PAPERS AND ORIGINALS

Observations on Clinical and Immunofluorescent Diagnosis of Parainfluenza Virus Infections

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Summary

Immunofluorescent techniques have been applied to nasopharyngeal secretions for the rapid diagnosis of parainfluenza virus types 1, 2, and 3 infections. Seventy-five infections were found by isolation techniques; 55 of these had nasopharyngeal secretions taken and 53 were positive by direct examination. A comparison of the results of 60 neutralization tests with immunofluorescence applied to monkey kidney isolations showed complete agreement. Immunofluorescence appeared to be a satisfactory method for differentiating the various haemadsorption viruses. The importance of parainfluenza viruses and respiratory syncytial virus in croup was noted and the association of the parainfluenza viruses with acute respiratory virus infection was confirmed. The clinical relationship between respiratory syncytial virus and parainfluenza virus type 3 is discussed.

Introduction

Since the first description of the parainfluenza virus group by Chanock *et al.* (1958, 1963) it has become evident that this group of viruses is a major cause of acute respiratory illness in the young, both for those admitted to hospital (Holzel *et al.*, 1963, 1965) and for those at home (Banatvala *et al.*, 1964), and in this country it is second in importance to respiratory syncytial (R.S.) virus. Over the past eight months there has been an increase in the occurrence of parainfluenza virus types 1, 2, and 3 in the Newcastle area, and it was considered opportune to re-examine both the clinical and the laboratory aspects of infection with this virus. The cases described were hospital admissions and showed a range of clinical syndromes varying from upper respiratory tract infection and croup to bronchiolitis and pneumonia. There was one death.

The diagnosis of parainfluenza virus infection has previously depended on the haemadsorption technique (Vogel and Shelokov, 1957) performed on monkey kidney cells, with confirmation of a virus isolate by either the haemadsorption neutralization or the haemadsorption inhibition test. During this study we introduced immunofluorescent techniques for the identification of parainfluenza virus types 1, 2, and 3, both for the direct examination of respiratory tract material and for the specific identification of these viruses on tissue culture. To date parainfluenza virus types 4a and 4b have not occurred in Newcastle, therefore immunofluorescent techniques for their identification have not yet been introduced.

This communication falls into two parts: laboratory details and certain clinical and epidemiological aspects.

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Materials and Methods

For a number of years all infants and children admitted with acute respiratory infections to Newcastle hospitals have been investigated for respiratory virus infection. This communication covers the period from February 1968 to November 1970. The clinical categories used and the precise method of obtaining both nasopharyngeal secretions and cough/nasal swabs have been described (Gardner *et al.*, 1960; Gardner, 1968; Sturdy *et al.*, 1969), and the immunofluorescent techniques are also identical with those used in our earlier studies (Gardner and McQuillin, 1968; McQuillin and Gardner, 1968; McQuillin *et al.*, 1970). During the period when the occurrence of parainfluenza virus infections were most frequent, between September 1969 and November 1970, all specimens suitable for examination by immunofluorescence were investigated for parainfluenza virus types 1, 2, and 3 when appropriate.

Reagents used for Immunofluorescence.—The reagents used were identical with those previously described for R. S. virus and influenza virus types A and B (Gardner and

McQuillin, 1968; McQuillin and Gardner, 1968; McQuillin *et al.*, 1970) except for the specific antisera for the parainfluenza viruses.

The preparation of these antisera was as follows:

Parainfluenza Virus Type 1.—A strain of Sendai virus was obtained from Dr. A. J. Beale (Glaxo Laboratories). The virus was grown in the allantoic cavity of fertile hens' eggs, and pooled allantoic fluid with a haemagglutinating titre of 1/1,024, which had been clarified by centrifugation, was used for rabbit inoculation. Human type 1 virus (Colindale) was not used because it did not grow on a continuous line of rhesus monkey kidney cells (LLC-MK2) or on fertile hen's eggs. It grew to high titre on rhesus monkey kidney cells but many batches of these contained simian viruses. The use of primary rhesus monkey kidney cells would also have created difficulties in absorbing the antiserum with this cell line. Moreover, preliminary experiments had shown that antiserum prepared against Sendai virus was equally effective in detecting both Sendai and human parainfluenza type 1 viruses in monkey kidney tissue culture.

