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The use of cough/nasal swabs in the rapid diagnosis of respiratory syncytial virus infection by the fluorescent antibody technique

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SUMMARY

Thirty-five consecutive infants admitted into hospital in Newcastle upon Tyne with acute respiratory disease had cough/nasal swabs and nasopharyngeal secretions taken. Both types of specimens were examined by the fluorescent antibody technique for respiratory syncytial virus; isolation techniques were also used. Twenty-eight specimens of nasopharyngeal secretion were positive, as were 26 of the corresponding cough/nasal swab preparations. Respiratory syncytial virus was isolated from all but one.

Sixteen consecutive children who were only suitable for examination by cough/nasal swab preparations were also investigated by isolation and fluorescent antibody techniques for respiratory syncytial virus. Respiratory syncytial virus was isolated from eight, seven of whom were positive by the fluorescent antibody technique. The use of cough/nasal swab preparations stained by the fluorescent antibody technique, although not as efficient as nasopharyngeal secretions, may have a place in the rapid diagnosis of respiratory virus infection in older children and children in general practice. The importance of rapid diagnosis for respiratory virus infection in relationship to antiviral therapy was also discussed.

INTRODUCTION

The rapid diagnosis of acute respiratory infection in childhood has made considerable progress over the last few years along two main lines. Some workers have examined nasopharyngeal secretions by electron microscopy and have attempted to make provisional diagnoses on the appearance of virus particles present (Doane *et al.* 1967; Joncas *et al.* 1969; Doane, Anderson, Zbitnew & Rhodes, 1969). Others have applied fluorescent antibody techniques to secretions from the nasopharynx; a rapid diagnosis for respiratory syncytial (R.S.) virus (McQuillin & Gardner, 1968; Gardner & McQuillin, 1968*a*), and for influenza (Hers, van der Kuip & Masurel, 1968) has been investigated in this way.

Collection of nasopharyngeal secretions is a technique only suitable for use with children who are less than 2 years of age and who are in hospital. It is not suitable for use outside hospital. Portable suction equipment is required unless each ward has suitable suction facilities. Claims have been made for a successful fluorescent

antibody technique for the rapid diagnosis of R.S. virus infection by smearing throat swabs directly on to slides (Gray, MacFarlane & Sommerville, 1968) but, on analysis, these results were not considered sufficiently reliable (Miller & Taylor, 1968; Gardner & McQuillin, 1968*b*). In 1968 McQuillin & Gardner had tried this method and found it unsuitable because of the absence of intact cells and the large amount of debris.

This communication describes a revised method for studying cells obtained from cough/nasal swabs after eluting, washing, concentrating and fixing them on slides, so producing preparations of intact cells suitable for staining by the fluorescent antibody technique. The results of staining cells taken on cough/nasal swabs in this way by the fluorescent antibody technique for R.S. virus have been compared with those of staining cells in materials taken by suction from the nasopharynx. Haire (1968), too, has recently reported the successful use of throat swabs stained by a similar technique for the rapid diagnosis of infection with rubella virus.

MATERIALS AND METHODS

Thirty-five cough/nasal swab preparations were taken from consecutive children who were also suitable patients for aspiration of nasopharyngeal secretions; in addition, 16 consecutive patients who were unsuitable for aspiration only had cough/nasal swabs taken. All specimens were examined both by isolation and by the fluorescent antibody techniques.

Nasopharyngeal secretions

The technique of taking and preparing nasopharyngeal secretions has been described fully elsewhere (Sturdy, McQuillin & Gardner, 1969).

Cough/nasal swabs

The patient's pharynx was swabbed thoroughly until he gagged and coughed onto the swab; in addition, a nasal swab was taken. The swabs were then broken in the same bijoux bottle of transport medium (Hanks' medium with 0·2 % bovine albumen and antibiotics) and the specimen transported to the laboratory on melting ice.

