of the preclinical studies. They stressed the importance of extending this teaching into the clinical years. Also regarded as essential was the more advanced teaching of immunology as a science in its own right in elective undergraduate courses and in postgraduate courses to serve as an introduction to research.

It was recommended that this teaching should be the responsibility of a group of immunologists constituting a distinct designated unit or subdepartment with established posts of senior academic standing up to and including professorial ranking. The immunology division could be associated with departments of biology, biochemistry, physiology, microbiology, pathology, or medicine. It would be beneficial, in fact, if the associations varied from university to university, for immunology has large intersections with all these subjects. Obviously immunopathology as such would be best promoted by close association with departments of pathology. In this respect I would prefer to see departments of "pathological and related studies" of somewhat elastic structure but with established professorial divisions or subdepartments of morbid anatomy, chemical pathology, experimental pathology, haematology, microbiology (bacteriology, virology, or parasitology), and immunology. This, I think, would accord well with the ideas of Payling Wright (1963) when he discussed the future of university pathology.

One of the main aims of the College of Pathologists is, I believe, that of ensuring the standards and professional status of what is essentially clinical pathology. This is to be done to a large extent, I gather, by prescribing professional training and by a system of examinations.

I am a little worried lest, while achieving this aim, many young persons may be deflected from following a more academic career in the sciences having these large intersections with pathology as generally defined. My more personal concern is,

of course, for young medical, veterinary, or science graduates coming into immunology. If they are subsequently to bring seminal ideas from the immunological side into immunopathology and to be able to lead and direct research in this field, then a more esoteric form of postgraduate training within immunology is most desirable. This may be done as B.Sc. or M.Sc. courses or by undertaking research under supervision for the Ph.D. degree, or, indeed, by simply doing good work within the field, and it seems to me this should not leave the pathologist, for such he may call himself, unaccredited in professional standing.

I am sure, however, that the College is very conscious of this very problem and will succeed, as is its avowed intention, in promoting not only the practice of pathology—and here the emphasis is on clinical pathology—but also the various contributing sciences which together establish so much of that body of knowledge we call pathology.

Figs 3 and 4 are reproduced by permission of Blackwell Scientific Publications.

REFERENCES

- Buxton, A., and Allan, D. (1963). *Immunology*, 6, 520.
 Buxton, A. and Davies, J. M. (1963). *Immunology*, 6, 530.
 Coombs, R. R. A., and Gell, P. G. H. (1963). In *Clinical Aspects of Immunology*, edited by P. G. H. Gell and R. R. A. Coombs, p. 317. Oxford.
 Coombs, R. R. A., and Smith, H. (1968). In *Clinical Aspects of Immunology*, edited by P. G. H. Gell and R. R. A. Coombs, 2nd ed., p. 423.
 Dingle, J. T., Fell, Honor B., and Coombs, R. R. A. (1967). *Int. Arch. Allergy*, 31, 282.
 Fell, Honor B., Coombs, R. R. A., and Dingle, J. T. (1966). *Int. Arch. Allergy*, 30, 146.
 Matsumura, T. (1962). *Gunma J. med. Sci.*, 11, 319.
 Rowley, D., Turner, K. J., and Jenkin, C. R. (1964). *Aust. J. exp. Biol. med. Sci.*, 42, 237.
 Webb, H. E., and Gordon Smith, C. E. (1966). *Brit. med. J.*, 2, 1179.
 W.H.O. (1959). *Wld Hlth Org. techn. Rep. Ser.*, No. 175.
 W.H.O. (1967). *Wld Hlth Org. techn. Rep. Ser.*, No. 175.
 Wright, G. P. (1963). *Lancet*, 2, 1177.

Rapid Diagnosis of Respiratory Syncytial Virus Infection by Immunofluorescent Antibody Techniques

JOYCE McQUILLIN,* B.Sc., F.I.M.L.T.; P. S. GARDNER,* M.D., DIP.BACT.

