J. Hyg., Camb. (1973), **71**, 151
Printed in Great Britain

Epstein–Barr virus antibody in cases and contacts of infectious mononucleosis; a family study

By F. J. NYE and H. P. LAMBERT
Communicable Diseases Unit, St George's Hospital, London, S.W. 17

(*Received* 28 *June* 1972)

SUMMARY

Serological investigations were carried out on 147 patients with Paul–Bunnell positive infectious mononucleosis (IM) from the general population. All possessed antibody to the Epstein–Barr virus (EBV) and 63% showed serological evidence of recent infection. Contacts of 132 patients, 306 in all, were followed serologically; within 6 months of the index cases' illness twenty one contacts developed evidence of EBV infection or re-infection and of these five developed overt IM. The secondary attack rate of EBV infection among susceptible contacts was at least 19%; the corresponding figure for clinically apparent IM was 6%. EBV antibody prevalence among patients' siblings was significantly lower than among age-matched controls, suggesting that cases of IM come from families with a lower than normal previous experience of the virus. Of thirteen patients with persistently Paul–Bunnell negative 'glandular fever' four had serological evidence of recent EBV infection and two had probable cytomegalovirus mononucleosis. Recent EBV infection may have been associated with the illnesses of five of the remaining patients.

INTRODUCTION

Although the aetiology of infectious mononucleosis (IM) is not firmly established, there is now a considerable amount of evidence incriminating the Epstein–Barr virus, a herpes-like agent, as the causative organism. The Epstein–Barr virus (EBV) has been particularly associated with Burkitt's lymphoma (Henle *et al.* 1969; Epstein, 1970), nasopharyngeal carcinoma (Henle, Henle & Diehl, 1968), sarcoidosis (Hirshaut *et al.* 1970), systemic lupus erythematosus (Evans, Rothfield & Niederman, 1971) and leprosy (Papageorgiou, Sorokin, Koutzouzakoglou & Glade, 1971), but IM remains the only condition in which there is a known temporal association between EB virus infection and the development of disease (Henle *et al.* 1968; Niederman, McCollum, Henle & Henle, 1968). It is also clear that although rising virus antibody titres have been observed in only a minority of patients with IM, virus antibody is always present in such patients. The disease does not develop in subjects with pre-existing EB virus antibody, and conversely only subjects lacking such antibody appear to be susceptible to the disease (Niederman *et al.* 1968; Evans, Niederman & McCollum, 1968).

In the past, understanding of IM has been hindered by ignorance of the nature and frequency of inapparent infection, and the presence of EB virus antibody now

provides the 'marker' of subclinical infection necessary for further epidemiological work. The majority of EB virus antibody studies so far reported have been made in student populations where IM is common, but there is little information about the epidemiology of apparent and inapparent EB virus infection in other groups. The aims of the present study were first, to seek evidence of recent EBV infection in patients with IM from the general population, and secondly to carry out a prospective study of the prevalance and spread of EBV infection among their contacts in the expectation that further light would be thrown on the aetiology and pathogenesis of IM.

PATIENTS AND METHODS

Index cases of IM were detected in three ways; admission to hospital, notifications by a group of general practitioners in the area served by St George's Hospital, S.W. 17, and requests to the hospital haematology laboratory for Paul–Bunnell tests. Index cases with a history and physical signs compatible with IM were admitted to the study if they developed a positive Paul–Bunnell–Davidsohn test or an atypical lymphocytosis. Five patients with neither of these abnormalities were also investigated as a result of highly suggestive clinical findings.

Patients and their families were visited as soon as possible after the onset of illness and clinical and epidemiological data recorded. Blood specimens were then taken from cases and contacts, and a record card was subsequently left on which the family were asked to record details of any illnesses occurring during follow up. Three months later, and in some cases at 6 and 9 months, the family was again visited, record cards collected and further specimens of blood taken from the index cases and contacts.

LABORATORY METHODS

Heterophile antibody

Patients' acute-stage sera were examined for heterophile antibody by a modification of a standard sheep-cell screening test (Brumfitt & O'Grady, 1957). The 'Monospot' slide test (Ortho Diagnostics), which detects heterophile antibody with the absorption pattern characteristic of IM, was also employed in the screening of both patients and contacts.

