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Rhinoviruses in Britain 1963–1973

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SUMMARY

The serological examination of over 900 rhinovirus strains isolated in Britain over a 10-year period has demonstrated a wide variety of different serotypes. Their seasonal and temporal distribution are described and discussed.

INTRODUCTION

Although the common cold is the most prevalent viral disease of man and the agent most commonly isolated from it is the rhinovirus, the natural existence of this ubiquitous virus is still imperfectly understood. This is partly because of the nature of the illness and the general behaviour of the virus itself.

Rhinoviruses are specially adapted for existence in the upper respiratory passages. They can be isolated throughout the year but undergo a seasonal variation. Many antigenic types are known and it is usual for several different serotypes to circulate in a community at the same time, each for a limited period.

Whilst a proportion of more serious respiratory illness, particularly acute exacerbations of chronic bronchitis in adults and bronchitis and bronchiolitis in young children, may be attributable to rhinovirus infection, its most characteristic clinical manifestations are upper respiratory in nature and often so fleeting and trivial that few sufferers bother to seek medical attention. As a result, most rhinovirus infections probably go undiagnosed (Hamre, 1968; Tyrrell, 1968; Stott, Grist & Eadie, 1968).

In this paper, rhinoviruses isolated throughout a decade from a wide variety of subjects in different parts of Britain are described and discussed.

MATERIALS AND METHODS

Rhinovirus strains

The viruses described in this study were isolated between January 1963 and the end of December 1973 from the sources listed below:

(*a*) An investigation of respiratory illness in a children's home on the outskirts of London (September 1965 to March 1968).

(*b*) A survey of respiratory illness occurring amongst workers in two factories in Staffordshire (October 1965 to April 1969).

(*c*) Respiratory illness occurring amongst children in a single general practice in south-west London. The isolation and typing of rhinoviruses formed only a small

part of a large clinical investigation which has been described elsewhere (September 1968 to December 1973) (Horn *et al.* 1975).

(*d*) Adults with bronchitis attending the chest clinic of a London hospital (September 1968 to December 1972).

(*e*) Routine throat/nose swabs from children admitted to hospital for any reason in a number of towns (Bristol, Leicester, Manchester, Edinburgh) (1966 to December 1973).

(*f*) Two outbreaks of respiratory infection amongst nurses and students in Oxford and London (1967–8, 1971).

(*g*) Post-mortem material mainly from infants who died unexpectedly.

(*h*) Random strains isolated in the course of routine diagnostic laboratory work (1963–73).

Most of the viruses were isolated in peripheral laboratories of the Public Health Laboratory Service and certain hospital laboratories. They were sent by post in tissue cultures to the Virus Reference Laboratory, Colindale, London, N.W. 9.

On receipt, each virus strain was passed into tissue cultures of WI-38 cells and a pool prepared. Identification was by means of neutralization with type-specific antisera.

Type-specific antisera

Standard neutralizing antisera were obtained from two sources. Up to the end of 1969 only 23 type-specific sera were in use. These were supplied by the Common Cold Research Centre, Harvard Hospital, Salisbury.

At the beginning of 1970, this number was supplemented by a further 28 from the Research Resources Branch of the National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

Neutralization test

Virus pools for neutralization were initially titrated in WI-38 tissue cultures and that dilution which gave 25–35 microplaques per tube at the end of 48 hr. incubation was selected for use in the test. It was soon found that most virus pools diluted 10^{-1} gave the desired plaque count and titration was abandoned except for strains which failed to conform.

All antisera were used at a dilution of 1/20. Sera were used individually in batteries of 6 at a time. Pooled sera were not employed.

In the test, 0·3 ml. of virus dilution was added to 0·3 ml. of diluted antiserum and the mixture shaken and left at room temperature for 2 hr. At the end of that time a volume of 0·2 ml. of the virus–serum mixture was inoculated into each of two WI-38 tissue culture tubes containing 1·0 ml. of maintenance medium. The inoculated tubes were incubated rolling for not longer than 4 days at 33° C. and examined daily. The test was read at the end of 48 hr., or 3 days if growth was poor.

Complete inhibition of virus growth was the only accepted criterion of neutralization.