The medium in the specimen bottle was pipetted over the swabs until the secretions adhering to them were suspended. The medium was transferred to another bottle and the original bottle containing the swabs was kept at a temperature of 4°C. The medium was centrifuged at 500 rev./min. for 10 min. in a refrigerated centrifuge to deposit the cells. The supernatant fluid was removed and returned to the original bottle (kept at a temperature of 4°C.), to be used for subsequent virus isolations. The cell deposit was re-suspended in phosphate buffered saline to wash the cells and again centrifuged at 500 rev./min. for 10 min. The resultant washed cell deposit was re-suspended in a small volume of phosphate buffered saline, sufficient to make two or three slides with three spots of cell deposit on each, the number of slides depending on the amount of cell deposit available. The cell spots were allowed to dry in air, then fixed in acetone at 4°C. for 10 min.;

they were then ready for staining by the fluorescent antibody technique, as described in previous publications (McQuillin & Gardner, 1968; Gardner & McQuillin, 1968*a*).

The isolation and identification of viruses

The supernatant fluids from cough/nasal swab specimens stored at 4°C. were used for attempted virus isolations. This material was inoculated in 0·2 ml. volumes on two tubes each of 'Bristol' HeLa, HEp 2 and rhesus monkey kidney cells and on one tube of W.I. 38 cells. The preparation of nasopharyngeal secretions has been described in previous publications, as has the isolation of viruses from them; the identification of virus strains has also been previously described (McQuillin & Gardner, 1968; Sturdy *et al.* 1969).

Fluorescent antibody methods for the identification of viruses

The fluorescent antibody technique used in this study to identify R.S. virus in exfoliated cells from the nasopharynx and to confirm the identity of iolations of R.S. virus were the same as those described elsewhere (McQuillin & Gardner, 1968; Gardner & McQuillin, 1968*a*).

All specimens of cough/nasal swabs and nasopharyngeal secretions stained by the fluorescent antibody technique were examined by two of the authors; the cough/nasal swabs were also re-examined under coded numbers without knowledge of the results of the previous examinations.

RESULTS

Surprisingly large numbers of intact cells were found on slides prepared from cough/nasal swab specimens, but these were mainly squamous cells and a great deal of debris was always present, some of which gave a uniform green non-particulate fluorescence and some a bright orange fluorescence, quite different from the specific particulate apple-green fluorescence of R.S. virus. Infected cells exhibiting specific fluorescence in cough/nasal swab preparations appeared to be small ones, probably macrophages and cells shed from the respiratory tract; occasionally, ciliated cells were observed. All these cells appeared to be the same type as those seen to be infected in nasopharyngeal secretions.

In this present series, infected cells were found in all the positively stained nasopharyngeal secretions within minutes of examining them under the fluorescent microscope. On the other hand, infected cells in cough/nasal swab preparations were very scanty and an intensive search, taking in many cases up to 20 min., was required to locate them. The fluorescence of positive cells in cough/nasal swab preparations was of lower intensity and it was more difficult to find an intact cell containing fluorescent particles or inclusions, which we have stipulated as being one of our basic requirements for making a positive diagnosis; in many cases, the cells exhibiting positive fluorescence appeared to be damaged. However, in all those cases except two, where the nasopharyngeal secretions were positive, cough/nasal swab preparations were also positive.

Table 1. *Results of examination of cough/nasal swabs and nasopharyngeal secretions stained by fluorescent antibody technique for respiratory syncytial virus, and virus isolation on tissue culture*