[WITH SPECIAL PLATE FACING THIS PAGE]

Brit. med. J., 1968, 1, 602-605

Immunofluorescent antibody techniques have been advocated as the most promising means of rapid diagnosis of virus infection. To be successful any technique devised must be easily adapted as a routine test by virus laboratories and the results be so precise that there is no danger of an incorrect diagnosis. A test which gives equivocal results or readings which vary with different observers is useless as a routine procedure. One of the first to achieve success was Liu (1956), who examined direct smears of nasal mucosa from patients with influenza; the results by fluorescent antibody technique compared favourably with isolation methods. Biegeleisen *et al.* (1959) used scrapings from vesicles for the rapid diagnosis of herpes simplex. In both these conditions success might be expected, as the material is taken direct from a site where the virus is present in great concentration.

The fluorescent antibody technique has also been used for the diagnosis of rabies in tissues by Goldwasser *et al.* (1959)

and by Hatch *et al.* (1961) for typing polioviruses grown in monkey kidney tissue culture.

If a fluorescent antibody technique is applied to the rapid diagnosis of virus respiratory infections in children two methods are possible. The first, which would be the more rapid, is the examination of direct smears or exudates from the throat and nasopharynx of patients. The second is the examination, at an early stage, of tissue culture cells inoculated with the specimen under investigation in order to detect the presence of virus antigen in the cells before the cytopathic effect, by which the virus is normally recognized, becomes obvious.

The main problem of investigating childhood respiratory disease is the wide range of causal or associated virus pathogens (Elderkin *et al.*, 1965), and a large number of highly specific antisera are required to examine such material. It is clear, however, from investigations over the last few years that respiratory syncytial virus is the most important cause of respiratory illness and respiratory death in childhood (Chanock *et al.*, 1961; Holzel *et al.*, 1963; Elderkin *et al.*, 1965; Gardner

* Department of Virology, Royal Victoria Infirmary, Newcastle upon Tyne 1.

JOYCE McQUILLIN AND P. S. GARDNER: RAPID DIAGNOSIS OF RESPIRATORY SYNCYTIAL VIRUS INFECTION

FIG. 1.—Uninfected Bristol HeLa cells showing non-specific fluorescence after treatment with unabsorbed respiratory syncytial virus antiserum and Fluoroscans Plus. ($\times 400$.)

FIG. 2.—Uninfected Bristol HeLa cells treated with absorbed respiratory syncytial virus antiserum and Fluoroscans Plus showing no fluorescence. ($\times 360$.)

FIG. 3.—Infected Bristol HeLa cells showing specific respiratory syncytial virus fluorescence with absorbed respiratory syncytial virus antiserum and Fluoroscans Plus. ($\times 250$.)

FIG. 5

FIG. 6

FIG. 5.—Cells in nasopharyngeal secretions showing specific respiratory syncytial virus fluorescence, with absorbed respiratory syncytial virus antiserum and Fluoroscans Plus. ($\times 400$.) FIG. 6.—Control for nasopharyngeal secretion in Fig. 5 showing no fluorescence with normal rabbit serum and Fluoroscans Plus. ($\times 400$.)

FIG. 4.—Control for infected Bristol HeLa cells for Fig. 3 showing no fluorescence when treated with normal rabbit serum and Fluoroscans Plus. ($\times 350$.)

Cervical appearances in Case 3.

R. R. WILLCOX: NECROTIC CERVICITIS

et al., 1967). Respiratory syncytial virus is comparatively slow-growing, and when isolated in tissue culture the neutralization test required to confirm its identity may take from four to six days. Laboratory reports on the presence or absence of this virus can therefore take up to 28 days.

Examination of tissue culture cells by fluorescent antibody technique presents practical difficulties. Antiserum produced in rabbits which have been inoculated with virus grown in human tissue culture cells contains antibodies against both virus and human cells. Uninfected human cells treated with this serum and with fluorescein-labelled anti-rabbit globulin will, because of the anti-human factor in the serum, fluoresce just as strongly as virus-infected cells, and, in the latter, specific virus fluorescence can be completely masked. Such sera must therefore be absorbed if consistent specific results are to be achieved. False-positives may also be obtained by unsatisfactory fluorescein-conjugates because of the presence of unreacted fluorescent material. The controls, which must be included to eliminate these sources of error, are normal cells treated with respiratory syncytial virus antiserum, and the cells under test are treated with normal rabbit serum.

