Endogenous Alpha Interferon Expression

in

Cot Deaths

by

ALISON ANN ARMSTRONG

BSc (Hons) Microbiology

University of Glasgow

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ABBREVIATIONS

CFT	complement fixation test
CMV	cytomegalovirus
CPE	cytopathic effect
CSF	cerebro-spinal fluid
ELISA	enzyme linked immunosorbent assay
FITC	fluorescein isothiocyanate
Hep2	continuous human epithelioma cells
HuIFN	human interferon alpha
HSV	Herpes simplex virus
125I	iodine 125
ICC	immunocytochemistry
IFN-α	alpha interferon
IFN-β	beta interferon
IFN-γ	gamma interferon
Ig A, E, G, M	immunoglobulins A, E, G, M
IRMA	immunoradiometric assay
IU/ml	international units of interferon per millilitre as reference standard
KP1	monoclonal antibody to macrophage antigen
MEM	eagle's minimum essential medium
MRC5	human embryonic lung
NK2	monoclonal antibody to Namalwa IFN-α
NPA/NPS	nasopharyngeal aspirate/secretions
NSS	normal swine serum
PBS	phosphate buffered saline
RHSC	Royal Hospital for Sick Children

RSV	respiratory syncytial virus
SIDS	Sudden Infant Death Syndrome
URTI	upper respiratory tract infection
VTM	viral transport medium
VZV	varicella-zoster virus
YOK5/19	monoclonal antibody to IFN
uci	microcuries

SUMMARY

During the period January 1987 to June 1989, 100 infant deaths (64 males, 36 females) aged between 17 days and 53 weeks (mean age, 16.4 weeks) were brought to the Pathology Department, R.H.S.C., Glasgow for post mortem examination. A similar group of 98 infant deaths (62 males, 36 females) aged between 2 days and 63 weeks (mean age, 22.6 weeks) were examined at the Paediatric Pathology Department, R.H.S.C., Edinburgh. These cases were categorised as suspected cot death cases using the individual pathologist's classification. Twenty seven paediatric deaths (14 males, 13 females) aged between 3 weeks and 3 years (mean age, 1.4 years) presenting with a variety of surgical disorders, mainly cardiac were investigated in parallel with the cot death group.

The 100 cot death cases from Glasgow over the two year period were collected from a substantial study population of 2.5 million persons (out of a total population of 5 million). A limited epidemiological analysis was carried out on the cot death cases from the two Scottish paediatric centres. The location of cases showed a random distribution pattern throughout both Glasgow and Edinburgh, with the highest numbers of cases present in areas of densely populated housing schemes, G33 Blackhill, G34 Easterhouse, EH16 Liberton and EH4 Silverknowes/Clermiston generally regarded as areas of social deprivation. However, the study did not include details of social conditions and status of the cot death families.

Seasonal distribution of cot death cases revealed a winter predominance (December to April) although the Glasgow cases tended to have higher numbers of cases in January and September, occurring earlier than the Edinburgh cot death cases. Case numbers in the summer months, June through to August were low in both populations. The number of infants dying of cot death at 9-16 weeks of age was greater than at any other period in the first year, with the mean age of 18 weeks. Mild respiratory infection was a clinical feature obtained by parental interview in 60% of cot death cases prior to death, although there did not appear to be any pathological evidence to support these findings.

A comprehensive study of the respiratory tract was included in the project. Exfoliated cells from nasopharyngeal aspirate (NPA) and also impression smears of various levels of the respiratory tract (pharynx, larynx, trachea, main bronchus, bronchioles, and peripheral lung) were investigated for the presence of respiratory viruses using a number of monoclonal antibodies incorporated in a direct immunofluorescence assay. Samples collected at autopsy from cot death cases and paediatric deaths for viral isolation included heart, lung, brain (temporal lobe), kidney, mediastinal lymph node, mesenteric lymph node, recto-sigmoid loop, post-nasal swab, urine, heart blood and cerebrospinal fluid. The stool samples were tested by ELISA for the presence of rotavirus antigen, and positives confirmed by electron microscopy.

Results revealed a 16% virus positivity rate for Glasgow cot deaths, 38% for Edinburgh cot deaths and 18% in paediatric deaths. A wide range of viruses were isolated/detected including influenza A, RSV, adenovirus, parainfluenza type 3, rhinovirus, rotavirus, echovirus and poliovirus. The respiratory viruses represented the largest number of isolates in cot death cases with the exception of the poliovirus group. The polio virus isolates were almost certainly vaccine strains as 50% of infants were immunised prior to death.

The serum phase was obtained from the heart blood and used in complement fixation testing and indirect immunofluorescence testing to detect antibodies against a range of viruses. These studies were unrewarding giving essentially negative results. Bacteriological examination of the cot death samples was also unrewarding as no positive correlation could be made between isolate and the pathological report.

An immunoradiometric assay was used to detect the presence of alpha interferon (IFN-α) in samples of NPA, serum and CSF obtained from cot death cases. This novel approach was taken to question the infection hypothesis with respect to viral disease, as this assay has already been shown to be both sensitive and specific for IFN-α determination in biological fluids and to be a good marker of virus disease. The assay evaluated on control and cot death samples was shown to be reproducible and specific throughout the duration of the study. The specificity of the assay on cot death samples was determined by a

neutralisation/blocking step, and data is provided to demonstrate that interferon present in specimens could be removed prior to testing.

The IFN-α value in NPA samples, was positive at levels >10 IU/ml in 35% of cases, while the values obtained from paediatric death samples were found to be substantially lower. In a comparative study of NPA IFN-α values from paediatric cases of bronchiolitis, upper respiratory tract infection, pneumonia and chronic lung disease, the latter two clinical conditions showed a similar range of IFN-α levels to those observed in cot death cases. It is possible that cot death cases are in the earliest stages of acute lower respiratory tract infection. Age distribution and post mortem interval were analysed with respect to IFN-α data and no positive correlation was found for these parameters. Analysis of virus positive and virus negative cot death cases demonstrated raised IFN-α levels in both groups of infants. Thus a positive correlation between virus positivity and high IFN-α values could not be made. It is possible that the limitations of conventional methods of virus detection account for this discrepancy and IFN-α tests may be a better guide to virus infection than currently available routine methods alone. Lung sections from various levels in the respiratory tract were investigated for the presence of IFN-α by an immunoperoxidase technique. Cells of the intra-alveolar macrophage lineage were confirmed as the cells expressing IFN-α in lung tissue. By further study, KP1 - positivity (see page 16) was observed within the macrophage cells in the peripheral lung. The intensity of staining for IFN-α and KPI was graded from 1 - 4.

The higher grading values corresponded to the pathologists report of congestion and oedematous infiltrate within the lower lung. However a positive correlation of in-situ IFN- α detection in peripheral lung and IFN- α by IRMA in NPA's could not be made.

IFN- α in CSF and serum samples from cot death cases and paediatric deaths was detected only at low levels of <10 IU/ml. Levels were not shown to be statistically significant between either of the study groups and the significance of its presence in CSF and serum at these levels is further considered.

The study uses the novel approach of IFN- α detection in cot death samples to further investigate the infection hypothesis. The combined epidemiological and interferon data suggests that an "as yet unidentified" microorganism, infecting the respiratory tract is still a possibility despite the absence of clinicopathological data to support this hypothesis. Although the field remains open for an explanation of the Cot Death Syndrome the data in this thesis has suggested that further infection-orientated programs are required before dismissing the infection hypothesis.

CHAPTER 1

INTRODUCTION

Part 1

Cot death is one of the major causes of mortality in infants during the first year of life in the developed world (Peterson et al., 1979). Sudden and unexplained death has become of increasing medical and media interest as the more common causes of childhood mortality have been substantially reduced. Despite improvements in social conditions and availability of medical care these benefits do not seem to have reduced the pattern of cot death. Historically, sudden death in infants formed a small proportion of the total number of infant deaths. At the turn of the century, the infant mortality rate was 150 per 1000 live births (15%) of which 148 of the 150 deaths were due to congenital malformation, infection and malnutrition and 2 deaths per 1000 (0.2%) were attributable to sudden unexplained death. Recent rates indicate that the infant mortality rate is 10 per 1000 live births (1%) of which 6 infants per 1000 are likely to die due to low birth weight and congenital defects. The remaining 4 deaths per 1000 live births occurred in infants between one month and one year of age, half of which are due to congenital abnormalities and infection and the other half, 2 deaths per 1000 live births occurred suddenly and unexpectedly (0.2%) (Pharaoh & MacFarlane, 1982).

Until a better understanding of the aetiological aspects of cot death have been elucidated, it is unlikely that there will be a decrease in the annual statistics for this syndrome.

1.1.1 HISTORICAL PROFILE OF COT DEATH

For thousands of years, the concept of "overlaying" of a child in their parents' bed was a popular explanation for sudden infant death. An early reference can be found in the Old Testament (First Kings, Chapter 3, vs19). At this time, infanticide was extremely common and no attempt was made by the authorities to distinguish between deliberate suffocation and overlaying. A retrospective study of the slave children of Virginia, USA suggested that the figures attributable to "overlaying" (16-20% of infant deaths less than one year of age) mimicked those of the modern term Sudden Infant Death Syndrome (SIDS) (Savitt, 1979).

Pathological investigation is only a recent innovation in the investigation of cases of sudden infant death. Earlier this century, several eminent physicians thought that the thymus was grossly enlarged in cot death cases in comparison to cases dying of more common illness - malnutrition and infection (Jessop, 1905). However, Werne and Garrow (1953) deduced that the thymus in these earlier cases of sudden death were in fact the normal size gland, and that enlargement of the thymus is not a consistent feature of this syndrome.

During the Second World War, one pathologist suggested that "dried milk kills babies" as the inhalation of milk caused a "carburettor effect of the larynx". However, this hypothesis was refuted on subsequent investigation by the Ministry of Health. Barrett noted that children dying from terminal anoxia regurgitated their stomach contents but concluded that the presence of milk in the airways was not a primary mechanism for the cause of death. In later studies, the theory of a possible sensitisation to cow's milk was also investigated as reported by Coombs (1974).

Over the past thirty years, other research areas have been explored. Discussions on various aspects of cot death research were held at the International Conferences in 1963 and 1969 (Bergman et al., 1970) in Seattle, USA and at the symposia in Cambridge, 1970 (Camps & Carpenter, 1974), Toronto, 1974, Baltimore, 1982 and Italy, 1987 (Schwartz et al., 1988). Various hypothesis on aspects of epidemiology, immunology, genetics, excess and deficiencies in metabolism were debated which has formed the foundation of numerous studies since.

A number of national organisations were set up to provide support for the families of victims and organise research funding specifically for SIDS. In the UK, The Foundation for the Study of Infant deaths and the Scottish Cot Death Trust were established in 1971 and 1985 respectively.

1.1.2 MODERN DEFINITION OF SUDDEN INFANT DEATH SYNDROME (SIDS)

In the literature several terms are used synonymously; "cot" death in Britain, "crib" death in the USA, sudden unexpected death in infancy (SUDI), sudden unexpected infant death (SUID) and now more commonly the term Sudden Infant Death Syndrome (SIDS).

J.B. Beckwith at the International Conference held in Seattle, 1969 defined the sudden infant death syndrome as **"The sudden death of an infant or young child which is unexpected by history and in whom a thorough necropsy examination fails to demonstrate an adequate cause of death".**

The definition is primarily a diagnosis of exclusion and therefore is limited by the current methods of pathological investigation and our present understanding of the syndrome. The term "sudden infant death cause unknown" was first accepted for this syndrome as the natural registerable cause of death in England and Wales in 1971. It was not until 1979 that the Sudden Infant Death Syndrome (SIDS) was included in the International Classification of Diseases, in the 9th revision.

1.1.3 INCIDENCE

Worldwide incidence rates for the Sudden Infant Death Syndrome are documented in Table 1.

Until relatively recently, few studies have been undertaken to ascertain the true incidence of sudden infant death, largely due to unreliable registration of SIDS as a cause of death and the lack of identifiable clinical or laboratory markers for this syndrome.

As post-neonatal mortality in the UK has decreased since the Second World War, cot death cases now account for more than half of the deaths recorded (Pharaoh & MacFarlane, 1982). Figures for cot death collated for the UK (Fedrick, 1973, Mason et al., 1980) and Australia (Beal, 1972, Hilton & Turner, 1976) show a general trend of between 1.6-4.1 per 1000 live births. In comparative studies these results were similiar to those calculated for North America, 2.1 per 1000 live births (Valdes-Dapena, 1980). The post neonatal mortality rate for Sweden has been recorded at 3.3 per 1000 live births and this low rate was reflected in the value of 0.6 per 1000 live births attributable to cot death (Karlberg et al., 1977).

Two studies carried out in Denmark gave conflicting results: Beiring-Sorensen (1978) observed a rate of 0.9 per 1000 live births but Zachau-Christiansen (1975) from a smaller study detected a mortality rate of 2.7 per 1000 live births. Taylor and Emery (1988) in the UK reported

TABLE 1

Worldwide Incidence Rates for Sudden Infant Death Syndrome (Fitzgerald, 1988)

COUNTRY	YEAR	AUTHOR	SIDS Incidence/1000 Live Births
Australia	1988	Newman	1.9 - 3.7
Great Britain	1985	Golding et al.	2.0 - 2.78
Canada	1971	Kraus et al.	3.0
Denmark	1978	Biering-Sorensen et al.	0.92
Finland	1986	Rintahaka & Hirvonen	0.31 - 0.51
France	1984	Wagner et al.	2.71
Germany	1980	Althoff	1.3
Ireland	1985	Matthews	2.67
Italy	1988	Rusinenti et al.	0.8 - 2.6
New Zealand	1986	Hassall	1.9
Scotland	1985	Arneil	2.71
Scotland	1988	Bartholemew & MacArthur	2.9
Sweden	1987	Norvenius	0.41 - 0.9
United States	1979	Peterson et al.	1.7 - 3.06

the results of a prospective study carried out between 1977-1987 which showed an increasing rate of unexpected infant death. The UK post-perinatal death rate increased in 1986 due to the increase in numbers of deaths registerable as SIDS. Although, the authors reported that this increase may be due to "random variation", they noted that the increase coincided with a steady rise in the unemployment rate and minor diseases in families. The same authors concluded that cot deaths were seen in all socio-economic groups and continued while the death rate for children with serious disease showed a downward trend.

Recent figures for Scotland (Arneil et al., 1985) from a study population of 244 infants indicated an infant mortality rate of 11.3 per 1000 live births, 2.7 per 1000 live births (27%) of which were attributable to sudden infant death.

1.1.4 EPIDEMIOLOGY

Epidemiological studies have assisted in the identification of an "at risk" group of infants within the population that are shown to be susceptible to cot death (reviewed by Kelly & Shannon, 1982). A number of Western countries have carried out substantial analysis of their SIDS data as indicated in Table 2.

TABLE 2

Published Epidemiological Data Related to SIDS

COUNTRY	YEAR	AUTHOR
Australia	1988	Newman
Europe		
Britain	1965	Cameron & Asher
	1969	Strimer et al.
	1971	Fedrick
	1985	Golding et al.
Finland	1986	Rintahaka & Hirvonen
France	1984	Wagner et al.
Germany	1980	Althoff
Italy	1988	Rusinenti et al.
Scotland	1985	Arneil et al.
Scotland	1987	Bartholemew et al.
Sweden	1987	Norvenius
United States		
	1976	Naeye et al.
	1979	Standfast et al.
	1980	Peterson
	1980	Valdes-Dapena
	1988	Hoffman et al.

Area/Place of Death

Although the syndrome has been termed "cot" death the place of death was not always the home or cot. Fedrick recorded 65% of cases occurred at home, 22% in hospital and 5% elsewhere. Peterson & Beckwith (1974) examined the possibility that hospital stay may protect against the likelihood of cot death and concluded that this was unlikely. Population studies have shown an increased incidence of cot death in the city environment compared to rural districts (Emery et al., 1959). Figures collated for the UK, Australia and Northern Ireland have shown similar trends but these were not noted in US surveys (Bergman et al., 1970). However, one large survey in the USA, detected an increased incidence on the Pacific Coast compared to the Atlantic. In Nebraska, the risk of cot death increased with altitude. No firm conclusions could be drawn from these results due to other risk factors also apparent in the study population (Getts & Hill, 1982).

Socio-economic Conditions

Social class status is not positively related to the likelihood of cot death (Fedrick, 1973), and this conclusion has been borne out by subsequent studies.

Age Distribution

Conclusions concerning age distribution in cot death were made on data collated from large studies (Jorgensen et al., 1979, Naeye et al., 1977). Althoff (1980) summarised his findings on 292 cases studied in Cologne observing that the majority of cases occurred in the first year of life, 89.1% before one year of age, 66.8% before the end of six months and 52.4% during the first four months of age. There was an increased risk of cot death as age increased from birth until 1 year of age (Jorgensen et at., 1979). The majority of deaths occurred between 4-20 weeks with a peak incidence in the 3rd month (Valdes-Dapena, 1968).

Sex Differences

A male preponderance (1.6:1male:female) was detected (Arneil et al., 1985). Analysis of cot death cases 8-15 weeks of age showed61.5%of boys died compared to 36% of girls. This finding was also noted for cot death cases by Arsenault (1980) during a study of maternal and antenatal factors.

Seasonal Variation

Published data has shown that the frequency of cot death increases in the Winter months in temperate climates (Nelson et al., 1975). However, a study carried out several years later indicated that

investigators had not taken into account that more babies are born at certain times of the year (Farber & Chandra, 1978). In Britain the peak birth rate is in the Spring but any variations in the number of infants born are slight compared to the numbers of sudden deaths in winter months. Zoglo and co authors (1979) concluded that variation of birth rate with season made no difference to the winter excess of cot deaths. Several investigators have attempted to correlate outbreaks of infectious disease (Nelson et al., 1975), air pollution levels (Kraus et al., 1967) and meteorologic cycles (Bonser et al.,1978) with the incidence of cot death in the community.

1.1.5 MATERNAL FACTORS

An American study undertaken by Arsenault (1980) noted that cot death occurred more frequently to mothers who are young i.e. 20 years of age particularly those who may have experienced previous fetal loss. The more children already born, the greater the risk of SIDS. Also Naeye (1976) reanalysed data from the US Collaborative study and indicated that a decrease in maternal blood pressure during the third trimester of pregnancy enhanced the likelihood of a cot death. The incidence of haemorrhage and anaemia were investigated but did not show any significant differences between the cot death group and the controls (Beal, 1987).

Drug Addiction

There are several studies carried out in the States relating drug addiction and SIDS incidence (Chavez et al., 1979, Finnegan, 1979, Rosen & Johnson, 1988). The chance of SIDS occurring to an opiate-addicted mother was enhanced by 20% i.e. by a factor of 5.

Murphy et al. (1982) in Cardiff indicated that the incidence of SIDS increased when the mother smoked during pregnancy. A non-smoker carried a risk value of 0.6 whereas a smoker of more than 20 cigarettes per day rose to 2.7 (Toubas et al., 1986). Two trans Atlantic studies showed that 68% and 39% of mothers had smoked during pregnancy compared to 39% and 21% respectively in the control groups (Naeye et al., 1976).

1.1.6 METABOLISM

Although studies have been carried out to assess levels of manganese, cobalt, iodine, iron, copper, zinc (Caddell et al., 1977, Erickson, 1983) no firm evidence exists as yet to suspect an aetiology of trace element deficiency or overload. Patrick & Logan (1988) published data on an investigation of the concentration of 27 amino acids in the vitreous humour from 120 cot death cases and concluded that their results could not show an aminoacidopathy in cot deaths.

Harpey and coworkers (1986) have suggested a relationship between fatty acid-oxidation defects and cot deaths. Eight siblings of cot death cases and 2 "near-miss" cases were studied. None of the cases were clinically normal and presented with an abnormal odour due to multiple acyl CoA dehydrogenase deficiency (1 case), ethylmalonic-adipic aciduria (4 cases) and carnitine deficiency (1 case). This work is still preliminary and further evidence is required to show the importance of this work to the large number of cases of cot deaths seen each year.

1.1.7 PATHOPHYSIOLOGY

Beckwith and colleagues (1970) highlighted the need for a detailed post-mortem investigation of infants (<1 year of age) who die suddenly and unexpectedly.

Anatomical studies have concentrated on the cardio-respiratory system. The respiratory tract histology has been extensively investigated (Tapp et al., 1975). Inflammation in the respiratory tract (trachea, bronchi, lungs) was categorised into four groups:

1. Acute tracheobronchitis, acute bronchitis both which cause death,
2. Less severe abnormalities presenting with oedema but no necrosis,
3. Minor lesions
4. No inflammation

From the 139 cot death cases, one third showed minor inflammation in lung parenchyma (group 3). Apnoea is a respiratory pattern abnormality common in preterm infants, although little is known of the physiologic mechanism. Steinschneider (1972) reasoned that apnoea or hypoxia may play a role in the sudden death of an infant. A number of papers appeared in the literature in the form of supportive evidence for the apnoea theory with the identification of "tissue markers" for hypoxia and hypoxaemia (Naeye, 1973, Naeye et al., 1976). Studies of respiratory control have identified abnormalities in groups at risk of cot death: prolonged sleep apnoea (Guilleminault et al., 1976), excessive periodic breathing (Kelly & Shannon, 1977) and impaired arousal responsiveness to hypercarbia or hypoxia (Hunt, 1981). Some protective response to these respiratory abnormalities would be expected to prevent sudden death during sleep.

An impaired arousal responsiveness would not necessarily be sufficient to cause cot death unless other factors caused sleep-related asphyxia. Brainstem structures may be affected by hypoxia hence altering the infant's autonomic control of ventilation and cardiac rhythm. Surfactant decreases the surface tension in the alveoli in the lungs and prevents collapse after birth. Data published by Morley and colleagues (1982) indicated that there was a decrease in the phospholipid and dipalmitoylphosphotidylcholine content in surfactant from lungs of SIDS victims compared to a control group. Several years later, Talbert & Southall (1985) suggested that abnormal surfactant content in SIDS may cause pulmonary atelectasis with hypoxemia during lung development.

1.1.8 CARDIAC

A prolonged QT interval may be related to SIDS. Gordon and co-workers (1982) analysed the heart rate and respiratory activity in eight babies who later died of cot death, and also included a control group in the study. They concluded that the eight infants who died had a defect in cardiorespiratory regulation. Marino and Kane (1985) documented results on the conduction system of seven infants who died suddenly. Schwartz and colleagues (1982) tested the sympathetic imbalance theory on new born infants over a range of age levels. The infants with cardiac arrhythmias or prolonged QT interval were tested. Thirteen thousand five hundred infants were enrolled in the study and data on 10,000 infants was collated. Ten SIDS deaths and 4 non SIDS deaths were reported. The 4 non SIDS victims had QT intervals within normal limits and 6 out of 10 (60%) SIDS victims had a prolonged QT interval. The risk factor of SIDS is 30 cases per 1,000 live births (Segantini et al., 1986).

The data collected indicated that 30% of infants who subsequently became a SIDS victim may have a prolonged QT interval on the fourth day of life and the investigators suggested this to be a valuable marker for risk of SIDS in infants (Schwartz, 1984). A development abnormality in the sympathetic nervous system was postulated (Haddad et al., 1981). The increased likelihood of arrhythmias linked with the autonomic nervous system and sudden cardiac death was reviewed by Schwartz and Stone (1982).

1.1.9 INFECTIVE ASPECTS

Following the upsurge in research in the 1960's, the role of infection as a possible mechanism in the pathogenesis of cot death was widely debated. Epidemiological evidence supported the view that infection may be a trigger mechanism in the final pathway leading to sudden death in infants (Urquhart & Grist, 1972, Uren et al., 1980).

Several epidemiological features associated with cot death have strengthened evidence for a viral aetiology; high seasonal incidence between November - March, coinciding with the respiratory epidemic in the community (Scott et al., 1978), respiratory and enteric infection apparent to parents and babysitters prior to death (Williams et al., 1984), cot deaths affecting twins dying on the same night (personal observation) and the increased frequency in cot deaths in larger families.

Gold and coworkers (1961) attempted viral isolation from post mortem tissue collected from a group of 48 sudden and unexpected infant deaths. Twelve viruses (25%) were isolated from the pharynx, stool and central nervous system tissues. These agents were identified as members of the enterovirus group i.e. Coxsackie A virus, poliovirus type 3.

A subsequent study carried out in Phoenix, Arizona, USA reported the isolation of viruses from 10 infants who died suddenly and unexpectedly. A total of 45 specimens including brain, lung, liver and stool were investigated for the presence of virus. Viruses were detected from one site in 7 (15%) of the 10 infants studied. ECHO virus types 7 and 22 and poliovirus types 1 and 2 were isolated from the stool specimens and tissues (Moore et al., 1964).

A four year study of 97 sudden infant deaths in New York, USA incorporated a "control" group of 33 infants aged 2 years of age who died of congenital heart defect, pneumonia and accidental injury. Fourteen (14%) viruses were isolated from the 97 sudden infant deaths and 1 (3%) virus from the "control" group. The viruses isolated were from the enterovirus group i.e. coxsackie A and B viruses, poliovirus and echovirus (Balduzzi & Greendyke, 1966).

Larger studies have shown increased isolation rates from infants dying suddenly and unexpectedly compared to explained deaths (Ray & Beckwith, 1970). A study of 72 sudden infant deaths and 34 explained deaths indicated a 42% and 29% viral isolation rate for the two study groups. The viruses isolated were approximately one half enteroviruses and one third adenoviruses. The authors concluded that the presence of virus in tissue was not sufficient clinical evidence to suggest an overwhelming viraemia (Urquhart & Grist, 1972).

As the isolation and detection of viruses became more specific and sensitivity was improved, the more fastidious respiratory viruses; influenza virus types A and B, parainfluenza virus types 1, 2, 3, respiratory syncitial virus (RSV) were detected in the cot death cases and in control groups (Scott et al., 1978). With the introduction of fluorescence antibody techniques by Gardner and colleagues (1970) in Newcastle-upon-Tyne, viruses have been identified in the respiratory tract of cot death cases at post mortem. Gardner identified RSV in impression smears and frozen sections of lung tissue prepared post necropsy examination.

Ferris and colleagues (1973) several years later, investigated the relationship between viruses and the histological picture in lung sections in cot death. The pathological findings from the lung tissue could be categorised into three groups:

Group 1 were characteristic of cot death cases indicating varying degrees of pulmonary congestion, alveolar haemorrhage and generalised alveolar oedema. Also present were petechial haemorrhage.

Group 2 showed the typical histological appearance of bronchitis that being inflammation affecting bronchioles with necrosis of bronchiolar epithelium.

Group 3 was typical of bacterial bronchopneumonia where inflammation was widespread in the bronchi and bronchioles.

Fifty one cot death cases were included in the study and the results indicated that 33 children were placed in group 1 and had no virus isolated. Sixteen children were placed in group 2 and in 13 children (25%) virus isolates were noted. Two children were categorised into group 3 and no viruses were apparent in this group. The virus and histological association in Group 2 was statistically significant. The viruses detected were RSV, influenza virus type A, parainfluenza virus type 3, adenovirus and rhinoviruses. Several observations made from these findings suggested that the respiratory viruses detected in the study were those associated with acute respiratory illness in childhood which could imply that viruses play a part in the process leading to death. The limitation of the study was the lack of a suitable "control" group.

Scott and co-workers (1978) detected RSV (11%) adenovirus (7%) and parainfluenza virus type 3 (3%) by immunofluorescence techniques in 52 cot deaths. The study revealed the presence of a respiratory virus infection in 25% of cot deaths. The overall viral isolation rate for sudden infant deaths in these studies ranged from 15-44%.

The incidence of cot death has been linked to the peak of the RSV epidemic in the community (Scott et al., 1978). The average age of RSV cases (mean age 3-4 months) has also been shown to be comparable in cot death cases (Hall et al., 1979). Apnoea has been observed as a presenting feature of RSV in 15-25% of infants and epidemiological

studies have shown this also to be a risk factor in cot deaths (Anas et al., 1982). Ray and Beckwith (1970) investigated 80 SIDS cases but failed to recover RSV from any of the samples tested. Brandt (1984) isolated RSV in 14% of the SIDS cases.

Investigators have been unable to link any one virus in the pathogenesis of sudden infant death although there are infrequent reports of such cases, for example, CMV was associated with interstitial pneumonitis in a cot death group. Similarly, CMV was detected in the brain tissue from 4 cases of sudden infant death via extensive pathological investigation indicating the presence of inclusion bearing cells pathognomic of infection (Variend et al., 1986). Rotavirus has also been implicated in a small number (3 cases) of suspected cot death cases and in 2 "near miss" infants. Enzyme immunoassay was employed to detect rotavirus in stool and in tracheal aspirates. Rotavirus was identified in all 5 cases but was not detected in 11 non SIDS control cases in the Boston area (Yolkin & Murphy, 1982). The identification of more than one virus isolate in cot death cases, as indicated, led to the hypothesis that the sudden infant death syndrome was not a feature of one individual virus but possibly the result of the host response to a viral infection.

