PAPERS AND ORIGINALS

Sudden and Unexpected Deaths in Infants: Histology and Virology

J. A. J. FERRIS, W. A. AHERNE, W. S. LOCKE, J. McQUILLIN, P. S. GARDNER

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Summary

The histological appearance of lungs of children whose death had been sudden and unexpected has been studied in relation with the viruses found at necropsy. Fifty-one children were investigated and in 33 the lungs had the histological appearance widely accepted as characteristic of the condition previously termed "cot death." Sixteen had the histological appearance of lymphocytic bronchiolitis, and it was from this group only that viruses were isolated. The viruses isolated were those associated with respiratory infections. Two patients showed the histological appearance of bacterial bronchopneumonia. Viruses are probably responsible for a substantial proportion of unexpected sudden deaths in infancy.

Introduction

Sudden and unexpected deaths in infants have been the subject of much speculation for many years (Froggatt *et al.*, 1968; Bergman *et al.*, 1970; Camps and Carpenter, 1972). Unfortunately, none of the many hypotheses put forward are supported by conclusive evidence concerning the fundamental process underlying the condition. It is known that viruses may be associated with some of these deaths (Aherne *et al.*, 1970), and this paper investigates the relation between the viruses isolated and the histological picture in what seems to be a defined sector of this tragic syndrome.

Methods

The deaths to be considered are those of infants who died with no apparent preceding illness, death being both sudden and unexpected. Our criteria for inclusion in the series were: (a) the child should be under 1 year of age; (b) the child must have died unexpectedly; (c) a necropsy must have been performed with histological examination of the lungs; (d) the virological examination described below must have been carried out; (e) there should be no obvious macroscopic cause of death (conditions such as congenital heart disease, pneumonia, bronchitis, and tracheobronchitis would eliminate the child from the series); and (f) there should be no histological changes in organs outside the respiratory system sufficient to account for deaths.

HISTOLOGY

The necropsies were carried out by one or other of three pathologists. All histological preparations were examined independently by two pathologists and complete agreement reached. Their findings enabled the pathological processes in the lung, which could have led to the infant's death, to be classified into the following three groups.

Group 1 had the histological features widely accepted as characteristic of the condition previously termed "cot death" (fig. 1). In this group a range of histological changes were found: varying degrees of pulmonary congestion, an increase in cellularity of the alveolar walls, alveolar haemorrhage, but most essential of all, a generalized alveolar oedema. There was no polymorphonuclear leucocyte infiltration. In several cases large mononuclear cells were seen within the alveolar spaces; peribronchiolar cuffing with inflammatory cells was never seen. The bronchi and bronchioles often contained desquamated epithelial cells, as described by Bodian and Heslop (1956), but in our opinion this is a frequent finding in necropsy material from other conditions and may represent post-mortem autolysis.

Group 2 had the histological appearance of bronchiolitis. The inflammation typically affected bronchioles with calibres of less than 300 μ m (figs. 2 and 3). The principal features were necrosis of bronchiolar epithelium and peribronchiolar lymphocytic infiltration. Lymphocytes were often seen within the

Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP
J. A. J. FERRIS, M.D., D.M.J., Lecturer in Forensic Pathology
W. A. AHERNE, M.D., M.R.C.PATH., Senior Lecturer in Pathology
J. McQUILLIN, B.Sc., Principal Scientific Officer and Honorary Lecturer,
Department of Virology
P. S. GARDNER, M.D., DIP.BACT., Professor of Clinical Virology

Queen Elizabeth Hospital, Gateshead
W. S. LOCKE, M.B., CH.B., Senior Consultant Pathologist

FIG. 1—Group 1. Congestion and increased cellularity of alveolar walls. Note absence of peribronchiolar lymphocyte cuffing. (H. and E. $\times 48$.)

FIG. 2—Complete destruction of bronchiolar epithelium with minimal peribronchiolar cellular response. Acute bronchiolitis, early stage. (H. and E. $\times 48$.)

FIG. 3—Peribronchiolar lymphoid infiltration in acute bronchiolitis. Note also the epithelial irregularity. Surrounding alveoli contain a little exudate only. (H. and E. $\times 35$.)

mucosal epithelial cells and often the smaller bronchioles were plugged with mucus and other cell debris (fig. 4). Often the alveolar walls were intensely congested and there were focal areas of collapse. The alveoli were free from inflammatory exudate and the appearance in some cases was compatible with obstructive emphysema during life. These changes have been described in more detail elsewhere (Aherne *et al.*, 1970).