In view of the predominant importance of respiratory syncytial virus, it was clearly necessary to assess the value of fluorescent antibody techniques for the rapid detection of this virus, both in tissue cultures and direct from material obtained from the nasopharynx.

Materials and Methods

Fluorescein-conjugated Anti-rabbit Globulin.—This material, Fluoroscans Plus, was supplied to us by Winthrop Biologicals Limited. It is an anti-rabbit fluorescent antibody conjugate prepared in dogs and contains fluorescein and an additive which counteracts non-specific staining. The details of its preparation will be published shortly. The method is based on that of Smith *et al.* (1959). The conjugate showed no non-specific staining activities and in our hands needed no further absorption.

Respiratory Syncytial Virus Antiserum.—Antisera were prepared in rabbits by intravenous inoculation of the Long strain of respiratory syncytial virus grown in Hep. II cells. The virus suspension was clarified by centrifugation to remove cell debris. Additional supplies of respiratory syncytial virus antiserum were also made available by Winthrop Biologicals Limited.

Absorption of Antisera.—Each 1 ml. of antiserum was absorbed with Bristol HeLa and Hep. II cells representing the contents of four confluent Roux flasks. Absorption was carried out for 90 minutes at 37° C. followed by 40 hours at 4° C., shaking mechanically. The cells were removed by centrifugation at 10,000 r.p.m. for 30 minutes in a refrigerated centrifuge. Absorbed sera were tested against uninfected Bristol HeLa and Hep. II cells to ascertain the complete removal of non-specific anti-human factors. Fig. 1 (Special Plate) shows uninfected Bristol HeLa cells treated with unabsorbed respiratory syncytial virus antiserum and Fluoroscans Plus, and shows the non-specific fluorescence in the cell cytoplasm. Fig. 2 (Special Plate) shows uninfected Bristol HeLa cells treated with absorbed serum and Fluoroscans Plus; there is no fluorescence.

Titration of Virus.—A suspension of the Long strain of respiratory syncytial virus was diluted in tenfold steps from 10^{-1} to 10^{-6} , and 0.1 ml. of each dilution was inoculated into each of four tubes. Four negative controls for the same batch of cells were included. The tubes were incubated at 37° C. They were inspected microscopically each day and one tube from each dilution was examined by fluorescent antibody technique on the second, third, fifth, and seventh days in parallel with a negative control.

Neutralization Tests and Tissue Culture Cell Lines.—These have already been described (Andrew and Gardner, 1963).

Specimens for Fluorescent Antibody Examination.—Cough swabs from infants with both upper and lower respiratory tract infection were collected in Hanks's medium containing antibiotics and 2% bovine albumin and transported to the laboratory on ice. They were immediately inoculated into pairs of Bristol HeLa and Hep. II tissue culture tubes as part of the routine procedure for the investigation of respiratory viruses. Our aim was to discover the earliest possible time when respiratory syncytial virus could be detected in tissue culture after inoculation; 16 specimens from children with bronchiolitis were therefore selected for more detailed study, since it is known that in most cases the condition is caused by this virus. Five tubes of either Bristol HeLa or Hep. II cells were inoculated with the specimens, three tubes being reserved for examination by fluorescent antibody technique within the first seven days at approximately two-day intervals, while the remaining pair were reserved for normal virus isolation. An additional cough swab was taken from these patients, smeared direct on to slides, and the fluorescent antibody technique applied. In six children with bronchiolitis aspirated nasopharyngeal secretions were examined direct by fluorescent antibody technique.

