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OCCURRENCE OF RESPIRATORY SYNCYTIAL VIRUS IN ACUTE RESPIRATORY DISEASES IN INFANCY

BY

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In 1959 a comprehensive study was carried out in Newcastle to correlate clinical, bacteriological, and virological findings in acute respiratory infection in children (Gardner *et al.*, 1960). One striking feature of this investigation was the failure to obtain evidence of virus infection in acute bronchiolitis.

Continuing observation in the succeeding years, 1960–2, has not thrown further light on this finding, but during this period workers in America (Beem *et al.*, 1960 ; Chanock *et al.*, 1961 ; Reilly *et al.*, 1961) have shown that the respiratory syncytial (R.S.) virus, originally isolated in 1956 as a coryza agent in chimpanzees (Morris *et al.*, 1956) and soon after from infants by Chanock *et al.* (1957), is an important causal agent of acute respiratory disease in children. In Britain so far there have been only two reports of the occurrence of this virus in association with respiratory disease (Peacock and Clarke, 1961 ; Holzel *et al.*, 1963).

We therefore describe briefly our findings in a study of acute bronchiolitis and pneumonia from December, 1962, to March, 1963.

Investigation

This survey was limited to children suffering from acute bronchiolitis and pneumonia, as previously defined by Gardner *et al.* (1960). Children entering the wards of three hospitals in Newcastle upon Tyne—the Royal Victoria Infirmary, the Walkergate Hospital, and, in February and March, the Fleming Memorial Hospital—were included. All hospitals drew patients from the same area of Newcastle and the adjacent communities, admission to a particular hospital being determined primarily by availability of beds. Because of the distances involved and occasionally from difficulties with the transport of specimens, it was not possible to include every child suffering from these conditions.

Whenever feasible, unfrozen cough swabs, broken into Hanks basic salt solution, were brought as quickly as possible to the virus laboratory for the attempted isolation of R.S. virus. Arrangements were made, too, for examination of frozen specimens for the established respiratory viruses which can be safely transported in this way. All specimens were dealt with in the virus laboratory of one hospital ; this gave us the opportunity to see if widely scattered hospitals could form a basis for a satisfactory investigation of the labile R.S. virus in one virus laboratory situated at a distance from some of the participating hospitals.

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Methods

All unfrozen specimens were inoculated on to " Bristol " line of HeLa cells if available (Peacock and Clarke, 1961), or Hep 2 cells. Frozen specimens were examined on fertile hens' eggs, monkey-kidney cells, and HeLa or Hep 2 cells. " Bristol " HeLa cells were grown and maintained in rabbit serum, while Hep 2 cells were grown and maintained in calf serum. During the latter part of the investigation suitable supplies of rabbit serum became difficult to obtain and most of the work was performed on Hep 2 cells grown and maintained in calf serum. Tissue-culture cells were maintained for at least 28 days before they were discarded as negative.

R.S. virus was suspected when the characteristic degeneration showing giant cells and syncytia was produced and when this phenomenon could be adequately passaged. Confirmation was obtained by the performance of a neutralization test for the R.S. virus. This test consists of standing a mixture of R.S. virus and antiserum at room temperature for an hour and then transferring the mixture into Hep 2 tissue-culture cells. R.S. virus was also confirmed by staining within the tubes, using Giemsa stain, for syncytia, giant cells, and inclusions. Techniques used for the isolation of other viruses were based on the methods previously described (Gardner *et al.*, 1960).

Results

Specimens were submitted from 43 children under the age of 2 years (20 boys and 23 girls).

There were 13 isolations of virus: R.S. virus from 10 patients and E.C.H.O. (type 11), para-influenza (type 3), and adenovirus (type 7) from one patient each. R.S. virus was found only in late January, February, and early March, and at this time Hep 2 cells were in use for attempted virus isolation from unfrozen specimens. During December and much of January, when " Bristol " HeLa cells were in use, no R.S. viruses were isolated, but it is difficult to say whether virus was absent in December and January or whether Hep 2 cells are a more suitable cell line. This problem is being further investigated. Table I shows the

total number of R.S. viruses isolated under frozen and unfrozen conditions.

