Infectious Mononucleosis at the United States Military Academy.
A Prospective Study of a Single Class Over Four Years

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A prospective study of EB virus infections was initiated in July, 1969 in the entering class of 1401 cadets, at the U.S. Military Academy at West Point, N.Y. and continued over 4 yr. On entry 63.5% possessed EBV antibody and 36.5 lacked EBV antibody. The rate of antibody prevalence varied with the geographic area from which the cadet originated.

Except in two cadets already ill on first bleeding no evidence of clinical infectious mononucleosis (I.M.) occurred over the 4 yr period in the 890 cadets entering the Academy with EBV antibody. Among 437 cadets without antibody on entry, 54 or 12.4% were infected (seroconverted) in the freshman year; 15 of these had clinical I.M., 12 had suggestive I.M., and 39 had no known mono-like illness. The annual infection rates in susceptible cadets in the second, third, and fourth years were 24.4, 15.1, and 30.8 per 100, respectively. Of 201 cadets infected with EBV over 4 yr only 26.4% were manifested by heterophile positive clinical infectious mononucleosis. Overall, 46% of the 437 cadets entering without EBV antibody became infected over 40 mo of serologic observation; definite clinical infectious mononucleosis developed in 53 cadets, a clinical attack rate of 12.1 per 100 for 4 yr. The EBV infection rate among exposed and susceptible roommates of known cases was no higher than in roommates not so exposed.

Elevations of EBV-specific and total IgM occurred during acute illness and dis-

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INTRODUCTION

Infectious mononucleosis in the Armed Forces has been a generally underrated and little studied disease. Important clinical analyses have been made in military populations (1) but there has been little appreciation of its high incidence, the degree and duration of disability and of lost time associated with it, or the epidemiologic features of the disease in this setting. Recently, a review of the incidence of infectious mononucleosis in the Armed Forces reported hospital admission rates from 140 to 228 per 100,000 (2). This places it among the first five infectious diseases of importance in the military and the fourth highest cause of days of hospitalization among Navy and Marine personnel. In these populations the lost time resulting from infectious mononucleosis was similar to that from viral hepatitis.

The present investigation uses antibody to EB virus as a marker of susceptibility, immunity, and infection in a prospective study of the disease among United States Military Academy cadets. These methods have been successfully employed in investigations of infectious mononucleosis among college students of the same age (3–8). A recent investigation by Lehane (9) has also utilized EBV antibody measurement to indicate infection rates of 166 per 1000 per year in Marine recruits at Parris Island.

The population described here consisted of the entering freshman class in 1969 at the United States Military Academy. This report records serial clinical and serologic observations made during their 4 yr at the Academy including the prevalence and incidence of EBV infection, the occurrence of infectious mononucleosis, and the contagiousness of the disease in roommates.

MATERIALS AND METHODS

Study Design

The entire entering class in 1969 was chosen for study. Blood samples and questionnaires were obtained from 1401 cadets in July 1969 on arrival at the Academy and on subsequent occasions as shown in Table 1. All cadets were observed for

| Date          | Status of Cadet                              | Group bled         | Number bled |
|---------------|----------------------------------------------|--------------------|-------------|
| July 1969     | Start of 4 yr program                        | Entire group       | 1401        |
| June 1970     | End of first academic year                   | Entire group available | 1148    |
| January 28, 1971 | After Christmas vacation and just prior to expected | Those lacking EBV antibody | 304    |
|               | February peak of I.M.                        |                    |             |
| April/May 1971| End of second academic year                  | Entire group available | 1046   |
| April 1972    | Toward end of third academic year            | Those lacking EBV antibody | 186    |
| October 1972  | Last available chance during fourth academic year | Entire group available | 937    |

* Bleedings had to be synchronized with annual physical examinations in most instances. Additional sera were collected during suspected infectious mononucleosis and in contacts.
infectious mononucleosis and monitored for other illness without knowledge of their entering EBV antibody status. Interval blood samples were collected during hospitalization of these cadets for acute respiratory disease or for infectious mononucleosis. Roommates of known I.M. cases occurring during the first 2 yr of the study were closely followed by serial bleedings and questionnaires over a period of 2 mo after exposure to determine the spread of infection to susceptible and exposed contacts.

**Laboratory Methods**

Serum samples were stored at \(-20^\circ\text{C}\) in 0.5 ml aliquots until tested. EBV antibody was measured by the indirect immunofluorescence technique utilizing the EB3 cell line (10) with four preparations on a slide. The sera were not inactivated. The test was read independently by two senior observers (ASE and JCN) with an agreement in titer within one dilution in over 90% of the sera examined. All sera with disputed readings or doubtful cell preparations were repeated. A single standard positive serum (titer 1:160) or a negative saline preparation was included with each three sera screened and in each run of quantitative titrations. Screening tests were made first at a 1:5 serum dilution and read at 250× magnification. Then all EBV antibody negative sera were retested simultaneously with serial serum samples obtained at the next bleedings to determine if seroconversion had occurred. When equivocal or definite evidence of seroconversion was seen, both sera were titrated quantitatively along with serum samples obtained during any interim illness. All preillness sera were retested undiluted, and at 1:2.5, 1:5, and 1:10 dilutions to insure complete absence of EBV antibody; all illness or postillness sera were titrated in doubling microtiter dilutions from 1:5 to 1:2560. The highest dilution yielding definitely positive cells at 400× magnification on which two readers independently agreed was taken as the titer.