Preparation of Specimens for Examination by Immunofluorescent Technique.—(a) *Direct Smears:*—Cough swabs were spotted direct on to slides, each swab being rolled gently in two places. These spots were allowed to dry, were fixed in cold acetone for five minutes at the patient's bedside, and were transported to the laboratory on ice. (b) *Nasopharyngeal Secretions:*—Secretions were aspirated from the nasopharynx by rubber tube and transported on ice to the laboratory. They were emulsified in 0.5 ml. of saline and then centrifuged at 1,000 r.p.m. for 10 minutes. The deposit was resuspended in phosphate-buffered saline and again centrifuged. The resultant washed deposit was resuspended in a few drops of the saline and two spots were placed on a slide and allowed to dry in air. These spots were then fixed in acetone for 10 minutes at 4° C. (c) *Tissue Culture:*—The medium was discarded from the tubes to be examined; the cell layer was washed once with phosphate-buffered saline. The cells were then gently scraped off the tube with a pipette and suspended in phosphate-buffered saline. The suspension was centrifuged at 500 r.p.m. for 10 minutes and the deposit was resuspended in a few drops of phosphate-buffered saline. Two spots were placed on a slide and allowed to dry in air. A third spot of negative cells from the same batch of tubes was included on the slide as an additional negative control. When dry, the spots were fixed in acetone for five minutes at 4° C.

Immunofluorescent Staining Procedure—Indirect Method

The slides were placed in Petri dishes in a moist atmosphere. Respiratory syncytial virus antiserum was applied to one of the duplicate spots and normal rabbit serum to the other. Respiratory syncytial virus antiserum was also applied to the negative cell control. The slides were incubated at 37° C. for 30 minutes, then rinsed for 30 minutes in three changes of phosphate-buffered saline. The slides were drained and Fluoroscans Plus was applied to all spots, after which the slides were again incubated for 30 minutes at 37° C. This was followed by a 30-minute rinse in three changes of phosphate-buffered saline and a final wash for two minutes in distilled water. The slides were allowed to dry in air, and were then examined for fluorescence.

Fluorescent Microscopy.—Specimens were examined under dark-ground illumination by blue light, using a Mazda ME/D 250 W bench mercury vapour lamp, a 6-mm. blue Chance OB10 exciter filter, a yellow barrier filter, and an ordinary Vickers Patholette II microscope with mirror.

Photography.—Photographs were taken with a Zeiss Standard R.A.38 fluorescent microscope, a BG12/4 exciter filter, 53/44 barrier filters, and 150-second exposure.

Results

Specificity of Antiserum

Bristol HeLa cells and Hep. II cells infected with poliovirus type 2, coxsackieviruses B1 and B5, echovirus type 30, herpesvirus hominis, adenoviruses types 1 and 5, and rhesus monkey kidney cells infected with parainfluenza type 3 and influenza B were tested against respiratory syncytial virus antiserum by fluorescent antibody technique. Respiratory syncytial virus infected cells were also included in each batch of tests. No fluorescence was observed against any other viruses than respiratory syncytial virus.

In all tests described below and recorded in Tables I and II non-specific fluorescence was completely absent from all controls.

TABLE I.—Time Interval Between Fluorescent Antibody (F.A.) Technique Results and Appearances of Cytopathic Effect (C.P.E.)

Specimen No.	Days Between Positive F.A. Results and Detection by Typical C.P.E.	Days for Positive Neutralization Test Result
1	0	4
2	5	4
3	2	Not tested
4	5	5
5	1	3
6	5	Not tested
7	2	5
8	7	5
9	7	7
10	14	4
11	5	5
12	7	4
13	7	4