Immunisation with diphtheria-tetanus-pertussis (DTP) vaccine has been identified as a possible risk factor in cot death. Griffin et al., (1988) studied a group of 129,834 children receiving a DTP vaccine

regime. Two hundred and four deaths were categorised as SIDS. The study concluded that there was no increased risk of SIDS post immunisation, with this 'triple' vaccine.

Theories regarding the immune response have also been suggested. Ogra and colleagues (1975) used a quantitative method to show immunoglobulin G (IgG) and immunoglobulin M (IgM) were present in lavage fluid, but secretory component was absent in pulmonary tissue. The levels of immunoglobulins G, M, D, E, A (IgG, IgM, IgD, IgE, IgA) in sera of cot deaths compared to a control group were not significantly different (Turner et al., 1975).

In a more recent study, Forsyth and colleagues (1989) indicated that in lung lavage specimens from 16 cot death cases high concentrations of IgG, IgM and to a lesser extent secretory IgA were detected compared to lower levels in the control samples. Positive cellular staining for the immunoglobulins was evident in the terminal airways and alveolar interstitium of cot death cases.

No consistent aetiological approach has yet emerged which takes account of all the epidemiological features suspected. Several main points are still debated in this syndrome:

- the lack of a decrease in SIDS numbers despite progress in decreasing the infant mortality rate
- the lack of an identifiable infectious agent , although there is an increase in SIDS cases during the Winter months
- a narrow age distribution with male predominance noted in cases
- an increased risk of cot death to mothers with poor pre/post natal care and an increase in risk to mothers who smoke.

The field is still wide open and the infectious hypothesis awaits either new diagnostic tests or even the discovery of a micro-organism as yet unidentified.

Part 2

1.2.1 CURRENT CONCEPTS OF INTERFERON

Interferon was discovered more than 25 years ago by Isaacs and Lindenmann (1957) during a study of viral interference. The work involved the incubation of heat inactivated influenza virus with chick chorioallantoic membrane. The cells were then washed and the monolayer challenged with live influenza virus. An interference phenomenon was observed and a hypothesis formulated suggesting that an interfering material was released into the fluid from the uninfected cells thus preventing subsequent infection. The diffusible substance produced was named 'interferon'. The historical discovery of interferon is reviewed by Burke (1985).

Interferon produced from a variety of cell types was shown to exhibit the property of species-specificity (Tyrell, 1959). Initial experiments suggested that the interferon was a single substance degradable by proteolytic enzymes, hence protein in nature. Detailed study using human cells enabled workers to describe the interferons as a family of proteins and glycoproteins secreted by virus infected cells (Beale, 1971).

It is now clear that there are at least three antigenically and chemically distinct classes of each interferon determined by the cell type from which they are produced: leucocyte for IFN- α , fibroblast

for IFN-β and lymphocyte for IFN-γ (Stewart et al., 1980). Although the precise biological role of each interferon species remains unclear, these interferons have been shown to exhibit diverse biological properties: antiviral action, cellular regulation, inhibition of cell proliferation and immunomodulatory capabilities (Moore, 1984).

The type of interferon produced depends on the method of induction of which there appears to be two types. Type 1 is induced by viruses, polyribonucleotides, endotoxins and comprises human leucocyte and fibroblast interferon. Significant quantities of human interferon-α were first made by exposure of buffy coat cells to Sendai virus, in vitro. Type 2 or "immune" interferon is produced when sensitised B or T lymphocytes are exposed to a suitable antigen. This Type 2 interferon has been compared to lymphokines and acts as an effector substance (Ho, 1982).

Interferon α, β and γ have now been synthesised by recombinant DNA technology and the resultant recombinant interferon preparations have contributed to the current knowledge of the biochemical and molecular components of natural interferon. Human IFN-α was purified using high performance liquid chromatography (Rubinstein et al., 1979). From gene and protein studies, more than 24 species of IFN-α have been identified. The molecular weights of these species range from 16000 to 27000 daltons (Pestka, 1984).

IFN- β was purified by Knight and colleagues (1984) from human diploid fibroblast cells. Two species of IFN- β have been isolated both of molecular weight 20000 daltons. The IFN- ∝ species all contain 166 amino acids with a high degree of conservation whereas the homology with IFN- β species is only 45%.

A single gene for IFN ϒ was identified by Gray et al.,(1982) coding for a polypeptide of 146 amino acids with a molecular weight of 15000 - 20000 daltons. Overall there is no significant homology between the three types of interferons (Toy, 1983).

1.2.2 INTERFERON INDUCTION DURING DISEASE

Interferon plays an important role in the host defence against disease. It has been suggested that interferon is the first line mechanism deployed by the infected host during recovery from viral infection. Interferon expression results in a delay in the spread of virus giving time for the immuneresponseto develop and hence eradicate virus (Ho, 1982, Billiou, 1984).

Interferon has been detected in various human and animal tissues and fluids during acute viral infections. Several studies have correlated the presence of interferon in acute infection with the establishment of a viral aetiology. The possibility of a virus being implicated in

illness of unknown aetiology has been enhanced by the presence of a raised interferon level (Wheelock & Sibley, 1964, Negreanu et al., 1983, Ho Yen & Carrington, 1987).

It is clear that certain viruses are better inducers of interferon than others i.e., 3% in RSV cases and up to 50% in influenza A infection. A series of experiments with mice indicated that primary infection with influenza virus produced peak levels of interferon in the lungs during virus replication (Iwasaki et al., 1977).

Detectable levels of IFN-α in nasal secretions during acute respiratory viral infection have been reported in paediatric patients where parainfluenza or influenza viruses have subsequently been isolated. However, Hall and colleagues (1978) found that all respiratory viruses were not all equal in their interferon induction capacity, and that RSV was a poorer inducer of IFN-α than influenza in children.

Serum studies have found that IFN-α determinations may be useful in establishing a viral aetiology (Matthews & Lawrence, 1979). The short half life of IFN-α was suggested as one explanation for variable levels in serum interferon found in cases of viral infection with or without viraemia measured over several days. Consequently, low levels of interferon in sera may not be an indication of an abnormal interferon response to viral disease (Levin & Hahn, 1981).

The central nervous system (CNS) is capable of an independent immune response to viral infection. The precise mechanism by which the host defence system eliminates virus from the CNS is still unclear, although Ichimura and colleagues (1985) have suggested that interferon may play an important role in cases of acute neurological illness. The IFN-α levels in CSF in cases of aseptic meningitis mainly due to enterovirus were significantly higher in acute phase specimens compared to convalescent CSF. In a recent study IFN-α was noted as a good indicator of virus in the CNS and hence is useful in determining viral from bacterial meningitis. The IFN-α determinations in the CSF were a better indication of virus in the CNS than the cell count and conventional cell culture techniques (Ho Yen & Carrington, 1987).

Kennedy reported the presence of IFN-α in the CSF of paediatric patients with acute encephalopathy. The pathogenesis of these encephalopathies was suggested as a feature of the host response as a range of viruses were detected (Kennedy et al., 1986).

The presence of interferon in cot death cases was investigated by Seto and Carver (1978). The detection of circulating interferon was investigated as a method of identifying cases of SIDS with suspected viral infection where conventional laboratory investigations were reported as unhelpful (Ray & Hebestreit, 1972). Using a biological assay system, the group detected raised serum levels of total interferon in 3 out of 44 SIDS cases and in only 1 out of 12 of the control infants. Although the numbers in the study were small

significant differences between the groups were not found. However, the conclusions drawn by the researchers suggested that with the subsequent isolation of viruses, it may have been helpful to have measured interferon where it may be produced locally rather than in serum.

Vanacek et al., (1986) has also studied the relationship between SIDS and interferon. The production of IFN-β from fibroblasts in the muscle tissue of SIDS victims was determined and compared with a control group of accident cases. The results indicated the presence of IFN-β at a level of 64 IU/ml in the SIDS group and no detectable level in the control group (Vanacek et al., 1985, Vanacek et al., 1986).

1.2.3 INTERFERON ASSAY SYSTEMS

Immunoassay

Studies prior to the 1980's to determine interferon levels in human and animal tissues and fluids used biological assays. These assays are based on the inhibition of challenge virus in culture fluids (Friedman et al., 1983). Biological assays measure "total" interferon effect and were unable to differentiate between the three species of interferon. The insensitivity of the biological assay i.e. limited to levels above 10 IU/ml also prompted many investigators to look for more sensitive and specific methods of measuring interferon (Parry & Parry, 1981, Secher & Burke, 1980).

Daubas and researchers used an inhibition assay for the detection of HuIFN-α . The specimens were incubated with rabbit anti HuIFN-α immunoglobin and also 125I radiolabelled HuIFN-α . The immune complex was incubated overnight and the bacterium, Staphylococcus aureus containing Protein A added. The radiolabelled 125I HuIFN-α-antiHuIFN-α immunoglobin complexed with Protein A and the radioactive level measured in a gamma counter (Daubus et al., 1982). The assay although not utilising a monoclonal antibody enabled a larger number of HuIFN-α species to be observed. The limit of sensitivity of the test was 10 U/ml.

Shiozawa et al., (1986) devised a radioimmunoassay for the detection of circulating IFN-α . Antibodies to IFN-α were raised in rabbits. The IFN-α produced from human BALL-1 cells on infection with Sendai virus was radioiodinated using the lactoperoxidase technique. The detection limit of the assay was 0.05ng/ml IFN-α .

Radioimmunoassays using monoclonal antibodies raised to IFN-α , IFN-β and IFN-γ increasedthe specificity of assay methods for detection of interferon. The production of two site immunoradiometric assay (IRMA) systems enabled increased rapidity of testing hence results were obtained within 24 hours of receipt of specimen (Wright et al., 1984).

The measurement of HuIFN-α concentrations in serum enabled evaluation of an IRMA using the monoclonal antibody (NK2) specific for HuIFN-α . HuIFN-α was purified by immunoadsorption chromatography using the

measurement of IFN-α were indicated by the differences in the IFN-α standards on testing by IRMA and bioassay. Direct comparison between biological and immunoassays indicated that the immunoassay was as reliable as the bioassay and easier to perform.

Immunocytochemistry

Immunocytochemistry (ICC), the in-situ localisation of tissue antigens using labelled antibodies has been utilised as an important area of pathological investigation.

Coons and co-workers (1941) developed the labelling of antibodies with fluorochrome dye - fluorescein isocyanate, to identify antigen in tissue. The concept that specific antigen-antibody complexes enabled positive identification of the relevant antigen in tissue was used. When activated by the appropriate light emissions in the UV range, fluorescein isothiocyanate (FITC) dye conjugated to antibody emitted a bright green fluorescent light at wavelength 490nm. An alternative red fluorescent dye, rhodamine isothiocynate was developed for identification of tissue constituents. Immunofluorescent techniques had several disadvantages due to instability of the label, expensive equipment for evaluation was required and quantitation was not possible. Enzyme labels such as peroxidase (Nakane & Pierce, 1966), alkaline phosphatase (Mason & Sammons, 1978) and glucose oxidase (Suffin et al., 1979) were suggested as alternatives to fluorescent dyes. Recently advances in immunogold-silver enhancement of peroxidase

staining has increased sensitivity of detection of antigen in fixed tissue (Lewis et al., 1987).

Immunohistological investigations using fluorochrome labelled antibodies were performed on fresh frozen, unfixed tissue as opposed to formalin paraffin processed material (Coons & Kaplan, 1950). Formalin fixed tissue rendered tissue unsuitable for such antibody investigation. However, immunoperoxidase methods were developed for the detection of several antigens; hormones (Kuhar & Uhl, 1979), enzymes, intracellular immunoglobulins (Van Noorden & Polak, 1983) and hepatitis surface and core antigen in formalin paraffin sections. Immunoperoxidase techniques enabled investigation by light microscopy, permanent stain via deposit of 3,3'-diaminobenzidine tetrahydrochloride (DAB) substituted fluorochrome label.

Immunocytochemical labelling using peroxidase was developed in three methods:

1. Direct: antigen in tissue was bound directly to HRP labelled antibody and the HRP reacted with DAB substrate to produce a brown pigment at the site of the antigen.

2. Indirect: antigen in tissue was reacted and bound to unlabelled antibody, treated with HRP labelled swine antibody and the complex reacted with DAB to produce a brown product.

3. Peroxidase anti-peroxidase method: a sensitive double immunoglobulin bridge was used and an unlabelled secondary antibody and third layer peroxidase anti peroxidase (PAP) antibody. The second layer antibody was added in excess to the primary antibody hence only one combining site of the secondary antibody was occupied by the initial antibody and thus one site was left free to react with the PAP complex.

Foulis and colleagues (1987) utilised the indirect immunoperoxidase technique to identify interferon-α , class 1 MHC heavy chain and insulin in patients with Type 1 diabetes. The antibody specific to interferon-α was raised in sheep using human lymphoblastoid interferon from the HuIFN-αLy Namalwa cell line (Wellferon, Beckenham, UK). The antibody was > 80% pure when tested against interferon and at least eight IFN-α subtypes identified. Immunoblot analysis showed that the antiserum reacted with IFN-α but not with recombinant IFN-β , thus indicating the test to be highly specific.

An immunocytochemical study was carried out by Howatson and co-authors (1988) using sheep IFN-α antiserum to identify the presence of IFN-α in human placental tissue. The immunocytochemical technique used was an indirect immunoperoxidase technique capable of determining the IFN-α antigens in wax embedded tissue sections. The antibody as described by

Foulis and co-workers was used in this study and the detection system was also a peroxidase conjugate identified by DAB substrate.

CHAPTER 2

PATIENTS, MATERIALS AND METHODS

2.1.1 PATIENTS AND EPIDEMIOLOGY

One hundred infant deaths (64 males, 36 females) aged between 17 days and 53 weeks (mean: 16.4 weeks) were brought to the Department of Pathology, Royal Hospital for Sick Children (RHSC), Glasgow for post mortem examination and ninety eight infants (62 males, 36 females) aged between 2 days and 63 weeks (mean: 22.6 weeks) were similarly examined at the Department of Paediatric Pathology, RHSC, Edinburgh as suspected cot death cases. These cases were included in the study group.

Cot death was defined as "the sudden and unexpected death of an infant who had previously been well or had only minor symptoms" and as such did not conform to the standard definition of the sudden infant death syndrome (SIDS), see section 1.1.2.

The Glasgow cot deaths were divided into four categories after thorough post mortem which included full histology, selected bacterial and viral culture as defined by Arneil and colleagues (1982) in a previous study of infant mortality: group 3A, deaths in which a definite cause was established; group 3B, subdivided into A. deaths in which definite findings were observed with may have contributed to death, B. deaths in which there were minor abnormalities which probably did not contribute

to the death, group 3C, deaths in which no significant abnormality was found after a thorough necropsy (true SIDS) and group 3D, deaths in which no definite cause was known and no necropsy undertaken and group 3E, deaths who subsequently died after a period of resuscitation. The Edinburgh cot deaths were similarly defined by Bartholemew and coworkers (1987) as group A, deaths in which a definite cause was ascertained, group AB, death where an associated disorder was detected as a cause of death, group B, deaths with an associated disorder present but not thought to be an adequate cause of death and group C, deaths in which no associated disorder was present.

The Glasgow cases separated into the categories as follows:
group 3A (14), group 3B A (15) B (30), group 3C (38), group 3D (0) and group 3E (3).

Edinburgh cases were categorised in the following groups:
group 3A (23), group 3B A (7) B (48), group 3C (20).

In separate studies:-

a) A group of 27 paediatric deaths (14 males, 13 females) aged between three weeks and 3 years (mean: 1.4 years) were investigated at the Royal Hospital for Sick Children, Glasgow. Twelve cases were identified as deaths occurring during or after cardiac surgery.

b) A group of 22 adult deaths (10 males, 12 females) aged between 38 years and 90 years (mean age 69.1 years) were examined at the Department of Pathology, Glasgow Royal Infirmary. A variety of post

mortem diagnosis were recorded including bronchopneumonia, myocardial infarction, adenocarcinoma of the bronchus, chronic lymphatic and chronic myeloid leukaemias.

2.1.2 SPECIMENS

Body Fluids

Nasopharyngeal aspirate (NPA) was collected by passing a feeding tube (5 pg) into the posterior nasopharynx through the nose and applying suction pressure using a 10 ml syringe. The tube containing the secretions was transported in a plastic universal container. These secretions were diluted to a concentration of 1:10 in viral transport medium (VTM) and centrifuged on a bench centrifuge (Heraeus Christ) at 3000 g for 10 minutes. The resultant supernatant was aliquoted for subsequent viral isolation and interferon-α assay. The pellet of exfoliated cellular material was examined by direct immunofluorescence using monoclonal antibodies to a range of respiratory viruses, see section 2.1.5.

Cerebro-spinal fluid (CSF) from the cot death cases and paediatric deaths was collected by lumbar or cisternal puncture. The CSF from adult cases was obtained from the space visible in front of the pons after parting the cerebral hemispheres. A sterile needle was used to remove the CSF and care was taken not to damage the small blood vessels

and hence avoid contamination of the specimen. Virological investigation included viral isolation and interferon-α assay.

The heart blood (HB) was withdrawn from the right side of the heart using a syringe inserted either under direct or indirect vision or indirectly through the fourth left intercostal space. The heart blood was centrifuged using a bench top centrifuge set at 2500 g for 15 minutes in order to obtain the serum phase. Serological analysis was carried out by complement fixation test and indirect immunofluorescence tests. Aliquots of serum for interferon-α testing were stored at -20°C.

Urine was obtained in small quantities by suprapubic puncture. The small quantity of this fluid enabled storage only for interferon-α assay.

Tissues

Specimens of NPA, CSF, heart blood and urine were taken prior to post mortem examination and samples for viral isolation were removed using aseptic techniques during the initial stages at autopsy prior to evisceration.

Samples collected for viral isolation included post-nasal swab, heart, lung, kidney, brain (temporal lobe), recto-sigmoid loop, mesenteric lymph node and mediastinal lymph node. The stool specimen obtained

from the recto-sigmoid loop was investigated for the presence of rotavirus antigen by an enzyme linked immunosorbent assay (ELISA) technique (Abbott Laboratories). Positive results by ELISA were sent to the Regional Virus Laboratory, Ruchill Hospital, Glasgow for confirmatory testing by electron microscopy.

2.1.3 VIRAL ISOLATION

The tissue samples were finely chopped with a sterile scalpel blade in Dulbecco's phosphate buffer solution (PBS) to a dilution of 1:10 and centrifuged in a bench centrifuge at 3000 g for 20 minutes.

0.2mls of supernatant from the tissue samples, CSF and urine, was inoculated into commercially prepared individual culture tubes (Flow Laboratories) containing the semi-continuous cell line MRC-5 (human embryonic lung), the primary cell line primary rhesus monkey kidney and a continuous cell line Hep-2. These were incubated at 37°C and observed for typical cytopathic effect (cpe) of viruses for a total of 18 days and if negative, discarded.

2.1.4 BACTERIAL EXAMINATION

Specimens collected at autopsy for bacterial investigation included lung fluid, intestinal swab, CSF and heart blood. The lung fluid was inoculated into Robertsons broth and onto a blood agar plate then incubated at 37 ° C in an atmosphere of 5-10% CO_2 for 48 hours. The intestinal swab was treated as a rectal specimen and inoculated onto MacConkey medium and Selenite medium and incubated at 37°C for 24 hours. XLD (xylose-lysine-deoxycholate) medium was inoculated with the intestinal material and subcultured at 48 hours and investigated for pathogens. The CSF was inoculated onto Chocolate blood agar and incubated at 37 ° C in an atmosphere of 5-10% CO_2 for 24 hours. Heartblood was inoculated into aerobic and anaerobic culture bottles and run through the appropriate programme in the Bactec (Becton Dickenson NR730) for a total of seven days. Positive cultures were plated out and pathogens identified.

2.1.5 IMMUNOFLUORESCENCE

Exfoliated cells from the NPA were resuspended in 1 ml of PBS and spotted onto a multiwell test slide using a capillary dropper. The slides were air dried and fixed in acetone for 15 minutes.

Commercially produced fluorescein isothiocyanate (FITC) conjugated murine monoclonal antibodies directed against respiratory viruses;

RSV, influenza A and B ("Imagen", Novo Nordisk Diagnostics Limited), adenovirus, parainfluenza types 1, 2, 3 (Whittaker Bioproducts) were provided at a working dilution along with a relevant counterstain-Evans blue. Monoclonal antibody (12 µl) was placed on the acetone fixed exfoliated cells and incubated in a humidified chamber at 37° C for 30 minutes. The slides were washed in PBS for 5 minutes, distilled water for 5 minutes and allowed to air dry before examination under the oil power field of a fluorescence microscope (Nikon Episcopic-Fluorescence attachment EF-D). The cells positive for the appropriate viral antigen gave the characteristic bright green expected from the fluorescein conjugate against a red background.

2.1.6 CYTOSPIN PREPARATIONS/IMPRESSION SMEARS

At autopsy, sterile swabs of six areas of lung tissues (pharynx, larynx, trachea, bronchus, bronchioles and peripheral lung) were obtained and placed into plastic universals containing 2 mls of PBS. These specimens were vortex mixed, the swabs discarded and the suspension centrifuged at 3000 g for 15 minutes.

The cell pellet was resuspended in PBS to a dilution determined by examination of one drop of suspension under low power (x 10) microscopy. A few drops of suspension were added to the sample chamber and spun at 1000 g for 10 minutes in a Shandon Cytospin 2. This method deposited cells evenly over a small area of a glass slide without distortion or cell damage. The slides were air dried and fixed in

acetone for 15 minutes.

Impression smears were also collected at autopsy from the six levels of lung tissue already defined in the cytospin preparation method. These slides were air dried and fixed in acetone for 15 minutes. Both cytospin preparations and impression smears were investigated for the presence of respiratory viral antigen by direct immunofluorescence, for method see section 2.1.5.

2.1.7 SEROLOGICAL ANALYSIS

The serum samples were tested for the presence of antibody titres to a range of viral antigens; mumps, measles, herpes simplex virus(HSV), cytomegalovirus (CMV), varicella zoster virus (VZV), RSV, adenovirus, using a direct immunofluorescence technique. Test slides with the appropriate cell overlay were produced by growing virus to a suitable infective level in cell culture flasks. A typical cytopathic effect (cpe) in cell culture was observed using the following techniques:

1. RSV, adenovirus were absorbed onto a monolayered 80cm^2 flask of Hep-2 cells for one hour and the cells maintained in minimal essential medium (MEM) supplemented with 1% foetal calf serum (FCS), penicillin/streptomycin and glutamine.

2. Mumps and measles infected cells were cocultivated with an 80 cm^2 flask of Vero cells for each virus and maintained with MEM supplemented with 10% FCS, penicillin/streptomycin and glutamine.

3. HSV was absorbed onto an 80 cm^2 flask of Vero cells for one hour and maintained with the appropriate medium (as above), for Vero cell line.

4. Stock CMV and VZV were used to infect an 80 cm^2 flask of MRC-5 cells and observed for cytopathic effect.

Cell suspensions for test slides were produced by washing the monolayer in PBS, eluting the monolayer with trypsin/versene and washing in PBS by centrifugation. The infected cells in suspension were set at an appropriate concentration by examination of one drop of cell suspension under low power microscopy (x 10). The cells were transferred onto multiwell slides using a capillary dropper. The slides were air dried and fixed in acetone for 15 minutes. Storage in the freezer at -20 ° C and quality control of the slides was carried out every month.

A series of doubling dilutions (neat, 4, 8, 16, 32, 64, 128, 256 units) of the serum sample were obtained and 12.5 µl of the appropriate dilution (IgM 4 units, IgG 8, 16, 128, 256 units) spotted onto the well containing the viral antigen. The slides were incubated in a humidified chamber at 37 ° C for one hour. The slides were washed thoroughly in PBS for 5 minutes and distilled water for a further 5 minutes. After air

drying, 12.5 µl of the anti-human conjugated, IgG (1:30) and IgM (1:30) (Scottish Antibody Production Unit, Law Hospital) with Evans Blue counterstain was placed on the wells. The slides were incubated for 30 minutes at 37°C and washed as previously described. The slides were examined under oil immersion lens (x 50) of a fluorescence microscope (Nikon Episcopic Fluorescence attachment EF-D).

If there was sufficient serum, an aliquot was sent to the Regional Virus Laboratory, Ruchill Hospital, Glasgow and latterly to the Virus Laboratory, Department of Microbiology, Glasgow Royal Infirmary for viral titres by the complement fixation test (CFT).

2.1.8 ALPHA INTERFERON ASSAY

Interferon-α levels were measured using a commercially produced two site immunoradiometric assay (IRMA) (Novo Nordisk Diagnostics Limited, formally Boots Celltech Limited). The test incorporated a monoclonal antibody (Yok5/19) specific to leukocyte IFN-α and radiolabelled with 125I. After further incubation with a polyclonal anti IFN-α bound to a solid phase (sepharose), the unbound IFN-α was separated from the bound fraction using a non centrifugal sucrose separation procedure ("Sucrosep"). The principle of the assay is diagrammatically represented in Figure 1.

INTERFERON ALPHA TEST

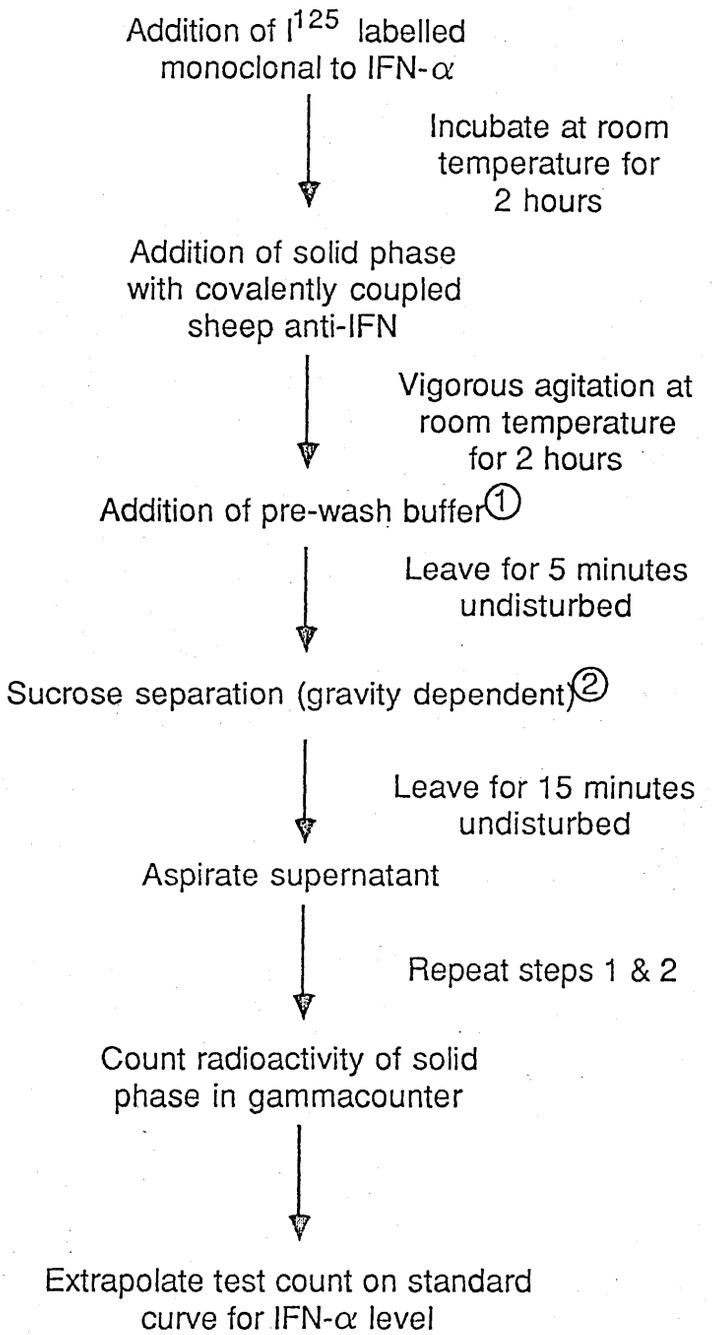


Figure 1

The specimens tested were clarified samples ie. serum, NPA at a dilution of 1:10 in VIM, CSF and urine.

The test procedure was carried out at room temperature in an area designated for radioactive work. The specimen (200µl) was incubated for two hours with the radiolabelled monoclonal antibody to IFN-α (50µl). The polyclonal anti IFN-α coupled to the solid phase (50µl) was added to the specimen/monoclonal antibody complex and further incubated for two hours on an orbital agitator at 300-350 rpm.