Patient	Diagnosis	Age (months)	Specimen	Result of F.A.	Result of Virus isolation	Day of isolation
M. M.	Bronchiolitis	1¼	C/swab	+	R.S.V.	11
			N.P.S.	+	R.S.V.	7
P. R.	Ac. bronchitis	4	C/swab	−	P. inf. 3	.
			N.P.S.	−	P. inf. 3	.
P. K.	Bronchiolitis	3	C/swab	+	R.S.V.	6
			N.P.S.	+	R.S.V.	7
M. A.	Ac. bronchitis	11	C/swab	+	R.S.V.	6
			N.P.S.	+	R.S.V.	6
G. C.	Croup	36	C/swab	−	Nil	.
			N.P.S.	−	Nil	.
J. S.	Pertussis	6	C/swab	−	Adeno 5	15
			N.P.S.	−	Adeno 5	11
G. W.	Ac. bronchitis	2½	C/swab	+	R.S.V.	13
			N.P.S.	+	R.S.V.	6
R. S.	Bronchiolitis	2½	C/swab	+	R.S.V.	13
			N.P.S.	+	R.S.V.	4
A. R.	Bronchitis	21	C/swab	−	Nil	.
			N.P.S.	−	Nil	.
S. R.	Bronchitis	7	C/swab	+	R.S.V.	16
			N.P.S.	+	R.S.V.	9
S. T.	Bronchitis	1½	C/swab	+	Nil	.
			N.P.S.	+	Nil	.
S. W.	Bronchitis	10	C/swab	+	R.S.V.	14
			N.P.S.	+	R.S.V.	8
K. C.	Bronchitis	10	C/swab	+	R.S.V.	17
			N.P.S.	+	R.S.V.	14
N. O.	Bronchitis	1¼	C/swab	−	P. inf. 3	.
			N.P.S.	−	P. inf. 3	.
S. H.	Bronchiolitis	9	C/swab	+	R.S.V.	8
			N.P.S.	+	R.S.V.	6
H. S.	Bronchiolitis	3	C/swab	+	R.S.V.	6
			N.P.S.	+	R.S.V.	6
H. W.	Bronchiolitis	7	C/swab	−	Nil	.
			N.P.S.	−	Nil	.
P. T.	Bronchiolitis	2	C/swab	+	R.S.V.	14
			N.P.S.	+	R.S.V.	6
A. B.	Bronchiolitis	4	C/swab	−	Nil	.
			N.P.S.	−	Nil	.
L. N.	Coryza	1	C/swab	+	R.S.V.	14
			N.P.S.	+	R.S.V.	12
K. P.	Bronchiolitis	5	C/swab	+	R.S.V.	13
			N.P.S.	+	R.S.V.	11
D. R.	Bronchiolitis	7	C/swab	+	R.S.V.	10
			N.P.S.	+	R.S.V.	4
D. K.	Bronchiolitis	1¼	C/swab	+	R.S.V.	11
			N.P.S.	+	R.S.V.	12
G. H.	Bronchiolitis	2½	C/swab	+	R.S.V.	15
			N.P.S.	+	R.S.V.	4
S. B.	Bronchiolitis	2¼	C/swab	+	R.S.V.	11
			N.P.S.	+	R.S.V.	4

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Table 1. (*cont.*)

Patient	Diagnosis	Age (months)	Specimen	Result of F.A.	Result of Virus isolation	Day of isolation
S. M.	Coryza	¾	C/swab	+	R.S.V.	10
			N.P.S.	+	R.S.V.	9
W. I.	Bronchiolitis	3	C/swab	+	R.S.V.	14
			N.P.S.	+	R.S.V.	7
P. M.	Bronchiolitis	1	C/swab	+	R.S.V.	11
			N.P.S.	+	R.S.V.	9
T. F.	Bronchitis	3	C/swab	−	R.S.V.	11
			N.P.S.	+	R.S.V.	11
C. C.	Bronchiolitis	3	C/swab	−	R.S.V.	18
			N.P.S.	+	R.S.V.	21
S. H.	Wheezy bronchitis	4	C/swab	+	R.S.V.	8
			N.P.S.	+	*Polio	.
R. R.	Bronchiolitis	15	C/swab	+	R.S.V.	9
			N.P.S.	+	R.S.V.	9
S. R.	Croup	21	C/swab	+	R.S.V.	18
			N.P.S.	+	R.S.V.	10
T. R.	Wheezy bronchitis	2½	C/swab	+	R.S.V.	15
			N.P.S.	+	R.S.V.	13
M. B.	Wheezy bronchitis	9	C/swab	+	R.S.V.	8
			N.P.S.	+	R.S.V.	8

* Oral polio vaccine recently given. Difficulty in isolating R.S.V. except in cough/nasal swab.