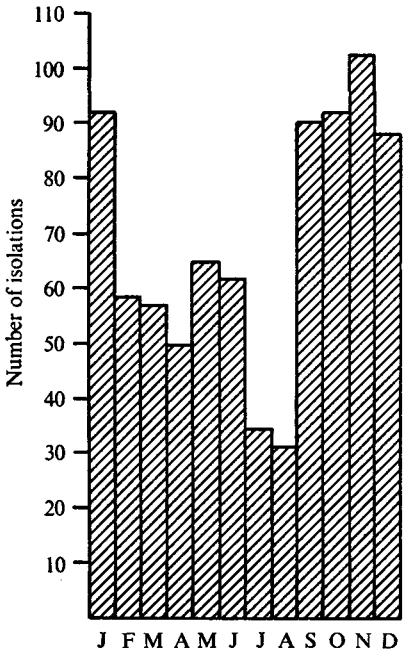

Fig. 1. Monthly isolation rate of rhinoviruses 1 January 1963 to 31 December 1973 (Total number 825).

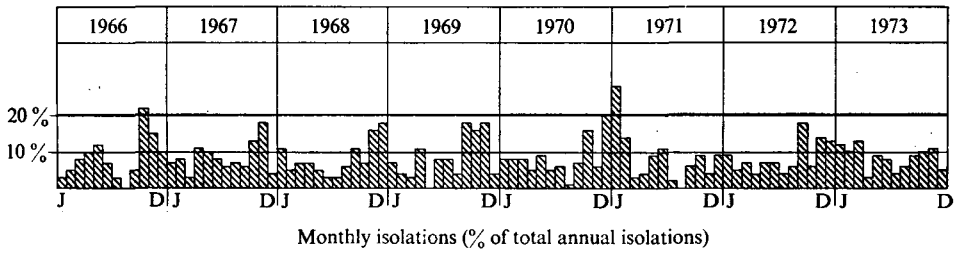

Fig. 2. Seasonal variation in isolation rate of rhinoviruses.

Between January 1963 and the end of December 1973, 903 rhinoviruses were examined and 868 (90 %) were typed.

RESULTS

Seasonal variation

The seasonal variation of 859 strains for which the date of onset of illness or the date of swabbing were known is shown in Fig. 1. The period covered is from January 1963 to December 1973. Isolations occur throughout the year, with the highest incidence in the autumn and early winter months. A smaller peak is apparent in the early summer. Between September and the end of January, 57 % of the annual isolations were made.

In Fig. 2 the seasonal variation for each year between 1966 and 1973 is illustrated. For comparison, the figures are expressed as a percentage of the annual isolations. The 3 years before 1966 have been omitted because the number of isolations

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was considered too small to be significant in this context. From January 1966 to December 1970 the seasonal trend is similar, with the peak isolation period falling between September and December. The 1970/1 autumn–winter peak is extended to the right by 2 months and the autumn–winter period succeeding it (1971/2) shows no peak. The isolation rate between September 1971 and August 1972 is remarkably uniform. The usual autumn–winter peak reappears in 1972/3, again extended to the right, this time by 3 months and followed by a fairly uniform pattern for the rest of the year.

Distribution of individual serotypes

Geographical

The number of recognized rhinovirus serotypes is 89. Antisera for 51 of these, ranging from 1A to 54, were available. The 868 typed strains comprised 42 serotypes. Those not detected were 25, 28, 33, 34, 43, 50, 51, 52 and 53. The commonest were 15, 1B, 4, 31, 1A, 2, 29 and 30. These 8 types constituted just over half of the 868 strains.

In Table 1 the distribution of individual serotypes in London, Leicester, Stafford and Edinburgh is given. The number at the head of each column is the total of typed isolates from that region and the figures below are a percentage of this number. Strains were not received from Stafford after 1969 and some antisera did not become available till 1970, therefore, the presence there of certain serotypes could not be detected. Rhinovirus studies were not made in Edinburgh until 1970.

Of the nationally commonest types, 1A, 1B, 4, and 15 are highly prevalent in each of the four towns. The remaining four (2, 29, 30 and 31) are well represented except in Edinburgh, where 2, 29 and 30 are among the least prevalent. It is clear, however, that all the 42 serotypes are widely disseminated with few regional differences, and there is no evidence to suggest that any particular types are associated with any one geographical area.