Parainfluenza Virus Types 2 and 3.—Standard strains of parainfluenza virus types 2 and 3 were obtained from the Standards Laboratory, Central Public Health Laboratory, Colindale. They were inoculated into LLC-MK2 cells obtained from Flow Laboratories Ltd. Both viruses produced a pronounced cytopathic effect on these cells. They were passaged several times to produce a virus of high titre between 10^6 and 10^7. Degenerate cells were scraped off into maintenance medium and disintegrated ultrasonically. Cell debris was removed by centrifugation at 10,000 r.p.m. for 30 minutes and the supernatant used as the rabbit inoculum.

Rabbits were inoculated three times at seven-day intervals. On the first occasion 1 ml of virus was inoculated intravenously, 2 ml intraperitoneally, and 1 ml of virus, together with Freund's complete adjuvant, subcutaneously. On the two subsequent occasions the virus was inoculated intravenously and intraperitoneally, the final inoculum being 4 ml and 5 ml respectively. The rabbits' serum was tested for the presence of the appropriate parainfluenza virus antibody on the seventh day after the final inoculation. If the immunofluorescent titre of antibody tested in secondary rhesus monkey kidney cells infected with parainfluenza virus was satisfactory ($\geqslant 1 : 80$) the rabbit was exsanguinated.

All three antisera were absorbed twice with LLC-MK2, Bristol, HeLa, and HEp 2 cells to remove all non-specific activity against monkey kidney and human cells. They were then titrated by immunofluorescence on rhesus monkey kidney cells infected with the appropriate virus, and the highest dilution showing maximum fluorescence was taken as the optimum dilution. The sera were also titrated on uninfected rhesus monkey kidney and human cells to ascertain that all non-specific activity had been removed. In this way an optimum dilution for immunofluorescent studies was obtained. These antisera were then tested at their optimum dilutions for non-specific activity, either in tissue culture or nasopharyngeal secretions, against the other two parainfluenza viruses, R.S. virus, influenza viruses A and B, mumps virus, adenovirus, SV5, and various types of Coxsackievirus and echovirus. None of the three antisera gave any cross-reaction against the other two types of parainfluenza viruses in monkey kidney tissue culture, or with the other viruses against which they were tested. To ensure that non-specific activity against human material was absent, the antisera were also tested against cell preparations from nasopharyngeal secretions and lungs from which no virus had been isolated.

Patients without respiratory infection could not be used as "controls" because of the absence of sufficient secretion in the nasopharynx. Figures for virus isolations obtained from the examination of cough swabs taken from children without respiratory infection are quoted under Results. Chanock *et al.* (1958, 1961, 1963) made it clear that R.S. virus and parainfluenza virus seldom occur in the respiratory tract of those without respiratory symptoms.

Results

LABORATORY ASPECTS

During the 22 months from February 1968 to November 1970 75 children were infected with one or other of the parainfluenza viruses. The first 20 children were diagnosed by culture only, but the results of the neutralization tests were confirmed by immunofluorescence. Nasopharyngeal secretions from the other 55 patients were tested for the presence of virus by both culture and direct examination by immunofluorescence. During the same period 133 nasopharyngeal secretions were tested for at least one of the parainfluenza viruses by immunofluorescence. Comparison of the results of isolation of the virus on monkey kidney cells with the results of the direct examination of specimens by immunofluorescence is shown in Table I. The first 20 parainfluenza viruses isolated, which are mentioned above, were seven type 1, four type 2, and nine type 3; direct material for immunofluorescence was not available from these patients.

Nasopharyngeal secretions taken from patients from whom parainfluenza viruses were not isolated were examined (Table II). There were no false-positive results, and the antisera showed no cross-reaction with the variety of viruses present in some of these secretions.