The complex was washed in prewash buffer (1ml) and allowed to settle for 5 minutes. The manufacturers "Sucropette" was placed in the tubes in the correct orientation and "Sucrosep" (2mls) added to the wells. The "Sucrosep" was allowed to drain through the "Sucropette" and the solution settled for 15 minutes. The top layer of unbound particles (green phase) was aspirated leaving 0.3 mls in the bottom of the tubes. The wash step was repeated once more. The radioactive level was measured using an LKB Beckman gamma scintillation counter.

A series of standard IFN-α preparations were placed in duplicate through the IRMA, see section 3.1.3. The counts per minute (cpm) were plotted against the standard IFN-α concentrations. The radioactive level in the specimens was calculated by extrapolation of the counts per minute against the standard preparations. The levels of IFN-α were expressed as International units per millilitre (IU/ml).

2.1.9 INDIRECT IMMUNOPEROXIDASE TECHNIQUE

An indirect immunoperoxidase technique (Foulis et al., 1987) specific for the measurement of IFN-α in tissue specimens was performed within the Pathology Department, Glasgow Royal Infirmary.

Formalin processed paraffin sections of the six levels of lung prepared post autopsy were cut using a microtome (4 μm) and placed on subbed slides (3-aminopropyltriethoxysilane coated) and placed through an in situ technique specific for the detection of IFN-α. The IFN-α antibody (H51) raised in sheep was obtained from Dr A. Meager, National Institute of Biological Standards and Control (NIBSC), London. The sheep anti IFN-α was raised using human lymphoblastoid IFN-α (HU IFN-αLy Namalwa Wellferon) as antigen. The antibody and normal sheep serum purified to minimise non specific binding by absorbing onto liver powders (guinea pig and porcine) and were thoroughly washed prior to the addition of antibody.

The sections were taken down to water through a series of alcohols, washed in tris saline (pH 7.5) for 10 minutes then endogenous peroxidase blocked by placing the sections in methanol/H_2O_2/HCl for 30 minutes. The slides were transferred to tris saline for a further 10 minute wash. The slides were blotted dry and the normal swine serum (NSS, 1:5 in tris buffer) placed on the sections for 15 minutes to reduce background staining. Excess NSS was removed and interferon-α

activity (1:100) added to the relevant sections.

The tissue sections were incubated overnight at 4 °C. Twenty four hours later the slides were washed in tris saline for 10 minutes, blotted dry and NSS (1:5 diluted in tris buffer) aliquoted using a capillary dropper onto the sections for 15 minutes. The secondary antibody, swine anti-sheep horse radish peroxidase (SWASH-HRP, 1:200) was added for 30 minutes. The slides were washed in tris saline and then placed in 3,3' diaminobenzidine (DAB) for 10 minutes. The sections were placed in counterstain-haematoxylin for 30 seconds, dehydrated and mounted for microscopic examination. The number of positive cells per five high power fields were counted. The high power field was calculated as 1400 square microns.

A negative control slide of normal sheep serum was run in parallel with the IFN-α sections. Controls to check the specificity of the IFN-α antibody were carried out using antiserum IFN-α (H51) incubated with Wellferon Hu IFN-α at 4 °C overnight. Staining of sections with was compared with absorbed anti sera and the IFN-α sera diluted in tris saline.

2.1.10 DETECTION OF MACROPHAGE CELL POPULATION USING MONOCLONAL ANTIBODY(KP 1)

The macrophage cell population in the lung sections was identified using a monoclonal antibody specific for macrophage/histiocytes in an

immunocytochemical technique, (Pulford et al., 1989).

The lung sections were placed through a series of alcohols, rehydrated to water then washed in tris saline for 10 minutes. Endogenous peroxidase was blocked by placing the slides in methanol/H_2O_2/HCl for 30 minutes at room temperature. The sections were washed in tris saline. They were then trypsinised for 20 minutes at 37 °C, the wash step was repeated, primary antibody, monoclonal antibody KP1 at a dilution of 1:20 placed over the sections and the slides incubated at 4 °C overnight.

The antibody was blotted and the sections washed in tris saline for 10 minutes. The secondary antibody: rabbit anti mouse horse radish peroxidase, (RAM-HRP, 1:50) was placed on the sections and incubated at room temperature for 30 minutes. The sections were washed in tris saline, placed in DAB for 10 minutes and counterstained in haematoxylin for 30 seconds. The sections were hydrated, mounted and examined for intracytoplasmic specific brown staining by light microscopy to determine the number of positive cells. The cells were counted per high power field (x40) which was defined as 1400 square microns.

STATISTICAL METHODS

The statistical methods for the non-parametric data analysed in this thesis included the Mann Whitney U-test and the Wilcoxon Rank Sum test.

CHAPTER 3

3.1 EXPERIMENTAL DATA SECTION

3.1.1 EVALUATION OF NEGATIVE CONTROL STANDARDS FOR SERUM AND VTM

Negative control standards as the serum diluent (0 IU/ml IFN-α) and VTM (0 IU/ml IFN-α) were aliquoted (200 ul) into ten labelled tubes and tested for endogenous alpha interferon using the "Sucrosep IFN-α", IRMA, see section 2.1.8. The mean value for total counts and standard deviation were calculated.

Results

The number of counts, standard deviation and coefficient of variation for the serum and VTM samples are indicated in Table 3. The mean values were 75 and 120 cpm for serum and VTM, respectively. The coefficient of variation was <10% for VTM samples and 10.6% when calculated for serum diluent. Only VTM was found to have an acceptable intra-assay reproducibility.

TABLE 3

Negative Control Standards
(0 IU/ml IFN-α) Assayed in
Serum and VTM Diluent using
Sucrosep IFN-α "IRMA"

	DILUENT	
Sample	Serum (cpm)	VTM (cpm)
1	75	104
2	70	110
3	84	121
4	69	132
5	85	125
6	70	120
7	83	131
8	60	124
9	75	113
10	80	119

	Serum	VTM
Mean	75	120
Standard Deviation (SD)	8.0	8.9
Coefficient of Variation (%)	10.6	7.4

3.1.2 EVALUATION OF THE IFN-α STANDARD CURVE IN SERUM AND VTM DILUENT

A series of IFN-α standard concentrations (0, 1, 4, 16, 64, 256, 1024 IU/ml) were prepared from the standard IFN-α preparation (1024 IU/ml IFN-α) (MRC 69/19 International Reference Preparation for the calibration of human leucocyte IFN-α) in serum and VTM. Each standard preparation was assayed in duplicate (200 μl) in the "Sucrosep IFN-α" IRMA, see section 2.1.8 and a radioactive signal finally measured as a quantitative measure of interferon found i.e. analysis of the counts per minute (cpm) obtained from the radiolabelled IFN-α monoclonal antibody reactive in the assay against the IFN-α standard concentration plotted on log/log paper. A standard curve was drawn through the points. A standard curve of the different IFN-α concentrations was prepared on the arrival of the new batch reagents every six weeks.

Throughout the study, the reproducibility of the standard curve was analysed by calculating the mean values for ten individual assays, of each standard concentration, in duplicate, from both serum and VTM curves. These values were plotted and the best fit line through the points analysed by regression analysis. The standard deviation, standard error and coefficient of variation were also calculated.

Results

The best fit line through the levels calculated for various IFN- α concentrations using the "Sucrosep IFN- α " IRMA in serum diluent is shown in figure 2 and for VTM in figure 3. The coefficient of variation is >10% in both sets of results but the levels in these interassay studies were within expected values for VTM (ie.11-20%) and within acceptable limits for serum (i.e.21-30%) (Tables 4 and 5).

3.1.3 MINIMUM DETECTION LIMITS IN BODY FLUIDS: NPA, SERUM AND CSF USING THE "SUCROSEP IFN- α " IRMA

The minimum detection limits for the measurement of IFN- α in body fluids using the "Sucrosep IFN- α " IRMA were evaluated within this laboratory, Virology Laboratory, RHSC, Glasgow in previously published studies. Since IFN- α tests under the same conditions and standardisation are currently in place at the Virology Laboratory, RHSC, it was thought appropriate to utilise this information and use the cut off levels predetermined for NPA, serum and CSF, in the cot death study.

Ho Yen and Carrington (1987) investigated the usefulness of IFN- α levels in CSF as an indicator of cases of viral meningitis as opposed to bacterial meningitis and determined levels of $\geqslant$1.5 IU/ml in CSF and serum to be of significance in clinical practice, however at this time a neutralisation blocking step was not included to confirm the specificity of interferon detected.

SERUM STANDARD CURVE OVER 10 ASSAYS USING
"SUCROSEP IFN-α" IRMA

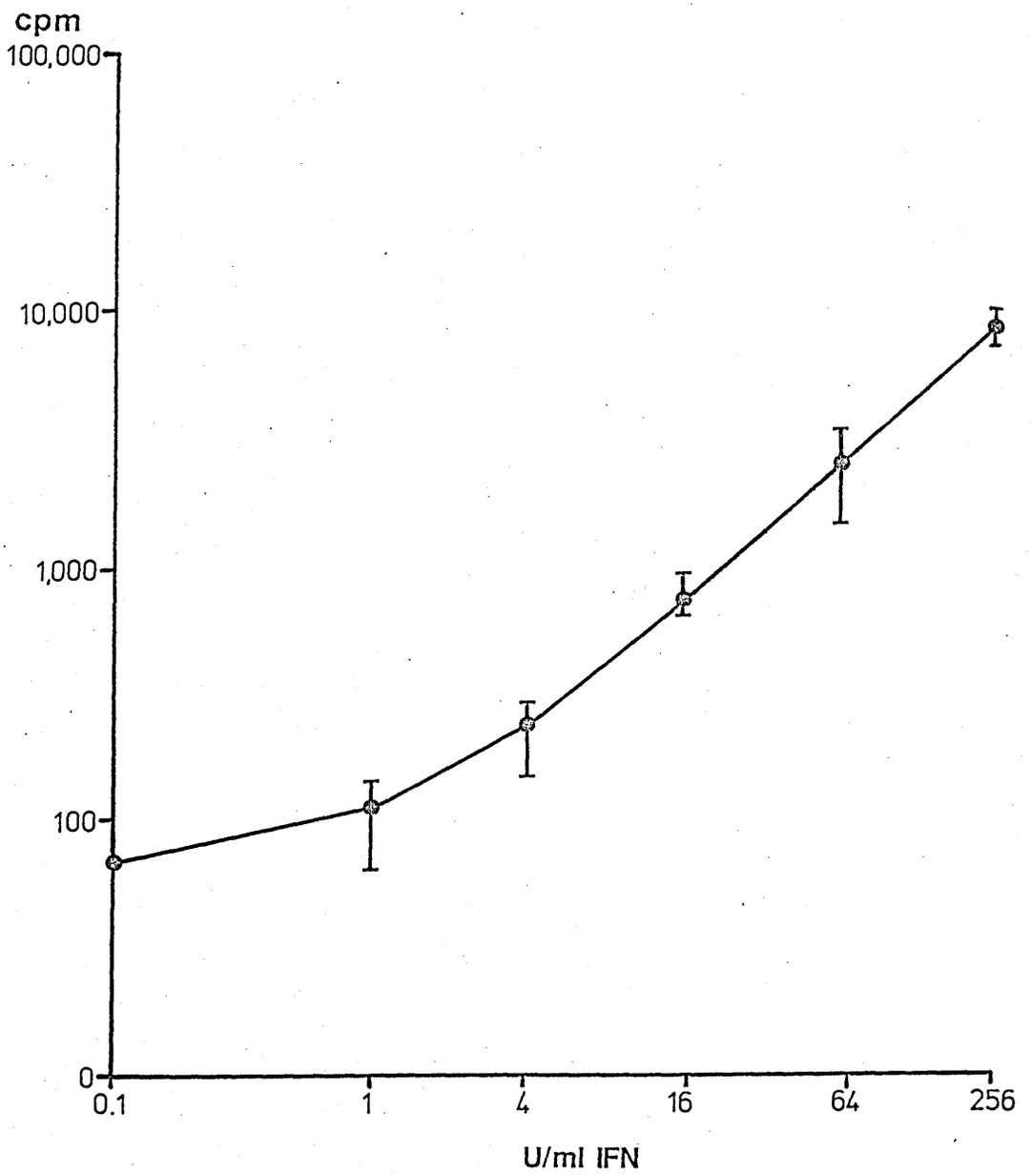


Figure 2

VTM STANDARD CURVE FOR IFNα VALUES (0-256U/ml) OVER 10 CONSECUTIVE ASSAYS USING "SUCROSEP IFN-α" IRMA

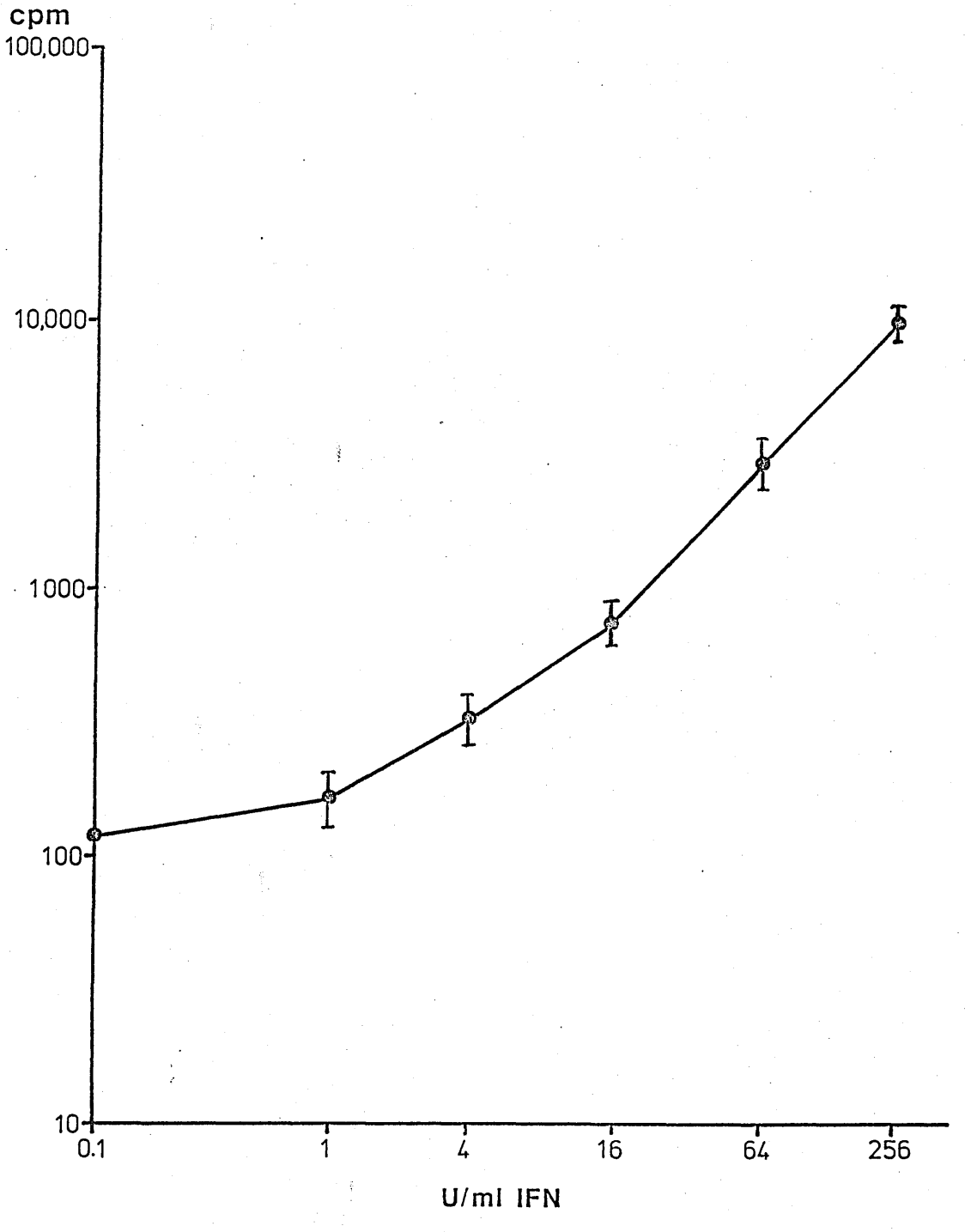


Figure 3

TABLE 4

Statistical Analysis of a Series of IFN- α Standard Levels (0-256 IU/ml) in Serum Diluent Measured by "Sucrosep IFN- α " IRMA

IU/ml IFN-α	Mean (cpm)	S.D.	S.E.	C.V. (%)
0	75	17	5.5	23
1	130	25	8.06	19.6
4	257	30	9.68	21.8
16	687.9	70	22.4	19
64	2561.7	573	181.5	22
256	8839	1717	542.8	19

S.D. = Standard Deviation
S.E. = Standard Error
C.V. = Coefficient of Variation

TABLE 5

Statistical Analysis of a Series of IFN-α Standard Levels (0-256 IU/ml) in VTM Measured by "Sucrosep IFN-α" IRMA

IU/ml IFN-α	Mean (cpm)	S.D.	S.E.	C.V. (%)
0	118.7	21.8	2	18
1	160	25.9	2	16
4	323.8	41.6	4	13
16	822	96.1	9	11
64	2972	362.7	36	12
256	9417.7	1134	113	12

S.D. = Standard Deviation
S.E. = Standard Error
C.V. = Coefficient of Variation

Salas M.P. (Glasgow University MSc Thesis 1988) investigated the IFN- α values measured in nasopharyngeal secretions (NPS) during an RSV epidemic. The specimen used was at a dilution of 1:10 in VIM and she observed that the level at which endogenous IFN- α could be neutralised was 0.2 IU/ml i.e. 2 IU/ml (undiluted specimens).

3.1.4. NEUTRALISED VALUES OF IFN- α STANDARDS FOR BOTH SERUM AND VIM DILUENT

In order to test the specificity of the IRMA technique, the IFN- α present in the standards/specimens was neutralised/blocked in a competitive assay by incubating with an excess of polyclonal sheep anti IFN- α prior to re-evaluation in the IRMA.

Method

IFN- α standard preparations (0, 1, 4, 16, 64, 256, 1024 IU/ml) and samples in duplicate aliquots of 200 μl were placed in labelled tubes. 50 μl of the polyclonal sheep anti IFN- α coupled to a solid phase (sepharose) was added to one of the labelled tubes. The standards/specimens were agitated on an orbital shaker for 2 hours at room temperature. The standards/specimens were centrifuged at 4000g for 20 minutes and the supernatant assayed for residual IFN- α activity as in section 2.1.8. The neutralised standards/specimens were assayed in parallel with the control standards/specimens placed in the second

labelled tube.

Results

The cpm of 125I versus the standard concentrations (0, 1, 4, 16, 64, 256,1024 IU/ml) of the neutralised and control (unneutralised) are plotted on the graphs in figures 4 and 5 for both serum and VTM diluents. The formula to denote the % IFN-α activity was:

$$\% \text{ IFN-}\alpha \text{ activity} = \frac{(\text{Unneutralised value} - \text{Neutralised value}) - \text{Background reactivity} \times 100}{\text{Unneutralised value} - \text{Background reactivity}}$$

The neutralised/blocked values of standard preparations are noted in Table 6. One hundred per cent of the IFN-α activity was neutralised/blocked in the 0, 1, 4, 16, IU/ml standard preparations diluted in the serum solution and found to be neutralised/blocked to 100% in VTM. Residual IFN-α activity of the order of 3%, 6% and 8% remained in the 64, 256 and 1024 IU/ml IFN-α standards in serum. Two per cent residual IFN-α activity remained in the 64 and 256 IU/ml IFN-α standard diluted in VTM. Six per cent IFN-α activity remained in the 1024 IU/ml IFN-α standard diluted in VTM. The implication was that in a standard preparation of 1024 IU/ml IFN-α 90 - 93% of the binding activity measured was specific for IFN-α.

NEUTRALISED / BLOCKED IFN-α STANDARD PREPARATIONS
IN SERUM DILUENT USING "SUCROSEP IFN-α" IRMA

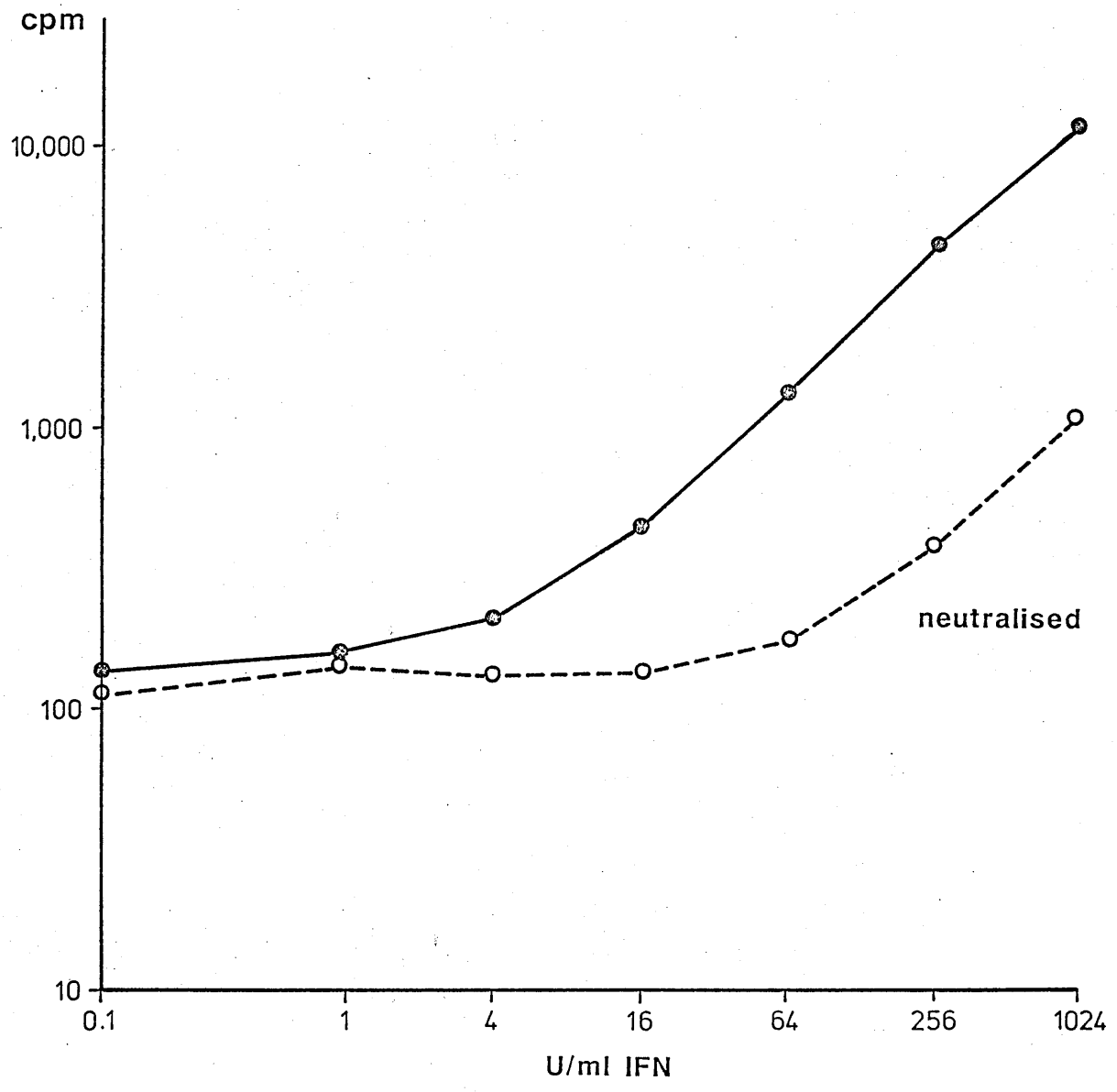


Figure 4

NEUTRALISED / BLOCKED IFN-α STANDARD PREPARATIONS
IN VTM USING "SUCROSEP IFN-α" IRMA

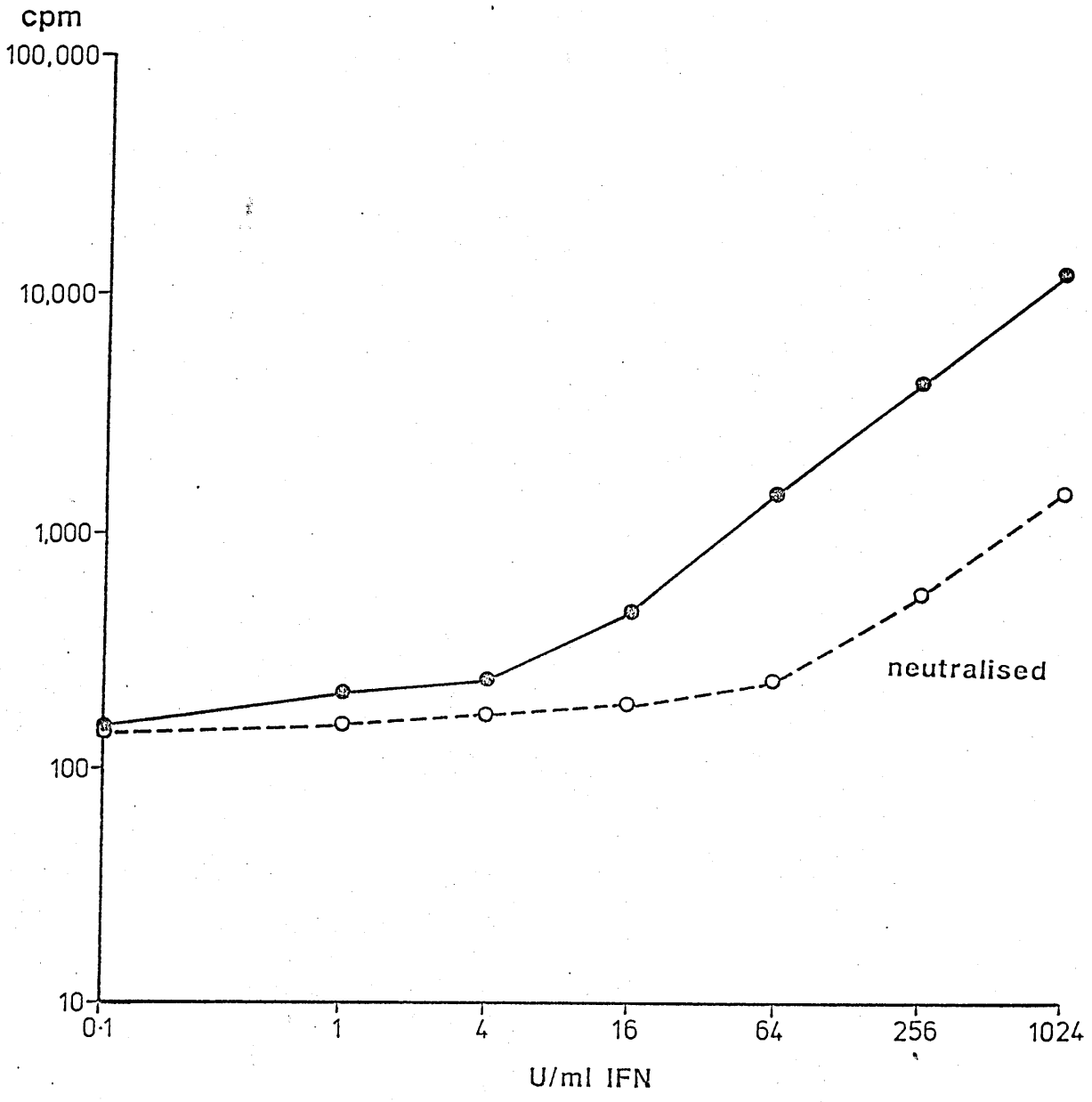


Figure 5

TABLE 6

Neutralised/blocked Values of Standard IFN-α Preparations in Serum and VIM Diluent Using "Sucrosep IFN-α" IRMA

IFN-α IU/ml Standard	Serum cpm	Serum Residual IFN-α Activity IU/ml	Serum (%) blocked	VIM cpm	VIM Residual IFN-α Activity IU/ml	VIM (%) blocked
0	70	0	100	120	0	100
1	125	0	100	160	0	100
4	225	0	100	285	0	100
16	615	0	100	750	0	100
64	2560	88	97	2566	65	98
256	8400	560	94	9745	240	98
1024	12200	980	92	14405	840	94

3.1.5 NEUTRALISATION OF IFN- α ACTIVITY IN COT DEATH SPECIMENS AND CSF SAMPLES FROM PAEDIATRIC AND ADULT DEATHS

Due to the small quantities of specimen available for testing only a limited number of the samples (NPA, serum and CSF) could be analysed in order to indicate that the IFN- α measured by the IRMA technique was not artefactual.

Method

The level of IFN- α was measured in the specimens after the additional neutralised/blocking step was carried out as in section 3.1.3. An aliquot of the original sample was evaluated in parallel with the neutralised specimen. The samples investigated had IFN- α levels measured by IRMA $\geqslant$1.5 IU/ml for serum and $\geqslant$0.5 IU/ml for CSF.