Temporal

Monthly isolations of the 42 antigenic types between 1966 and 1973 are shown in Fig. 3. The most prevalent, already indicated, can be picked out easily. The least common are 7, 11, 17, 18, 37, 39 and 42.

Perhaps the most striking feature of this chart is the absence of a regular epidemiological pattern for any individual serotype.

However, two groupings can be made on the basis of behaviour which could be described as 'persistent' and 'intermittent', the 'persistent' group being the larger. This group encompasses not only numerically superior types such as 1B, 4 and 15 but also types like 3, 5, 6, 10 and 12, which constitute only a small proportion of the whole. The common feature of these types is a pattern of 'persistent' distribution in time. Consecutive isolations of types 1B, 4 and 15 have been recorded over periods of 6 and even 10 months. Periods during which virus was not isolated are of the same order, varying between 2 and 10 months. Small numbers of isolates are fairly evenly distributed from month to month with an occasional peak at irregular intervals.

Table 1. *Geographical distribution of individual serotypes*

Serotype	London, total: 342 (%)	Leicester, total: 103 (%)	Stafford, total: 106 (%)	Edinburgh, total: 109 (%)
1A	4·4	4·8	8·4	2·7
1B	5·8	4·8	10·3	2·7
2	4·9	2·9	2·8	0·9
3	3·5	1·9	1·8	2·7
4	9·0	7·7	10·3	7·3
5	2·6	2·9	1·8	3·6
6	1·5	3·8	2·8	0·9
7	0·3	0·9	0·0	0·9
8	5·5	2·9	1·8	1·8
10	1·5	0·0	0·9	1·8
11	0·0	0·0	1·8	0·0
12	2·9	3·8	3·7	2·7
13	4·1	7·7	2·8	4·5
14	2·0	3·8	2·8	3·6
15	9·6	15·5	14·5	14·6
16	2·0	1·9	4·7	0·9
17	0·3	0·0	0·0	0·0
18	0·6	0·0	0·9	0·0
19	4·4	5·8	NT	14·6
20	0·3	0·0	NT	0·0
21	0·0	0·0	NT	0·9
22	1·8	0·0	NT	0·9
23	1·8	4·8	NT	4·5
24	0·3	0·0	NT	0·0
26	6·4	1·9	3·7	1·8
29	3·2	4·8	8·4	0·9
30	4·7	1·9	7·5	0·9
31	5·3	2·9	6·6	1·8
32	2·9	2·9	0·0	1·8
36	3·2	2·9	NT	7·3
37	1·2	0·0	0·0	0·0
38	0·6	0·0	0·0	0·0
39	0·3	0·9	NT	0·0
41	0·3	0·0	NT	0·0
42	0·3	0·9	0·9	0·9
44	1·8	1·9	NT	0·0
45	0·6	0·9	NT	0·9
46	0·0	0·0	NT	1·8
48	0·0	0·9	NT	5·5
49	0·0	0·0	NT	1·8
54	0·0	0·0	NT	0·9

* NT, Not tested.

The 'intermittent' group is exemplified by type 1A, which has a time distribution in blocks over periods of 3–5 months and wide gaps of 10–20 months during which virus isolations were not recorded. Types 19 and 36 also probably belong to this group, although a study period of 4 years is undoubtedly too short to be certain of this. However, if type 36 is compared with type 23, which has been studied for only 4 years, a difference in pattern can be seen. During this period 19 strains of