TABLE I—*Direct Examination of Specimens from Patients by Immunofluorescence from Whom Parainfluenza Viruses Were Isolated. September* 1969 *to October* 1970

Type of Parainfluenza Virus	Total No. Isolated	Immunofluorescent Result	
		Positive	Negative
1	25	23	2
2	5	5	0
3	25	25	0
Total	55	53	2

Parainfluenza virus type 1: Co-positivity 23/25 (92%), co-negativity 128/128 (100%), overall agreement 151/153 (98·7%).
Parainfluenza virus type 2: Co-positivity 5/5 (100%), co-negativity 87/87 (100%), overall agreement 92/92 (100%).
Parainfluenza virus type 3: Co-positivity 25/25 (100%), co-negativity 133/133 (100%), overall agreement 158/158 (100%).
Combined parainfluenza virus: Co-positivity 53/55 (96·4%), co-negativity 348/348 (100%), overall agreement 401/403 (99·5%).
Figures for co-negativity obtained from Table II (After Buck and Gart, 1966)

TABLE II—*Immunofluorescent Examination of Nasopharyngeal Secretion from which Parainfluenza Viruses Were not Isolated. September* 1969 *to October* 1970

Type of Parainfluenza Virus Antisera against Each Specimen Tested	No. of Viruses other than Parainfluenza Virus Isolated						Total Negative by Culture	Total Tested Negative by Culture and Immunofluorescence for Parainfluenza Virus
	R.S. Virus	Adenovirus	Influenza B	H-strain Rhinovirus	M-strain Rhinovirus	Enterovirus		
1	13	*7	*2	*4	2	3	99	128
2	7	*4	*2	*3	*2	1	70	87
3	9	*8	*2	*4	2	3	107	133

*One patient had a multiple infection with an H-strain rhinovirus, adenovirus type 2, and influenza B.

Of the three types of parainfluenza virus, type 1 was the most difficult to recognize in nasopharyngeal secretions because, on occasions, specimens showed only faint intracellular, though specific, fluorescence.

FLUORESCENT APPEARANCES

Fluorescence of the antigen in the three types of parainfluenza viruses in monkey kidney cells is restricted to the cytoplasm and appears not only as fine or coarse particles but frequently as strands of fluorescence which form a reticulum. A monkey kidney cell infected with parainfluenza

virus type 1, examined 36 hours after inoculation with a nasopharyngeal secretion, is shown in Fig. 1.

In nasopharyngeal secretions most cells showing fluorescence are ciliated columnar epithelial cells. The fluorescence is always cytoplasmic but varies in form in different specimens. In the few nasopharyngeal secretions infected with parainfluenza virus type 2 fluorescence is very similar to that shown by R.S. virus, with fine particles and large spherical or oval inclusion-like bodies (Fig. 2). Parainfluenza virus type 1 often produces strands of fluorescence (Fig. 3) and coarse aggregates, but some specimens have also shown cells with fine particles and large inclusions. Parainfluenza virus type 3 often produces the particulate type of fluorescence seen with R.S. virus and parainfluenza virus type 2, but a number of secretions have shown coarse aggregates and a few have shown strands of fluorescence in some cells (Fig. 4). The nasopharyngeal secretion cells infected with parainfluenza virus type 3 illustrated in Fig. 4 show coarse aggregates and fine particles of virus antigen fluorescence. In the preparations of cells from the tracheal and bronchial secretions of a patient who died, and from whom parainfluenza virus type 1 was isolated, large numbers of ciliated epithelial cells were found containing one or more very large spherical, oval, or irregular inclusion-like bodies, all of which fluoresced with varying degrees of intensity. In some cells fine particles were distributed throughout the cytoplasm. The fluorescence was strongest in the cells from the trachea (Fig. 5). Necropsy appearance was that of an acute laryngotracheobronchitis with no microscopical or gross abnormality of the lungs.