F.A. = Fluorescent antibody. C/swab = Cough/nasal swab. N.P.S. = Nasopharyngeal secretion. R.S.V. = Respiratory synctial virus. P. inf. 3 = Parainfluenza virus type 3. Adeno. 5 = Adenovirus type 5.

In those 16 children from whom only cough/nasal swabs were taken, seven were positive by direct fluorescence for R.S. virus; all were confirmed by virus isolation. One R.S. virus isolation was made from a child in this group in whom the cough/nasal swab preparation was negative by the fluorescent antibody technique. Plate 1, figs. 1 and 2 show cells eluted from the cough/nasal swabs of two infants with bronchiolitis and stained by the indirect fluorescent antibody technique for R.S. virus. Plate 1, fig. 1 shows a considerable amount of non-specific fluorescence, but nevertheless specific fluorescence is still detectable in two cells; Pl. 1, fig. 2 shows that occasional cells showing positive fluorescence are as easily detected as they are in nasopharyngeal secretions. Plate 1, fig. 3 shows cells in a nasopharyngeal secretion stained in the same way; there is little background non-specific staining.

Table 1 summarizes the results obtained by examination of the nasopharyngeal secretions and cough/nasal swabs in parallel from our series of infants. Table 2 summarizes the results in those patients from whom only cough/nasal swabs were taken. The results of virus isolations are also included, as is the time taken for identification of R.S. virus in tissue culture. The average time taken for the isolation of R.S. virus from cough/nasal swabs is longer than that from nasopharyngeal secretions and confirms our observations as to the relative scantiness of infected cells in cough/nasal swab preparations and also our previous comparison of cough/nasal swabs and nasopharyngeal secretions (Sturdy *et al.* 1969). Table 3 compares

the efficacy of cough/nasal swab preparations with nasopharyngeal secretions, both stained by the fluorescent antibody technique for R.S. virus. Table 4 compares the efficiency of cough/nasal swab preparations with the standard isolation techniques in those children from whom it was impossible to obtain nasopharyngeal secretions.

Table 2. *A comparison of virus isolation on tissue culture, and the examination of cells on cough/nasal swabs for respiratory syncytial virus by the fluorescent antibody technique*

Patient	Diagnosis	Age	Result of F.A. for R.S.V.	Result of Virus isolation	Day of isolation
B. D.	Pneumonia	2 years	+	R.S.V.	8
W. C.	Pneumonia	4 years	+	R.S.V.	11
S. K.	Pneumonia	4 years	+	R.S.V.	11
A. B.	Febrile convulsions	2 years	+	R.S.V.	5
M. G.	U.R.T.I.* and febrile convulsions	14 months	+	R.S.V.	10
L. H.	U.R.T.I.	5 years	+	R.S.V.	8
L. D.	Bronchitis	2 years	−	—	.
M. B.	U.R.T.I.	5 years	−	—	.
L. H.	Pneumonia	2 years	−	—	.
G. M.	Pneumonia	6 years	+	R.S.V.	15
T. F.	Bronchitis	2½ years	−	—	.
T. C.	U.R.T.I.	5 weeks	−	—	.
J. H.	U.R.T.I.	3 years	−	—	.
B. R.	Bronchitis	20 months	−	—	.
D. J.	Croup	6 years	−	R.S.V.	8
I. M.	Croup	3 years	−	P. inf. 1.	.

* U.R.T.I. = Upper respiratory tract infection. F.A. = Fluorescent antibody. R.S.V. = Respiratory syncytial virus. P. inf. 1 = Parainfluenza virus type 1.