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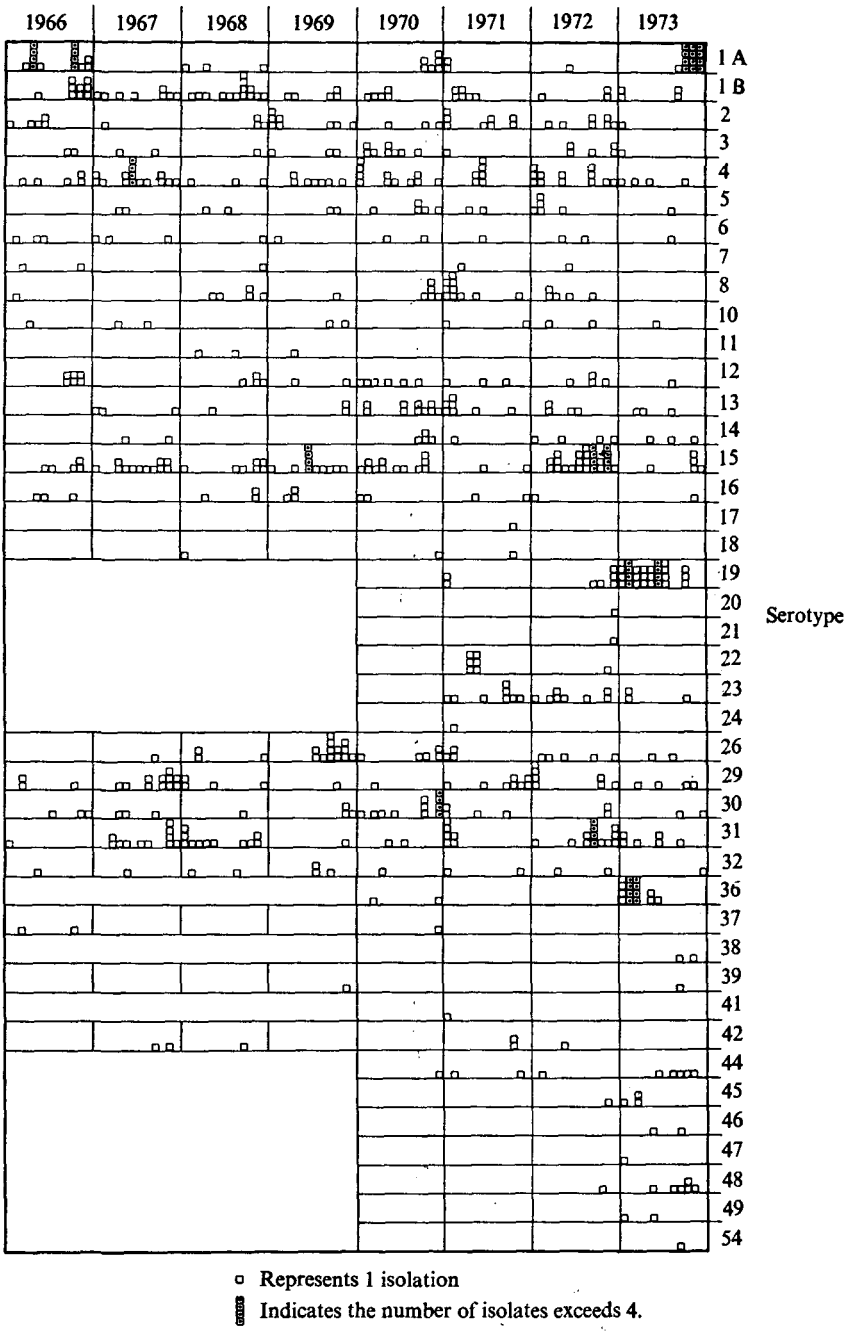

Fig. 3. Monthly isolations of antigenic types of rhinovirus.

type 23 and 22 strains of type 36 have been examined. Of the type 23 isolates, 18/19 are evenly spread over a period of 26 months whereas 20/22 of type 36 are concentrated in a period of 6 months. A similar comparison can be made between types 8 and 12.