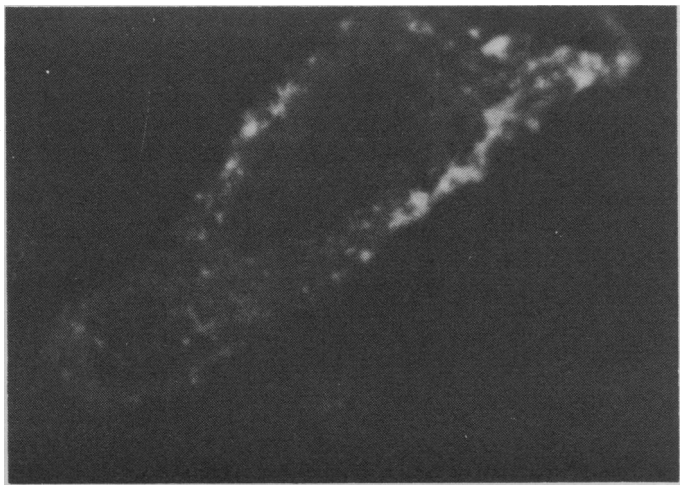

FIG. 1—Monkey kidney cell showing cytoplasmic fluorescence of parainfluenza virus type 1 antigen 36 hours after inoculation with a specimen of nasopharyngeal secretion. (× 2,710).

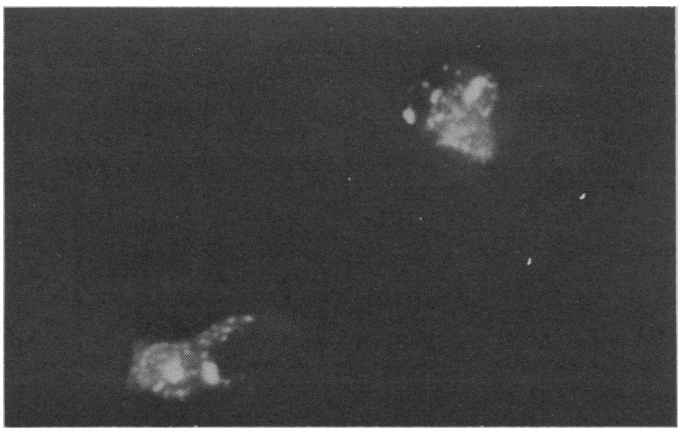

FIG. 2—Parainfluenza virus type 2 antigen in two ciliated epithelial cells in a nasopharyngeal secretion showing fine particles and larger inclusion-like bodies fluorescing in the cytoplasm. (× 2,400).

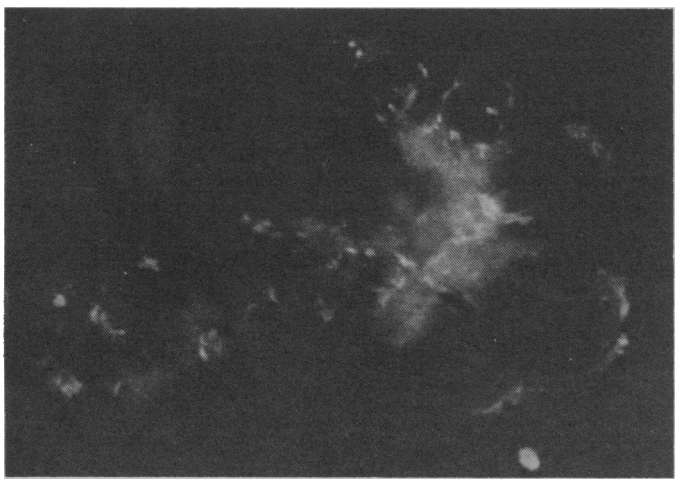

FIG. 3—Parainfluenza virus type 1 antigen in a group of ciliated epithelial cells in nasopharyngeal secretion showing cytoplasmic fluorescence in stranded. form (× 1,870).

FIG. 4—Parainfluenza virus type 3 antigen in cells of a nasopharyngeal secretion showing cytoplasmic fluorescence in the form of coarse aggregates, fine particles, and occasional strands. (× 1,735).