Sera giving a positive sheep-cell screening test or a positive 'Monospot' were tested by the full Paul–Bunnell–Davidsohn differential absorption procedure, using the method of Davidsohn & Henry (1968). This method uses doubling dilutions starting at 1/7. When sheep erythrocytes were used in this test, results were interpreted by the criteria of Davidsohn & Lee (1969). The test is positive provided that (i) the agglutinin titre after absorption with guinea-pig kidney is not more than eight-fold less than the unabsorbed titre and (ii) the titre after absorption with ox cells is at least sixteen-fold less than the unabsorbed titre. When the unabsorbed titre is 28 (all titres are expressed as reciprocals), any incomplete removal with guinea-pig kidney and complete removal with ox cells is interpreted as positive.

When horse erythrocytes were used the criteria for a positive test was that of Lee, Davidsohn & Slaby (1968), viz. that the titre after guinea-pig-kidney absorp-

tion should be higher than the titre after ox-cell absorption, irrespective of the unabsorbed titre.

The term 'Paul–Bunnell-positive' is used in this paper to denote a positive result in the Paul–Bunnell–Davidsohn differential absorption test using the criteria stated.

Atypical lymphocytes

Total and differential white cell counts were performed on all IM patients, and blood films were examined for atypical lymphocytosis. Blood films from family contacts were also so examined. Blood specimens were taken into EDTA tubes and films were prepared within six hours. The Jenner–Giemsa staining technique was used.

EBV antibody

Sera from index cases and contacts were examined for EBV antibody by an indirect immunofluorescence technique (Henle & Henle, 1966) using a modification of the method employed by the Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London N.W. 9 (Pereira, Blake & Macrae, 1969).

The EB3 line of Burkitt lymphoma cells was used as a source of antigen. Cells suspended in phosphate-buffered saline were transferred to antigen wells on glass microscope slides and air-dried. The preparations were fixed in acetone and stored at $-20°$ C.

For testing, sera were inactivated at 56° C. for 30 min. and then diluted, initially 1/8 in phosphate-buffered saline. Doubling dilutions were then prepared using a Microtiter technique. A drop of each dilution was spread over each antigen well and the slides incubated at 37° C. for 1 hr. The slides were then washed thoroughly in three 10 min. changes of 0·9 % saline and air-dried. A drop of fluorescein-labelled sheep anti-human immunoglobulin (Wellcome Reagents Ltd.) was spread over each antigen well and the slides incubated a further hour at 37° C. After the second incubation slides were washed in three 10 min. changes of phosphate-buffered saline (with mechanical agitation), air-dried, and mounted in phosphate-buffered glycerol.

The preparations were examined by ultraviolet microscopy. Known positive and negative control sera were included with each batch and the end-point read as the titre above which strongly fluorescent cells were no longer seen.

Cytomegalovirus, adenovirus and toxoplasma antibodies

Antibodies to these agents were measured in sera from all index cases. Complement-fixing antibody to cytomegalovirus was measured by the method of Stern & Elek (1965).

A standard method was used for the adenovirus complement-fixation test, using overnight fixation. Sera were examined for antibodies to *Toxoplasma gondii* by the dye test of Sabin & Feldman (1948). Sera from index cases with Paul–Bunnell negative IM which failed to show rising titres to EB virus, cytomegalovirus,

adenovirus or toxoplasma were tested for rubella haemagglutination-inhibition antibody (Stewart *et al.* 1967).

For all antibody measurements fourfold (or greater) rises or falls in titre were regarded as significant.

RESULTS

Patients with Paul–Bunnell positive IM

A total of 147 Paul–Bunnell positive patients was investigated. Their ages ranged from 3 to 48 years with a mean age of 18·6 years and a modal age of 16 years; 105 (71 %) were aged 14–23 years. Seventy-seven patients were male and 70 were female.

Epstein–Barr virus antibody results

All patients possessed EB virus antibody or developed it during or after illness. Antibody measurements were also carried out in a control group of 322 hospital surgical inpatients (without known IM, malignant disease, or other conditions known to be associated with high EBV antibody titres). Peak antibody titres from 100 IM patients were compared with the antibody titres in 100 age- and sex-matched controls. In contrast to the 100 % antibody prevalence in the IM group, only 78 % of the controls were antibody positive. However, the geometric mean titre of the patients' sera (73) was similar to that of sera from antibody-positive controls (76). High titres of antibody (equalling or exceeding 256) were also of similar frequency in both groups, being found in 11 % of patients and 14 % of antibody-positive controls.