Results

Samples with sufficient quantity of material for further evaluation included 50 NPA samples, 45 serum samples and 24 CSF samples from cot death cases. Samples from paediatric deaths included 10 NPA specimens, 8 serum specimens and 10 CSF samples. A group of adult deaths (22 CSF specimens, mean IFN- α value 1.94 IU/ml) were evaluated in parallel with the cot deaths specimens. The specimens analysed are shown in Tables 7 and 8.

TABLE 7

Neutralisation of IFN-α Activity Measured in NPA and Serum in Cot Deaths and Paediatric Deaths

Cases	Number of Cases	Number of IFN-α tested	(%)	Number >50% reduction in IFN-α	(%)
NPA					
Cot deaths	97	50	(52)	45/50	(90)
Paediatric deaths	24	10	(42)	9/10	(90)
SERUM					
Cot deaths	99	45	(45)	44/45	(97)
Paediatric deaths	25	8	(32)	7/8	(87)

TABLE 8

Neutralisation of IFN-α Activity Measured in CSF Samples from Cot Death Cases, Paediatric Deaths and Adult Deaths

Cases	Total Number of Cases	Number of IFN-α Tested $\geqslant$0.5 IU/ml	(%)	Number >50% redn IFN-α	(%)
Cot deaths	99	24	(24)	24/24	(100)
Paediatric deaths	26	10	(38)	8/10	(80)
Adult deaths	22	22	(100)	18/22	(81)

The samples of serum and CSF from cot death cases when tested indicated that between 97-100% of the samples could be reduced 50% in IFN-α value after neutralisation/blocking experimentation. The paediatric death samples of serum and CSF and also the CSF samples from adult deaths showed similar numbers of cases with a significant reduction in IFN-α level (80%). In 5 cases the NPA samples from cot death cases showed residual IFN-α activity in post neutralisation/blocking tests. The IFN-α values on initial testing suggested levels of >16 IU/ml which were finally shown to have 10% IFN-α activity i.e. 1.6 IU/ml in sample (as analysed earlier for standard preparation, see section 3.1.4).

Although cases were seen in paediatric and adult deaths where false positive interferon levels were recorded when compared with cot death data (Table 8), no significant difference was found i.e. there was no greater tendency for false positives to occur in any age group.

In order to evaluate the sensitivity and specificity of the "Sucrosep IFN- α" IRMA in the clinical situation, studies were carried out to determine the level of IFN- α present in serum samples.

3.1.6 INVESTIGATION OF SERUM IFN- α IN PAEDIATRIC PATIENTS

A group of 53 serum samples obtained from paediatric patients (29 females: 24 males, mean age 5 years) with no suspected infectious illness were obtained from the RHSC, Glasgow blood bank. The specimens were evaluated for the presence of IFN- α using the Sucrosep IFN- α IRMA as demonstrated in section 2.1.8. Labelled tubes for background reading, total count and a control standard of 4 IU/ml IFN- α were analysed in the assay run.

Results

The levels of IFN- α present in serum are illustrated in Table 9. Five sera, representing 9% of the total number of samples, gave detectable levels of IFN- α above the minimum detection limit set at 1.5 IU/ml (see section 3.1.3). The mean level of IFN- α present in positive samples was 2.9 IU/ml. The mean level of total samples was 0.5 IU/ml IFN- α .

TABLE 9

Serum IFN-α in
Paediatric Patients,
RHSC, Glasgow

Cases	IFN-α Level (IU/ml)	Cases	IFN-α Level (IU/ml)
1	1.5	6-32	0
2	2.0	34	0.2
3	2.0	35-38	0.3
4	3.2	39-40	0.4
5	6.0	41-43	0.5
		44-45	0.6
		46-47	0.8
		48-49	1.0
		50-53	1.1
Mean	2.9	Mean of Total Samples	0.5

CHAPTER 4

4.1 CONCLUSIONS OF THE EXPERIMENTAL SECTION

Published data on the sensitivity and specificity of IFN-α immunoassays has been analysed with particular reference to the clinical situation and samples taken (Parry & Parry, 1981, Taylor et al., 1985). As part of this study, it has been important to evaluate the IRMA immunoassay used here to verify the presence of alpha interferon in specimens received from the cot death post mortem cases.

The assay was carried out in a supervised radiation area approved by the Radiation Protection Officer and safety standards met the International Committee on Radiation Protection (ICRP) code of practice. Particular care was taken with monitoring the radioactive area and records kept of the isotope received and used. The diagnostic reagents arrived every six weeks, one week after radiolabelling of the monoclonal antibody with 125I. Due to the half life of 125I (i.e. 59.6 days), the test reagents had a short shelf life.

The standard preparations were aliquoted in 300 µl quantities and stored at -70°C. Specimens prepared for assay were also aliquoted and stored at -20°C or -70°C depending on the length of time until testing. Thawing and refreezing of samples was avoided as the IFN-α activity decreased over a period of time.

The NPA samples were diluted in viral transport medium in order to obtain sufficient specimen for assay in the IRMA test. As NPA samples contained mucous material and CSF samples were occasionally bloodstained these specimens werecentrifugedprior to testing as sample debris was occasionally associated with false positive results.

The within or inter assay capability of the assay was tested on ten sequential samples of neat (0 IU/ml IFN-α) in serum diluent and VTM see Table 3. The coefficient of variation for serum diluent was 10.6% and for VTM diluent 7.5%. Using the same assay system Chard and co-workers (1986) and Ho Yen and Carrington (1987) detected a coefficient of variation of 4.2% and <10%, respectively for a series of samples of serum diluent analysed in the same assay on the same day. The results obtained in this study were therefore considered compatible with other data.

The between or intra assay reproducibility was tested over ten consecutive standard curves. Any variation between each reagent batch was not statistically significant. On receipt of the new diagnostic reagents, the standard preparations were assayed in duplicate for each diluent. Chard (1986) again noted low variability between assays of 6% when using serum as diluent. The results from this study demonstrated acceptable limits during intra and interassay reproducibility- sufficient to believe that over the two year study period the assay performed reliably.

Further experimentation to detect the specificity of the "Sucrosep IFN-α " IRMA was carried out in the study. Although a monoclonal antibody specific to IFN-α (Yok 5/19) was incorporated in the assay system, the ability of the monoclonal antibody to detect IFN-α was investigated using a neutralisation/blocking technique. The term neutralisation/blocking is used in the thesis to describe the extra experimental step carried out prior to the IRMA testing.

The assay utilises excess polyclonal anti-IFN-α to react with the IFN-α sites available on the samples hence the test does not adhere to a virological definition of neutralisation in an infectivity sense, but rather a blocking of the free IFN-α antigen sites on the standards and samples.

Results indicated that the recombinant IFN-α (Standard preparation MRC 69/19) was completely neutralised/blocked (100% removed) in standards up to 16 IU/ml and almost completely blocked (90% removed) in samples up to 1000 IU/ml (Figures 4 and 5, Table 6) in both serum and VTM diluents. Analysis of the clinical samples, NPA, serum and CSF using the additional competitive step in the assay, prior to testing for IFN-α confirmed the specificity of the test to detect 'non artefactual' IFN-α (Tables 7 and 8). The number of cot death specimens analysed in this way were limited due to the small quantity of samples available from post mortem. Salas (1987) indicated similar results by using the neutralisation/blocking assay prior to IRMA analysis of endogenous IFN-α in NPA specimens in VTM obtained during

an RSV epidemic and confirmed excellent specificity in the test.

Studies of the recombinant monoclonal antibody to IFN- α (Yok 5/19) have shown the ability of this monoclonal antibody to detect at least seven subtypes of IFN- α A (2), C, D (1), G (5), I, J (7) and K (6), although more than 24 species of IFN- α are known. However a number of IFN- α subtypes may be missed in clinical specimens evaluated in this IRMA.

The evaluation of IFN- α levels in the cot death situation required knowledge of IFN- α values present in specimens received from children who had no clinical evidence of viral disease. For ethical reasons, it was not possible to analyse samples from infants in the community setting therefore samples of serum from the hospital blood bank were thought to represent a reasonable group of serum samples from age matched infants. Children admitted for general surgery were chosen in this study. The results from normal children in the present study indicated that 9% of serum samples assayed had detectable levels of IFN- α (Table 9). This detection rate from an infant population was similar in number to the 6% of serum samples also with detectable levels of IFN- α noted by Chard in a similar study using the "Sucrosep IFN- α" IRMA. The IFN- α levels were detected at a low level in these specimens however their presence was above the cut off level and considered positive for IFN- α.

It is a widely held view that IFN is not normally found in the serum from healthy individuals, however researchers have used biological assay to identify leucocyte IFN- α (Green et al.,1982, Chisholm & Cartwright, 1987) and the ability to detect levels of IFN- α <10 IU/ml was questionable. The increased sensitivity of the assay i.e. $\geqslant 1$ IU/ml used may explain the interferon yield in this study.

The IRMA used in the study showed high sensitivity and specificity to IFN- α . Large numbers of samples could be evaluated rapidly and the results obtained within twenty four hours of receipt of specimen.

CHAPTER 5

ANALYSIS OF THE EPIDEMIOLOGICAL FEATURES ASSOCIATED WITH COT DEATHS

PART 1

Glasgow Data

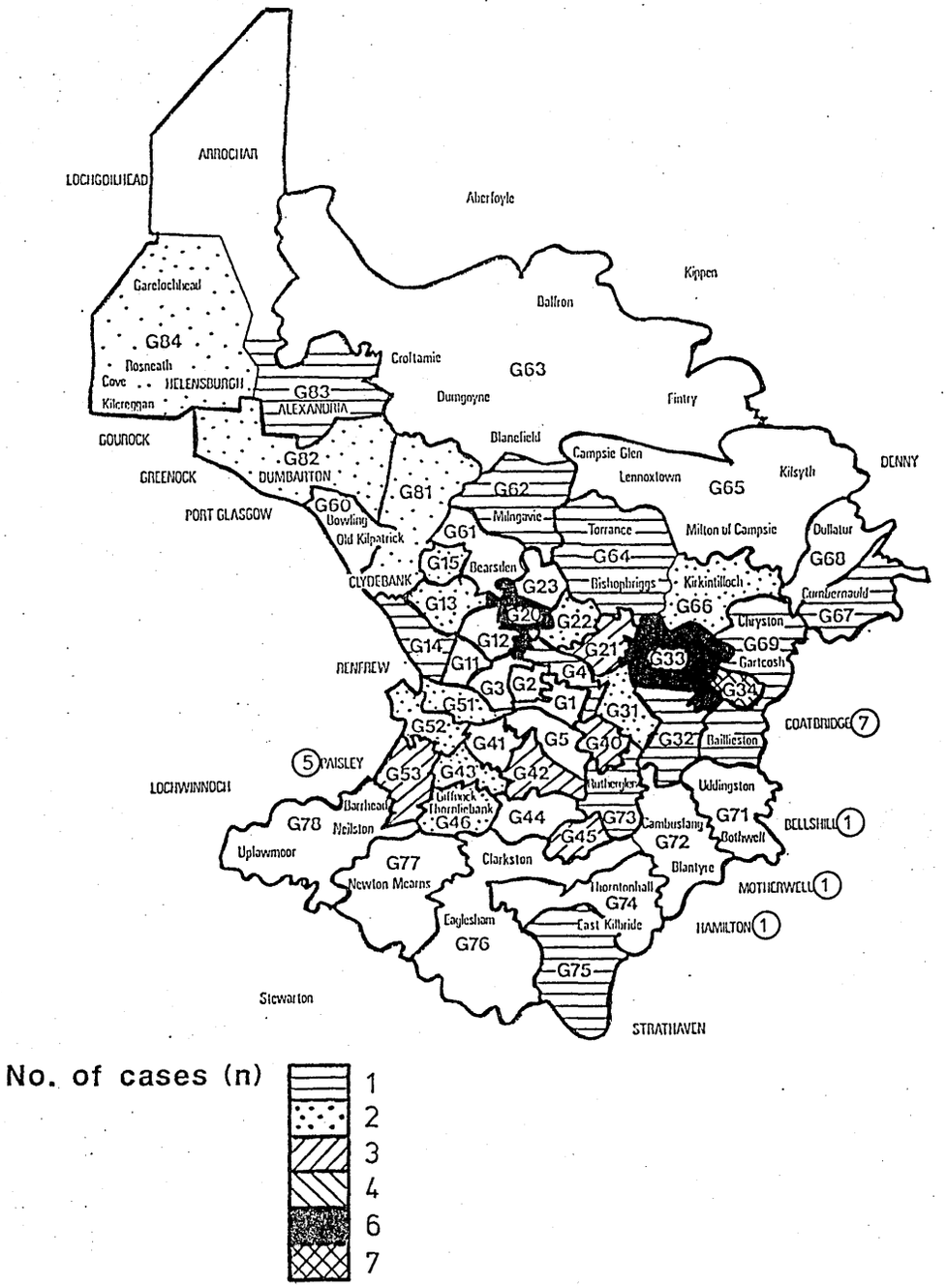
During the study period, January 1987 to March 1989, brief clinical histories from one hundred suspected cases of cot death referred from the Procurator Fiscal Office, Glasgow were collected and analysed.

Although data was obtained from the post mortem reports and other sources e.g. parent interviews, police reports in many cases this was incomplete.

5.1.1 GEOGRAPHICAL LOCATION OF COT DEATH CASES

The home address and place of death of the cases referred to the Pathology Department, RHSC were principally from within the Glasgow City boundary, see figure 6. The number of cases indicated on the map was 73 (73%) of the total cases analysed as documented in Table 10. The remaining cases were located in Airdrie/Coatbridge (7), Paisley (5), Bridge of Weir (2), Holytown (1), Drumry (1), Spittal (1), Wishaw (1) and Argyll (3), Table 11. The areas with highest numbers of cot death cases are denoted by the heavily shaded/black areas on the map:

DISTRIBUTION OF COT DEATH CASES
JANUARY 1987 - MARCH 1989
IN GLASGOW AREA, R.H.S.C., GLASGOW



No. of cases (n)

1
2
3
4
6
7

Figure 6

TABLE 10

Distribution of Cot Death Cases by Postcode Area, Glasgow

Post Code	Areas	Number of Cases
G4	Townhead	1
G13	Kelvindale	2
G14	Knightswood	1
G15	Drumchapel	2
G20	Maryhill	6
G21	Woodlands	3
G22	N. Kelvinside/High Possil	3
G31	Parkhead	2
G32	Carntyne/Shettleston	1
G33	Blackhill/Riddrie/Ruchazie	6
G34	Easterhouse	7
G40	Bridgeton/Dalmarnock	3
G41	Dumbreck/Pollockshields	2
G42	Toryglen	3
G43	Pollockshaws/Newlands	2
G45	Castlemilk	3
G46	Giffnock/Thornliebank	2
G51	Ibrox/Govan	2
G52	Penilee/Cardonald	2
G53	Pollock/Nitshill	3
G62	Milngavie	1
G64	Bishopbriggs	1
G66	Kirkintilloch	2
G67	Cumbernauld	1
G69	Baillieston	1
G73	Rutherglen	1
G75	East Kilbride	1
G81	Clydebank	4
G82	Dumbarton	2
G83	Alexandria	1
G84	Helensburgh	2

TABLE 11

Distribution of Cot Death Cases Outwith Postcode Area, Glasgow

Area	No. of Cases
Airdrie/Coatbridge	7
Drumry	1
Paisley	5
Spittal	1
Holytown	1
Bellshill	1
Bridge of Weir	2
Argyll	3
Hamilton	2
Motherwell	1
Wishaw	1

G20 Maryhill (6), G33 Blackhill (6), G34 Easterhouse (7), Airdrie/Coatbridge (7) and Paisley (5). The figure in the brackets represented the percentage of total cases. The location of the cot death cases follows a concentric pattern around the city centre similar in appearance to the location of the main housing areas of the city.

5.1.2 MONTHLY DISTRIBUTION OF COT DEATH CASES

The monthly distribution of the 100 cases examined during the study period is represented on the bar chart in figure 7. For each year i.e. 36 in 1987, 42 in 1988 and 22 in the early months of 1989, the highest distribution of cases was found to be between January and April each year (1987, 1988, 1989) at 17%, 18% and 20%, respectively. A lower percentage of cases was noted between May to August for each year studied i.e. 5% and 10%, and the month with the lowest number of cases was June, in both years. Although the data is limited, to a two year analysis, it would support a June and October low with January and September peaks in a twice yearly cycle.

The number of respiratory virus infections reported to the Communicable Diseases Unit, Ruchill Hospital, Glasgow each month during the study period from the Glasgow Virology Laboratories were numerically analysed and plotted on a graph beside the number of cot death cases as in figures 8a and 8b. A similar pattern of presentation each month was noted with highest numbers of cases detected during the winter months.

MONTHLY DISTRIBUTION OF GLASGOW COT DEATH CASES BETWEEN JANUARY 1987 - MARCH 1989 ROYAL HOSPITAL FOR SICK CHILDREN (RHSC), GLASGOW

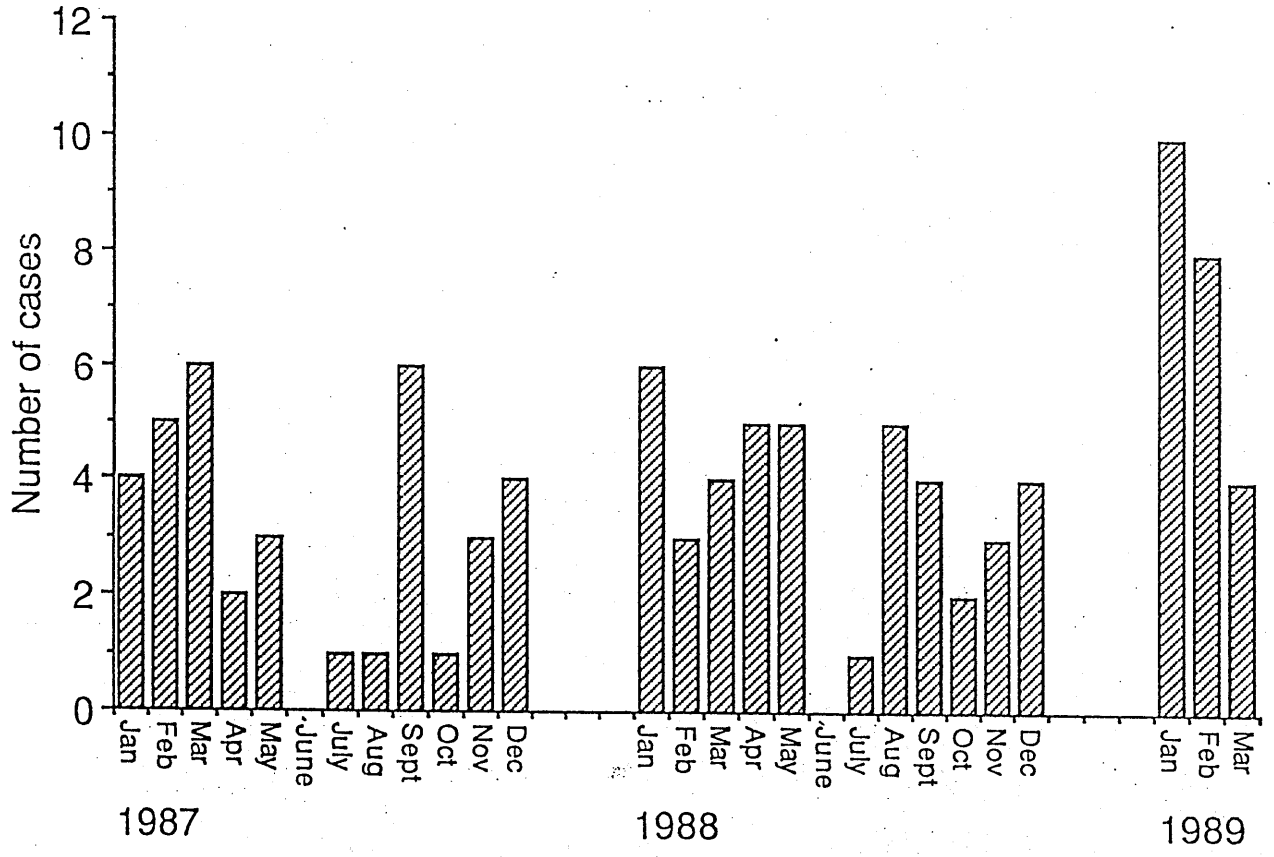


Figure 7

DISTRIBUTION OF GLASGOW COT DEATH CASES COMPARED TO TOTAL NUMBER OF RESPIRATORY VIRUS INFECTIONS (CDS SCOTLAND, 1987)

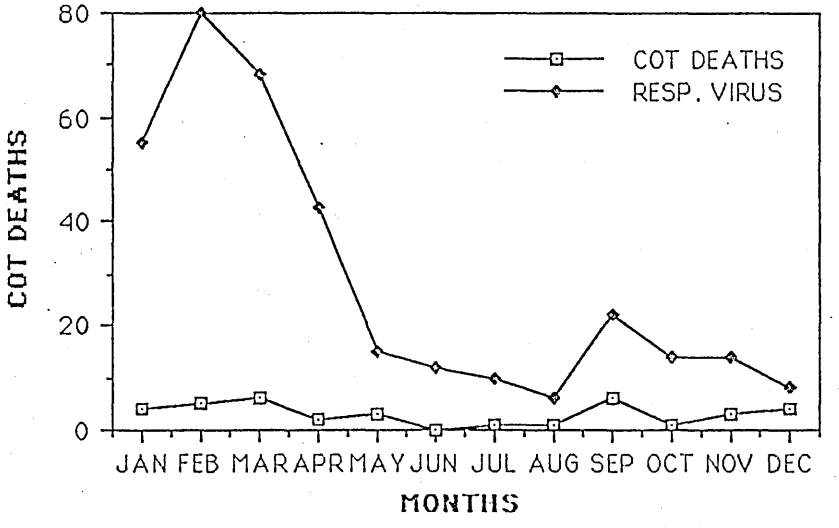


Figure 8a

DISTRIBUTION OF GLASGOW COT DEATH CASES COMPARED TO TOTAL NUMBER OF RESPIRATORY VIRUS INFECTIONS (CDS SCOTLAND, 1988)

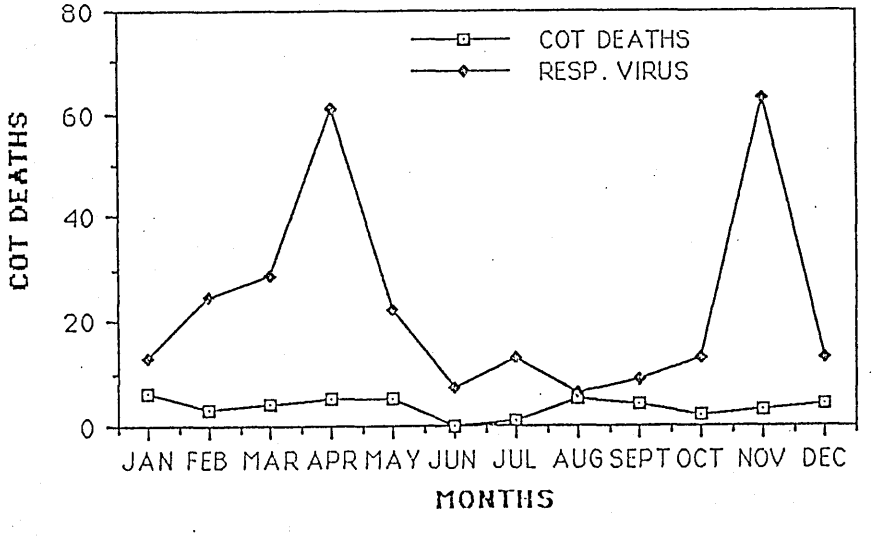


Figure 8b

5.1.3 AGE DISTRIBUTION OF COT DEATH CASES

The mean age of the one hundred suspected cot death cases was calculated as 16.6 weeks from within the age range 17 days and 53 weeks.

Analysis of the age distribution is tabulated in Table 12 . Twenty three infants were aged 1-18 weeks, 39 infants were 9-16 weeks of age, 22 infants were aged 17-26 weeks and 16 infants in the 27-53 week age group i.e. the age range with highest incidence of cot death in this study was in infants between 2-4 months of age, with 62% of deaths in the first 6 months of the year. The mean age of paediatric deaths (non cot death) in this study was 1.4 years.

5.1.4 DETAILS RELATING TO SIBLINGS

The position of the cot death victim in relation to other infants in the family unit was analysed. The results are shown on Table 13. Eight infants were from twin births and one infant was from a set of triplets. Thirty eight infants were shown to be the only child in the family group. Eighteen cases had one additional infant in the family. Fifteen cases had two additional infants and sixteen families had 3 additional infants in the family. In summary 58 cot deaths occurred in families where other siblings were present compared to 38 as the only child.

TABLE 12

Age Distribution of Cot Death Cases, RHSC, Glasgow

Age Range (weeks)	No. of Cases
1-18	23
9-16	39
17-26	22
27-53	16

TABLE 13

Position of Cot Death Victim in Family Unit

No. of Infants in Household	No. of Cot Death Cases
0 (only child)	38
1	18
2	15
3+	16
Twin	8
Triplet	1

5.1.5 ILLNESS PRIOR TO DEATH IN COT DEATH CASES

Fourteen cases (14%) were admitted to hospital for a period of time but all the infants were discharged and at home prior to death. The infants were referred to a hospital paediatric unit for a variety of disorders including bronchitis, suspected meningitis, acute renal failure, stenosis and hole in the heart. The number of infants admitted to hospital with respiratory disorders was calculated as seven infants (50%) of the infants with recorded stay in hospital. These included infants with upper and lower respiratory tract infection. Details of the illness were fairly limited as the histories were taken shortly after the death of the infant.

The remaining infants in the study not hospitalised prior to death were 86 cases. Seventy nine cases had informative clinical histories and no evidence of serious illness. There were, therefore, seven infants from the total eighty six cases with no clinical details available due to their location outwith the city boundary.

However, a total of36/93(39%)infants had history of respiratory infection, cold, cough, congested, snuffly and 4/93(4%) infants presented with diarrhoea and vomiting a few days before death. Hence, the total number of infants with illness prior to death was 54/93(58%).

The number of infants with no obvious signs of illness was 39/93(42%), All of the infants attended the baby clinics after birth.

The paediatric deaths were all brought to the Pathology Department from the hospital wards. The largest group (12/27) were cardiac cases who died less than 10 days after surgery.

5.1.6 DETAILS OF VACCINATION

The number of cases in the study group eligible for first triple vaccine to diphtheria, tetanus and pertussis (DTP) and oral polio vaccine came to a total of 52/93(56%). The clinical histories were examined on the 93 cases with clinical details and 28/52(54%) cases had received vaccination. From the 65 remaining, a total of 24 (37%) cases were unprotected. However, 12 cases out of the 65 had no record of vaccination. One case had details from the general practitioner (GP) advising against vaccination at the appropriate age due to ill health of the infant. This left 29 cases not eligible for vaccination.

PART 2

Edinburgh Cases

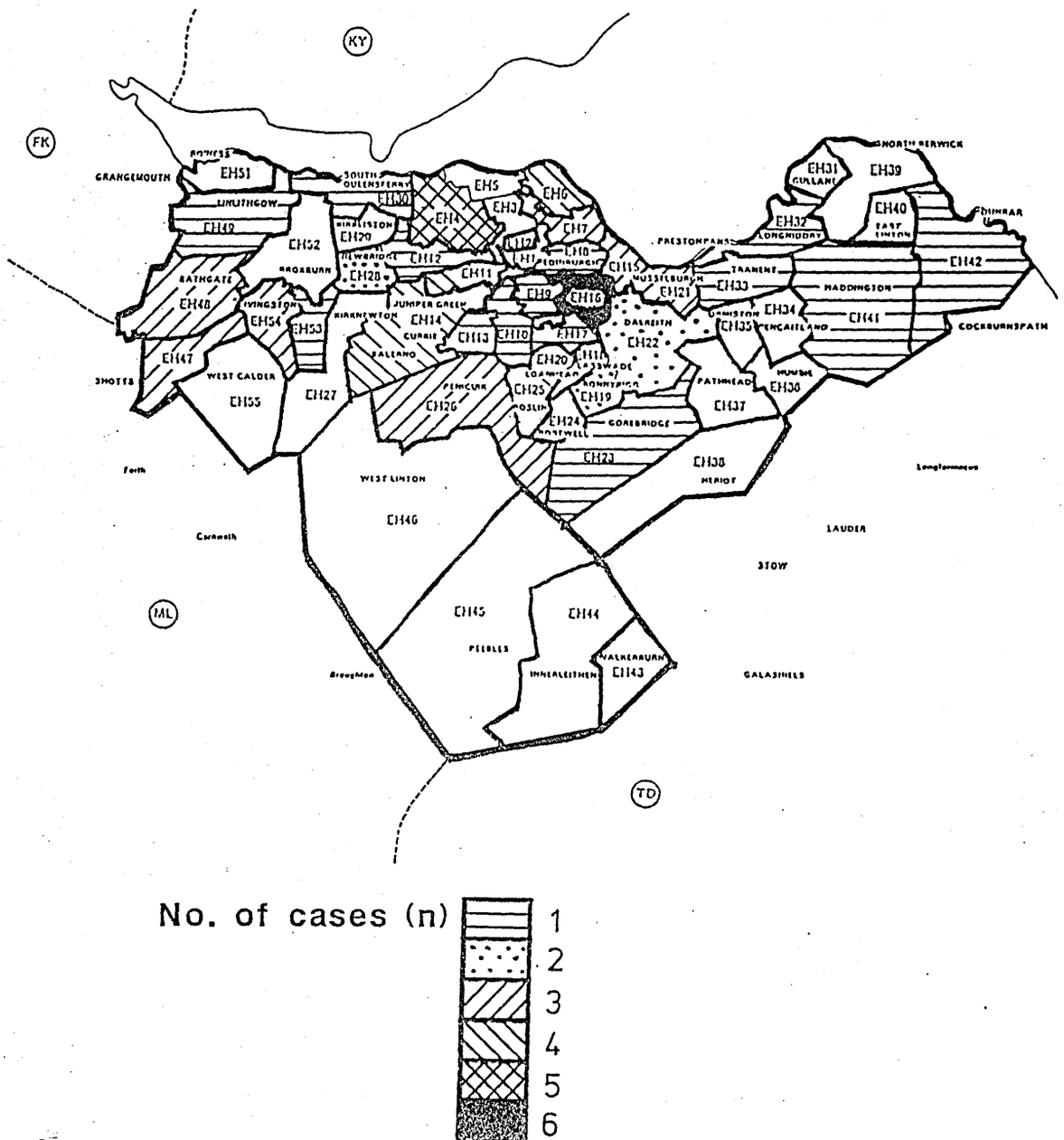
The relevant information for a limited epidemiological study on the ninety eight suspected cot death cases brought to the Pathology Department, RHSC, Edinburgh was collected. Specimens for IFN-α assay from these cases were sent to Glasgow during the period March 1987 to June 1989.