DISCUSSION

It is less distressing and more practicable to take a cough/nasal swab than to aspirate the nasopharynx in children over the age of 1 year. Moreover, cough/nasal swabs can be taken from children of all ages, whether at home or in hospital, whereas nasopharyngeal secretions can only be obtained easily from patients under the age of 2 years in hospital.

There is no doubt that the examination of nasopharyngeal secretions is the method of choice and where possible they should be used, for, not only are the infected cells far more abundant, but the material is also a much better source of virus for isolation procedures (Sturdy *et al.* 1969). Preparations of nasopharyngeal secretions take far less time to examine than the corresponding specimen of cough/nasal swab. However, in the very young infant or the older child where nasopharyngeal secretions cannot be obtained, an alternative specimen for rapid diagnosis has to be found. We have shown that, although the method has certain

Table 3. *Nasopharyngeal secretions examined for respiratory syncytial virus by fluorescent antibody technique, compared with cough/nasal swab preparations examined by the same method*

Diagnosis	Number examined	N.P.S.+		N.P.S.−	
		C/swab+	C/swab−	C/swab+	C/swab−
Bronchiolitis	17	14	1	—	2
Bronchitis	13	9	1	0	3*
Croup	2	1	—	—	1
U.R.T.I.	2	2	—	—	—
Pertussis	1	0	0	0	1†
Totals	35	26	2	0	7

* Parainfluenza virus type 3 isolated from two cases.

† Adenovirus type 5 isolated.

There was only one case in which both cough/nasal swab and nasopharyngeal secretion were positive by the F.A. technique but virus isolation failed (S. T., of Table 1).

Copositivity: 26/28 = 93 %; conegativity: 7/7 = 100 %; overall agreement: 33/35 = 94 %. After Buck & Gart (1966).

N.P.S. = nasopharyngeal swab; C/swab = cough/nasal swab; U.R.T.I. = upper respiratory tract infection; F.A. = fluorescent antibody.

Table 4. *Cough/nasal swab preparations examined for R.S.V. by fluorescent antibody technique compared with isolations*

Diagnosis	No. of C/N swabs examined	R.S.V. culture positive		R.S.V. culture negative	
		F.A. positive	F.A. negative	F.A. positive	F.A. negative
Pneumonia	5	4	0	0	1
Bronchitis	3	0	0	0	3
U.R.T.I.	6	3	0	0	3
Croup	2	0	1	0	1*
Totals	16	7	1	0	8

* Parainfluenza virus Type 1 isolated.

Copositivity: 7/8 = 88 %; conegativity: 8/8 = 100 %; overall agreement: 15/16 = 94 %. After Buck & Gart (1966).

R.S.V. = Respiratory syncytial virus. C/N = Cough/nasal. F. A. = Fluorescent antibody.

disadvantages, cough/nasal swab preparations can be used dependably for this purpose. Intact cells, which are essential for diagnosis, are very scanty in number, therefore much more time has to be spent in examining the stained specimens under the fluorescent microscope. In addition, reliable discrimination requires considerable experience because of the amount of non-specific fluorescence evident in these preparations. When examined by an experienced worker, however, a correct positive diagnosis can be made, as has been proved here by examining parallel nasopharyngeal secretions and cough/nasal swabs by fluorescent antibody technique and confirming positive results by the subsequent isolation of the virus. It must be emphasized again that the technique that we have devised and described in this paper is quite different from those previously used as a means of early detection of R.S. virus in throat swabs (Gray *et al.* 1968); the purpose of

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swabbing the patient is to remove intact cells for examination by the fluorescent antibody technique.