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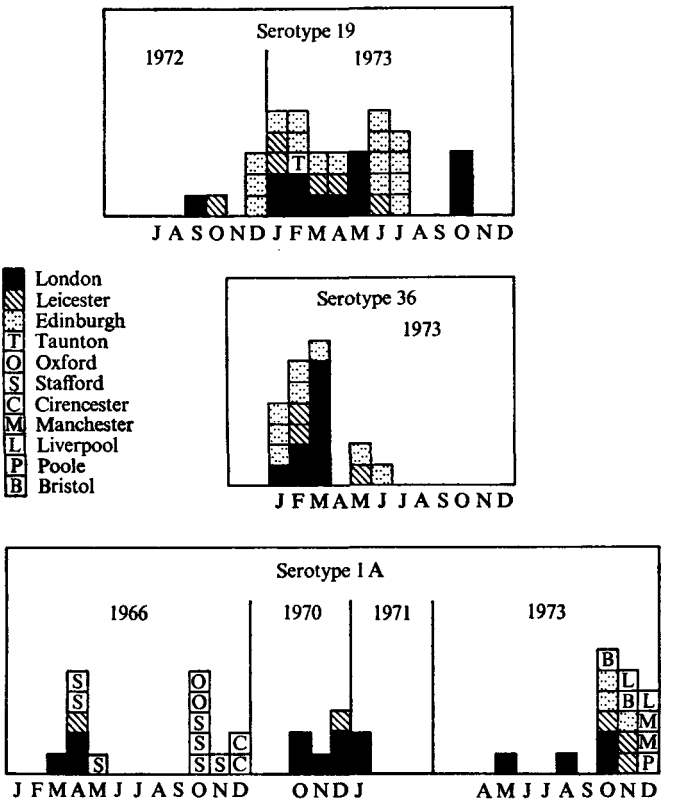

Fig. 4. Synchronous appearance of certain rhinovirus serotypes in different geographical areas.

Synchronous appearance

It has been observed that single rhinovirus serotypes sometimes appear simultaneously in widely separated geographical areas (Stott, 1969). This has been confirmed during the course of the present study, especially with regard to types 19 and 36, and is illustrated in Fig. 4. Between December 1972 and July 1973 no fewer than 31 isolations of type 19 were made from four different places. In the 4-month period from January to April, during each month isolations were made in three separate regions. In the two previous years, two strains were identified in 1971. There were no isolations in 1970.

The isolation pattern of type 36 is similar. Following a period of 2 years during which this virus was not encountered, 20 strains were isolated between January and June 1973, 17 of them occurring throughout the first 3 months in 3 different towns – London, Leicester and Edinburgh.

In 1966, 15 strains of 1 A appeared during two periods of 3 months, the first from March to May in both London and Stafford and the second from October to December when this serotype was reported in Oxford, Cirencester and Stafford. A period of 45 months followed during which three strains appeared sporadically. Between October 1970 and January 1971 eight strains were isolated, of which

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seven were from south-west London. A further 27 months elapsed. Then 17 strains of 1A were encountered between May and December 1973. Of these, 15 occurred in seven different towns within 3 months.

Clinical source of virus strains

Rhinoviruses were isolated from patients of all ages and from every grade of illness from minimal coryza and rhinorrhoea to fatal broncho-pneumonia. A few strains were isolated from subjects who were clinically well at the time of swabbing.

Patients have been divided according to age into infants (0–4 years), school-children (5–14 years) and adults (15 years and over). The associated clinical conditions have also been divided into three groups as follows:

(*a*) Upper respiratory.

(*b*) Lower respiratory – included in this group are any illness described clinically as 'influenza' and unexpected deaths in infants, generally described as 'cot deaths'. The latter form a small group, some of which manifested respiratory signs before death and others gave no such history.

(*c*) Non-respiratory.

Infants

The number of rhinoviruses isolated from this group was 279 (35 serotypes). Of these, 123 (45%) were from upper respiratory infections which presented as 'coryza' or 'rhinorrhoea' (102), 'otitis media' (6) and 'sore throat' (15). There were two isolations from children who were not ill.

Strains associated with lower respiratory illness numbered 103 (37%). These presented as 'bronchitis/asthma' (68) and 'bronchiolitis/pneumonia' (35), the latter group including 6 'cot deaths' and 3 double infections where the rhinovirus was associated with another virus (RS, parainfluenza 4B and cytomegalovirus, respectively).

Non-respiratory illnesses yielded 51 rhinoviruses (18%). Vomiting and diarrhoea accounted for half of these. Of the remaining 26, conditions relating to the central nervous system, 'meningitis', 'encephalitis' and 'convulsions' were the source of 13; and 13 were recovered from miscellaneous illnesses, mostly of a long-standing and debilitating nature.

The total number of serotypes represented was 35, but 5 of these (15, 1B, 4, 13 and 19) accounted for 131 isolates (47%).