5.2.1 GEOGRAPHICAL LOCATION OF COT DEATH CASES

The largest proportion of suspected cot death cases (64) brought to the Pathology Department, RHSC, Edinburgh were from within the Edinburgh city limit, see map in figure 9. The RHSC Department, Edinburgh was the main East coast centre for post mortem analysis of sudden death in infants (<1 year) hence cases were also brought for autopsy from Fife (27) and the Borders (7), Tables 14 and 15.

The hatched and solid black areas on the map are those with higher numbers of cot death cases and includes segments of the city denoted by post code areas EH14, Balerno/Currie/Riccarton (6), EH4, Silverknowes/Clermiston (5), EH6, Leith (4). There was also an area in Fife district which had an increased number of cot deaths i.e. 8 deaths (8%) during the study period.

DISTRIBUTION OF COT DEATH CASES
MARCH 1987 - JUNE 1989
IN EDINBURGH AREA, R.H.S.C., EDINBURGH



No. of cases (n)

Figure 9

TABLE 14

Distribution of Cot Death Cases by Edinburgh Postcode Areas

Postcode	Areas	Number of Cases
EH4	Silverknowes/Clermiston	5
EH6	Leith	4
EH7	Abbeyhill	3
EH8	Pleasance	1
EH9	Southside	1
EH10	Morningside	1
EH11	Polwarth/Sighthill	4
EH12	Murrayfield	1
EH13	Collington	1
EH14	Balerno/Currie/Riccarton	4
EH15	Portobello	3
EH16	Liberton	6
EH17	Gilmorton	1
EH19	Bonnycraig	2
EH20	Musselburgh	3
EH22	Dalkeith	2
EH23	Gorebridge	1
EH26	Penicuik	3
EH28	Newbridge	2
EH29	South Queensferry	1
EH32	Longniddry	1
EH41	Haddington	1
EH42	Dunbar	1
EH47	Whitburn	3
EH48	Bathgate	3
EH49	Linlithgow	1
EH53	East Calder	1
EH54	Livingston	3

TABLE 15

Analysis of Cot Death Cases Outwith Edinburgh City Boundary

Postcode/Area	Number of Cases
Borders	
TD1	2
TD5	1
TD7 Galashiels	2
TD13	1
TD14	1
Fife	
KY1	3
KY2	4
KY4	3
KY6	3
KY7	3
KY11	8
KY12	3

The distribution of cot deaths in the postcode areas of the city again follow a similar pattern to that of the geographical location of housing estates radiating from the city centre.

5.2.2 MONTHLY DISTRIBUTION OF COT DEATH CASES

The distribution of cases by month is presented on the graph in figure 10. A trend was noted in the number of infants dying each year: 36% of total infants in study in 1987, 40% in 1988 and 24% in the early part of 1989. When the year was separated into four monthly parts a pattern of the number of infants dying during each year emerged. During January to April for 1988 and 1989 15% and 20% of cases were brought for post mortem. The study started in March 1987 hence only 5% of infants died during March and April of that year. Lower percentages were observed in the "Summer" months - May to August during 1987 and 1988 as shown by the following figures, 10% and 9%. The study ended in June 1989 hence only 4% of cases were noted for May to June 1989. During the latter part of the year, September to December in 1987 22% of cases were collected and 16% in 1988.

The number of cot death cases and the number of respiratory virus infections reported to the Communicable Diseases Unit at Ruchill Hospital , Glasgow from the Edinburgh Virus Laboratories throughout the study were analysed as shown in figures 11a and 11b. Although cot death numbers are low, the winter excess was coincident with the winter increases of respiratory virus infections reported.

MONTHLY DISTRIBUTION OF EDINBURGH COT DEATH CASES
BETWEEN MARCH 1987 - JUNE 1989
ROYAL HOSPITAL FOR SICK CHILDREN (RHSC), EDINBURGH

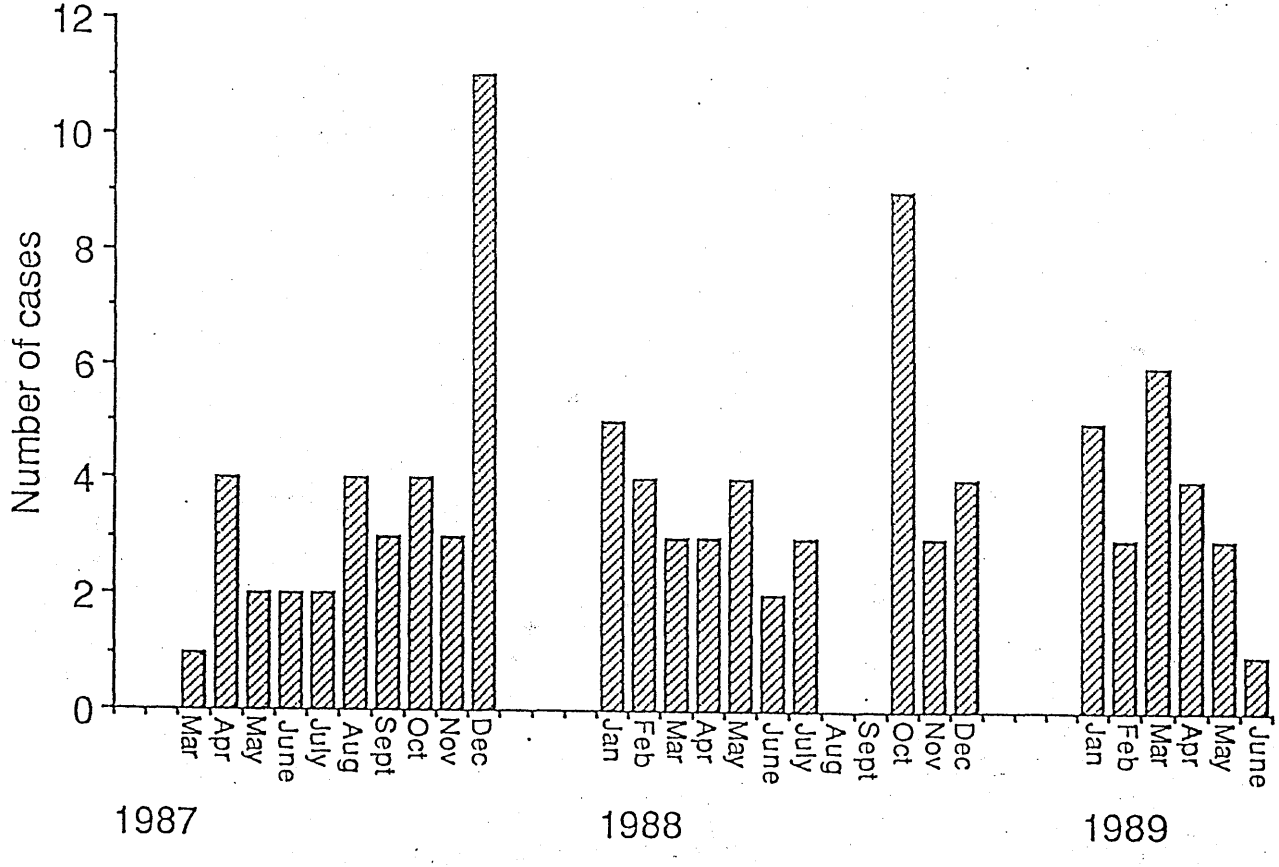


Figure 10

DISTRIBUTION OF EDINBURGH COT DEATH CASES COMPARED TO TOTAL NUMBER OF RESPIRATORY VIRUS INFECTIONS (CDS SCOTLAND, 1987)

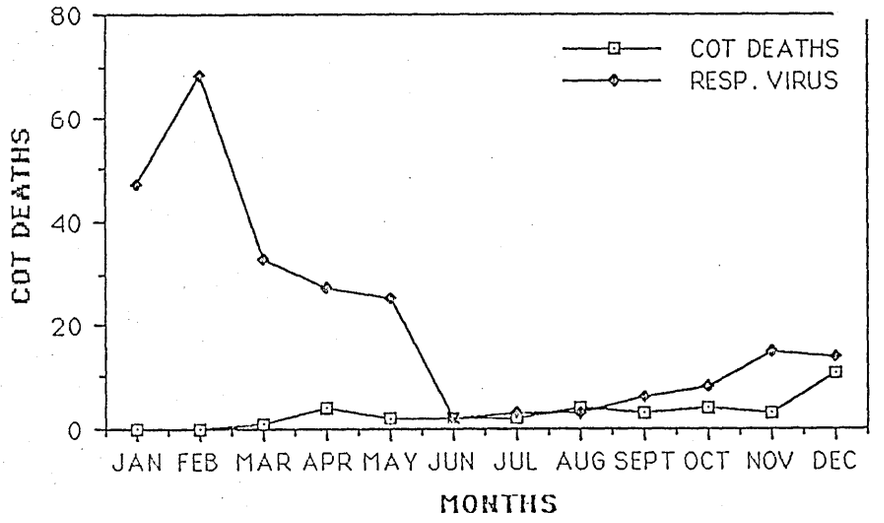


Figure 11a

DISTRIBUTION OF EDINBURGH COT DEATH CASES COMPARED TO TOTAL NUMBER OF RESPIRATORY VIRUS INFECTIONS (CDS SCOTLAND, 1988)

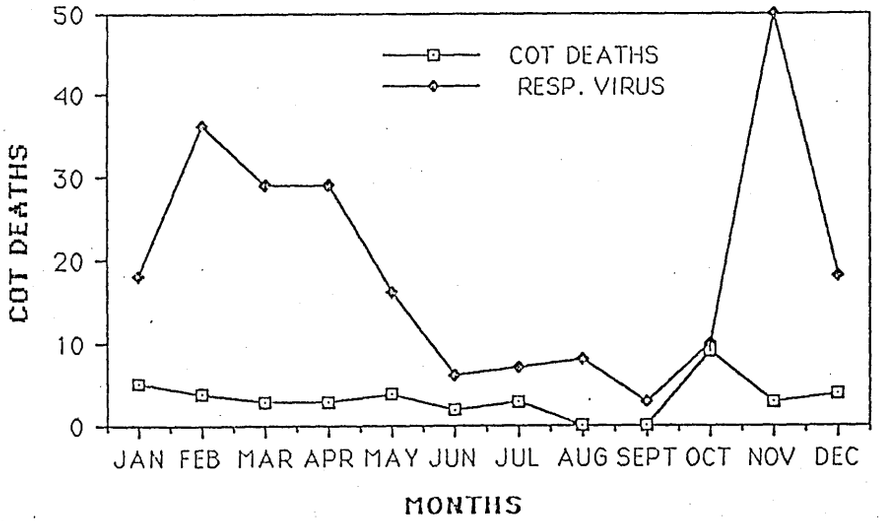


Figure 11b

5.2.3 AGE DISTRIBUTION OF COT DEATH CASES

The mean age distribution of the Edinburgh cot death cases was 21 weeks as calculated from the age range 1 week to 124 weeks (Table 16). Nine cases from the group were found >52 (range 64 weeks to 124 weeks) weeks and hence did not conform to the standard categorisation of cot death infant i.e. < 1year, however the data was collated on these infants. The details therefore have been included in the thesis with particular note to the different age range. Analysis of the cases showed that 18 infants were in the low age range of 1-8 weeks, 34 infants were in the 9-16 week group, 21 infants in the 17-26 week age range, 16 infants in the 27-52 weeks age group and 9 infants > 52 weeks of age. The age range with highest numbers of cot deaths was the 9-16 week group. The number of infants dying aged 1-16 weeks was 52 (53%) and in the upper age range 17-52 weeks, 46 cases (47%).

TABLE 16

Age Distribution of Cot Death Cases, Edinburgh

Age Range (weeks)	No. of Cases
1-8	18
9-16	34
17-26	21
27-52	16
> 52	9

CHAPTER 6

6.1 CONCLUSION OF THE DEMOGRAPHIC STUDY

Two major Scottish centres, Glasgow and Edinburgh covering 2.5 million people out of a total population of 5 million represented a substantial study population. The study group of 198 cot death cases were thus representative of a large population group. It was appropriate to include a limited epidemiological analysis of the clinical and environmental results. Epidemiological studies of cot death data over the past fifteen years hasenabled "risk" groups of infants to be identified within the community (reviewed by Peterson, 1984). The collection of data from this multi-centre study resulted in detailed information related to social and clinical aspects of both Glasgow and Edinburgh cot death cases .

The geographical location of cot death cases throughout the two Scottish cities (Figures 6 and 9; Glasgow and Edinburgh) included in the study, showed a similar distribution pattern. Higher numbers of cot death cases were indicated in several postcode areas in Glasgow the location of which were in densely populated housing schemes, (Easterhouse, Blackhill, Castlemilk, Paisley, Airdrie and Coatbridge). A number of these post code areas, (G33, G34) were identified as having housing of low grade as defined by Greater Glasgow Health Board, 1986. The numbers of cot death cases in Edinburgh in the postcode areas were spread out throughout the city. Higher numbers were noted in areas such

as Silverknowes/Clermiston, Balerno/Currie and also Sighthill (Table 14).

Other factors of social deprivation were not analysed in the study, hence no conclusions were recorded concerning the social conditions of the families in the survey. It was noted by Emery and colleagues that in order to draw conclusions concerning the social status of the families in the cot death group required a set of parameters prior to initiation of the study.

As both city and rural areas were investigated in the study it was noted that the largest number of cot death cases were found in the city location, and particularly with city housing developments.

Although published data relating geographical location of cot death victims within cities in Scotland is limited (Arneil et al., 1985, Bartholemew et al., 1987) studies representing the rest of the UK, from Cardiff, Sheffield (Emery & Taylor, 1988) and London/Cambridge (Golding et al., 1985) have shown increased numbers of cot death cases to be in densely populated areas of these cities rather than rural areas, however population statistics for the two areas cancelled this factor. Studies carried out in the USA (Kelly & Shannon, 1982) have looked at geographical location on a much broader perspective.

The monthly distribution of the study cases indicated that a winter predominance, December-April, was likely although several discrepancies were noted i.e. a larger number of cases occurred in Glasgow in

September 1987 which coincided with an unusually early start to the respiratory epidemic for that year as confirmed by the Virology Laboratory reports, RHSC, 1987. Most cot deaths were recorded in January and September in the Glasgow area, and December and October respectively in the Edinburgh data.

Data collected by the Communicable Diseases Unit, Ruchill Hospital for Scotland enabled the respiratory epidemics in the Glasgow and Edinburgh areas (Figures 8a, 8b, 11a and 11b) to be monitered resulting in the conclusion that the paediatric respiratory epidemics tended to increase earlier in the Glasgow area before the main respiratory epidemic, a feature not noted in the Edinburgh area during the two years of the study (CDS Scotland Weekly Reports, 1987-1988). This would coincide with the early increase in September in cot death cases brought to the Pathology Department, RHSC, Glasgow.

The low numbers of cot death cases in June was observed in both Glasgow and Edinburgh. The high number of cases detected between November and April and low or zero cases presented during June-August was a feature of cot death epidemiology noted by Nelson and colleagues during a survey carried out in the mid 1970's.

Previous studies carried out in the Pathology Department, RHSC, Glasgow have indicated that the number of infants dying of cot death at 9-16 weeks of age was significantly greater than in the younger or older age groups (Patrick et al., 1989). The results obtained in this study from

both Glasgow and Edinburgh correlated with this finding (Tables 12 and 16). The mean age of the study group was 18 weeks which was similar to results published by Valdes-Dapena (1980) and Kelly & Shannon (1982).

A high proportion of cot death infants in this study had various non-specific clinical symptoms in the weeks prior to death (56%) (Table 14). A small percentage of infants were admitted to hospital and these included infants suffering from bronchitis or chest infection. The remaining infants had more serious illnesses but were discharged prior to death. A proportion of infants had mild respiratory (35%) symptoms/infection as documented by the parental interviews. Although some of the infants were unwell prior to death their condition did not give cause for concern as it was considered natural for an infant to to have mild and recurrent respiratory infection at a young age. There was no correlation between the clinical symptoms and the pathologists post mortem report.

Beckwith at the International Symposium, 1970 also noted that 50% of cases of cot death had respiratory illness two weeks prior to death. The non SIDS group used for comparison was hospital-based infants preventing meaningful analysis of the data. Similarly the paediatric death group in the present study were not analysed with respect to illness prior to death as all the infants were hospital based and their illnesses thought to be of non infectious origin.

A relatively small proportion of the total cases were eligible for triple vaccine and oral polio vaccine and even smaller number of infants had received vaccination. The records of vaccination were however limited and most infants who were vaccinated had been several weeks prior to death. Further discussion of poliovirus vaccination in cot death cases is to be found in Chapter 8.

This particular section of the thesis details the background information of the cases studied. The increase in number of cases in particular months of the year i.e. winter months and the infection prior to death were of interest for the subsequent analysis of the micro-organisms detected in the cot death cases and the endogenous alpha interferon levels. Further analysis has been left to the following chapter.

CHAPTER 7

RESULTS OF EXPERIMENTAL DATA TO DETERMINE THE PRESENCE OF ENDOGENOUS ALPHA INTERFERON IN COT DEATH CASES

Glasgow Cases

7.1.1 ANALYSIS OF POST MORTEM INTERVAL

The post mortem interval was defined for the purpose of this study as the time between death and commencing the autopsy. Before the body was opened, body fluid specimens, i.e. NPA, heart blood, CSF, urine, were taken by aspiration. In some cases these specimens were taken several hours before the autopsy proper. The post mortem interval for the cot death cases is presented in Table 17. The largest number of samples were obtained between 25-48 hours.

7.1.2 DETECTION AND ISOLATION OF VIRUS

A total of over 1000 samples, post nasal swab, heart, lung, kidney, brain, rectosigmoid loop, mesenteric lymph node, mediastinal lymph node, CSF and urine were taken, processed and finally inoculated into cell culture tubes with usually 5 tests carried out on each specimen to identify the presence of virus. A summary of the positive viral results is indicated on Table 18. Positive viral results were obtained in sixteen cot death cases (16%). A total of 19 viruses were isolated

TABLE 17

Post Mortem Interval for Glasgow Cot Death Cases

Post mortem Interval (hours)	Number of Cases
< 24	24
25-48	40
49-72	20
73-96+	16

TABLE 18

Positive Virus Results by Isolation for Glasgow Cot Death Cases and Paediatric Deaths

	Cot Death Cases	Paediatric Cases
Number of Cases	100	27
Number of virus positive	16 (16%)	5 (18%)
Number virus isolated/detected	19	0
Number with double infection	3	0
RSV	3	3
Influenza A	1	0
Adenovirus	2	0
Rotavirus	4	1
CMV	1	1
Poliovirus type 1	3	0
Poliovirus type 2	3	0
Poliovirus type 3	1	0
Echovirus type 22	1	0

from these cases. Double infection was noted in three cases: influenza A/rotavirus, adenovirus/rotavirus and adenovirus/poliovirus.

The respiratory viruses isolated/detected included RSV found in the exfoliated cells from NPA samples from 3 cases using a direct immunofluorescence technique (see section 2.1.5). One case of influenza A from an infant 53 weeks of age was similarly detected by a monoclonal antibody specific to influenza A incorporated in a direct immunofluorescence technique.

Seven viral antigens: influenza virus types A and B, adenovirus, RSV, parainfluenza virus types 1, 2 and 3, were used to screen the 100 NPA samples obtained from cot death cases for respiratory infection. The number of positive samples as indicated above shows that 3% of total cases were positive for RSV, and only 1% for influenza virus type A.

Adenovirus was isolated in two cases from the post nasal swab and also in the mesenteric lymph node in one case. The adenovirus isolated was not typed.

Rotavirus was detected in stool samples obtained from rectosigmoid loop in a total of four cases. These positive results were confirmed by electron microscopy carried out at Regional Virus Laboratory, Ruchill.

CMV was isolated in one case (1%) from a variety of sites i.e. brain, kidney, lung, urine.

Several poliovirus isolates of types 1 (3 cases), 2 (3 cases) and 3 (1 case) obtained from the sigmoid loop and nasal swab. In this study, polioviruses represent 7/16 (43%) of the total positive virus cases. Echovirus type 22 was isolated from the brain, mesenteric lymph node, sigmoid loop and kidney in one case.

The paediatric deaths were investigated in a similar manner to that of the cot deaths. Positive results were obtained in 5 cases (5/27, 18%). RSV was detected in 3 cases from the NPA samples. Two cases had detectable levels of antigen to rotavirus in stool samples and in one case CMV was isolated in the kidney.

All the paediatric death NPA samples were screened for the presence of respiratory virus using a direct immunofluorescent technique (section 2.1.5). The three positive cases of RSV were detected by this method.

7.1.3 CYTOSPIN PREPARATIONS/IMPRESSION SMEARS

The individual anatomic levels of lung as described in section 2.1.6 from both cot death cases and paediatric deaths were studied at post mortem.

In one cot death case RSV was detected in the lower respiratory tract i.e. bronchioles and peripheral lung. Ninety nine cases of cot death specimens were negative for RSV as were 15 out of the 27 paediatric

patients examined using the fluorescence technique for this virus.

7.1.4 SEROLOGICAL ANALYSIS

The number of cases tested for an antibody response to a range of viral antigens: CMV, HSV, VZV, influenza A, adenovirus, mycoplasma pneumonia, mumps, measles, was limited by the quantity of serum available. Sixty three cot death cases were tested by the complement fixation test and yielded no significant titres on 62 cases i.e. titres of < 16 were noted to all viruses analysed. From the case in which CMV infection was proven by viral isolation, a titre of 512 was recorded.

Indirect immunofluorescence tests to a range of viral antigens; HSV, VZV, RSV, CMV, mumps, measles were used to detect and quantify levels of specific IgM titres in a total of 65 cot death cases, however no significant IgM titres were detected in these cases.

The paediatric deaths (25 cases) were also tested by complement fixation test and indirect immunofluorescence.
The presence of IgM was not detected in serum samples.

7.1.5 BACTERIOLOGICAL EXAMINATION OF POST MORTEM MATERIAL

Routine bacteriology was carried out on the cot death samples. From the 100 cot death samples, microorganisms were recovered by culture from either lung fluid, heart blood, CSF or tissue samples in 56 infants. No evidence of growth was noted on the remaining 44 cases in the study after 7 days incubation.

The organisms recovered included 23 cases of coagulase negative Staphylococcus (42%). Nineteen of these bacteria were cultured from the heart blood, 2 from lung fluid, 1 in CSF and 1 in a brain sample. Haemophilus influenzae was isolated in 4 cases, 3 in lung fluid and 1 from a CSF sample. Streptococcus pneumoniae was recovered from the lung fluid and heart blood from five cot death cases. Three cases of a group B Streptococcus were cultured from the lung fluid specimens.

The cot death cases with evidence of bacterial culture and pathological reports to support minor abnormalities in histological sections were categorised by the pathologists into grouping 3BB i.e. where the findings were not thought to be sufficient to cause the death.

Edinburgh Cases

7.1.6 ANALYSIS OF POST MORTEM INTERVAL

The post mortem interval calculated for fluid specimens obtained from Edinburgh cases of cot deaths is represented in Table 19. Forty four cases out of the total 98 cases (44%) of samples were collected between 25-48 hours, as previously observed in the Glasgow data.

7.1.7 DETECTION AND ISOLATION OF VIRUS

The body fluids and tissue specimen collected as detailed in section 2.1.2. were analysed for the presence of virus at the Regional Virus Laboratory, City Hospital, Edinburgh.

The positive viral results are presented in Table 20. A total of 39 viruses were isolated from 33 positive viral cases (38%) from the 87 cot death cases studied. Six cases presented with double infection: Rhinovirus/Echovirus type 22, CMV/Polio 2, Rhinovirus/Coxsackie A, Echovirus type 22/RSV, CMV/Polio 2 and Polio type 1/Polio type 3.

Respiratory viruses detected from the specimens collected included, 6 cases of RSV isolated from the tracheal ring, NPA and nasal/throat swab. Adenovirus was noted in 4 cases: 2 isolated from one site only i.e. the ileal loop and bronchial swab, and in two other cases from a variety of sites.

TABLE 19

Post Mortem Interval for Edinburgh Cot Death Cases

Post mortem Interval (hours)	Number of Cases
< 24	20
25-48	44
49-72	20
73-96+	13

TABLE 20

Positive Virus Isolation Results for Cot Death Cases, Edinburgh

	Cot Death Cases
Number of Cases	87
Number of Virus Positive Cases	33 (38%)
Number of viruses isolated	39
Number with double infection	6
RSV	6
Adenovirus	4
Parainfluenza type 3	2
Rhinovirus	4
Rotavirus	1
CMV	3
Poliovirus type 1	4
Poliovirus type 2	4
Poliovirus type 3	2
Coxsackie A*	1
Coxsackie B*	1
Echo Virus type 11	3
Echo Virus type 22	4

* = type unknown

Parainfluenza type 3 was detected in the bronchial swabs from two cot death cases. Rhinovirus was isolated from the upper respiratory tract, nasal swab and tracheal ring in 4 cases. Rotavirus was detected in the ileal loop in only one case.

In three cot death cases CMV was isolated from kidney specimens.

The Coxsackie virus types A and B represented one case each. These viruses were isolated/detected in ileal loop and bronchial swab.

Echovirus typed as 11 and 22 were also noted in 3 cases and 4 cases, respectively and were isolated from the tracheal ring sample, ileal loop and faeces. The poliovirus type 1 (4 cases), type 2 (4 cases) and type 3 (2 cases) represented 9 cases from the total 33 cot death cases(27%) with positive virus isolation.

ANALYSIS OF INTERFERON- α LEVELS IN GLASGOW AND EDINBURGH COT DEATH CASES AND PAEDIATRIC DEATHS

The measurement of IFN- α levels in body fluids, serum, CSF, NPA was carried out using "Sucrosep IFN- α " IRMA as in section 2.1.8. Levels of IFN- α detected in the cot death specimens collected from Glasgow and Edinburgh are plotted in figures 12 and 13. A group of paediatric deaths were analysed in parallel with the cot death cases and the IFN- α levels in fluid samples are shown in figure 14.

7.2.1 INTERFERON- α LEVELS IN NASOPHARYNGEAL ASPIRATE (NPA)

Nasopharyngeal aspirate samples diluted 1:10 in VTM were collected from 97 cot death cases from Glasgow, 90 cases of cot death from the Edinburgh area and a group of 22 paediatric deaths and investigated for the presence of IFN- α . The number of cot death cases and paediatric cases in the individual IFN- α ranges 0 - 1 IU/ml, 1.1 - 10 IU/ml, 10.1 - 100 IU/ml and >100 IU/ml were analysed (Table 21). The highest number of Glasgow cases (37%) were noted in the 10.1 - 100 IU/ml IFN- α range, however, the 0 - 1 IU/ml and 1.1 - 10 IU/ml IFN- α were shown to have 34% and 27% of cases, respectively. The number of cases in each IFN- α range were similar.