Although it can also be found in less severe respiratory illnesses, R.S. virus causes severe infection in children mainly under the age of 2 years and is capable of causing bronchiolitis, pneumonia and death (Channock *et al.* 1961; Holzel *et al.* 1963; Elderkin, Gardner, Turk & White, 1965; Holzel *et al.* 1965; Gardner *et al.* 1967; Aherne *et al.* 1970). Even in this present small series of cases, 35 children mainly under the age of 1 year from whom both cough/nasal swabs and nasopharyngeal secretions were taken, and a group of 16 older children from whom only cough/nasal swabs were taken, R.S. virus was detected in 28 of the first series and eight of the second series (80 % and 50 % respectively). The total virus isolation rate was 89 % in the first series and 56 % in the second series. The failure rate for the detection of R.S. virus in cough/nasal swab preparations when compared with nasopharyngeal secretion was only two out of 28; the failure rate for cough/nasal swabs alone, as judged by virus isolation, was 1 out of 8. There was 1 patient (S. T., Table 1) in which both cough/nasal swab preparations and nasopharyngeal secretions were positive but no R.S. virus was isolated. This child had been ill for a fortnight before a specimen had been taken. Although specific R.S. virus fluorescence was found in both preparations, the fluorescence was duller and gave the appearance of perhaps being blocked by the child's own antibody production. We have observed on a number of occasions this sort of fluorescence in secretions taken from patients some days after their first positive specimen and have found difficulty in isolating the virus from the second specimen which exhibits this type of fluorescence (P. S. Gardner & J. McQuillin, unpublished results). These are all hospital studies and are, perhaps, biased in that only the child who is sufficiently ill with a respiratory infection to necessitate admission is examined.

The typical history of a bronchiolitis is of a severe illness which is usually preceded by a mild cold. It is therefore the young baby in the susceptible age group acquiring an upper respiratory infection who is in danger of developing a severe lower respiratory infection when R.S. virus is the infecting agent. It would be an advantage if these children could be investigated by a rapid diagnostic technique during the early stages of their upper respiratory tract infection when they are under the care of the general practitioner at home. It is well within the scope of the general practitioner to take a cough/nasal swab and send it to the virus laboratory for examination, provided facilities for transport of the specimen are available. Once we are able to diagnose R.S. virus infection at this early stage, it is possible that bronchiolitis might be prevented as antiviral agents become available. The success of the technique depends on the cough/nasal swabs being efficiently taken with much adhering secretion and cells; preparations containing too few cells could lead to false negative results.

Viruses other than R.S. virus are implicated in respiratory infections of older children and adults. It is known that influenza A can be seen in secretions of the respiratory tract using the fluorescent antibody technique (Hers *et al.* 1968), and it is possible that the cough/nasal swabs could become the method of choice in diagnosing infections in older children and adults.

Croup, principally caused by the parainfluenza group of viruses, is an infection of children between the ages 1 and 5 (Chanock *et al.* 1963). Techniques for the rapid diagnosis of parainfluenza virus infections are being developed at this Centre and perhaps could be applied to cough/nasal swab preparations (Gardner, 1969).

If cough/nasal swab preparations can be successfully used for the rapid diagnosis of these viruses, then the virus laboratory could carry out a diagnostic service for a much wider section of the population, should it be considered necessary to do so. This would be particularly important should antiviral agents become available.

The series of 35 children of the younger age group has shown, when isolation and fluorescent techniques are used together, that an 89% diagnostic rate can be achieved; 80% of these infections were due to R.S. virus, for which fluorescent techniques are available, and all of the children in this series were diagnosed on the day of admission to hospital. The second series of 16 children, from whom only cough/nasal swab preparations were taken, has shown that even in children over the age of 2 a high diagnostic rate can be obtained on the day of admission.

Should rapid diagnosis of parainfluenza, influenza A and adenovirus infections become possible in the future with cough/nasal swab preparations, then with relatively simple techniques, a diagnosis, within hours of onset of respiratory illness, could be confidently expected in between 80 and 90% of infants and in a high percentage of older children and adults with respiratory virus infection.

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EXPLANATION OF PLATE

Fig. 1. Two cells in a cough/nasal swab preparation stained by the fluorescent antibody technique for R.S. virus. There is a considerable amount of non-specific fluorescence. Magnification: × 1900.