TABLE II.—Time of Diagnosis with Fluorescent Antibody and Tissue Culture Techniques									
Case No.	First Cough Swab							Second Nasopharyngeal Cough Swab	Nasopharyngeal Secretion
	Tube 1		Tube 2		Tube 3		Tubes 4 and 5	F.A. Result for Direct Preparation	
	Day of F.A. Test	Result	Day of F.A. Test	Result	Day of F.A. Test	Result	Day when Typical CPE Observed		
1	2	—	4	—	6	+	10	—	N.T.
2	2	—	3	—	4	+	18	—	”
3	2	—	4	+	6	+++	9	—	”
4	2	—	4	—	6	—	13	—	”
5	3	—	5	—	7	—	—	—	”
6	3	++	5	+++	7	++++	10	—	”
7	3	—	5	—	7	—	—	—	”
8	2	—	4	—	7	—	16	—	”
9	2	+	4	++	6	++++	9	—	N.T.
10	2	+	5	++	7	++	10	Doubtful	+++
11	2	+	4	++	6	+++	7	”	+++
12	2	—	4	—	6	N.T.	26	+	—
13	2	—	4	—	6	—	27	—	N.T.
14	2	C	4	C	6	C	C	—	—
15	2	—	4	—	6	D	Echovirus type 19	—	—
16	2	—	4	—	7	+	14	—	N.T.

+, Weak positive. ++, Moderately strong positive. +++, Strong positive. + + + +, Very strong positive. N.T., Not tested. D, Cytopathic effect too advanced for test. C, Contaminated. —, Negative.

Sensitivity of Test

Preliminary tests by virus titration experiments in Bristol HeLa and Hep. II cells recorded at seven days indicated that respiratory syncytial virus could be detected by fluorescent antibody technique in tissue culture cells at dilutions of 10⁻⁴ to 10⁻⁵, while observation of cytopathic effect could be detected only at dilutions of 10⁻² to 10⁻³; this was a hundredfold increase in sensitivity.

Identification of Virus by Fluorescent Antibody Technique

Examination by fluorescent antibody technique of tissue culture tubes, either Bristol HeLa or Hep. II, inoculated with respiratory specimens was carried out when the presence of respiratory syncytial virus was suspected. During routine reading of tubes, one tube of any pair showing early signs of possible respiratory syncytial virus degeneration was selected for examination. The partner-tube was allowed to remain until typical giant-cell degeneration was observed. The majority of isolations were confirmed by neutralization tests.

Fig. 3 (Special Plate) shows particulate cytoplasmic fluorescence due to respiratory syncytial virus in Bristol HeLa cells stained with absorbed respiratory syncytial virus antiserum and Fluoroscans Plus. Fig. 4 (Special Plate) shows the complete absence of fluorescence in the same infected cells when stained with normal rabbit serum and Fluoroscans Plus.

Table I shows the time lag between the day on which a positive fluorescent antibody result was obtained and the day the presence of virus could be recognized by typical cytopathic effect. The number of days taken to confirm cytopathic effect by neutralization is also recorded.

As can be seen from Table I, the delay between a positive fluorescent antibody result and positive identification by routine methods varied from 4 to 18 days when neutralization tests were included.

Nine tubes showing non-specific degenerative changes gave negative fluorescent antibody results, and in all nine respiratory syncytial virus was not isolated from the specimen after a minimum incubation of 28 days.

Early Diagnosis of Children with Bronchiolitis

Table II shows the diagnostic time with fluorescent antibody techniques compared with conventional isolation techniques. Direct smears from cough swabs were least satisfactory because of the non-specific fluorescence of background debris. Moreover, intact cells were rarely found. Two doubtful positives were observed, but since there was no certainty of the intracellular localization of the fluorescence a confident diagnosis could not have been made on the result of these direct smears. Early examination of tissue cultures inoculated with cough swabs showed that three were first recognized as positive on the second day, one on the third day, two on the fourth day, one on the sixth day, and one on the seventh day.

Examination of the six nasopharyngeal aspirates showed that three were positive by fluorescent antibody techniques, all within 24 hours of admission to hospital.

Fig. 5 (Special Plate) shows cytoplasmic fluorescence due to respiratory syncytial virus in the cells from nasopharyngeal secretion in Case 10; Fig. 6 (Special Plate) shows the absence of fluorescence in the same secretion stained with normal rabbit serum and Fluoroscans Plus.