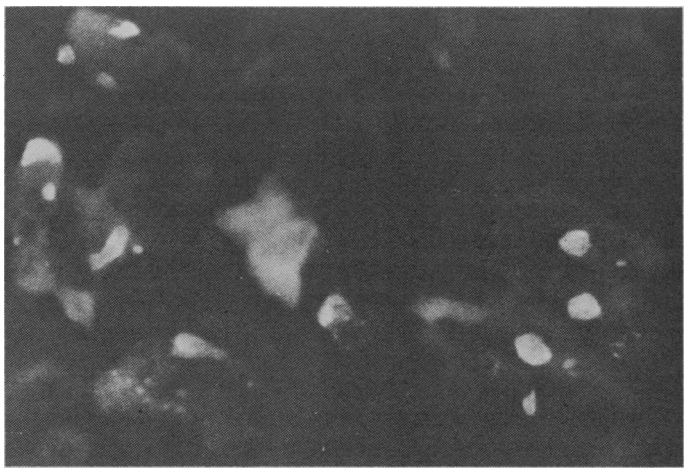

FIG. 5—Parainfluenza virus type 1 antigen in ciliated epithelial cells in post-mortem specimen of tracheal secretion showing bright cytoplasmic fluorescence in the form of large spherical, oval, or elongated inclusion-like bodies and small particles. (× 1,735).

Cells eluted from cough swabs and stained by the immunofluorescent technique for the three parainfluenza viruses can be used in diagnosis. The appearance of parainfluenza virus types 1 and 3 in cough swab preparations is identical with that seen in nasopharyngeal secretions. So far six out of six cough swabs for parainfluenza virus type 3 and one out of three cough swabs for parainfluenza virus type 1 have been successfully diagnosed by direct immunofluorescence on eluted cells.

TABLE III—*Comparison between the Haemadsorption Neutralization Tests and the Fluorescent Antibody Technique on Monkey Kidney Cells*

Type of Parainfluenza Virus	Result of Fluorescent Antibody and Neutralization Tests on Monkey Kidney Cells		
	No. Isolated Which Were Tested by Both Techniques	Neutralization Tests	Fluorescent Antibody Technique
1	26	26	26
2	9	9	9
3	25	25	25
Total	60	60	60

A comparison of the results of immunofluorescence with the standard haemadsorption neutralization tests (Table III) shows that there was complete agreement between the two methods. It was decided to limit the comparison to about 25 for each type of virus, by which time the reliability of the fluorescent antibody technique would either be proved or disproved for use as a routine test.

CLINICAL ASPECTS

Parainfluenza viruses are associated with about 8% of all acute respiratory infections in children admitted to hospital. Their relative importance is shown in Table IV, which also confirms the strong association of parainfluenza viruses with croup, and shows that parainfluenza virus type 3 is far more often associated with lower respiratory tract infections than types 1 and 2.

TABLE IV—*Association between Clinical Category and Parainfluenza Virus Isolation*

Clinical Category	Total No. of Children Tested	Parainfluenza Virus			% Associated with Parainfluenza Virus
		Type 1	Type 2	Type 3	
Pneumonia ..	189	2	0	6	4·2
Bronchiolitis ..	227	1	0	10	4·8
Bronchitis	262	4	0	7	4·2
Croup	94	18	10	5	35·1
Upper respiratory tract infection ..	154	4	0	7	7·1
Total	926	29	10	35	8·0

TABLE V—*Viruses Associated with 94 Cases of Croup*

Virus	No. of Cases	% of Total
Parainfluenza 1	18	19·1
Parainfluenza 2	10	10·6
Parainfluenza 3	5	5·3
R.S. virus	10	10·6
Rhinovirus H	2	2·1
Rhinovirus M	1	1·1
Adenovirus 6	1	1·1
Influenza A	3	3·2
Coxsackie B4	1	1·1
Echovirus type 21	1	1·1
No virus isolated	42	44·7
Total	94	100·0

The association of all viruses, including the parainfluenza viruses, with the clinical condition of croup is shown in Table V. Though parainfluenza viruses are the main causal agents, R.S. virus plays a significant part. The severity of illness in croup varies from mild to severe, depending on the degree and speed of development of laryngeal obstruction. It may well be an important observation that out of 33 patients infected with parainfluenza virus croup was considered severe in 28 (84%), whereas in 51 children with croup from whom parainfluenza virus was not isolated the illness was severe in only 22 (44%) ($\chi^2=9.7$; $P<0.005$). When patients with croup, and infected with parainfluenza virus, were compared for severity of illness with those infected with other viruses the difference was again highly significant ($\chi^2=16.5$; $P<0.005$).