FIG. 4—Plug of exudate, mucus, and cell debris. Rim of patency is a shrinkage artefact; occlusion of this small bronchus may have been complete. Note epithelial abnormality and transmigrating lymphocytes. Acute bronchiolitis. (H. and E. $\times 175$.)

Group 3 had the histological appearance of a bronchopneumonia (fig. 5). The inflammatory infiltration involved the bronchi and bronchioles and the surrounding alveolar spaces were packed with inflammatory exudate. The inflammatory cell was principally the polymorphonuclear leucocyte, which clearly distinguished the inflammatory reaction from that seen in groups 1 and 2. Occasionally the inflammation was associated with minute foci of necrosis and often the overlying pleura was microscopically infiltrated with polymorphonuclear leucocytes.

FIG. 5—Section of bronchiole in acute bronchopneumonia. The lumen and the surrounding lung alveoli contain pus cells. The mucosa (artefactually separated from its basement membrane) is not abnormal. ($\times 300$.)

These features are typically those of bacterial bronchopneumonia. Macroscopically there were no obvious lesions in any of the three groups, with one exception: subpericardial or thymic petechial haemorrhages were always present in group 1, occasionally in group 2, and never in group 3. It must also be emphasized that although the picture in group 3 is called bronchopneumonia, this was a histological finding; consolidation and pus in bronchi were never obvious at necropsy.

VIROLOGY

At necropsy—which was carried out at the earliest opportunity, usually within 24 hours and rarely as late as 72 hours after death—secretions were aspirated from the respiratory tract, swabs taken from the trachea and bronchi for the collection of epithelial cells, and small portions of lung removed.

Slide preparations of cells from the tracheal and bronchial swabs were prepared by essentially the same method as described for the examination of cells from cough/nasal swabs taken in life (Gardner *et al.*, 1970). The culture methods were those used by Sturdy *et al.*, 1969.

Preparation of the lung for culture was as described by Aherne and his colleagues (1970), except for one point; the tissue was ground in 10 times its own volume of transport medium, using a Griffiths' tube in preference to an electrically operated homogenizer because this method allowed more rapid preparation of the tissue for culture.

Impression smears from the lung tissue for examination by indirect fluorescent antibody technique were made in the way described by Gardner *et al.*, (1970). Cryostat sections were prepared from small portions of lung snap frozen in dry ice and alcohol. Frozen sections, 0.6 μ m thick, were cut at a temperature of -40°C in a Slee cryostat, they were fixed in acetone at 4°C for 10 minutes, and dried at 37°C for 30 minutes before staining by the indirect fluorescent antibody technique referred to above. Preparations were stained for the presence of respiratory syncytial virus in all cases, and influenza A and parainfluenza virus types 1 and 3 in selected specimens. Control preparations treated with normal preinoculation serum from the rabbits in which all four antisera were prepared showed no staining on application of fluorescein-labelled anti-rabbit globulin. All antisera were absorbed with human tissue culture cells and tested for specificity of staining and for the absence of non-specific staining properties before being used for diagnostic tests. Specificity was accepted on the grounds that non-specific staining did not occur with the four antisera in any of the many specimens of lung tissue which were negative on culture, or from which viruses other than the specific virus being investigated had been isolated. The presence of virus was confirmed by culture in most cases.

Results

Fifty-one children fulfilled the criteria given above and were included in the series by definition. Their distribution among the three histological groups and the virus associations were: group 1 (33 children) no viruses; group 2 (16 children) 13 viruses; group 3 (two children) no viruses. The association of

viruses with group 2 was highly significant ($\chi^2 = 36.5$; $P < 0.0005$) and could not be due to chance. In one child included in group 2 but from whom virus was not isolated, there was a family history of an influenza-like illness; in another there was a delay of three days between death and post-mortem examination. The types of virus which were identified in the lungs of these

FIG. 7—Impression smear of lung showing ciliated epithelial cells infected by parainfluenza virus type 3. (Stained by indirect fluorescent antibody technique. $\times 840$.)

FIG. 8—Section of lung showing respiratory syncytial virus-infected cells in mucus plug of a bronchus; a segment of wall of bronchiole can be seen at top left of photograph. (Stained by indirect fluorescent antibody technique. $\times 840$.)