The possibility that pus cells in nasopharyngeal secretions might show non-specific fluorescence was thought to be unlikely; it was absent in the three negative nasopharyngeal secretions which contained many pus cells and in a number of adult sputa similarly examined.

Comments and Conclusions

Few workers apart from Kisch *et al.* (1962) and Bennett and Hamre (1962) have studied the intracellular distribution of respiratory syncytial virus in tissue culture. Schieble *et al.* (1965) described an immunofluorescent staining method for the rapid identification of respiratory syncytial virus, but the method involved making cover-slip cultures of cells, infecting them with a virus isolate, and finally staining them by a

fluorescent antibody technique after 24 to 48 hours' incubation. We have adapted and simplified this technique, and a positive result can be obtained on the same day that the tissue culture is suspected of showing a respiratory syncytial virus type of degeneration. The reliability and simplicity of this method suggests that the neutralization test could well be abandoned; with the curtailment of isolation procedures, reports could be issued on an average of 10 days earlier than at present.

Before embarking on this project we decided to test the sensitivity of the method by comparing virus titration by microscopical observation of cytopathic effect with the fluorescent antibody technique. Both cell lines, Hep. II and Bristol HeLa, showed at least a hundredfold greater sensitivity by the fluorescent antibody technique. Once this had been established it seemed probable that specific fluorescence might be detected in tissue culture tubes some days before the first cytopathic effect could be observed. As bronchiolitis is in most cases due to respiratory syncytial virus, material from children with this disease was studied; in half the cases the diagnosis was established within the first week, and in a quarter as early as the second day. In no instance did the fluorescent antibody technique prove positive without subsequent isolation of virus. In three cases, however, the converse proved true (Cases 4, 8, and 13), but this might be expected with a very low virus inoculum.

Direct smears made from cough swabs were disappointing; in no instance could a firm diagnosis be given, and their further investigation did not seem justified.

The investigation of nasopharyngeal secretions appears, at present, to be the most hopeful method of obtaining a rapid diagnosis. Nasopharyngeal secretions have previously been used for rapid diagnosis of paramyxovirus infections by electron microscopy (Doane *et al.*, 1967). The method we have devised resulted in a large number of intact cells in the preparation, and we would stress this essential requisite for the diagnosis of virus infection by the fluorescent antibody technique. It is clearly unwise to draw final conclusions from the few cases of a preliminary communication. It does, however, appear practical to diagnose infection with respiratory syncytial virus in young children with greater speed than by any previous method and within the period when bacterial isolations and sensitivities would be forthcoming.

We shall from now on investigate all severe lower respiratory disease in children by inoculating cough swabs on to five tissue culture tubes, examining three of them at intervals during the first week by the fluorescent antibody technique, allowing the remaining two to continue for 28 days, should the fluorescent antibody technique prove negative. Where possible, aspirates of the nasopharynx will be examined as direct preparations by fluorescent antibody technique and also by culture. By these methods we believe we can increasingly provide the paediatrician with early evidence of respiratory syncytial virus infection.

In the absence of pathogenic bacteria, antibiotics are unnecessary, and other aspects of treatment, recently emphasized, must be attended to (Simpson and Flenley, 1967; Holdaway *et al.*, 1967). Our main concern in seeking the more rapid diagnosis of respiratory syncytial virus infections is that it will be essential when antiviral drugs become available. The techniques we have described would also be important for the investigation of such substances.

The failure to reach a confident virus diagnosis in the early stages of severe lower respiratory infection in children has remained an unsatisfactory and potentially serious gap in the understanding required for rational management.

We do not wish at this preliminary stage to overstate our case. Our findings need confirmation by ourselves and others in a larger number of children. If, as we expect, this is forthcoming it could lead to the more discriminating use of anti-

biotics, an attitude which should be firmly established before antiviral agents are available for treatment.