To place these findings in perspective the viruses isolated over one year of the same period from 304 children with acute lower respiratory tract infection but without croup are reported. There were 124 R.S. viruses (40·8%), 13 parainfluenza viruses (4·3%), 12 adenoviruses (4%), 8 rhinoviruses (2·6%), 6 influenza viruses (2%), 5 herpesviruses (1·6%), and 4 echo and coxsackieviruses (1·3%). During investigations of acute respiratory infections in Newcastle cough swabs were taken from 109 children without respiratory infection and were used as "controls." From them five adenoviruses, two herpesviruses, and three Coxsackieviruses were isolated. Neither R.S. virus nor parainfluenza viruses were isolated from this group.

It has already been noted that parainfluenza virus type 3 is often associated with lower respiratory tract infection. It was considered important to compare the age distribution of lower respiratory tract infection caused by this virus with R.S. virus and parainfluenza virus types 1 and 2. This is given in Fig. 6,

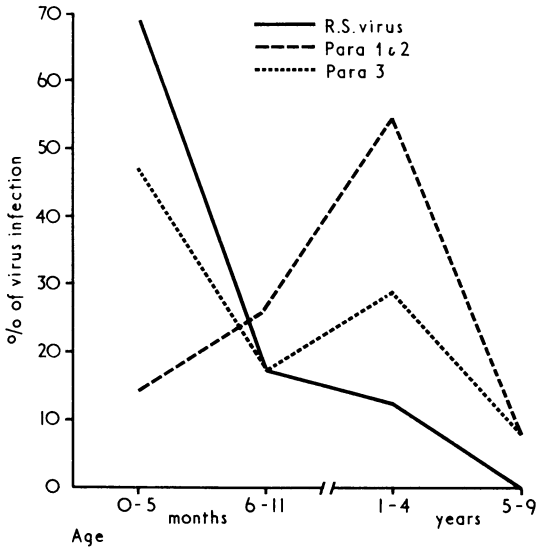

FIG. 6—Age incidence of R.S. virus and parainfluenza virus infection in children. February 1968 to October 1970.

which shows the similar age distribution of parainfluenza virus type 3 and R.S. virus and contrasts sharply with that for parainfluenza virus types 1 and 2. The small number of patients between 1 and 4 years of age infected with parainfluenza virus type 3 are those with croup.

Discussion

An effective and reliable method for the rapid diagnosis of parainfluenza virus infections by immunofluorescence within 24 hours of the patient's admission to hospital has been established by using cells from nasopharyngeal secretions; co-positivity and co-negativity estimates have confirmed the reliability of the test (Buck and Gart, 1966). Cells eluted from cough swabs could also be stained, but this method appeared to be less sensitive. The conventional method for the isolation of parainfluenza virus is on primary or secondary monkey kidney cells, the haemadsorption test for detection and the haemadsorption neutralization test for identification being used. Monkey kidney cells are not always available and are occasionally contaminated with simian haemadsorption viruses (SV5 and SV41), which makes detection of human parainfluenza viruses difficult. Furthermore, parainfluenza viruses in specimens are not easy to store, and attempts to

reisolate a virus from a stored specimen often fail. For these reasons, too, the use of immunofluorescence for the diagnosis of infection with parainfluenza virus has proved valuable.

It is now possible to diagnose by immunofluorescence all the known major virus pathogens causing acute respiratory infections in children, and a technique applicable to all of them is now available (Gardner and McQuillin, 1968; McQuillin and Gardner, 1968; McQuillin *et al.*, 1970). Immunofluorescence for the rapid diagnosis of acute respiratory infection must be used with discretion. To make it economic, factors such as local prevalence of a virus, the clinical condition, season of year, and age of patient must be considered. For example, R.S. virus infects children mainly under the age of 1 year; the most frequent cause of bronchiolitis is R.S. virus, and though parainfluenza virus infections by types 1 and 3 may occur in epidemics they are less frequent than those caused by R.S. virus and less often restricted to winter. With such discrimination immunofluorescence can become an invaluable technique in diagnostic virology.