At least two serum specimens were available from each patient. Of the 147 patients 93 (63 %) showed a significant change in antibody titre; 46 (31 %) with seroconversion, 44 (30 %) with a significant rise in titre and 3 (2 %) with a significant fall in titre. Although there was a wide scatter of results, seroconversion or a significant rise in titre was more likely when the first serum specimen was taken early in the illness. The first serum was taken within 10 days of onset in 41 of 90 patients (46 %) showing seroconversion, compared with 15 of 54 patients (28 %) whose antibody titres remained unchanged. Antibody was found in 69 % of sera taken during the first week of illness, 75 % of sera taken during the second and third weeks and 93 % of sera taken during the fourth and fifth weeks after onset. Detectable antibody was found in all sera taken after the fifth week. One patient was still EBV antibody negative on the 35th day after onset but developed a titre of 32 in a serum specimen taken on the 47th day after onset. A Paul–Bunnell–Davidsohn test was carried out for the first time on the 19th day and was positive.

Serial specimens were taken from twenty patients over a period of up to 16 weeks. The antibody titres of these patients in relation to the onset of illness are shown in Fig. 1. Titres rose until 6–8 weeks after onset; thereafter they showed no tendency to fall during the period of observation.

A pre-illness serum specimen was taken from five contacts who later developed IM, and fortuitously from two index cases. Of these seven patients six had no detectable EBV antibody before the onset of illness while the seventh had a low titre (8) in a serum specimen taken 45 days before onset.

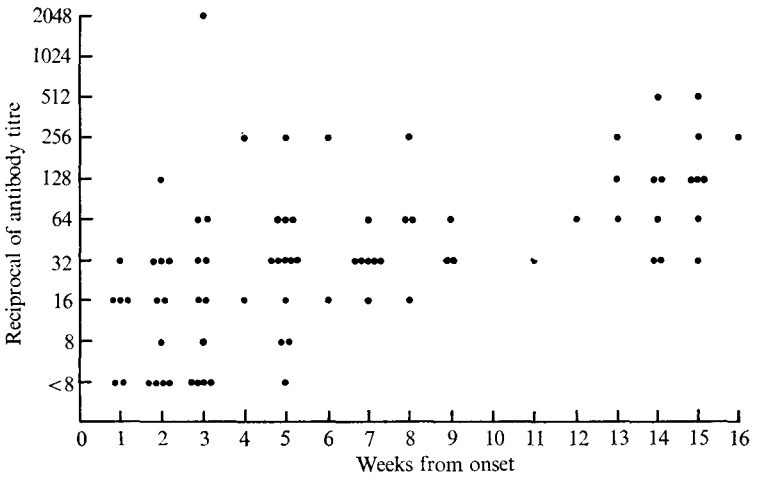

Fig. 1. Serial Epstein–Barr virus antibody measurements in twenty cases of infectious mononucleosis.

Toxoplasma antibody

Twenty-two of 146 patients (15%) had detectable antibody to *Toxoplasma gondii* at a titre of 16 or above. Of these, six had dye test titres of 512 or greater but in no case was there any significant change in titre.

Cytomegalovirus antibody

Three patients showed serological evidence of recent cytomegalovirus (CMV) infection during the first three months of follow-up. One of these acquired antibody to both CMV and EBV, a second showed a simultaneous rise in antibody to both these agents, and the third acquired antibody to CMV but had an unchanging EBV titre. This patient showed a strongly positive 'Monospot' test but there was insufficient serum for the Paul–Bunnell–Davidsohn titration.

Adenovirus antibody

Eighty-four of 137 patients (61%) had detectable adenovirus at a titre of 8 or above. Seventeen patients (12%) had a significant change in antibody titre; 8 with seroconversion, five with a significant rise and four with a significant fall.

Patients with Paul–Bunnell negative IM

Thirteen persistently Paul–Bunnell negative patients with 'glandular-fever-like' illnesses were studied; their ages ranged between 4 and 47 years.

Eight patients had an atypical lymphocytosis in the peripheral blood. Of these, three had serological evidence of recent EBV infection (two with rising titres and one with seroconversion) and two had serological evidence of recent cytomegalovirus infection (one with a rising titre and one with seroconversion).

Five patients had no atypical lymphocytosis; one of these showed a rising EBV antibody titre.