Summary

Present methods of diagnosing respiratory syncytial virus infection by isolation and identification in tissue culture takes from 10 to 28 days. An immunofluorescent technique was used for the rapid detection of respiratory syncytial virus in: (a) cells scraped from three inoculated tissue culture tubes at intervals during the first seven days of culture; (b) direct smears from throat swabs; and (c) aspirated nasopharyngeal secretions from children with bronchiolitis. A fluorescent antibody technique was also used to confirm that early cytopathic effects were due to respiratory syncytial virus. Materials used for the fluorescent antibody technique were: acetone for fixation; a respiratory syncytial virus antiserum, prepared in rabbits, absorbed with human tissue culture cells; and a fluorescein labelled anti-rabbit globulin (Fluorescan Plus). Three out of six nasopharyngeal secretions gave positive fluorescent antibody results on the same day that the patient was admitted to hospital. Five tissue culture tubes were inoculated with cough swab material from each of the 16 cases. Two tubes were used for conventional isolation techniques and three were examined by fluorescent antibody technique. Twenty-five per cent. of the latter were positive on the second day and 50% within seven days by the fluorescent antibody technique. Examination of direct smears by fluorescent antibody techniques were unsatisfactory. Preliminary results suggested that, by the early examination of nasopharyngeal secretions and tissue culture cells by the fluorescent antibody technique, a virological report could be available as early as a report on bacterial isolations and sensitivities. This would give early guidance on management of cases, and will also be of particular use if anti-viral drugs become available.

We wish to thank Professor S. D. M. Court for his help and encouragement in this work. We are grateful to the paediatricians at all hospitals and to their ward sisters and medical staff for their co-operation and to Miss P. M. Sturdy for the collection of specimens. Special thanks are due to the Scientific and Research Subcommittee of the Royal Victoria Infirmary and to the Medical Research Council for grants which made this work possible. We also wish to thank Winthrop Biologicals Limited for their reagents.

REFERENCES

- Andrew, J. D., and Gardner, P. S. (1963). *Brit. med. J.*, **2**, 1447.
- Bennett, C. R., jun., and Hamre, D. (1962). *J. infect. Dis.*, **110**, 8.
- Biegeleisen, J. Z., jun., Scott, L. V., and Lewis, V., jun. (1959). *Science*, **129**, 640.
- Chanock, R. M., *et al.* (1961). *J. Amer. med. Ass.*, **176**, 647.
- Doane, F. W., Anderson, N., Chatiyononda, K., Bannatyne, R. M., McLean, D. M., and Rhodes, A. J. (1967). *Lancet*, **2**, 751.
- Elderkin, F. M., Gardner, P. S., Turk, D. C., and White, A. C. (1965). *Brit. med. J.*, **2**, 722.
- Gardner, P. S., Turk, D. C., Aherne, W. A., Bird, T., Holdaway, M. D., and Court, S. D. M. (1967). *Brit. med. J.*, **4**, 316.
- Goldwasser, R., Kissling, R. E., Carski, T. R., and Hosti, T. S. (1959). *Bull. Wild Hlth Org.*, **20**, 579.
- Hatch, M. H., Kalter, S. S., and Ajello, G. W. (1961). *Proc. Soc. exp. Biol. (N.Y.)*, **107**, 1.
- Holdaway D., Romer A. C., and Gardner, P. S. (1967). *Pediatrics*, **39**, 924.
- Holzel, A., Parker, L., Patterson, W. H., White, L. L. R., Thompson, K. M., and Tobin, J. O'H. (1963). *Lancet*, **1**, 295.
- Kisch, A. L., Johnson, K. M., and Chanock, R. M. (1962). *Virology*, **16**, 177.
- Liu, C. (1956). *Proc. Soc. exp. Biol. (N.Y.)*, **92**, 883.
- Schieble J. H., Lennette, E. H., and Kase, A. (1965). *Proc. Soc. exp. Biol.*, **120**, 203.
- Simpson, H., and Flenley, D. C. (1967). *Lancet*, **1**, 7.
- Smith, C. W., Marshall, J. D., jun., and Eveland, W. C. (1959). *Proc. Soc. exp. Biol. (N.Y.)*, **102**, 179.