The Edinburgh cot death samples of NPA in each IFN- α range, 35% in 0 - 1 IU/ml and 31% in 1.1 - 10 IU/ml and 10.1 - 100 IU/ml ranges, showed a similar pattern of case distribution in IFN- α levels as

LEVELS OF ALPHA-INTERFERON IN NASOPHARYNGEAL ASPIRATE (NPA), SERUM, CSF
FROM COT DEATH CASES,
ROYAL HOSPITAL FOR SICK CHILDREN, GLASGOW

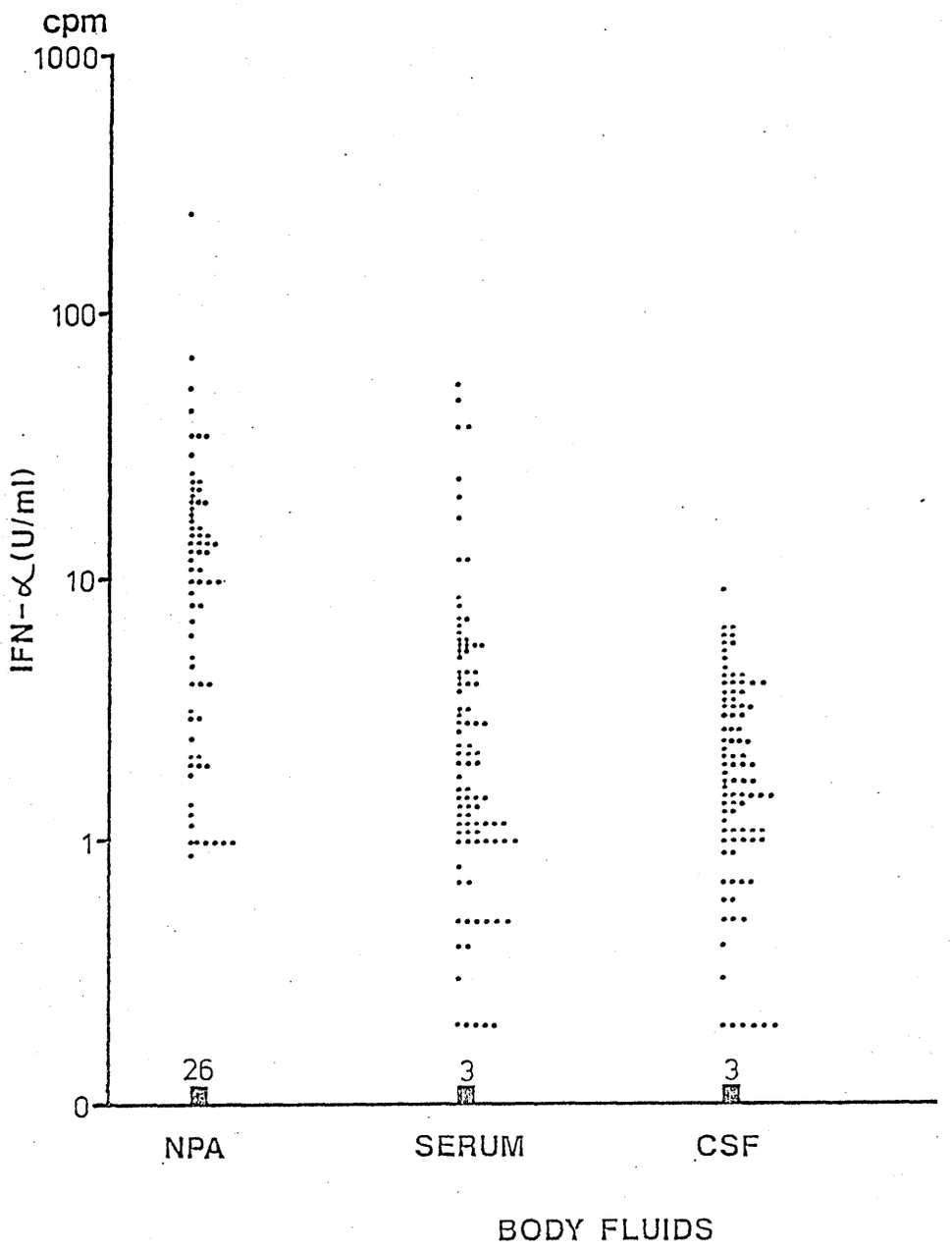


Figure 12

LEVELS OF ALPHA-INTERFERON IN NASOPHARYNGEAL ASPIRATE (NPA), SERUM AND CSF FROM COT DEATH CASES, ROYAL HOSPITAL FOR SICK CHILDREN, EDINBURGH

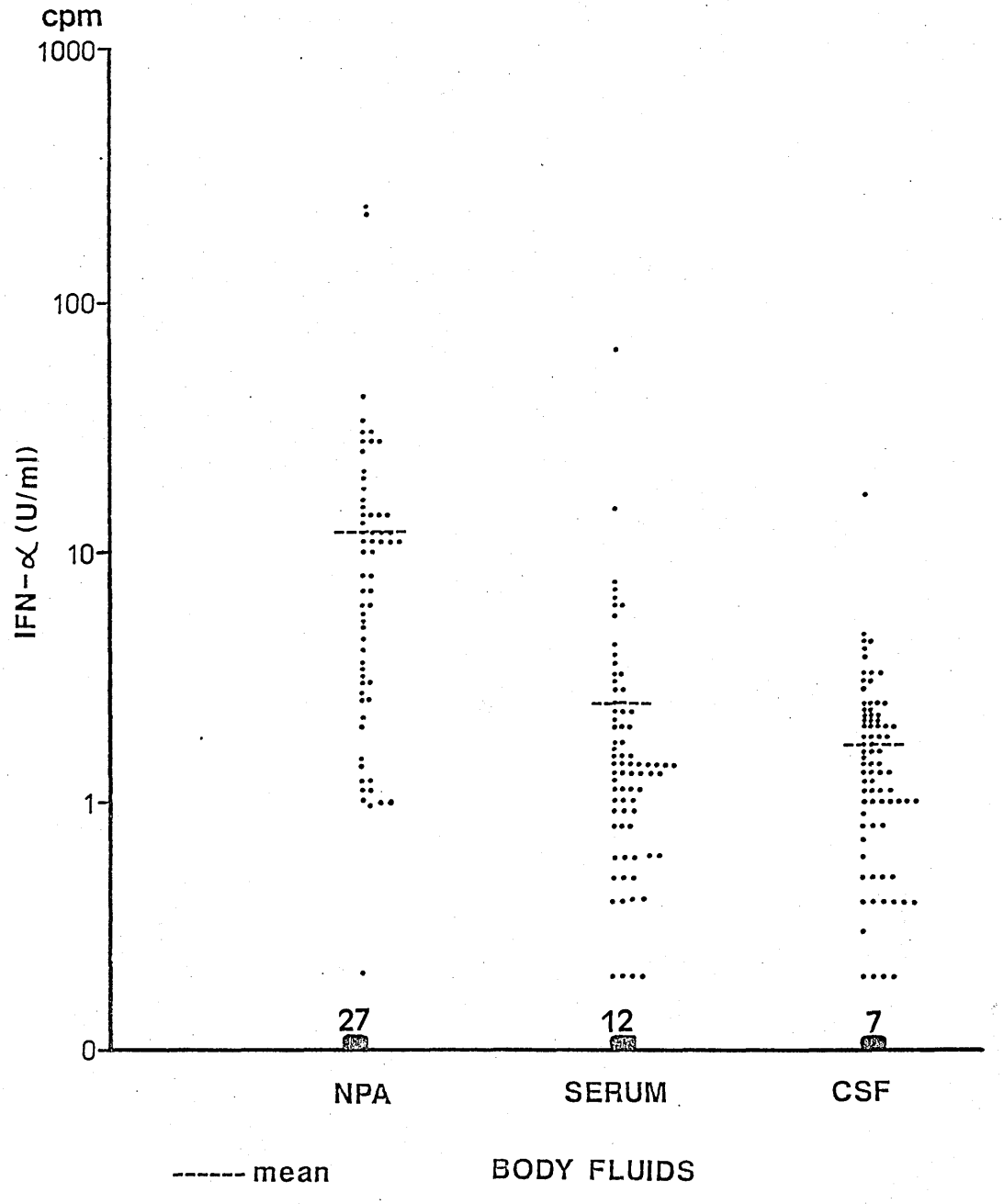


Figure 13

LEVELS OF ALPHA-INTERFERON IN NASOPHARYNGEAL ASPIRATE (NPA), SERUM AND CSF FROM PAEDIATRIC DEATHS, ROYAL HOSPITAL FOR SICK CHILDREN, GLASGOW

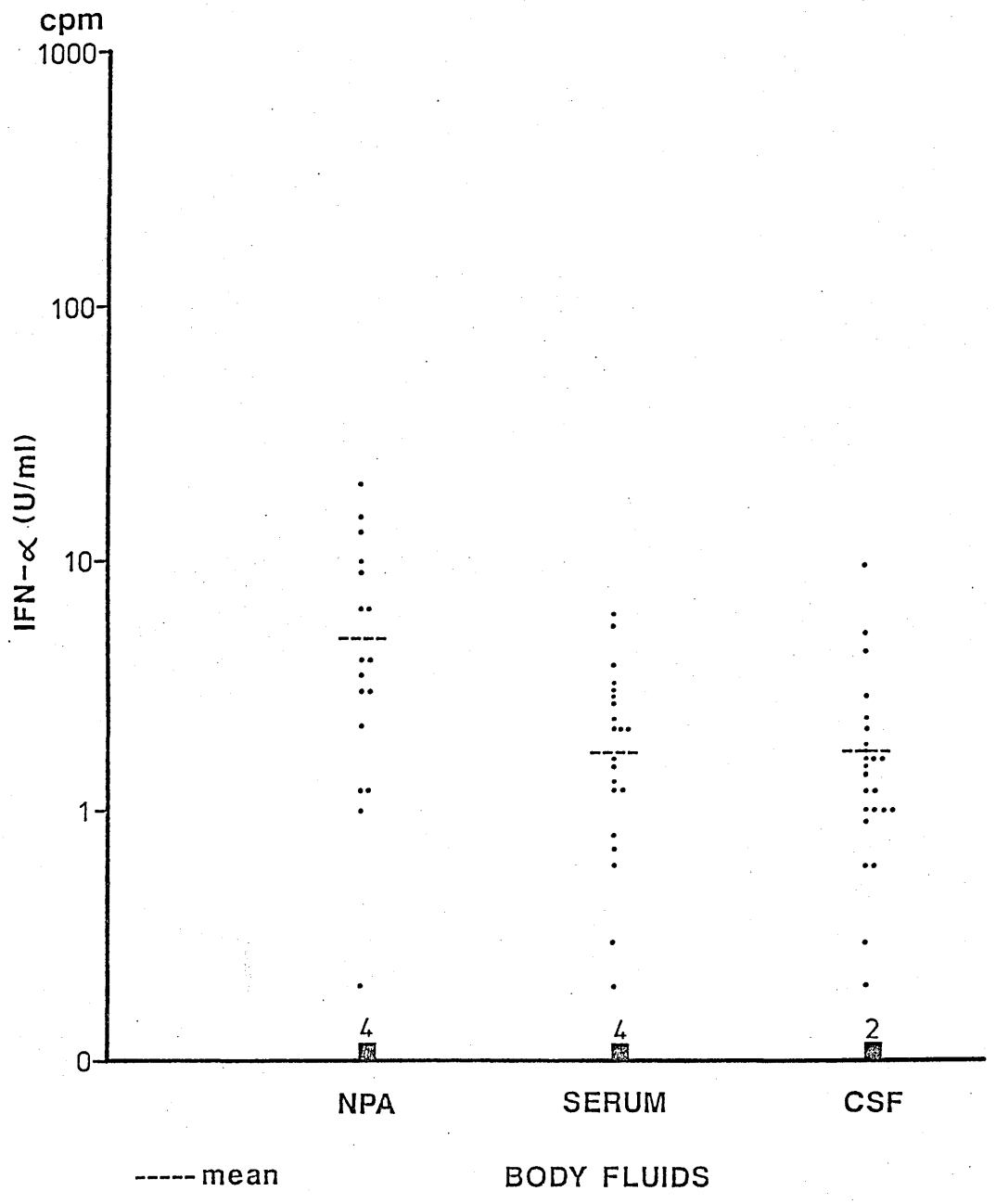


Figure 14

TABLE 21

Range of IFN-α Level Present in NPA in Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

IFN-α IU/ml	CASES					
	Glasgow	(%)	Edinburgh	(%)	Paediatric	(%)
0 - 1	33/97	(34)	32/90	(35)	6/62	(28)
1.1 - 10	26/97	(27)	28/90	(31)	12/21	(57)
10.1 - 100	36/97	(37)	28/90	(31)	3/21	(14)
> 100	1/97	(1)	2/90	(2)	0/21	(0)

noted for the Glasgow cases.

Analysis of the total cot death group (186 cases) indicated that 64 cases (34%) had IFN-α values >10 IU/ml and hence these samples would be positive for IFN-α by any assay system.

Paediatric deaths were evaluated over the IFN-α range 0 - 12 IU/ml. The number of cases detected in 1.1 - 10 IU/ml range were 12/21 (57%), however only 3 cases (14%) of cases had levels of IFN-α >10 IU/ml.

Using the previously derived minimum detection limits (see section 3.1.3) , the number of NPA samples with IFN-α values ≥ 2 IU/ml in the Glasgow cot death cases was 59 (60%) and in cases collected from Edinburgh, 51 (53%), see Table 22. The paediatric death data was analysed similarly and indicated that 14 (63%) of NPA samples showed the presence of IFN-α above 2 IU/ml. The mean values of IFN-α in NPA ≥ 2 IU/ml were 20.56 IU/ml for Glasgow cot death cases, 20.5 IU/ml for Edinburgh cot death cases and 7.6 IU/ml for paediatric cases and these results highlight the IFN-α range data shown in Table 23.

Statistical analysis was carried out using the Mann-Whitney U-test. No statistical significance ($P > 0.05$) was observed between the values of IFN-α detected in the NPA from cot death specimens and paediatric deaths.

TABLE 22

Analysis of IFN-α Levels in Nasopharyngeal Aspirate in Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

CASES	NUMBER OF CASES STUDIED	NUMBER OF CASES $\geq$2 IU/ml	(%)	MEAN VALUE of IFN-α	P VALUE	
GLASGOW COT DEATHS [1]	97	59	(60)	20.56	>0.05	>0.05 [1, 3]
EDINBURGH COT DEATHS [2]	90	51	(53)	20.5	>0.05	
PAEDIATRIC DEATHS [3]	22	14	(63)	7.6		

TABLE 23

Comparison of NPA IFN-α Levels in Cot Death Cases and Paediatric Patients Presenting with Acute Respiratory Infection

IFN-α IU/ml	Glasgow/Edinburgh Cot Death (1987-89)	(%)	Glasgow 1985-86 (Salas, 1988) Pneumonia	(%)	Chronic Lung Disease	(%)
0 - 1	65/187	(35)	15/51	(29)	8/15	(53)
1.1 - 10	54/187	(29)	13/51	(26)	2/15	(13)
10.1 - 100	64/187	(34)	15/51	(29)	4/15	(27)
> 100	3/187	(2)	7/51	(14)	1/15	(6)

Comparisons of the IFN- α levels in NPA samples obtained from paediatric patients during a repiratory virus epidemic (Salas, 1988) and the current NPA IFN- α data collected from the combined Glasgow and Edinburgh cot death group are documented in Tables 23 and 24 . The IFN- α values were obtained using identical laboratory techniques and under the same conditions at the Virus Laboratory, RHSC, Glasgow. The data revealed a striking similarity of IFN- α levels in the cot death cases and the paediatric patient presenting with pneumonia and chronic lung disease (Table 23) compared to the bronchiolitis/URTI group(Table 24). Both the study group and the pneumonic cases had IFN- α levels 10.1 - 100 IU/ml in the order of 30% suggesting significant virus associated lower respiratory tract infection in both groups. This result is surprising in view of the lack of significant clinical disease or pathology in the cot death cases.

TABLE 24

Comparison of NPA IFN-α Levels in Cot Death Cases and Paediatric Patients Presenting with Respiratory Illness

IFN-α IU/ml	Glasgow/Edinburgh Cot Death (1987-89)	(%)	Glasgow 1985-86 (Salas, 1988) Bronchiolitis	(%)	URTI	(%)	Others	(%)
0 - 1	65/187	(35)	39/95	(41)	20/29	(69)	12/18	(67)
1.1 - 10	54/187	(29)	28/95	(29)	4/29	(13)	4/18	(22)
10.1 - 100	64/187	(34)	18/95	(19)	2/29	(7)	1/18	(5)
> 100	3/87	(2)	7/95	(7)	4/29	(13)	1/18	(5)

7.2.2 INTERFERON- α LEVELS IN SERUM

A total of 99 Glasgow cot death cases, 92 Edinburgh cot death cases and 26 paediatric deaths were tested for the presence of IFN- α in serum samples.

The IFN- α data was analysed with respect to the number of cases categorised into several IFN- α ranges of the cot death cases and paediatric deaths as indicated in Table 25. Similar trends in the number of cases of Glasgow, Edinburgh cot death and paediatric deaths were noted in the IFN- α ranges 0 - 1 IU/ml and 1.1 - 10 IU/ml. The highest proportion of cases 54% for the combined levels of IFN- α in the cot death group and 62% for the paediatric deaths was noted in the IFN- α range 12.1 - 100 IU/ml. Only the cot death cases had levels >10 IU/ml in serum samples (5%).

Further analysis of serum IFN- α values (Table 26) indicated that 58 specimens (58%) of Glasgow cot death cases had IFN- α levels $\geqslant 1.5$ IU/ml. Similarly, 31 (34%) of serum samples from Edinburgh cot death cases were $\geqslant 1.5$ IU/ml IFN- α. In the paediatric deaths group 12 (46%) samples had IFN- α levels $\geqslant 1.5$ IU/ml. The mean levels of specimens $\geqslant 1.5$ IU/ml was 8.06 IU/ml for Glasgow cases, 5.9 IU/ml for cases of cot death from Edinburgh and 2.96 IU/ml for paediatric deaths. Statistical analysis was carried out on the total IFN- α levels in serum samples in the three study groups and found not to be significant by Mann Whitney U-test ($P > 0.05$).

TABLE 25

Range of Interferon α Levels in Serum in Glasgow and Edinburgh Cot Deaths and Paediatric Deaths

	CASES					
Range of IFN-α IU/ml	Glasgow	(%)	Edinburgh	(%)	Paediatric	(%)
0 - 1	29/99	(29)	37/92	(40)	10/26	(38)
1.1 - 10	61/99	(62)	52/92	(56)	16/26	(62)
10.1 - 100	9/99	(9)	2/92	(2)	0/26	(0)
>100	0/99	(0)	0/92	(0)	0/26	(0)

TABLE 26

Analysis of IFN-α Levels in Serum Samples in Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

Cases	Number of Cases Studied	Number of Cases $\geq$1.5 IU/ml	(%)	Mean Value of IFN-α $\geq$1.5 IU.ml	P Value	
Glasgow [1] Cot Deaths	99	58	(58)	8.06	>0.05	1, 3 >0.05
Edinburgh [2] Cot Deaths	92	31	(34)	5.9	>0.05	
Paediatric [3] Deaths	26	12	(46)	2.96		

7.2.3 INTERFERON- α LEVELS IN CSF

Samples of CSF from 99 cases of Glasgow cot deaths, 96 Edinburgh cot death cases and a group of 26 paediatric deaths were examined for the presence of IFN- α. The number of cases analysed in each individual IFN- α range is indicated in Table 27 and 28.

The combined analysis of the Glasgow and Edinburgh cot death groups indicated that the number of cases with IFN- α levels between 1.1 - 10 IU/ml in CSF was double (66%) the number of cases observed in the 0 - 1 IU/ml range (32%). The paediatric death data concurs with the results of the cot death cases. One level of IFN- α was detected in the 10.1 - 100 IU/ml range for a CSF sample from a cot death. IFN- α levels were not detected in the paediatric death group at levels > 10 IU/ml.

The minimum detection limit of $\geqslant 1.5$ IU/ml was used in the study for IFN- α in CSF samples (see section 3.1.3). Sixty (61%) of the Glasgow samples had levels of $\geqslant 1.5$ IU/ml IFN- α and 41 (34%) of CSF specimens from Edinburgh cot deaths had similar raised levels. Paediatric deaths were also analysed and showed 12 (46%) cases had IFN- α levels $\geqslant 1.5$ IU/ml.

The mean levels evaluated for the CSF samples $\geqslant 1.5$ IU/ml IFN- α were 3.08 IU/ml in Glasgow cot death cases, 2.0 IU/ml in Edinburgh cot death cases and 3 IU/ml in paediatric cases. The Mann Whitney U- test was used to statistically analyse the CSF IFN- α data.

TABLE 27

Range of Interferon α Present in
CSF Samples from Glasgow and Edinburgh
Cot Deaths and Paediatric Deaths

	CASES					
IFN-α IU/ml	Glasgow	(%)	Edinburgh	(%)	Paediatrics	(%)
0 - 1	26/99	(26)	36/93	(39)	11/26	(42)
1.1 - 10	72/99	(73)	56/93	(60)	15/26	(58)
10.1 - 100	0/99	(0)	1/93	(1)	0/26	(0)
> 100	0/99	(0)	0/93	(0)	0/26	(0)

TABLE 28

Analysis of IFN-α Levels in CSF Samples in Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

Cases	Number of Cases Studied	Number of Cases $\geq$1.5 IU/ml	(%)	Mean Value of IFN-α	P Value	
Glasgow Cot Deaths [1]	99	60	(61)	3.08	>0.05	1, 3
Edinburgh Cot Deaths [2]	93	41	(34)	2.8	>0.05	>0.05
Paediatric Deaths [3]	26	12	(46)	3.0		

No difference in overall IFN- α values was noted between the two cot death groups or on comparison with the paediatric death group ($P > 0.05$).

7.2.4 INTERFERON- α RESULTS FOR VIRUS POSITIVE COT DEATH CASES; GLASGOW AND EDINBURGH AND PAEDIATRIC DEATHS

A range of viruses were detected/isolated in both cot death cases from the Glasgow and Edinburgh cases as well as paediatric deaths as documented in section 7.1.2 and the IFN- α levels are documented in Tables 29, 30 and 31.

Samples of NPA, serum and CSF from the 16 (16%) virus positive cases of Glasgow cot deaths and 30 (34%) of the 33 cases of cot death from Edinburgh had sufficient specimen for IFN- α testing. Five (18%) of the 27 paediatric deaths were found to be positive for virus. Paediatric deaths were investigated for IFN- α in parallel with the cot death cases.

The range of IFN- α levels observed in virus positive cases of Glasgow cot death cases are illustrated in figure 15. A similar figure was collated for the Edinburgh cot death data, see figure 16. The number of cases in each IFN- α range for cot deaths and paediatric deaths are shown in Tables 32 and 33.

The range of IFN- α values for NPA samples from Glasgow and Edinburgh cot deaths was between 0 - >100 IU/ml, with 2 cases >100 IU/ml. The

ALPHA INTERFERON LEVELS IN NPA, SERUM, CSF IN
VIRUS POSITIVE COT DEATH CASES,
ROYAL HOSPITAL FOR SICK CHILDREN, GLASGOW

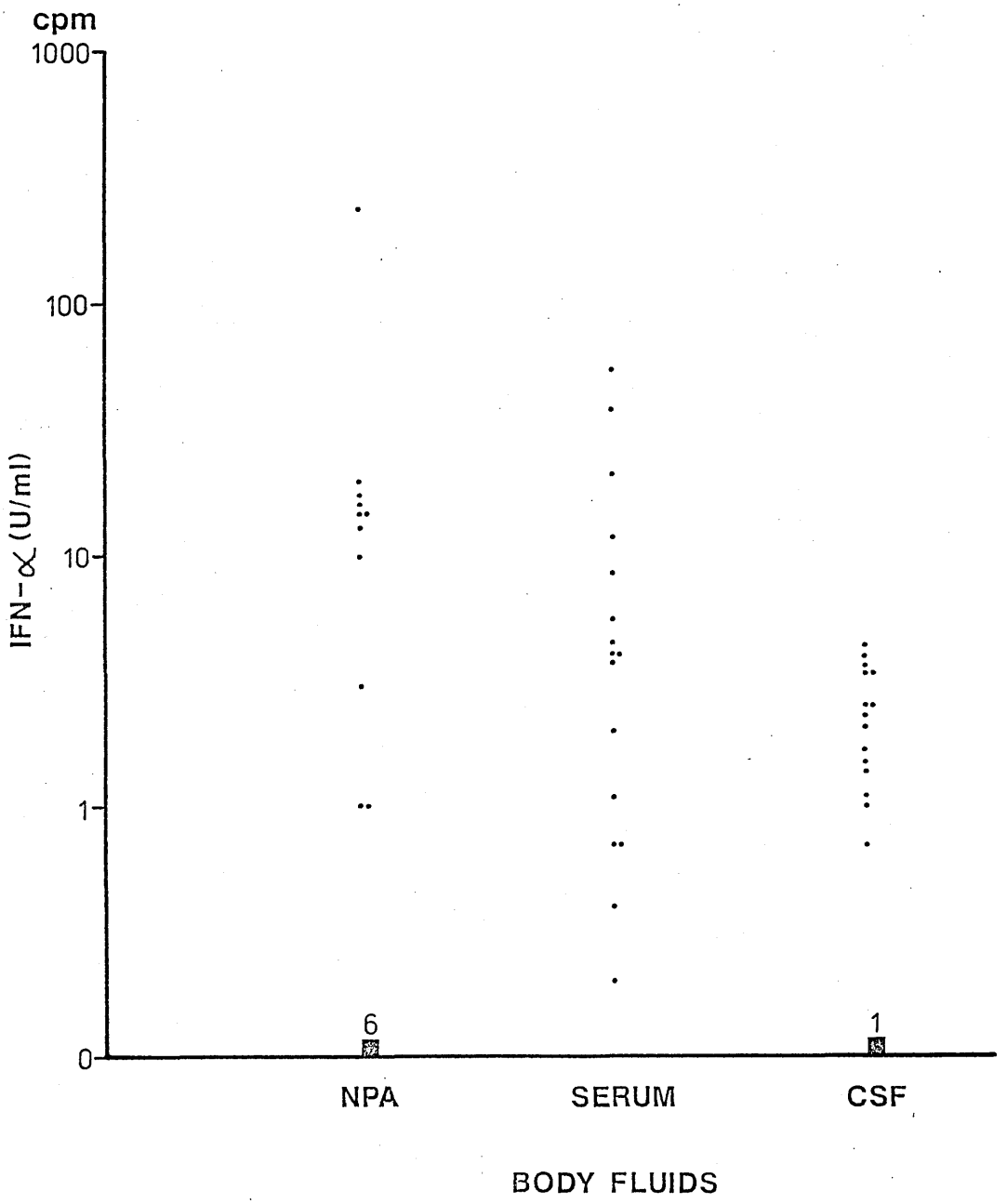


Figure 15

ALPHA-INTERFERON LEVELS IN NPA, SERUM, CSF IN VIRUS POSITIVE COT DEATH CASES
ROYAL HOSPITAL FOR SICK CHILDREN, EDINBURGH

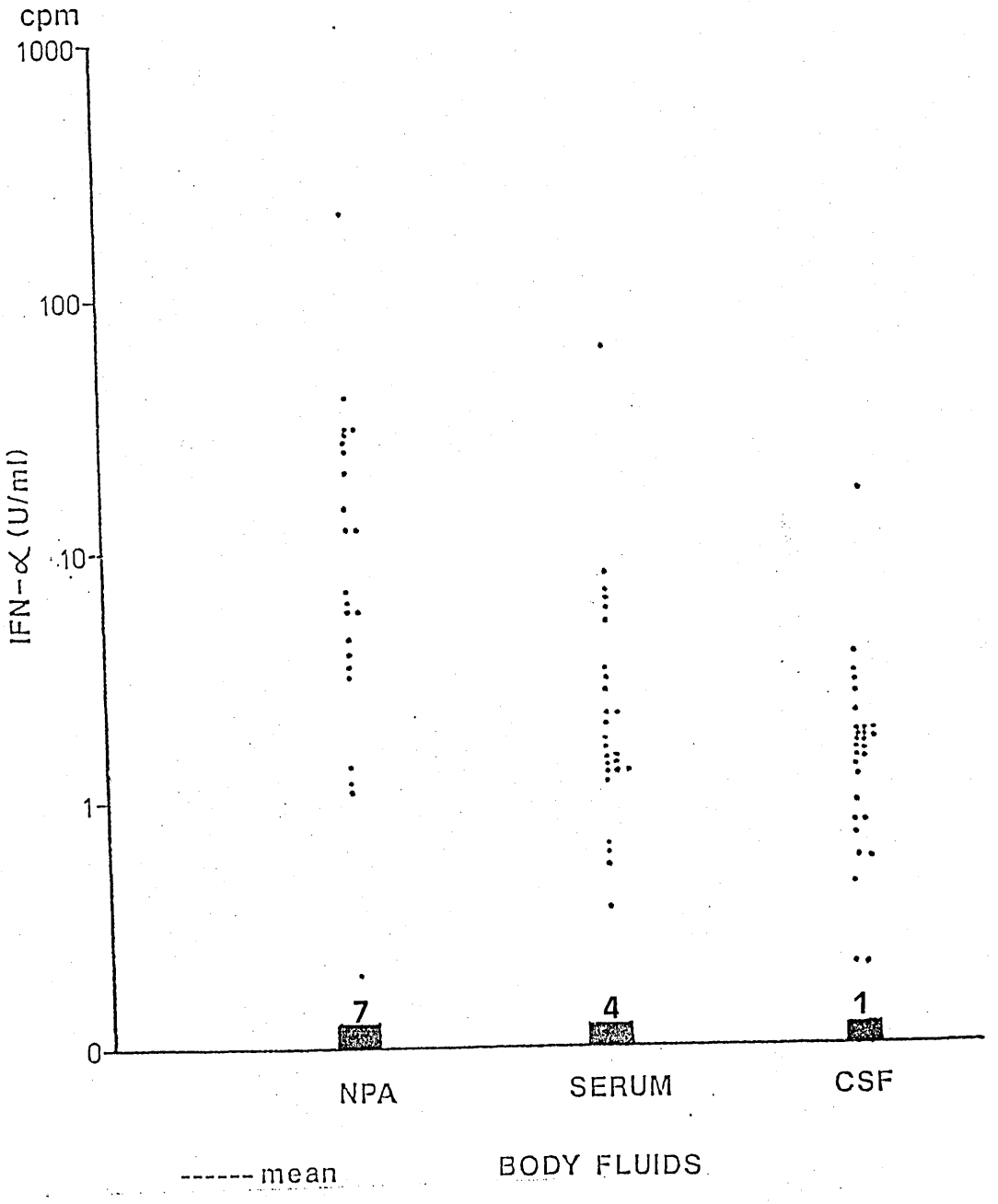


Figure 16

TABLE 29

Alpha Interferon Levels from Virus Positive Glasgow Cot Death Cases

Number of Case	Virus Detected	IFN-α (IU/ml) NPA	SERUM	CSF
1	RSV	0	8.4	3.6
2	RSV	0	5.6	0
3	RSV	16	21	3.4
4	Rotavirus	1	4.6	2.3
5	Rotavirus	0	38	1.5
6	Echo type 22	0	2	1
7	CMV	17	0.4	2.5
8	Polio type 1	15	0.1	0.7
9	Polio type 1	20	0.7	1.4
10	Polio type 2	1	12	3.4
11	Polio type 2	0	0.7	4.2
12	Polio type 3	3	1.1	2.5
13	Influenza A/Rotavirus	240	55	4
14	Adeno/Rotavirus	0	3.8	2.1
15	Adeno/Polio	15	4	1.7
16	Polio types 1 and 2	10	4	1.1

