Thus, no definite conclusions can be drawn if these results were compared with those of cord blood studies. However, our finding that neonates with high IgE

values in filter paper blood and an atopic family history results in a high incidence of atopic disease in infants, agrees with those of cord blood studies (3, 6, 7, 8, 10, 13, 15). In cases with low IgE levels in filter paper blood, atopic disease developed in about 50 % of those with a family history and in 24.7 % of those with no family history. The relative incidence of atopic disease appeared to be high because infants with no atopic diseases did not come to the hospital for examination at 18 months and parents did not answer the questionnaire.

More recently, it has been reported that the incidence of atopic disease in infants can be reduced by a hypoallergic diet in infants and mothers of breast-fed infants (1). It has also been reported that interleukin-2 reactivity to mites is reduced by the hypoallergic diet (1). Food allergens are an important problem in neonates. According to the authors' results, it will be necessary in the future to perform a prospective study to determine whether a hypoallergic diet, such as exclusion of hens' eggs and early administration of anti-allergic agents in infants, reduces the incidence of atopic disease in cases with high IgE levels in filter paper blood.

Early prediction of the onset of atopic disease in infants is important in order to actively prevent atopic disease at the nursing stage. Since the collection of filter paper blood is a routine in Japan, the checking of IgE levels in these samples should be widely performed for the prediction of the onset of atopic disease in infants.

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