The distribution of serotypes through all the categories of illness was quite random and there was no suggestion that any particular type was linked to any individual clinical condition. The 6 cases of otitis media and the 6 'cot deaths' each yielded 5 serotypes. There were 2 serotypes associated with 3 cases of 'influenza' and no fewer than 13 with the 25 gastro-enteritis syndromes. A further 9 types were isolated from the CNS-type illnesses.

Schoolchildren

A total of 184 rhinoviruses was isolated from children of school age (30 serotypes).

Upper respiratory infections yielded 97 (53%). Lower respiratory disease accounted for 72 (41%) and non-respiratory conditions for 12 (6%). There were 3 strains from children who were not ill. The number of serotypes isolated from this group was 30, and again a mere 5 (types 4, 15, 30, 1B and 31) formed 47% of the total. Not unexpectedly, the illness-associated distribution of serotypes was similar to that in the younger children.

Adults

Rhinoviruses from this age group numbered 376 (35 serotypes), of which 263 (70%) were isolated from upper respiratory conditions. Strains associated with lower respiratory illness numbered 105 (27%) of which 37 were isolated from clinical 'influenza' and the remaining 68 from acute exacerbations of bronchitis occurring in patients with long-standing bronchial disease. Non-respiratory illness accounted for 4 strains and another 4 were from subjects who were well.

In this group also, although 35 serotypes were identified, 5 types (1A, 31, 1B, 2 and 29) represented 46% of the isolates.

In each of the three age groups, the same rhinovirus serotypes occur with similar frequency. The most significant finding is a change in the pattern of illness. In the younger children, rhinovirus infection embraces a wide variety of clinical conditions often of a serious nature whereas in the adult, its manifestations are predominantly upper respiratory and mild.

DISCUSSION

The brief clinical account summarizing the origins of the rhinovirus strains confirms the findings of other observers.

With regard to seasonal variation, the results are in accord with those already described both in the United States and in Britain (Higgins, 1967; Hamre, 1968; Stott, 1969). They indicate that although the seasonal ebb and flow follows a loose general pattern from year to year, it is by no means immutable and deviations are not infrequent. Continuous studies over many years would seem to be necessary to see this pattern fully in perspective. It seems certain that it is connected with meteorological events despite the inconclusive results of all efforts to find a correlation.

That the geographical distribution of all the 42 serotypes is equally wide is perhaps not surprising in a small country with good communications and a mobile population.

The pattern of spread of individual types described as 'persistent' or 'intermittent' is consistent with a difference in infectivity or communicability. Such differences have been noted by Monto & Johnson (1968) and Monto & Cavallaro (1972).

The simultaneous appearance of certain serotypes in different regions is inexplicable but conforms to a general pattern observed most dramatically in outbreaks caused by influenza viruses.

It is difficult to explain why some serotypes are more prevalent than others. Types 15, 1B, 4, 31, 1A, 2, 29 and 30, which together form about half of the strains

herein described, have one characteristic in common: all grow exceedingly well in tissue culture. It is interesting to note in this connexion that 22 out of 24 rhinovirus strains isolated in Prague between 1965 and 1967 belonged to types 1A (5), 2 (1), 30 (9) and 31 (7) (Strizová, Vojtisková, Grünwald & Haasová, 1970).

Many of the less prevalent types although numerically insignificant when compared with the 'common' ones, are remarkably constant in their appearance year after year although it may consist of a mere one or two isolates. This seems to suggest that such types may be more prevalent than is indicated by the isolation rate.

Some rhinoviruses are undoubtedly commoner than others, but it should not be assumed that the serotype patterns discovered by present laboratory procedures are necessarily an accurate reflexion of those occurring in nature. Preliminary studies (to be published) have shown that antibody to certain serotypes is more prevalent than would be expected from the infrequency of isolation.

I should like to thank all the virologists who provided me with rhinovirus strains, particularly Dr W. L. Hooper, Public Health Laboratory, Poole, Dorset, Dr J. M. Inglis, Regional Virus Laboratory, Edinburgh, Dr Hélène J. Mair, Public Health Laboratory, Leicester and Mrs Susan J. Yealland (formerly) Virus Laboratory, Brompton Hospital, London.

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