FIG. 6—Section of lung showing respiratory syncytial virus-infected ciliated epithelial cells lining a bronchiole. (Stained by indirect fluorescent antibody technique. $\times 840$.)

FIG. 9—Section of lung showing respiratory syncytial virus-infected distal epithelial cell of an alveolar duct. (Stained by indirect fluorescent antibody technique. $\times 840$.)

children were: respiratory syncytial virus (six children), influenza A (two), parainfluenza virus type 3 (one), H-strain rhinovirus (one), and adenovirus type 1 or 5 (three). We would emphasize, however, that in all 13 cases only small amounts of virus could be found in the lungs. The brunt of the infection involved the ciliated epithelial cells of the smaller bronchi and bronchioles, and figs. 6 and 7 show cells infected with respiratory syncytial virus and parainfluenza virus type 3 respectively. Very occasionally, infected cells were seen in a plug of cell debris situated within the bronchiole (fig. 8). The distal epithelial cells were rarely involved but fig. 9 shows the involvement of such cells by respiratory syncytial virus infection.

Discussion

In cases of sudden and unexpected death in infancy where no obvious cause can be found at necropsy, histological examination of the lungs allows the grouping of the pathological features in a meaningful way. The vascular congestion, alveolar and subpleural haemorrhage, and lobular collapse seen in group 1 are features commonly present in deaths thought to be due to an acute allergic reaction. If this were the case for those 33 children, then we have no evidence of the nature of the sensitizing agent. Such a reaction could account for the hypoxic changes found in the conducting tissue of the heart in the "sudden death in infancy syndrome" described by Ferris (1973).

Group 2 has most meaning for us at this early stage of our studies. Diligent scrutiny showed the histological features of lymphocytic bronchiolitis, and in 13 of the 16 children respiratory viruses were present in the lungs. This means that nearly one-third of all the sudden and unexpected deaths in this series had histological evidence of virus infection. Nine of the viruses associated with these deaths are of the type which appear from our studies and those of others to be the cause of acute respiratory illness of childhood and seldom, if ever, carried by children without illness (Chanock *et al.*, 1961; Gardner, 1968). The assumption may be made, therefore, that these viruses played a part in the process leading to the death of these children. This view is strengthened by the demonstration by immunofluorescence of virus in the distal epithelial cells of the alveolar ducts and the ciliated cells of the smaller air passages in those deaths

where the histological features vary from minimal lesions shown in fig. 2 to a fully developed bronchiolitis, as depicted in fig. 3.

Speculations on the pathogenesis of respiratory virus infection in young children suggest that bronchiolitis may be a type 1 allergic reaction (Gardner *et al.*, 1970). In this study about one-third of sudden and unexpected deaths in the series were associated histologically with features characteristic of virus infection and this was confirmed in four out of five cases by the physical presence of virus. This correlation of histological response and isolation of virus must surely be significant. This conviction is not lessened by the scanty distribution of virus in group 2, which resembles certain deaths from bronchiolitis we have previously described (Gardner *et al.*, 1967; Gardner *et al.*, 1970). The finding of minimal invasion of distal epithelial cells, the involvement of ciliated epithelial cells in the lower respiratory tract, and the occurrence of infected cells in occasional plugs of debris in the bronchioles further substantiates this similarity. That viruses are involved in a proportion of sudden and unexpected deaths seems to us to be established, but the precise mechanisms leading to death are still a matter of speculation.

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Epilepsy and Pregnancy: A Report from the Oxford Record Linkage Study

JEAN FEDRICK

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Summary

The files of the Oxford Record Linkage Study were used to identify 223 infants delivered to 168 epileptic women as the result of 218 pregnancies. There were six stillbirths, two of which were grossly malformed. It was shown that the population of epileptic mothers differed significantly from the total reproducing population in respect of social class. Each pregnancy resulting in a livebirth was

therefore matched exactly for social class, civil status, maternal age, parity, hospital, and year of delivery with three control deliveries resulting in livebirths. The defects noted at birth were abstracted from the Record Linkage files, and any subsequent hospital admissions or deaths of the children were also abstracted.

There were highly significant excesses of congenital abnormalities among the infants born to epileptic mothers (13.8% of livebirths had some degree of defect of congenital origin compared with 5.6% of controls, $P < 0.0005$). It was shown that neither the frequency with which the mother had fits nor the length of time she had had the epilepsy seemed to bear any relation to the frequency of defects in the offspring—with the exception of the two mothers who developed epilepsy in the first