Immunofluorescence has also proved reliable for the detection of parainfluenza viruses in monkey kidney tissue culture. We have found no significant cross-reaction between the three types investigated on monkey kidney cells and a minimal cross-reaction between parainfluenza virus type 1 antiserum and parainfluenza type 3 virus in cells of nasopharyngeal secretions; therefore, both antisera should be used in parallel. So far there are no reports by other workers of direct examination of nasopharyngeal secretions by immunofluorescence for parainfluenza viruses. Chin (1963) and Fedová and Zelenková (1969) found some cross-reaction between the parainfluenza virus types and other haemadsorption viruses on monkey kidney cells. The identification of a haemadsorption virus is time-consuming when the haemadsorption neutralization test is used, but the same identification can be made as effectively in a few hours by immunofluorescence. In routine virology a frequent problem is the identification of a haemadsorption virus on monkey kidney cells.

The following haemadsorption viruses can now be identified immediately: parainfluenza virus types 1, 2, and 3, and influenza virus A and B. These are the most likely pathogens to give a positive haemadsorption reaction. They show no cross-reaction by immunofluorescence with influenza C, mumps, or SV5, which are also haemadsorption viruses. We have no information about their cross-reaction with parainfluenza virus types 4a and 4b. Mumps and influenza C can be identified by a complement fixation test and SV5 with parainfluenza virus types 4a and 4b by a haemadsorption neutralization test. This system has simplified the identification of a haemadsorption virus.

Variation in the types of cytoplasmic fluorescence is seen with different parainfluenza viruses in nasopharyngeal cells from patients. No significance can be attached to these findings without the investigation of a larger number of parainfluenza virus infections and having available the precise details of the stage of illness at the time when nasopharyngeal specimens are examined. Differences in the appearance of fluorescence of parainfluenza virus antigens in various tissue culture cells have been described by many workers, with reference to the development of the type of antigen (S soluble or V virus particle) in different sites of the cell, according to the stage of infection (Zhdanov *et al.*, 1965; Howe *et al.*, 1967; Sominina *et al.*, 1967; Fedová and Zelenková, 1969).

The cause of the weak fluorescence occasionally observed in the cells of nasopharyngeal secretions in parainfluenza virus type 1 infections has yet to be determined. It may be due to an antigenic difference between the infecting strain and the strain of parainfluenza virus type 1 (Sendai) used to prepare the antiserum. Alternatively, it could be due to a "blocking" of staining by local antibody in a later stage of illness, similar to that observed in R.S. virus infections (Gardner *et al.*, 1970).

Parainfluenza viruses may also be associated with fatal respiratory illness. Parainfluenza virus type 3 was found associated with pneumonia in an infant death (Aherne *et al.*, 1970), and in the present series parainfluenza virus type 1 was associated with death in an infant with acute laryngotracheobronchitis. In this second child virus was found by both immunofluorescence and isolation techniques in the nasopharyngeal secretions before death and in tracheal and bronchial secretions after death, but not in the lungs. This was in agreement with the necropsy findings when the lungs appeared normal, both macroscopically and microscopically. Parainfluenza virus type 1 variety Sendai was first described as a cause of death in the newborn by Sano *et al.* (1953) and Kuroyo *et al.* (1953), but the relationship of virus to illness was obscured by the isolation of virus in mice, known on occasions to carry this virus.

CLINICAL IMPORTANCE

Chanock *et al.* (1963) first showed the clinical importance of these three parainfluenza viruses in a five-year investigation of children admitted to hospital with acute respiratory infections and comparing them with controls. They found that 29% of croup cases was caused by these viruses and also a small but significant number of acute lower respiratory infections. Parainfluenza viruses were also associated with 6% of all acute respiratory infections; a similar incidence was found by Van der Veen Smeur (1961) in Holland. The results obtained in the present study are similar to those previously recorded, but the importance of parainfluenza virus types 1 and 2 as causes of croup is noteworthy; parainfluenza virus type 2 was not associated with any other acute respiratory syndrome. The severity of croup also appeared to be related to the causal organism, the illness being more severe in patients infected with parainfluenza viruses than in those infected with other viruses or in those in whom no virus was isolated. Parainfluenza virus type 3 and, to a smaller extent, type 1 caused acute lower respiratory infections.