Of the thirteen patients with Paul–Bunnell negative IM, four showed serological evidence of recent EBV infection. Recent EBV infection may have been associated

Table 1. *Prevalence of EBV antibody*

Family contacts of Paul–Bunnell positive IM compared with controls

	Contacts					Controls				
Age	No.	No. pos.	Pos. (%)	Geometric mean titre of pos. sera	No. of sera with titres ⩾ 1/256	No.	No. pos.	Pos. (%)	Geometric mean titre of pos. sera	No. of sera with titres ⩾ 1/256
10–19	65	29	45	41	Nil	67	44	66	71	6
20–29	50	39	78	51	3	51	44	86	80	6
30–39	31	28	90	67	6	37	33	89	60	3
40–49	89	78	88	67	12	91	84	92	73	8
50–59	63	60	95	67	6	63	59	94	64	6
60–69	12	10	83	104	1	13	12	92	54	7
Totals										
10–19	65	29	45	41	Nil	67	44	66	71	6
20–69	245	215	88	NE*	28	255	232	91	NE	30
All ages	310	244	79	NE	28	322	276	86	NE	36

* NE = Not estimated. Pos. = positive.

with the illnesses of five more patients but changing titres were not recorded. Cytomegalovirus mononucleosis was established in two patients, and in the remaining two patients, who had persistently negative EBV antibody titres, no aetiology was established.

Contacts of Paul–Bunnell positive patients

Prevalence of EBV Antibody

We investigated 335 contacts of 132 patients, of whom 265 (79%) possessed EBV antibody. Antibody prevalence among *family* contacts in a number of age-groups was compared with that in controls (Table 1). A significant difference was observed only in the decade 10–19; 45% of contacts in this age group (mostly patients' siblings) were antibody positive compared with 66% of controls ($\chi^2 = 5{\cdot}9$; $0{\cdot}02 > P > 0{\cdot}01$). Antibody titres among contacts in this age group were lower than among the corresponding controls (mean ages 15·1 and 15·0 years respectively).

Recent EBV infection among contacts

Three hundred and six contacts were followed for periods of up to 9 months. Twelve of 63 contacts initially lacking EBV antibody underwent sero-conversion, seven during the first 13 weeks and five during the second 13 weeks of follow-up. In addition, nine antibody-positive contacts showed a significant change in antibody titre during the first 15 weeks of follow-up. The relationship between changing EBV antibody titres at different ages and the development of Paul–Bunnell positive IM, is shown in Table 2.

Heterophile antibody and atypical lymphocytosis

Initial and final sera from 218 contacts were examined by the 'Monospot' test. Seven specimens were 'Monospot' positive; five of these were taken in the acute

Table 2. *Recent EBV infection among contacts of Paul–Bunnell positive IM*

Age group	No. investigated	Contacts with recent EBV infection: No. lacking EBV antibody at first visit	Contacts with recent EBV infection: No. with changing EBV antibody titre	Contacts with recent EBV infection: No. developing Paul–Bunnell positive IM	Ages of contacts with recent EBV infection: Developing IM	Ages of contacts with recent EBV infection: Not developing IM
10–29	115	45	10, (7, 3)*	3	20, 21, 22	12, 13, 18, 18, 20, 22, 23
30–69	191	18	11, (5, 6)	2	31, 47	40, 42, 46, 52, 54, 58, 59, 61
Totals 10–69	306	63	21, (12, 9)	5	—	—

* The first figure in parentheses represents contacts with sero-conversion, the second represents those with a significant rise or fall in titre.

stage of illness from contacts who developed Paul–Bunnell positive IM. The final serum from a contact aged 20 with subclinical EBV antibody acquisition was also 'Monospot' positive; this serum gave a positive result in the Paul–Bunnell–Davidsohn test. Another positive was obtained on testing the initial serum from the wife of a patient with IM. This contact had had a sore throat three weeks before her husband's illness, but although she possessed EB virus antibody there was no serological evidence of recent infection.

Blood films were made from the initial and final specimens of 176 contacts. Apart from the secondary cases of IM already mentioned only one contact was found to have an atypical lymphocytosis. This was noted in the initial blood specimen from a 34-year-old female contact who was asymptomatic and had an unchanging EB virus antibody titre.

Contacts of Paul–Bunnell negative patients

Twenty-six contacts of the thirteen Paul–Bunnell negative patients were investigated; in no case were significant clinical, serological or haematological changes observed.