TABLE 30

Alpha Interferon Levels from Virus Positive Edinburgh Cot Death Cases

Number of Case	Virus Detected/Isolated	IFN-α (U/ml) NPA	SERUM	CSF
1	RSV	17	1.2	2.2
2	RSV	0	0.2	0.2
3	RSV	3.6	0.4	1.8
4	RSV	4	0	1.05
5	RSV	0	0	3.1
6	Adenovirus	20	6.5	2.8
7	Adenovirus	11	1.3	1.7
8	Adenovirus	6	2.2	1.1
9	Adenovirus	6	3.2	0.2
10	Parainfluenzae 3	0	6	3
11	Rhinovirus 3	7	1.4	1.6
12	Rhino	0	2.1	0.4
13	Rotavirus	1.0	1.1	1.0
14	CMV	42	0.6	1.6
15	Polio type 1	0	0	0.5
16	Polio type 1	28	0.5	0.5
17	Polio type 1	28	2.8	1.3
18	Polio type 2	11	0.6	2.2
19	Polio type 2	3.2	6.1	2.2
20	Polio type 3	0	1.1	1.3
21	Coxsackie B	240	1.2	17
22	Echo type 11	1.1	2.2	0.7
23	Echo type 11	21	3	0.1
24	Echo type 22	0.1	0	0
25	Echo type 22	14	7.5	1.4
26	Polio type 1/3	26	64	0.3
27	CMV/Polio 2	0	1.4	1.8
28	Rhino/Coxsackie A	8	1.6	2
29	RSV/Echo type 11	3	5.5	4
30	CMV/Polio 2	1.5	1.2	2

TABLE 31

Alpha Interferon Levels from Virus Positive Paediatric Deaths

Number of Case	Virus Detected/Isolated	IFN-α (U/ml) NPA	SERUM	CSF
1	RSV	1.2	0.7	1.7
2	RSV	20	5.4	0.12
3	RSV	0	6	1.2
4	CMV	0	2.3	1.5
5	Rotavirus	1.2	NT	1.8

NT = not tested

TABLE 32

Analysis of Range of IFN-α Results for Virus Positive Glasgow and Edinburgh Cot Deaths

IFN-α IU/ml	NPA G	(%)	NPA E	(%)	SERUM G	(%)	SERUM E	(%)	CSF G	(%)	CSF E	(%)
0 – 1	8/16	(50)	10/30	(32)	4/16	(25)	10/30	(32)	3/16	(19)	11/30	(35)
1.1 – 10	2/16	(12)	11/30	(35)	8/16	(50)	20/30	(65)	13/16	(81)	19/30	(61)
10.1 – 100	5/16	(31)	9/30	(29)	4/16	(25)	1/30	(3)	0/16	(0)	1/30	(3)
>100	1/16	(6)	1/30	(3)	0/16	(0)	0/30	(0)	0/16	(0)	0/30	(0)

G = Glasgow
E = Edinburgh

TABLE 33

Range of Interferon α Levels for
Virus Positive Paediatric Deaths

IFN-α (IU/ml)	NPA	(%)	SERUM	(%)	CSF	(%)
0 - 1	2/5	(40)	1/4	(25)	1/5	(20)
1.1 - 10	2/5	(40)	3/4	(75)	4/5	(80)
10.1 - 100	1/5	(20)	0/4	(0)	0/5	(0)
$>$100	0/5	(0)	0/4	(0)	0/5)	(0)

largest number of cases was found between 0 - 10 IU/ml i.e. 62% and 67% respectively. In 46 cot death cases from Glasgow and Edinburgh 30% of NPA samples had IFN- α levels $>$ 10 IU/ml. Serum levels of IFN- α were also noted between 0 - 100 IU/ml with the largest number of cases (57%) observed in the 1.1 - 10 IU/ml range. Fourteen per cent of the total cot death group had IFN- α values in the range 10.1 - 100 IU/ml. CSF levels of IFN- α for the cot death cases were noted between 0 - 10 IU/ml with the largest number of cases in the IFN- α range 1.1 - 10 IU/ml i.e. 32/46 (71%) of total cot death cases and only 1 case (3%) had IFN- α $>$10 IU/ml.

The serum and CSF samples in virus positive paediatric deaths were similarly noted as in the cot death cases to be between 0 and 10 IU/ml, with increased numbers of 75% and 80% in cases in the 1.1 - 10 IU/ml IFN- α range. The NPA samples showed IFN- α levels between 0 - 100 IU/ml with 20% of cases with IFN- α levels of 10.1 - 100 IU/ml.

Analysis of the IFN- α data for virus positive cot deaths and paediatric deaths is presented in Table 34. Levels greater than the minimum detection limit for IFN- α $\geqslant$2 IU/ml in the NPA were detected in 8 (50%) cot death cases from Glasgow. Twenty Edinburgh cot death cases (67%) were above the cut level of 2 IU/ml and only one case in the paediatric death group was noted. The mean levels of IFN- α detected above 2 IU/ml in the NPA were 42, 23.6 and 20 IU/ml for Glasgow, Edinburgh cot death cases and paediatric deaths. The number of virus

TABLE 34

Analysis of Alpha Interferon Results for Virus Positive Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

Cases	Samples Studied	Number of Cases	Number of Cases IFN-α Positive	(%)	Mean Value (U/ml)
Glasgow Cot Death	NPA	16	8	(50)	42
	Serum	16	11	(68)	14.4
	CSF	16	11	(68)	2.8
Edinburgh Cot Deaths	NPA	30	20	(67)	23.6
	Serum	30	13	(43)	8.6
	CSF	30	16	(53)	3.2
Paediatric Deaths	NPA	5	1	(20)	20
	Serum	4	3	(75)	4.5
	CSF	5	3	(60)	1.6

positive paediatric deaths is too small for meaningful analysis.

Evaluation of the levels of IFN- α present in serum, $\geq$1.5 IU/ml were carried out on 11 (68%), 13 (43%) and 3 (75%) of virus positive Glasgow, Edinburgh cot deaths and paediatric deaths. The mean levels were calculated as 14.4, 8.6 and 4.5 IU/ml IFN- α for the cot death groups and paediatric group.

Similarly CSF samples $\geq$1.5 IU/ml IFN- α were analysed. The number of cases above the minimum detection limit were shown to be 11 (68%), 16 (53%) and 3 (60%) for Glasgow, Edinburgh cot death and paediatric cases.

7.2.5 INTERFERON- α RESULTS FOR VIRUS NEGATIVE COT DEATH CASES, GLASGOW AND EDINBURGH AND PAEDIATRIC DEATHS

Specimens: NPA, serum and CSF were tested for the presence of IFN- α from the cot death cases and paediatric cases in which no viral isolate was obtained. The range of IFN- α values obtained from Glasgow and Edinburgh virus negative cot death cases are shown in figures 17 and 18. The number of cases were analysed in each IFN- α range for cot deaths and paediatric deaths 0 - 1 IU/ml, 1.1 - 10 IU/ml, 10.1 - 100 IU/ml and $>$100 IU/ml are illustrated in Tables 35 and 36 .

The NPA samples from 81 Glasgow cot death cases and 62 cases of cot death collected from Edinburgh indicated a range of IFN- α levels between 0 - 100 IU/ml. The number of cases in the individual ranges

ALPHA-INTERFERON LEVELS IN BODY FLUIDS FROM
VIRUS NEGATIVE COT DEATH CASES,
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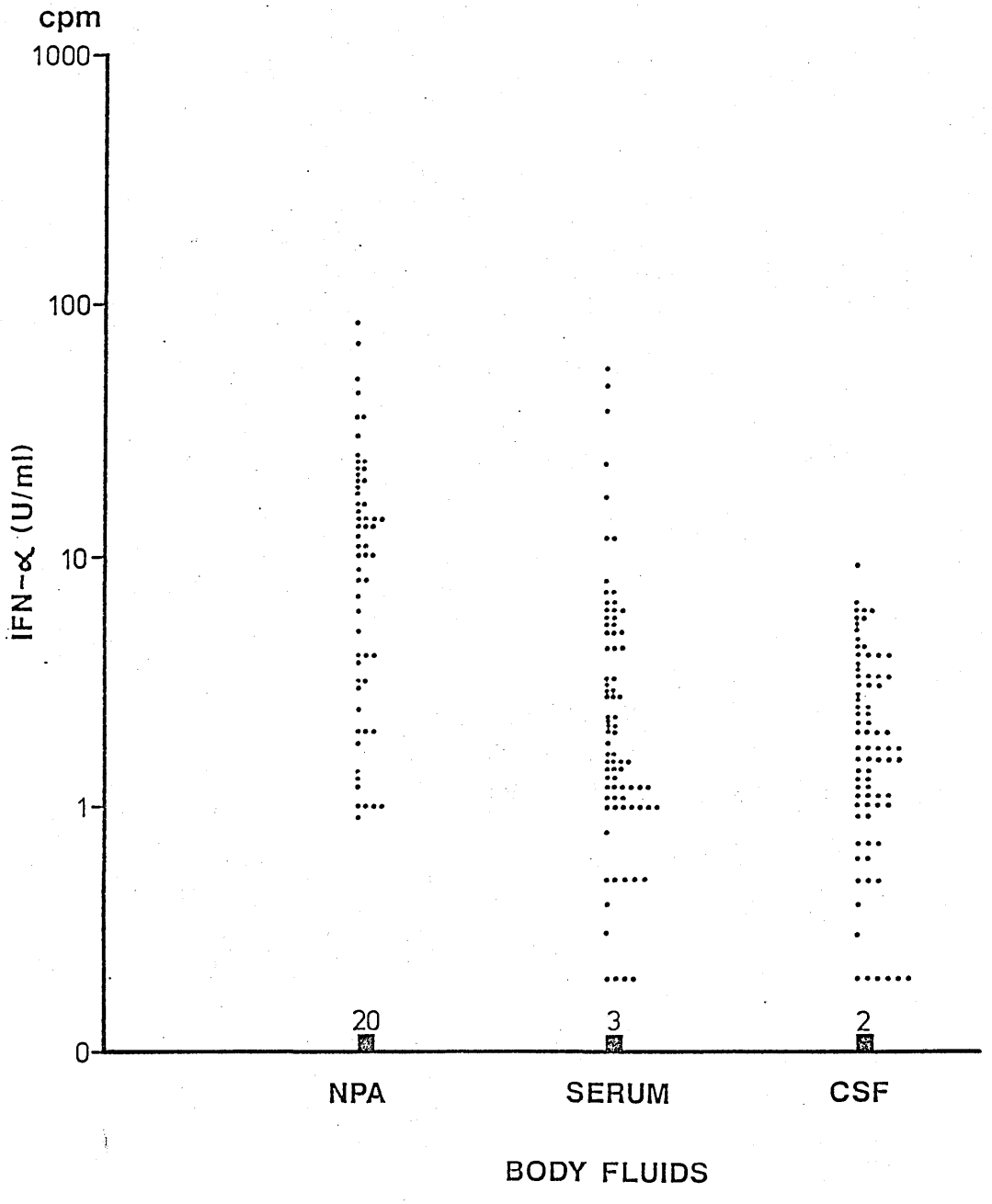

Figure 17

ALPHA-INTERFERON LEVELS IN NPA,SERUM, CSF IN VIRUS NEGATIVE COT DEATH CASES
ROYAL HOSPITAL FOR SICK CHILDREN, EDINBURGH

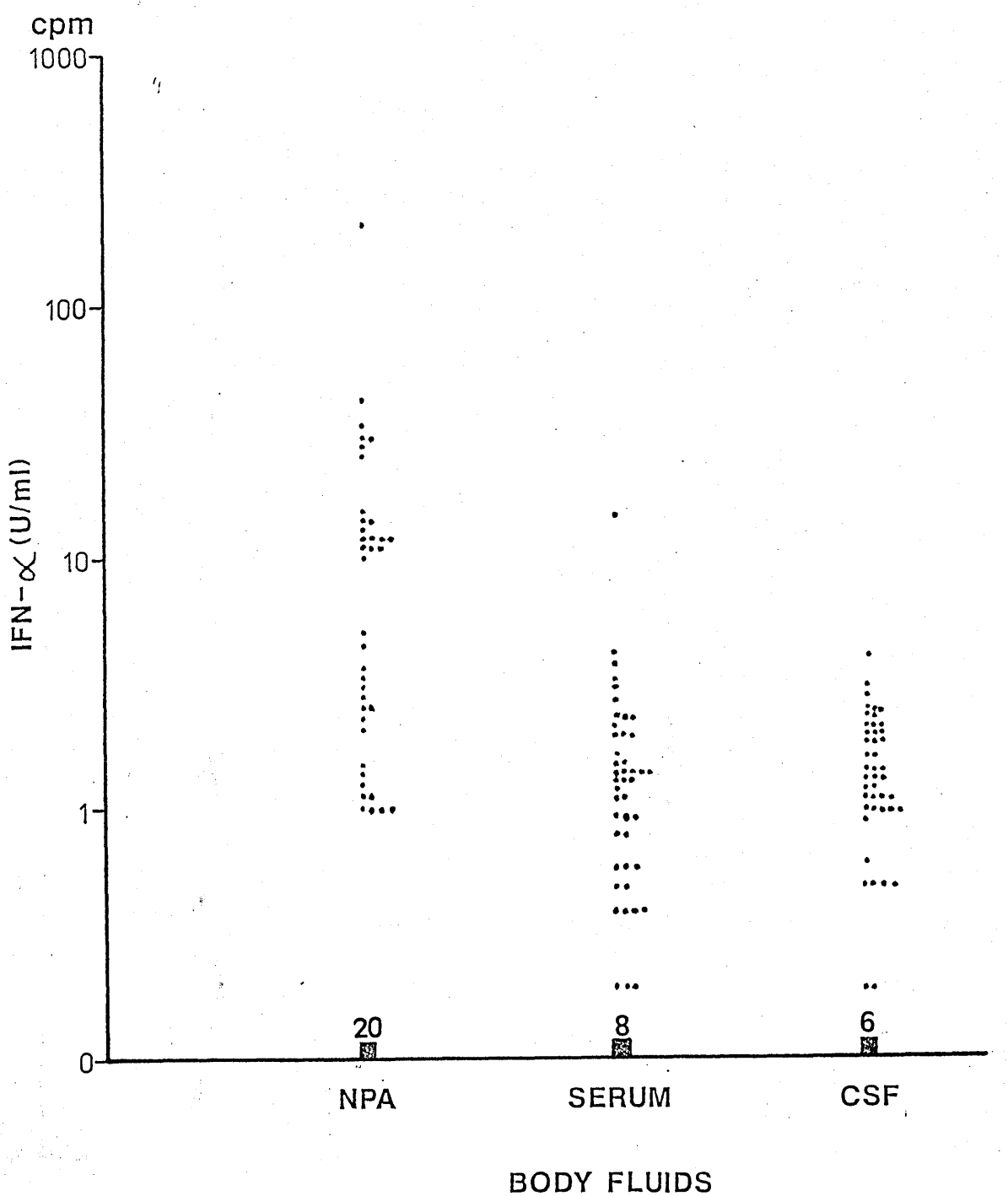

Figure 18

TABLE 35

Range of IFN-α Results for Virus Negative Glasgow and Edinburgh Cot Deaths

IFN-α IU/ml	NPA G	(%)	NPA E	(%)	SERUM G	(%)	SERUM E	(%)	CSF G	(%)	CSF E	(%)
0 – 1	25/81	(31)	22/59	(37)	25/83	(30)	27/60	(44)	23/82	(25)	25/62	(40)
1.1 – 10	24/81	(30)	17/59	(29)	53/83	(64)	32/60	(52)	59/82	(71)	37/62	(60)
10.1 – 100	31/81	(39)	19/59	(32)	5/83	(6)	1/16	(2)	0/82	(0)	0/62	(0)
>100	0/81	(0)	1/59	(2)	0/83	(0)	0/60	(0)	0/82	(0)	0/62	(0)

G = Glasgow
E = Edinburgh

TABLE 36

Range of Alpha Interferon Results for
Virus Negative Paediatric Deaths

IFN-α IU/ml	NPA	(%)	SERUM	(%)	CSF	(%)
0 - 1	4/16	(25)	9/12	(41)	10/21	(48)
1.1 - 10	10.16	(62)	13/22	(59)	11/21	(52)
10.1 - 100	2/16	(12)	0/22	(0)	0/21	(0)
>100	0/16	(0)	0/22	(0)	0/21	(0)

for both cot death groups showed a constant pattern of between 30 - 40%.

The IFN- α level in serum was tested in 83 Glasgow cot death cases and 55 Edinburgh cot death cases. The range of IFN- α values in virus negative cot death cases was between 0 - 100 IU/ml, with peak numbers of cases noted in the 1.1 - 10 IU/ml grouping, 58% of total cot death cases.

The CSF samples from virus negative cot death cases from Glasgow (83 cases) and Edinburgh (59 cases) had IFN- α levels of 1.1 - 10 IU/ml in 71 and 60% of cases, respectively. No IFN- α levels were detected in the CSF specimens above 10 IU/ml.

The cot deaths showed IFN- α values between 0 - 100 IU/ml in NPA specimens (16 cases) from virus negative cases, 12% of cases in the range 10 - 100 IU/ml. The serum and CSF samples (21 cases each) had levels of IFN-α in the range 0 - 10 IU/ml. The largest proportion of cases with IFN- α found in the all three samples was detected between 1.1 - 10 IU/ml i.e. 62%, 59% and 52% in NPA, serum and CSF.

An indication of the number of Glasgow and Edinburgh cot death cases with IFN- α levels above the minimum detection limits previously set for NPA, serum and CSF samples are shown in Table 37. The mean values of IFN- α were obtained on a smaller number of cases therefore increasing the IFN- α detected in NPA i.e. 17.8 IU/ml and 18.5 IU/ml, serum 6.36 IU/ml and 3.64 IU/ml and CSF 2.9 IU/ml and 2.3 IU/ml. The

TABLE 37

Analysis of IFN-α Results for Virus Negative Glasgow and Edinburgh Cot Death Cases and Paediatric Deaths

Cases	Samples Studied	Number of Cases	Number of Cases >2 IU/ml	Number of Cases >1.5 IU/ml	(%)	Mean Value U/ml
	NPA	81	51		(63)	17.8
Glasgow	Serum	83		45	(54)	6.36
Cot Deaths	CSF	83		50	(60)	2.9
	NPA	62	32		(52)	18.5
Edinburgh	Serum	55		20	(36)	3.64
Cot Deaths	CSF	59		29	(49)	2.3
	NPA	16	12		(75)	6.6
Paediatric	Serum	21		10	(48)	2.49
Deaths	CSF	21		9	(43)	3.4

IFN- α values from samples obtained from negative cot death cases in the study showed similar ranges of level of IFN- α present. The IFN- α levels in NPA samples from paediatric deaths were lower than the cot death cases. The mean IFN- α level was 6.6 IU/ml. The serum and CSF IFN- α levels in paediatric deaths were similar to those detected in cot death samples.

Analysis of the virus positive and virus negative cases of cot death using the Wilcoxon Rank Sum test indicated that there was no significant difference between the levels of IFN- α in NPA obtained in viral positive and viral negative cases, although the total mean IFN- α values appear to be higher in viral positive cases i.e. 21.11 and 11.3 IU/ml. The high mean IFN- α values in NPA were due to the several values $>$ 200 IU/ml which increased the range of detectable levels. No significant difference was noted in the serum and CSF levels of IFN- α of the virus positive and negative case of Glasgow and Edinburgh cot death cases.

The mean IFN- α values of samples from the paediatric deaths were lower than those noted for cot death cases, but again no statistical difference was noted between the IFN- α levels in NPA, serum and CSF in paediatric viral positive or negative cases and the viral positive and negative cases of cot death.

7.3.1 INTERFERON- α LEVELS AND AGE DISTRIBUTION IN GLASGOW COT DEATH CASES

The Glasgow cot death cases were investigated for positive IFN- α levels in the fluid samples; NPA, serum and CSF and the age distribution of the infants as shown in Table 38. The number of cases with raised IFN- α values in the NPA and CSF were similar over the age ranges 1 - 8 weeks to 27 - 52 weeks. i.e. Fifty per cent of cases. The serum samples with detectable levels of IFN- α had increased numbers of cases presented in the 9 - 16 week age group.

Despite the above trends, on statistical evaluation using Wilcoxon Rank test, no evidence was found to suggest that the age of the cot death victims was a factor in the increase of IFN- α levels detected in the fluid samples obtained from the body.

Edinburgh Cot Death Cases

The detection of IFN- α levels in the body fluids in Edinburgh cot death cases was analysed with respect to the age of the infant at the time of sampling. The data collected for numbers of cot death cases in the age ranges 1 - 8, 9 - 16, 17 - 26 and 27- 52 weeks and increased values of IFN- are detailed in Table 39. It was noted that the number of infants with increased IFN- α levels in the NPA occurred between 9 - 16 weeks and 17 - 26 weeks although no increase in IFN- α values was noted for CSF over the age ranges. There was an increase in

TABLE 38

Interferon α Levels and Age Distribution of Glasgow Cot Death Cases

Age (weeks)	Body Fluids NPA ⩾2 IU/ml	(%)	SERUM ⩾1.5 IU/ml	(%)	CSF ⩾1.5 IU/ml	(%)
1-8	15/23	(65)	11/23	(48)	12/23	(52)
9-16	21/39	(54)	27/39	(69)	22/39	(56)
17-26	13/22	(59)	7/22	(32)	10/22	(45)
27-52	9/16	(56)	9/16	(56)	11/16	(69)

TABLE 39

Interferon ∝ Levels and Age Distribution of Edinburgh Cot Death Cases

	Body Fluids					
Age (weeks)	NPA ⩾2 IU/ml	(%)	SERUM ⩾1.5 IU/ml	(%)	CSF ⩾1.5 IU/ml	(%)
1-8	5/18	(28)	5/18	(28)	8/18	(44)
9-16	25/34	(73)	20/34	(29)	15/34	(44)
17-26	14/21	(66)	9/21	(43)	10/21	(47)
27-52	8/16	(50)	7/16	(44)	8/16	(50)

the number of cases with raised IFN-α in the 17 - 26 week age group.

7.3.2 INTERFERON-α RESULTS AND POST MORTEM INTERVAL FOR GLASGOW COT DEATH CASES

Analysis of the post mortem interval and the number of cot death cases with IFN-α above the minimum detection limits in the body fluid specimens are shown in Table 40.

Similar number of cases were observed in the 24 hours and 25 - 48 hour intervals for NPA, serum and CSF sampling. The cases with raised serum IFN-α levels in 49 - 72 hours interval were higher in number than in NPA or CSF. This trend was also noted in the CSF IFN-α values in the 73 - 96+ hour post mortem interval.

Statistical analysis using the Wilcoxon Rank test indicated that there was no significant differences in the IFN-α levels observed in any of the specimens compared with the time interval at which the samples were taken. This result was despite apparent increases in the number of specimens with raised IFN-α in CSF and serum and 49 - 72 hours and 73 - 96+ sampling after death of the cot death victim.

Edinburgh Cot Death Cases

The number of cases of Edinburgh cot death specimens with IFN-α detected $\geqslant$2 IU/ml for NPA and $\geqslant$1.5 IU/ml in serum and CSF

TABLE 40

Positive Levels of IFN α and Post Mortem Interval for Glasgow Cot Death Cases

PM Interval (Hrs)	Body Fluids IFN-α NPA ⩾2 IU/ml	(%)	SERUM ⩾1.5 IU/ml	(%)	CSF ⩾1.5 IU/ml	(%)
24	6/12	(50)	6/12	(50)	6/12	(50)
25-48	15/29	(52)	14/29	(48)	16/29	(55)
49-72	5/9	(55)	7/9	(77)	6/9	(66)
73-96	4/7	(57)	4/7	(57)	6/7	(85)

samples are presented in Table 41.

No real difference was noted in the number of cases with raised IFN-α in each post mortem range in the samples tested. A larger proportion of cases tested for IFN-α in NPA specimens were detected in the 49 - 72 hour post mortem interval (50%) compared to the serum and CSF samples at the same time interval. A similar trend was noted for IFN-α levels in NPA samples in the 25 - 48 hour post mortem interval.

7.4.1 IMMUNOCYTOCHEMICAL ANALYSIS OF IFN-α IN LUNG SECTIONS

From the Glasgow cot death cases and paediatric deaths serial sections of lung specimens - pharynx, larynx, trachea, bronchus, bronchioles and peripheral lung see section 2.1.6 were cut into 4 μm sections and placed on coated slides. The positive cells in the sections were observed by microscopic examination and scored on a system based on visual analysis.

The scoring system ranged from 0 to >16 positive cells per high power field (x40, 1400 square microns) as a mean value over a series of 5 microscope fields. The number of positive cells observed were categorised into 0, 1 - 5, 6 - 10, 11 - 15, >16 cells and given the graded values 0 to 4 for each grouping of positivity, see Table 43.

From a total of 100 Glasgow cot death cases, 94 cases were processed using the 'in situ' technique. Five paediatric deaths were evaluated

TABLE 41

Positive Levels of IFN-α and Post Mortem Interval for Edinburgh Cot Death Cases

PM Interval (hrs)	Body Fluids IFN-α NPA ⩾2 IU/ml	(%)	SERUM ⩾1.5 IU/ml	(%)	CSF ⩾1.5 IU/ml	(%)
< 24	9/20	(45)	6/20	(30)	8/20	(40)
25 - 48	26/44	(59)	14/44	(32)	22/44	(50)
49 - 72	10/20	(50)	6/20	(30)	7/20	(35)
73 - 96+	6/13	(46)	5/13	(38)	4/13	(31)

similarly to the cot death cases.

The lung sections: pharynx, larynx, trachea, bronchus and bronchioles were tested and the results of the positivity specific to IFN- α in the cells is detailed in Table 42.