Fig. 2. One cell in a cough/nasal swab preparation showing good specific fluorescence when stained by the fluorescent antibody technique for R.S. virus. Considerable non-specific fluorescence still visible. Magnification: × 1900.

Fig. 3. Two cells in a nasopharyngeal secretion stained by the fluorescent antibody technique for R.S. virus. There is little specific background fluorescence. Magnification: × 1900.

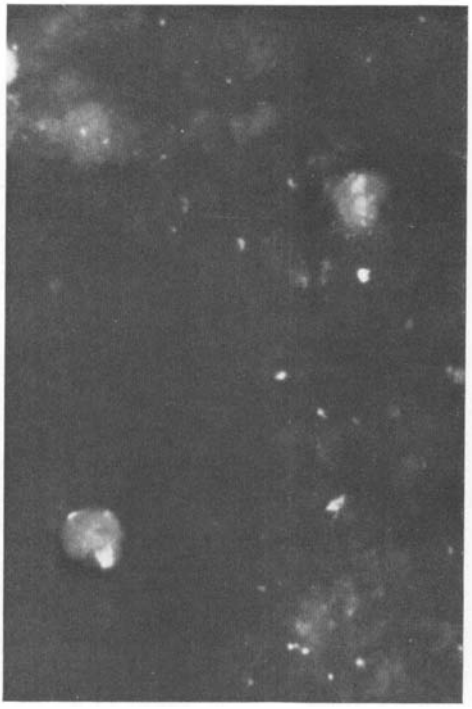

Fig. 1

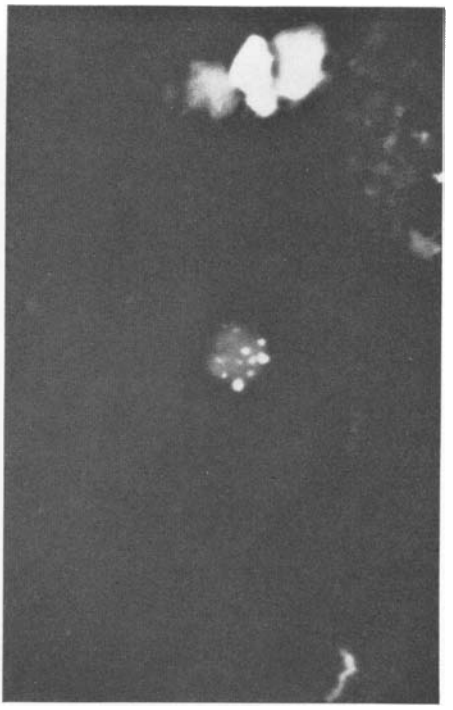

Fig. 2

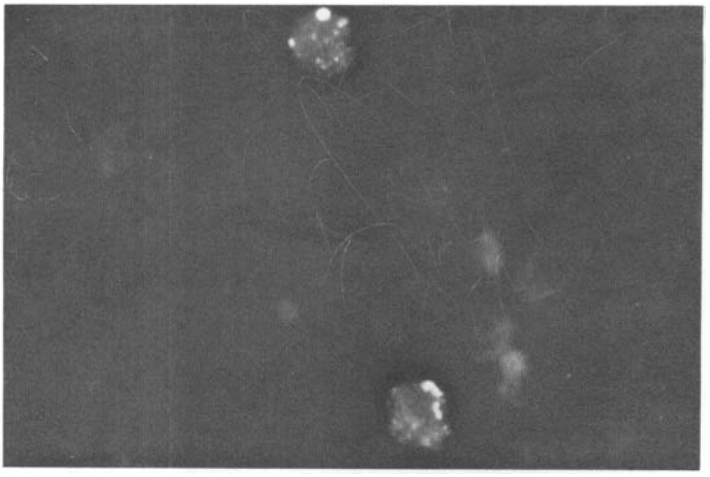

Fig. 3

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