Incubation period of IM

Seven patients developed Paul–Bunnell positive IM after a documented contact with the disease. Six of these secondary cases occurred in family members (four siblings and two spouses) who had been in continuous contact with a patient suffering from IM. In these instances the interval between the onset in the 'source' case and the onset in the 'secondary' case ranged from 6 to 23 weeks (mean; 14 weeks). The seventh patient became ill after intermittent contact with an IM patient for 8 weeks, the first contact occurring when the 'source' case had been ill for 6 days.

Fourteen other patients with Paul–Bunnell positive IM gave a history of possible contact but *confirmation of the diagnosis in the 'source' case was only available* in one instance.

DISCUSSION

This study confirms the constant association between Paul–Bunnell positive IM and the presence of EB virus antibody in a general population in Great Britain. Antibody was already present at first test or was shown to develop in all the patients, in contrast to an antibody prevalence of 78% in controls. Evidence that EB virus was acquired at about the time of the illness was obtained in a considerably higher proportion of cases, 63%, than in previous studies, since Niederman *et al.* (1968) found evidence of current infection in 7/29 of their patients and Joncas & Mitnyan (1970) showed a rising titre in only 15 of 129 cases. Although EB virus antibody usually appears early its late appearance has occasionally been described (Niederman *et al.* 1968). In this series 75% of the patients had developed EBV antibody by the 3rd week of illness and all were positive after the 5th week.

The study of family contacts also demonstrated the association between susceptibility to IM and lack of EB virus antibody, since evidence of recent acquisition of virus was obtained in all five patients who developed the disease. Four of them acquired antibody *de novo* and one showed a rising titre over the period in which the disease became manifest.

Patients with clinical or haematological features of IM in whom the Paul–Bunnell (heterophile antibody) test is negative form an interesting group of diverse aetiology. Of thirteen patients who failed to develop heterophile antibody evidence of recent EBV infection was obtained in four, and of cytomegalovirus infection in two. Five of the thirteen patients showed no atypical mononucleosis and were included in the study because they had clinically typical glandular fever. It is notable that a rising EB virus antibody titre was shown in one patient who had neither heterophile antibody nor atypical lymphocytosis. Five of the thirteen patients showed no evidence of recent EB virus or of recent cytomegalovirus infection; they all possessed EB virus antibody, and some, of course, may have been infected shortly before the first serum specimen was obtained.

Epidemiological studies have shown that EB virus is acquired rapidly in all communities, and that the rate of acquisition is higher in poor than in rich communities (Porter, Wimberley & Benyesh-Melnick, 1969). Since frank infectious mononucleosis is rare both in childhood and in older adults it is of interest to note whether any particular syndromes could be associated with EB virus in age groups other than those liable to IM. Henle & Henle (1970) re-examined some of the sera from the Cleveland family survey, and found a great paucity of illnesses in these careful records which could have corresponded to acquisition of EB virus. It was possible, for example, to calculate that if EB virus were a cause of non-streptococcal pharyngitis, it could not have accounted for more than about 2% of such illnesses. The susceptible contacts in our study, too, showed very little evidence of minor IM-like illnesses or haematological changes. Of twenty-one contacts with changing EBV antibody titres five developed Paul–Bunnell positive IM. Of the others,

eleven remained perfectly well during the 3–6 month period during which seroconversion was detected and five had an episode of minor upper respiratory tract infection which could easily, of course, have been caused by one of many other agents. Haematological changes were not found in the contacts with any frequency, only one positive Paul–Bunnell–Davidsohn test being detected in a contact with subclinical EB virus infection, together with another positive 'Monospot' test in a contact with a history of recent sore throat who possessed EBV antibody at first test. Since these haematological changes are transient we might have found them more often with more frequent tests. Few of our contacts were young children, and the age of the infected contacts who acquired EB antibody without illness ranged from 12 to 69. It is notable that eleven patients over 40 showed evidence of recent infection although only one of them developed mononucleosis. Similarly, Wahren, Lantorp, Sterner & Espmark (1970) found four seroconversions among twelve parents in their family study. The relative immunity to IM of the older age groups cannot therefore be accounted for solely by the high prevalence of pre-existing antibody in this group.