The clinical features of acute lower respiratory tract infections caused by parainfluenza virus type 3 are similar to those associated with R.S. virus infections. A comparison was therefore made of the age distribution of parainfluenza virus type 3 infections with those caused by R.S. virus (Fig. 6). They both caused severe lower respiratory infections in those under 6 months, which was not a feature of parainfluenza virus type 1 and 2 infections. All three parainfluenza viruses and R.S. virus may cause croup in those over 1 year of age.

Chanock *et al.* (1970) speculated that a type 3 allergic reaction (Gell and Coombs, 1968) involving the formation of toxic complexes by virus and circulating maternal IgG may be a factor in the pathogenesis of severe R.S. virus infections of infancy. Gardner *et al.* (1970), though not ruling out a type 3 reaction, suggested a type 1 reaction involving sensitization by previous exposure to the ubiquitous R.S. virus as a more likely explanation. Though severe lower respiratory infections of infancy may have similar pathogenesis in the two virus infections, parainfluenza virus type 3 epidemics are less frequent than those due to R.S. virus. This may account for minor differences in the percentage distribution of the two infections in older children (Fig. 6).

This communication has shown the position of the parainfluenza viruses as pathogenic agents in acute respiratory infections in children and the techniques which can be reliably used in their rapid diagnosis and identification. The similarity in the clinical behaviour of parainfluenza virus type 3 and R.S. virus suggests the need for the further investigation of the pathogenesis of illness associated with these two viruses.

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Coagulation and Fibrinolytic Systems in Pre-eclampsia and Eclampsia

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Summary

The coagulation and fibrinolytic mechanisms were investigated in a group of patients with severe pre-eclampsia and eclampsia and the findings were compared with those of healthy women in late pregnancy. In patients with pre-eclampsia the following significant differences were found: (1) greater depression of plasma fibrinolytic activity (euglobulin lysis time) than in normal pregnancy, (2) a higher level of inhibitor to urokinase-induced lysis, (3) increased levels of serum fibrin degradation products, and (4) reduced platelet counts.

In patients with eclampsia a progressive increase of the level of serum fibrin degradation products was found over the three days following eclamptic seizures. No such increase occurred after grand mal seizures in late pregnancy. The findings in this study support the view that intravascular clotting is taking place in pre-eclampsia and that this disturbance of the balance between coagulation and fibrinolysis may be localized to certain areas of the vascular compartment, particularly the placental and renal circulations. Fibrin deposition in the maternal vessels supplying the placenta would impair the placental blood flow, which may explain the placental insufficiency which occurs in pre-eclampsia. Likewise fibrin deposition in the renal vasculature will result in glomerular damage and proteinuria. Hypertension may be related to the renal ischaemic changes or a compensatory response to the presence of fibrin deposition in the vascular compartment. This evidence of intravascular fibrin deposition raises the question of the possible therapeutic value of antithrombotic agents to inhibit the clotting process. On a theoretical basis such treatment might be expected to improve blood flow to the placenta and thereby fetal growth.

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Introduction

The aetiology of pre-eclampsia remains obscure. Jeffcoate (1966) summarized many of the pathological factors known to be associated with pre-eclampsia and affirmed that it was a disease of theories; the possible role of the coagulation and fibrinolytic systems was not included and this aspect has received little attention. In some fatal cases of eclampsia, however, a prominent finding has been widespread fibrin deposition (McKay *et al.*, 1953). Electron microscopical study of tissue obtained by renal biopsy from patients with pre-eclampsia has revealed swelling of the glomerular capillary endothelium and deposition of an amorphous fibrinoid material within the cells and beneath the basement membrane (Pollak and Nettles, 1960; Altchek, 1961). Morris *et al.* (1964) using immunofluorescent techniques showed that the material in the glomeruli was identical to fibrin.

Recently evidence has been accumulating that the fibrinolytic system may be implicated in the mechanisms which influence blood pressure and blood flow (Niewiarowski, 1968). The physiological role of plasminogen activator is most likely to keep the blood vessels free of fibrin deposits, and in situations where the secretion or action of plasminogen activator is impaired fibrin deposition may be more likely to occur. To our knowledge there have been no detailed and serial studies of components of the fibrinolytic enzyme system in a well-defined group of patients with severe pre-eclampsia and eclampsia. The following investigation was undertaken to