TABLE I.—*Isolation of R.S. Virus on Hep 2 Cells*

Unfrozen Specimen Examined on Hep 2 Cells		Frozen Specimen Examined on Hep 2 Cells	
Total	R.S. Virus Isolations	Total	R.S. Virus Isolations
11	8	32	2

R.S. virus was isolated from specimens submitted from patients in all three hospitals, the proportion of isolations from the hospital in which the virus laboratory is located (four isolations from 15 patients) being of the same order as from those hospitals from which specimens had to be transported from a distance (six from 28 patients).

The number of children entering the investigation remained at a fairly constant level in December, January, and February, with a diminution in March, but the isolation of R.S. virus was restricted to patients admitted during late January, February, and early March (Table II). This suggests that there may have been an outbreak of infection

TABLE II.—*Occurence of Illness by Months*

	December	January	February	March
Number of patients ..	14	12	14	3
Isolation of R.S. virus	0	2	7	1
,, ,, other ,,	1	2	0	0

by this virus, limited in duration, as has been described by Beem *et al.* (1960) and McClelland *et al.* (1961), but it is possible that the localization of isolation to these months might be due to the use of Hep 2 cells rather than " Bristol " HeLa cells for unfrozen specimens. This is unlikely, as other workers have had success with " Bristol " HeLa cells (Peacock and Clarke, 1961 ; Holzel *et al.*, 1963), and all specimens, whether frozen or not, were inoculated on to Hep 2 cells. We would have expected at least a few of these specimens in December and January to have yielded some virus in spite of freezing (Hamparian *et al.*, 1961 ; Hilleman *et al.*, 1962).

The isolation rate of viruses from children up to 6 months old has in this region in the past been very low—6% in the study by Gardner *et al.* (1960) in which unfrozen specimens were not taken and Hep 2 tissue culture cells were not used. From Table III it is clear that the present

TABLE III.—*Virus Isolation Related to Age and Clinical Picture*

	Bronchiolitis		Pneumonia	
	0–6 mths	7–23 mths	0–6 mths	7–23 mths
Number of patients ..	22	5	12	4
Isolation of R.S. virus	6	0	2	2
,, ,, other ,,	2	0	1	0

isolation rate is much higher, 32%, and that this fivefold difference is due primarily to the R.S. virus.

It was a striking feature of this earlier investigation that from none of the 27 cases of acute bronchiolitis was any virus isolated. In the present group of 27 cases of acute bronchiolitis R.S. virus was isolated from six, a recovery rate of 22%, and E.C.H.O. (type 11) and adenovirus (type 7) were recovered from two of the others. The virus recovery rate from the 16 cases of pneumonia is of the same order (Table III).

Discussion

In 1959 we were unable to isolate any viruses from our children with acute bronchiolitis. Subsequent work in America (Chanock *et al.*, 1961 ; Reilly *et al.*, 1961) and in this country (Holzel *et al.*, 1963) has shown that their absence might be accounted for by recovery of the R.S. virus when specimens are suitably collected and examined. Our results now suggest that this is so and that R.S. virus may be an important cause of bronchiolitis.

As this investigation was in the nature of a search for the presence of R.S. virus, no controls were taken to see if normal subjects harbour this virus. Previous workers have done this (Chanock *et al.*, 1961 ; Reilly *et al.*, 1961) and found that only a very low percentage of normal controls yielded R.S. virus.

Our experience confirms the view that virus isolations must be attempted so far as is possible on unfrozen material, and as this presents great difficulty in hospitals where there is no virus laboratory means must be found of bringing specimens rapidly to the laboratory for the immediate inoculation of suitable tissue cultures. We have found, in contrast to workers quoted by Holzel *et al.* (1963), that the virus can be isolated effectively in calf serum. This difference is probably due to the length of time taken for some of our viruses to appear ; some did not appear until the 28th day. It is possible, therefore, that calf serum has some inhibitory effect on virus growth, but with adequate examination of tissue culture it can be used satisfactorily.

Besides confirming that R.S. virus was an important cause of severe respiratory illness in young children in Newcastle in the winter months of 1963, this investigation has given us information on how the virus may be isolated from hospitals over a wide area. We hope that it will lead to a fuller investigation, in subsequent winters, of the part that R.S. virus plays in the total respiratory infections of infancy and childhood.

Summary

Evidence is presented that R.S. virus was associated with pneumonia and acute bronchiolitis in young children in Newcastle upon Tyne during the winter months of 1963. Methods of isolation of this labile virus are discussed.

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