The epidemiology of EB virus infection has been studied mainly in populations of young adults in which the disease is common. Indeed, the evidence suggesting that EB virus is a necessary cause of IM was obtained from a study of such a group in the U.S.A. (Niederman *et al.* 1968; Evans *et al.* 1968) and similar findings have recently been reported in Britain (University Health Physicians and P.H.L.S. Joint Investigation, 1971). Little is known of the epidemiology of IM in civilian populations and the immunofluorescent technique for the detection of antibody to EB virus has provided an epidemiological tool by which the spread of this agent may be traced. Wahren *et al.* (1970) noted change in antibody titre in nine of twenty family contacts and Joncas & Mitnyan (1970) demonstrated seven seroconversions among 67 antibody negative contacts of a group of 129 patients with IM. In our study 12 of 63 (19%) of the initially negative contacts became infected during the 6 months after the index case was detected, while another nine contacts, already possessing antibody at first test, showed a change in titre. This rate of infection (or re-infection) among contacts suggests that virus is being excreted in the vicinity of the index case. Moreover, this estimate must represent a minimum value, since EBV antibody titres tend to level out soon after infection, so that we are unable to say how many of the group initially antibody positive had been recently infected. We hope to obtain further evidence on this point by other methods. This relatively high infectivity of the virus is not, of course, expressed clinically with any frequency, and only five contacts developed the disease. The secondary attack rate of IM was 6% (4 of 63) among those not possessing pre-existing antibody, or 4% (2 of 45) among the susceptibles aged 10–29 years. Yale students showed a remarkably high ratio of illness to inapparent infection of 2:1 (Niederman, Evans, Subrahmanyan & McCollum, 1970) while British students showed a ratio of 1:1 (University Health Physicians and P.H.L.S., 1971). In our contacts the overall ratio of illness to inapparent infection was approximately 1:3.

The evidence of causal association between EB virus infection and IM is based

on the insusceptibility to the disease in those already possessing antibody to the virus, and the finding that, conversely, all patients developing IM are derived from the group previously lacking antibody. The transmission of EB virus to cell cultures previously free from the virus has been claimed, using material from throat swabs or washings from patients with IM (Golden, Chang, Lou & Cooper, 1971; Pereira *et al.* 1972), but the problems of handling EB virus have made it difficult to prove unequivocally a causal association, and other interpretations of the association of EB virus with IM have been proffered (Hirshaut *et al.* 1969). One finding in this family study does provide support for the view that recent EB virus infection is a necessary cause of IM. The young family contacts, mostly sibs, of the index cases of IM showed a significantly lower prevalence of antibody than did their age-matched controls, suggesting that the cases of IM come from families with a lower than normal previous experience of this virus. For reasons already stated, moreover, the difference observed between two test and control groups is likely to be an underestimate. Antibody measurements in a normal community then tend to confirm the evidence of causal association between EB virus and IM, obtained from the study of student populations.

It is a pleasure to thank Professor Harold Stern and Dr Douglas Fleck for much valuable help, Miss Yvonne Tryhorn for expert technical assistance, and the many family doctors who referred patients for their generous cooperation. Our thanks are due also to the Royal College of Physicians of London, who, through the London Fever Hospital Research Fund, provided financial support for the project.

REFERENCES

BRUMFITT, W. & O'GRADY, F. (1957). Slide screening test for Glandular Fever. *Journal of Clinical Pathology* **10**, 243.

DAVIDSOHN, I. & HENRY, J. (1968). In *Clinical Diagnosis by Laboratory Methods*. 14th Ed. (Philadelphia: Saunders).

DAVIDSOHN, I. & LEE, C. L. (1969). In *Infectious Mononucleosis*. Ed. R. L. Carter and H. G. Penman. Oxford and Edinburgh: Blackwell Scientific Publications.

EPSTEIN, M. A. (1970). Aspects of the EB virus. *Advances in Cancer Research* **3**, 383.

EVANS, A. S., NIEDERMAN, J. C. & MCCOLLUM, R. W. (1968). Sero-epidemiologic studies of Infectious Mononucleosis with EB virus. *New England Journal of Medicine* **279**, 1121.

EVANS, A. S., ROTHFIELD, N. F. & NIEDERMAN, J. C. (1971). Raised antibody titres to EB virus in systemic lupus erythematosus. *Lancet* i, 167.

GOLDEN, H. D., CHANG, R. S., LOU, J. J. & COOPER, T. Y. (1971). A filterable agent in throat washings of patients with infectious mononucleosis. *Journal of Infectious Diseases* **124**, 422.