No IFN- α positive cells were noted in the pharynx, larynx, trachea or bronchus lung section in either cot death cases or paediatric death. Fifty four cot death cases showed positive staining to IFN- α in large nuclear phagocytic cells located within the alveoli on sections of bronchiole. The highest number of cases showed positivity in 1 - 5 cells (36%) in cot death cases and the paediatric deaths indicated 2/5 (40%) of cases were IFN- α positive in the alveoli.

The positive staining indicative of IFN- α in the peripheral lung section of cot death cases and paediatric deaths is shown in figure 19. Non specific staining was noted in the epithelial cells surrounding the alveolar spaces. The control slide containing normal sheep serum as primary antibody is shown in figure 20.
The number of cases of cot death and paediatric death with positive staining in the form of granular brown pigment, specific for IFN- α is evaluated in Table 43.

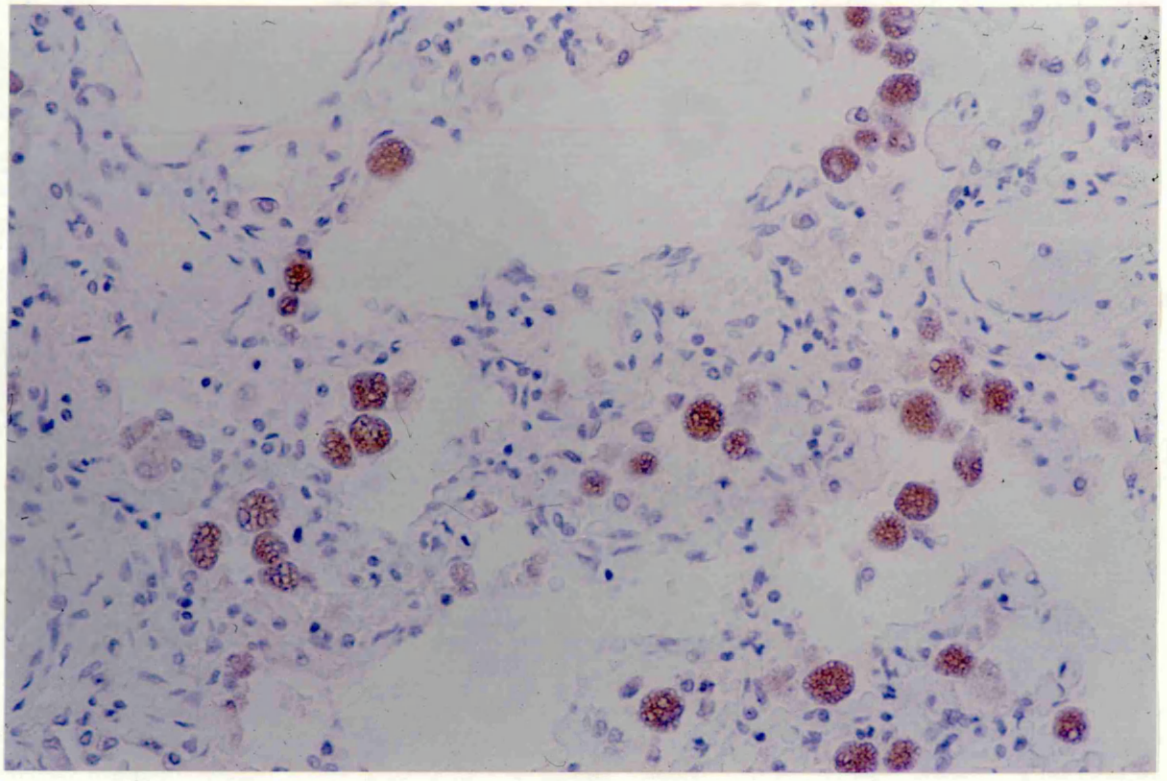

Figure 19 Peripheral lung section from Glasgow cot death case (PM 348/88) stained by the immunoperoxidase technique against IFN-α (x40)

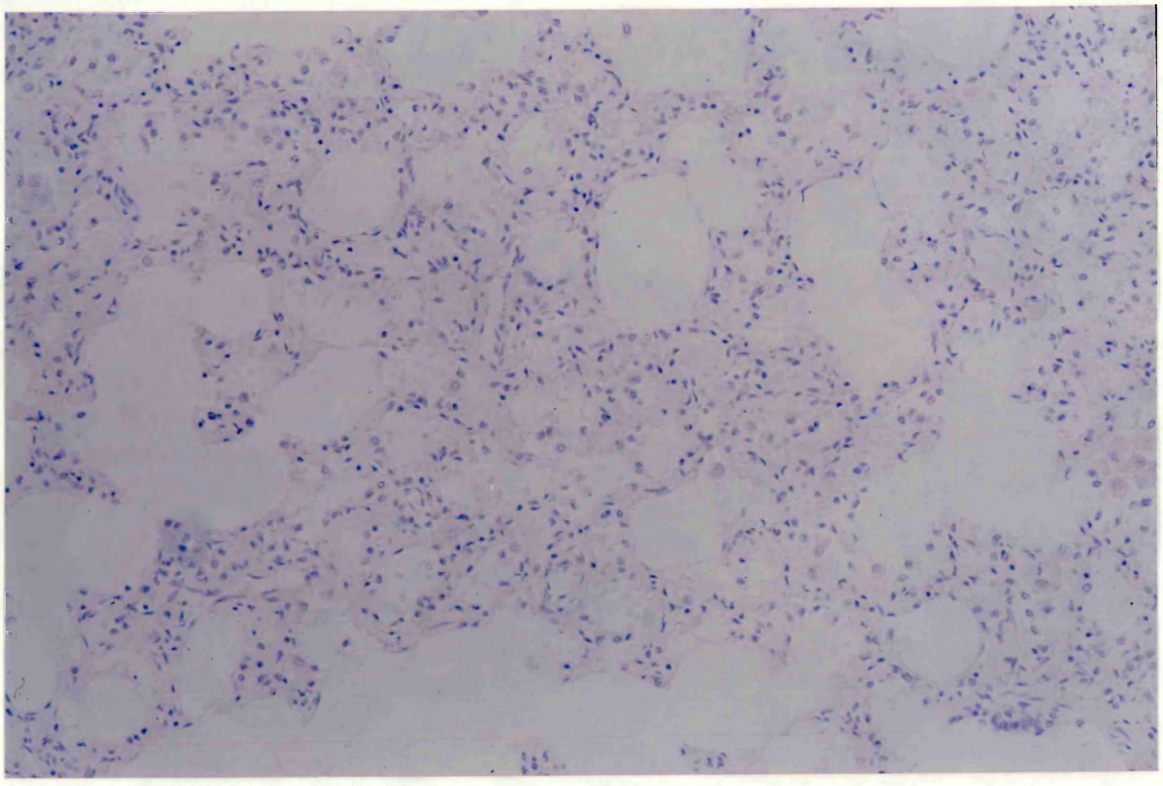

Figure 20 Immunoperoxidase staining for sheep antibody in peripheral lung section of Glasgow cot death case, PM 348/88 (x10)

TABLE 42

Analysis of the Indirect Immunoperoxidase Technique Specific for IFN-α in Lung Sections: pharynx, larynx, trachea, bronchus, bronchioles from 94 Glasgow Cot Death Cases and 5 Paediatric Deaths

Case	Section of Lung	Grade Values of Cases with Cells Positive IFN-α									
		0	(%)	1	(%)	2	(%)	3	(%)	4	(%)
Glasgow Cot Deaths	pharynx	94		0		0		0		0	
	larynx	94		0		0		0		0	
	trachea	94		0		0		0		0	
	bronchus	94		0		0		0		0	
	bronchioles	40	(43)	34	(36)	20	(21)	0		0	
Paediatric Deaths	pharynx	5		0		0		0		0	
	larynx	5		0		0		0		0	
	trachea	5		0		0		0		0	
	bronchus	5		0		0		0		0	
	bronchioles	3	(60)	2	(40)	0		0		0	

TABLE 43

Positive Staining by Indirect Immunoperoxidase Technique Specific for IFN-α in Lung Sections from Glasgow Cot Deaths and Paediatric Deaths

PERIPHERAL LUNG

	GRADES									
Case	0	(%)	1	(%)	2	(%)	3	(%)	4	(%)
Glasgow Cot Deaths	7	(7)	41	(44)	24	(25)	15	(16)	7	(7)
Paediatric Deaths	2	(40)	2	(40)	1	(20)	0		0	

High power field defined as 1400 square micron

		Number of positive/cells high power field	
Grading System	0	0	or negative (-)
	1	1 - 5	
	2	6 - 10	
	3	11 - 15	
	4	16+	

The highest number of positive cells observed in the peripheral lung sections from cot deaths was between 1 - 10 cells i.e. grade 1 and 2 comprising 65 cases (69%). Positive cells were noted in the range of 16 (grade 4) per high power field in the cot death cases but not found in the paediatric cases. Two of the 7 cot death cases (28%) with IFN-α positivity in the grade 4 grouping were also noted as virus positive cases i.e. RSV, Polio 2. Five virus positive cot death (33%) cases were categorised as 11 - 15 (grade 3) positive IFN cells/high power field i.e. RSV, Adeno/Polio 3 and Influenza A. The remaining 6 virus positive cot death cases were categorised as 2 cases in the grade 1 range of positivity e.g. Rotavirus, Adeno and 4 cases with no positive cells for IFN- α e.g. Rotavirus, Polio 2, CMV, Polio/RSV.

No correlation between IFN- α levels (IU/ml) measured by IRMA in the NPA from cot death cases and the staining specific for IFN- α in the lung sections was noted. Hence as the IFN- α levels in IU/ml increased this did not necessarily correlate with a higher positive ratio of cells present in the peripheral lung section. The in situ technique and IRMA methods of detecting endogenous IFN- α used a quanlitative and quantitative analysis therefore analysis was limited. From a small study population the paediatric deaths were grouped in 1 - 5 positive cells for IFN- α (40%) i.e. grade 1 and included one virus positive case i.e. RSV. The remaining cases were virus negative and also were in the grade 1 category of staining. The cases indicated 0 IU/ml IFN- α in the NPA as measured by IRMA.

7.4.2 IDENTIFICATION OF MACROPHAGE CELL POPULATION BY MONOCLONAL ANTIBODY KP1

The large nuclear cellular particles positively staining for IFN- α in the alveolar species in the peripheral lung sections from cot death and paediatric death cases were identified by using a marker specific to the macrophage cell population. The results confirmed that the cells positively staining for IFN- α in the lung sections were from the alveolar macrophage cell line. The number of cells staining with the brown/blue pigment were counted and an average number obtained over five high power fields (x40). The number of macrophage positive cells was compared with the number of IFN- α positive cells and a ratio obtained which indicated IFN- α positivity in macrophage cells i.e. the ratio <1 indicated that macrophage cell line were cells positive for IFN- α. A value >1 suggested that another group of cells were staining positively for IFN- α. The results of ratios obtained on a range of peripheral lung sections are shown in Table 44. All the ratios obtained in the cases in this study were <1. The IFN- α values in 8 cot death cases and 2 paediatric deaths along with the immunocytochemistry and ratio of number of positive IFN- α cells on comparison with the number of macrophage cells present in the peripheral lung sections were shown with isolation/detection of virus. The staining pattern obtained with the monoclonal antibody KP1 in alveolar macrophages is illustrated in figure 21.

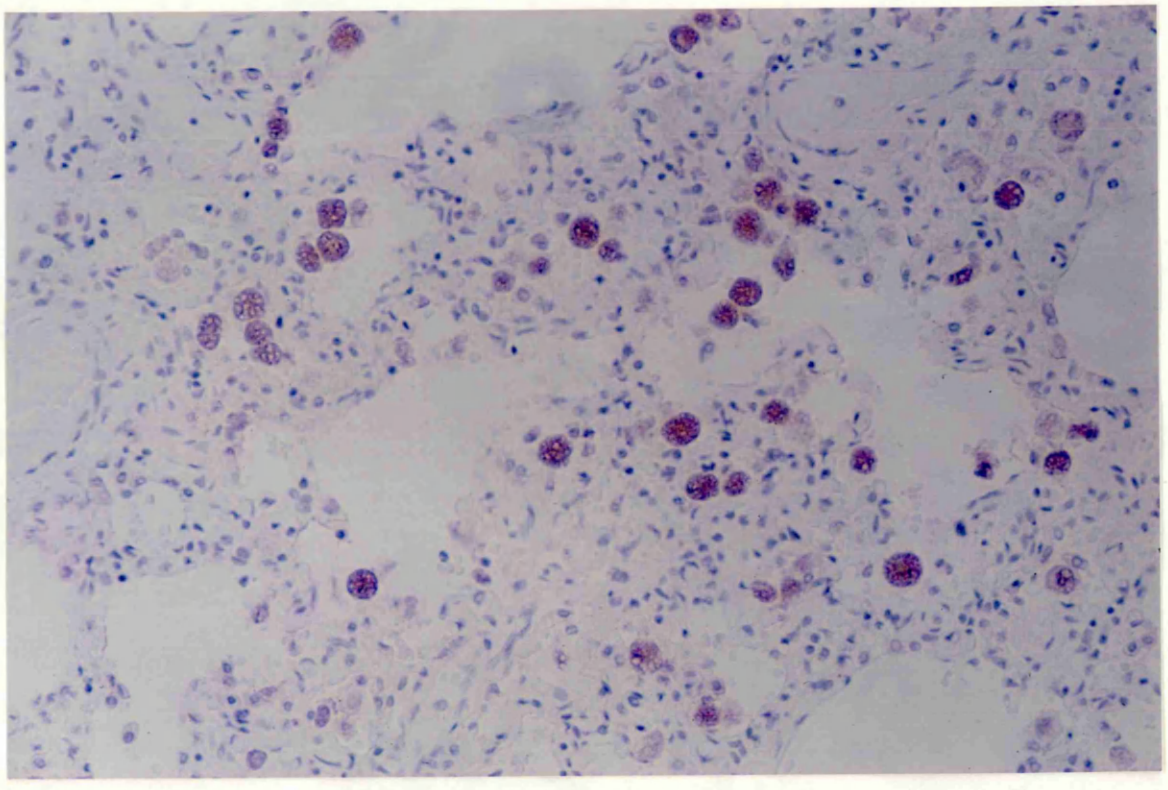

Figure 21 Staining of alveolar macrophage cells with monoclonal antibody KP1 in peripheral lung section of Glasgow cot death case, PM 348/88 (x20)

TABLE 44

Overall Analysis of Eight Glasgow Cot Death Cases and Two Paediatric Deaths on Investigation of Lung Section for IFN- α.

	Case	Virus Present	IFN- α (U/ml) in NPA	Grade of Staining	Number of Macrophage	Ratio
Cot Deaths	1	Influenza A	240	3 (12)	25	0.48
	2	RSV	20	3 (11)	21	0.52
	3	Adeno/Polio	15	3 (13)	19	0.68
	4	RSV	0	3 (14)	31	0.45
	5	Polio	0	0	22	0
	6	Negative	52	4 (21)	40	0.52
	7	Negative	0	0	2	0
	8	Adeno/Rota	0	1 (2)	12	0.16
Paediatric Deaths	9	RSV	20	1 (3)	9	0.3
	10	Negative	0	0	6	0

7.4.3 PATHOLOGICAL AND IMMUNOCYTOCHEMICAL ANALYSIS

Histology reports, obtained from the pathologists, relating to the individual lung sections: pharynx, larynx, trachea, bronchus, bronchioles and peripheral lung from Glasgow cot death cases were analysed with respect to the results of IFN- α detection by the in situ technique as in section 2.1.9.

No pathology of note was detected in the pharynx, larynx, trachea, bronchus and bronchioles. The reports indicated that these sections had no significant abnormality, normal submucosal lymphoid tissue and mild lymphoplasmacytic submucosal infiltrate. The cot death cases with IFN- α detection by the immunoperoxidase method in grades 1 and 2 of positivity in cells were reported as normal lung parenchyma, lymphocytic infiltrate and subpleural petechiae.

However, the cases with grades 3 and 4 staining in the peripheral lung section were categorised as congested, oedematous with eosinophilic necrosis, lobular consolidation and early bronchopneumonia. From the 22 cases in the category grades 3 and 4, 18 cases (75%) indicated positive findings by histological observation in the peripheral lung sections.

CHAPTER 8

FINAL DISCUSSION

Research workers looking for the aetiological causes of the sudden infant death syndrome or unexplained death have followed two lines of enquiry. Initially, epidemiological surveys were carried out to assess the factors associated with a higher risk of cot death. These have not been conclusive, but have been helpful to bring the syndrome into perspective. The second pathway has searched for a defined cause for sudden death in infants < 1 year of age, and utilised clinical and pathological investigations in multidisciplinary research programs.

The present multicentre study merged both pathways in order to gain further knowledge and data on the infection theory proposed for cot death cases. The suggested hypothesis that infection by an undefined microorganism may lead to the triggering of a mechanism in the final pathway leading to sudden unexplained death in infants was popularised in the 1970's (Scott et al.,1978, Uren et al., 1980). The theory had lost some favour with research workers due to the unrewarding results of virological studies in cot death cases.

In the present study, the detection of alpha interferon has been applied to samples obtained from cot death and paediatric death cases.

The additional pathological investigations of impression smears and cytospin preparations were used to aid the detection of respiratory viruses in lung sections. However, the viral positivity rate for cot death cases i.e. 16% in Glasgow cases and 38% in Edinburgh cases were not dissimilar from the documented values found elsewhere. A feature of note was the high proportion of respiratory viruses eg. RSV, influenza virus type A, rhinovirus, parainfluenza type 3 in the total viruses isolated/detected (Tables 18 and 20).

In the paediatric death group an 18% viral positive rate was found (Table 18) and again the largest proportion of viruses detected were from the respiratory tract. The RSV positive cases of cot death and paediatric deaths presented during the RSV epidemic of the winter of 1987 and 1988 in Scotland. The significance of the respiratory results will be dealt with in more detail on discussion of the demographic survey.

The poliovirus isolates as a group accounted for more recovered viruses than any other isolate obtained from the cot death cases. These isolates were almost certainly vaccine strains. Persistent excretion of oral polio vaccine strains for several weeks is widely accepted (Philip Minor, personal communication). Evidence relating the Glasgow infants who had been immunised to the positive poliovirus isolation results was obtained despite the small number of complete reports of vaccine history.

The number of poliovirus positive cot death cases per annum in this study were similar to those documented in the pathological reports, RHSC, Glasgow, 1979-86 (Patrick et al., 1989).

The significance of finding poliovirus in specimens from cot deaths was considered to be doubtful from the results presented in this thesis. These findings correlated with Roberts (1987) published data supporting the view that there was no link between sudden infant death and DTP-polio vaccination.

The present analysis recorded a wide variety of viruses for cot death cases. This factor highlighted the observation by Urquhart and Grist that no one particular virus was responsible for cot deaths. The serological analysis undertaken gave no valuable insight into a possible virus aetiology. Although the number of viruses screened was small, they were those routinely tested in virus laboratories as responsible for clinically significant disease.

As part of the analysis of this prospective study a demographic survey of the cot death data from both Glasgow and Edinburgh was documented. The survey pinpointed several useful parameters as background information to the study group. Cases brought to the Pathology Departments as suspected cot death were consistent in numbers for each year studied i.e. 40 cases in 1987 and 1988, and therefore confirmed

incidence rates for Scotland as published by Arneil and colleagues (1985).

The cases were categorised (see section 2.1) as in previous studies to indicate the "true" cot death group and enable cross reference with retrospective and prospective data.

The cot death cases, all less than one year of age covered a wide age distribution within the twelve month span. The highest number of cases were in the age grouping 9-16 weeks, a factor noted by Patrick and coworkers (1989) at the Pathology Department, RHSC, Glasgow.

Evidence of increased monthly distribution during the winter months November - April and early increase in numbers of cases in the months September and October for Glasgow and Edinburgh cases are presented. A significant decrease in the numbers of cot death during the months June-July further highlighted the winter increase. This coincided with the paediatric respiratory epidemic of RSV during the two year study period, although relatively small numbers of RSV positive cases were detected in the samples from cot deaths. From these observations it would seem unlikely that cot deaths are caused principally by RSV but does not rule out an as yet unidentified virus prevalent in winter months. These conclusions are ratified in the literature (Nelson et al., 1975).

Mild infection in 60% of cot death cases before death was reported. This figure would not be unreasonable in this situation as infants are susceptable to a wide range of pathogens from the earliest age.

The location of cot death within the cities investigated showed a random distribution pattern. The increase in numbers of cases in areas with dense populations was noted in postcode areas G33, Blackhill/Riddrie, G34, Easterhouse,EH16 , Liberton, EH4, Silverknowes/Clermiston. The population statistics for these postcode areas of Glasgow and Edinburgh contradicted any increases in numbers of cot deaths due to high residency and higher birth rates in these locations (Figures 6 and 9).

Comparisons of the cot death group and the adult and paediatric death specimens were useful in a study of this type in order to make some limited observations of the normal population. The present study included a paediatric death group, referred to as such and not as a specific "control" group due to the lack of common factors.
It is the long held view of workers in the field that no appropriate "control" group exists for the study of cot death. For the purposes of the present study, the most appropriate "control" group would have been a group of infection free infants, age and sex matched to the cot deaths. For ethical reasons, taking clinical specimens from infants apparently healthy was not possible.

The group of infants thought to be a good comparative group for the cot death infants are " near miss" infants, however the numbers are small (Golding et al., 1985). The paediatric death cases analysed were all hospital based prior to death and hence had some intrinsic abnormality or disease.

The additional work and relative novel approach presented in this thesis corresponded to the detection and analysis of IFN- α in biological fluids from cot death cases. Interferon as an acute phase protein is induced by a variety of stimuli including viruses and has been shown to be part of the initial response of the host to acute viral infection.

The assay system used in this project to detect the presence of IFN- α was evaluated for sensitivity and specificity during a series of experimental investigations(Tables 4, 5, and 6). The specificity of the assay was investigated by a neutralisation/blocking assay on standard preparations (Figures 4 and 5, Table 6) and subsequently on the test samples (Tables 7 and 8). The results demonstrated the monoclonal antibody to IFN- α to be specific in standard preparations and the specimens tested.

Values of IFN- α in samples from cot deaths and paediatric deaths were evaluated and found to be reliable at levels of >1 IU/ml. The sensitivity of the IRMA was greater than biological assay systems used in other projects relating interferon in cot death cases

(Seto & Carver, 1978, Vanacek et al., 1986).

The detection of IFN- α at relatively low levels i.e. <10 IU/ml (Table 27) in cerebrospinal fluid samples from cot death cases and paediatric cases was interesting as the viral culture results from the brain and CSF samples were negative (Tables 18 and 20). The highest numbers of cot deaths and paediatric deaths were observed in the same IFN- α range 1.1 - 10 IU/ml and these groups indicated comparable mean values of IFN- α (Table 26 and 27). Conclusions from these observations showed no significant difference in CSF IFN- α values for the two study groups. No identifiable cause of acute infection was found although the levels were comparable with those detected in a group of patients presenting with viral meningitis(Ho Yen & Carrington, 1987). However other pathological features of meningitis were absent from the cot death group or the paediatric group and it is uncertain as to why the interferon level was raised in these groups.

The data presented in this thesis is consistent with the view that as IFN- α is detected in the cuboidal epithelium of the choroid plexus. The IFN- α may be synthesised at this location and released into the CSF during viral infection(Khan et al., 1989), as opposed to release by inflammatory cells recruited into the area of infection.

The blood brain barrier is a selectively permeable membrane which does not normally allow leakage of interferon in either direction. This implies the endogenous generation of interferon within the CNS, i.e. during virus infection or other triggers. The significance of the presence of IFN-α in the choroid plexus is at present not understood and may have a metabolic rather than anti-viral function. However, during leakage of serum fluids over the blood-brain barrier during meningitis, tissue injury or during death, IFN-α may gain access to the CSF. Interestingly, data generated from the testing of CSF samples obtained from adult deaths indicated that 80% of cases had detectable levels of IFN-α present. These cases were reported as "infection free" hence the presence of IFN-α highlights the hypothesis that this protein may be produced as part of a metabolic event during death. It is clear that IFN-α present in CSF in life and after death may be derived by alternative mechanisms which may not be always infection related.

The infants investigated in the study were analysed for age distribution as a contribution to the presence of increased IFN-α levels in the variuos fluid samples tested. No conclusive evidence was found to suggest a positive relationship with age (Tables 36 and 37).

A small percentage of cot death cases have serum IFN-α levels >10 IU/ml (5%) a feature not noted for the paediatric death group. The low levels of serum IFN-α in the cot death cases is not likely to be retained from the placental transfer or amniotic fluid in late pregnancy

especially as the half life of IFN- α is a matter of hours. However, the possibility of a neonatal or congenital virus infection inducing circulating IFN- α cannot be ruled out to account for some cases with high IFN- α levels.

The NPA samples collected from cot death cases, both Glasgow and Edinburgh were positive at levels >10 IU/ml in 35% of the cases, although paediatric deaths showed evidence at 14% for IFN-α >10 IU/ml (Table 21). These results were confirmed in the mean values of IFN- α documented in Table 22. Evaluation of the cot death data in conjunction with the clinical data generated at the Virology Laboratory, RHSC, Glasgow by Salas (1988) indicated a similar range of IFN- α values to those in pneumonia and chronic lung disease. Bronchiolitis cases and upper respiratory tract infection similarly analysed did not show a similar range of IFN- α values when compared with cot death NPA samples. These results are remarkable as they may suggest that the cot death cases were in the very earliest stages of acute respiratory infection, prior to significant clinical symptomatology and inflammatory disease. This data provides an interesting perspective to this syndrome which has so far failed to provide an identifiable marker of disease.

The qualitative detection of IFN- α in positively stained cells in the peripheral lung by immunocytochemical methods did not correlate

with the raised IFN- α values noted by IRMA (Table 44). Samples of NPA for IRMA testing were obtained from the upper respiratory tract while sections of the lower respiratory tract were investigated by immunocytochemical staining. Qualitative evidence of IFN- α staining therefore could not be directly correlated with the quantitative IFN- α values observed in the NPA.

IFN- α was detected in this study in the alveolar macrophages in the peripheral lung sections(Figure 21). The source and role of these interferons are still unclear. Recent work has investigated the role of IFN- α from the mononuclear cells and indicated that these cells may be the main immunological cells of the primary response to viral infection, thereby releasing low levels of endogenous IFN- α . Published data showed that the presence of lymphocytes and mononuclear cells, in particular the alveolar macrophages in the tissues would enable local production of IFN-αin vivo by these cells after induction by the appropriate agents (Bocci, 1988).

The analysis of raised IFN- α values in the NPA, serum and CSF and the 'post mortem interval' indicated that there was no increased likelihood of obtaining high levels of IFN- α as time progresses after body death. These results counter the suggestion that the presence of IFN- α in the cot death cases was due to tissue injury and subsequent release of interferons(directly or indirectly).

The presence of endogenous IFN- α in virus positive cot death cases and paediatric deaths was documented in detail (Tables 32 and 33). Significant levels of IFN- α (i.e. $>$ 10 IU/ml) were observed in NPA but not in serum or CSF. Specimens tested for IFN- α during proven acute viral infection have indicated raised levels in these clinical samples, and its presence in cot death specimens raises further suspicion in support of the infection hypothesis. On first analysis raised IFN- α values in the NPA samples would appear to indicate that the 30% positivity rate for respiratory viruses in the cot death cases were confirming the isolation data. However, the raised IFN- α levels were not exclusively from the proven respiratory cases suggesting that isolation/virus detection systems are underestimating the total virus load in true respiratory tract infection of these infants.
It was clear from the results that as the cot death cases and paediatric deaths reported as virus positive had raised IFN- α values in the body fluids in only 50% of cases it was equally likely not to find IFN-α in these cases. This is a worrying statistic if this assay was used as the sole marker of viral disease.

It was surprising that in virus negative cases raised IFN- α levels were seen in all three samples tested(Tables 35 and 36). This is further evidence to highlight the need to consider viruses as yet "not identified", or conventional diseases at an early stage before detection by the clinician, pathologist or virologist.

It is clear from this study that conventional approaches to virus detection by tissue culture techniques and serology provide little useful data to the aetiology of cot death. A wide range of viruses is seen with no predominant type. However, the winter increase of cases and the summer low is such a regular feature of cot deaths throughout the world that a microorganism transmitted in the winter season must still be considered. The interferon data provided in this study goes some way to suggest that an as yet unidentified agent may exist, and if so, it is likely to be respiratory pathogen.

Raised interferon levels were consistently found in NPA samples from cot death cases in the two geographical locations and over two years of study. These levels are comparable with studies where clinical lower repiratory tract infection is suspected or proven, despite the absence of clinical or pathological signs of disease in cot deaths. This dilemma highlights the dilemma of the sudden infant death syndrome- a death in an apparently healthy baby.

Although this thesis is by no means conclusive, it points to interferon inducing agents released around the time of death in the majority of cot death cases and thus requires an explanation. An unidentified virus is as plausible as ever, but clearly it would be one with unusual properties and disasterous consequences. It is premature to discount the infection hypothesis.

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