HENLE, G. & HENLE, W. (1966). Immunofluorescence in cells derived from Burkitt's lymphoma. *Journal of Bacteriology* **91**, 1248.

HENLE, G. & HENLE, W. (1970). Observations on childhood infections with Epstein–Barr virus. *Journal of Infectious Diseases* **121**, 303.

HENLE, G., HENLE, W. & DIEHL, V. (1968). Relation of Burkitt's tumour – associated Herpes-type virus to infectious mononucleosis. *Proceedings of the National Academy of Sciences of the U.S.A.* **59**, 94.

HENLE, G., HENLE, W., CLIFFORD, P., DIEHL, V., KAFUKO, G. W., KIRYA, B. G., KLEIN, G., MORROW, R. H., MUNUBE, G. M. R., PIKE, P., TUKEI, P. M. & ZIEGLER, J. L. (1969). Antibodies to Epstein–Barr virus in Burkitt's lymphoma and control groups. *Journal of the National Cancer Institute* **43**, 1147.

HIRSHAUT, Y., GLADE, P., MOSES, H., MANAKER, R. & CHESSIN, L. (1969). Association of Herpes-like virus infection with infectious mononucleosis. *American Journal of Medicine* **47**, 520.

HIRSHAUT, Y., GLADE, P., VIEIRA, L. O. B. D., AINBENDER, E., DVORAK, B. & SILTZBACH, L. E. (1970). Sarcoidosis, another disease associated with serologic evidence for herpes-like infection. *New England Journal of Medicine* **283**, 502.

JONCAS, J. & MITNYAN, C. (1970). Serological response of the EBV antibodies in pediatric cases of infectious mononucleosis and in their contacts. *Canadian Medical Association Journal* **102**, 1260.

LEE, C. L., DAVIDSOHN, I. & SLABY, R. (1968). Horse agglutinins in infectious mononucleosis. *American Journal of Clinical Pathology* **49**, 3.

NIEDERMAN, J. C., MCCOLLUM, R. W., HENLE, G. & HENLE, W. (1968). Infectious Mononucleosis. Clinical manifestations in relation to EB virus antibodies. *Journal of the American Medical Association* **203**, 139.

NIEDERMAN, J. C., EVANS, A. S., SUBRAHMANYAN, L. & MCCOLLUM, R. W. (1970). Prevalence, incidence, and persistence of EB virus antibody in young adults. *American Journal of Medicine* **282**, 361.

PAPAGEORGIOU, P. S., SOROKIN, C., KOUTZOUZAKOGLOU, K. & GLADE, P. R. (1971). Herpes-like Epstein–Barr virus in leprosy. *Nature, London* **231**, 47.

PEREIRA, M. S., BLAKE, J. M. & MACRAE, A. D. (1969). EB virus antibody at different ages. *British Medical Journal* iv, 526.

PEREIRA, M. S., FIELD, A. M., BLAKE, J. M., RODGERS, F. G., BAILEY, L. A. & DAVIES, J. R. (1972). Evidence for oral excretion of EB virus in infectious mononucleosis. *Lancet* i, 710.

PORTER, D. D., WIMBERLEY, I. & BENYESH-MELNICK, M. (1969). Prevalence of antibodies to EB virus and other herpes viruses. *Journal of the American Medical Association* **208**, 1675.

SABIN, A. B. & FELDMAN, H. A. (1948). Dyes and microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). *Science, New York* **108**, 660.

STERN, H. & ELEK, S. D. (1965). The incidence of infection with cytomegalovirus in a normal population. A serological study in Greater London. *Journal of Hygiene* **63**, 79.

STEWART, G. L., PARKMAN, P. D., HOPPS, H. E., DOUGLAS, R. D., HAMILTON, J. P. & MEYER, H. M. (1967). Rubella-virus haemagglutination-inhibition test. *New England Journal of Medicine* **276**, 554.

UNIVERSITY HEALTH PHYSICIANS AND P.H.L.S. JOINT INVESTIGATION (1971). Infectious mononucleosis and its relationship to EB virus antibody. *British Medical Journal* iv, 643.

WAHREN, B., LANTORP, K., STERNER, G. & ESPMARK, A. (1970). EBV antibodies in family contacts of patients with Infectious Mononucleosis. *Proceedings of the Society for Experimental Biology and Medicine* **133**, 934.