Two Leitz Labolux fluorescence binocular microscopes with 200 W Osram light sources and dry dark-field condensers were used. Screening was done at 250 magnification. BG12 (heat) and OG1 (exciter) filters were employed near the light source and K420 (barrier) in the oculars.\(^1\) The fluorescein labeled conjugate employed was an anti-IgG (human) serum prepared in rabbits\(^2\) and was titrated against a known reference conjugate provided through the courtesy of the Special Leukemia Project, National Cancer Institute, using the known standard serum in a checkerboard titration. Very similar patterns were seen with both conjugates; usually a conjugate dilution of 1:30 was employed.

EBV-specific IgM determinations followed a three layer indirect immunofluorescence method described by Schmitz and Scherer (11); these were read under oil (100×) using a KP 490 exciter filter and a K510 barrier filter.

Heterophile antibody determinations were made routinely by a quantitative titration before and after absorption with guinea pig kidney and carried out on microtiter plates according to methods of the Center for Disease Control and confirmed by ox-cell hemolysin tests performed at the Connecticut State Health Department through the courtesy of Mrs. Bernice West.

Immunoglobulin determinations for IgG, IgM, and IgA were made on immunodiffusion plates.\(^3\) All IgM tests were done in duplicate and the average taken. The

\(^1\) More recently we have used a BG#2 (heat), KP490 (exciter), and K510 (barrier) filter for better illumination.

\(^2\) Obtained from Hyland Laboratories, Costa Mesa, CA, 92626.
sera had been stored at −20°C and all sera from a single cadet were titrated simultaneously to provide uniformity of results. Standard preparations of known content were included.

**Statistical and Computer Methods**

All data were entered into an IBM computer 7094 system, through a precoded questionnaire or standard laboratory code sheet. Two by two tables for analysis of data were made through a statistical analysis program. Computer cards listing many characteristics of the entering West Point class were made available through the Computer Center at the United States Military Academy, West Point.

**Diagnosis**

Criteria of diagnosis of I.M. included (1) a clinical picture of sore throat, fever, and cervical lymphadenopathy; (2) a total lymphocyte and monocyte count of 50% or more with at least 10% atypical lymphocytes; (3) elevated heterophile antibody titer (≥1:40) after guinea pig kidney absorption or beef hemolysin titer (≥1:80) and; (4) the development of EBV antibody between pre- and postillness sera.

**Clinical Surveillance**

Two of the authors saw all cases suspected of infectious mononucleosis and/or reviewed the records. In addition, at the end of the 4 yr study the records of all cadets in the study were reviewed to identify outpatient visits or hospitalizations for illnesses suggestive of infectious mononucleosis. These were all tabulated without knowledge of EBV antibody status and later correlated with initial antibody status and with EBV antibody seroconversion in the 2 sera which bracketed the suspected illness in time.

**RESULTS**

**Background Data**

The United States Military Academy was selected because of certain features of its training program, the numerous studies of infectious mononucleosis at this institution in the past, the disciplined military environment, the close daily contacts of roommates, and the regulated diet and exercise. Above all was a willingness and interest of the medical and administrative staff to cooperate in the study and the participation of the cadets. In the first year at the Academy cadet leaves are generally limited to Christmas and spring vacations and to supervised weekend events. Increasing leave privileges were granted in the second and subsequent years.

The study of infectious mononucleosis at West Point has been a traditional area of inquiry through the work of Col. Robert S. Hoagland, M. C. (Ret.). In addition to extensive clinical studies, it was here that he suggested kissing as an important means of spread (12) and estimated the incubation period to be between 30 and 49 days (13).

There were an average of 58.5 cases of infectious mononucleosis per year at United States Military Academy in the 5 yr preceding the present study. The yearly rate approximated 1000 per 100,000. The distribution by month showed a sharp and constant peak in February. This reflected the occurrence of cases about 6 wk following the cadets' Christmas leave. An analysis of cases by company and squad gave no clear-cut indication of clustering of cases.
Prevalence of EBV Antibody on Entry

Sera from 1401 first year cadets obtained on their entry into the preacademic summer program of the Academy in June, 1969 were tested for EBV antibody at a 1:5 dilution: 63.5% possessed antibody and 36.5% lacked detectable antibody. The 890 subjects with antibody were regarded as having had an EBV infection in the past but only 5.6% of these gave a history of known infectious mononucleosis. The 511 cadets lacking EBV antibody were considered susceptibles; 3% of these also gave a history of earlier "infectious mononucleosis." In neither group was the past history accepted as reliable because of errors in recall or in diagnosis. Eighty-one percent of 42 black cadets and 62% of 1357 white cadets had detectable serum antibody to EBV \( (P = < 0.02) \). The home state of the cadet was analyzed in relation to EBV antibody status for those 1281 subjects who had previously resided in the same state for at least 6 yr before entering the Academy. To obtain meaningful numbers, states were grouped into larger geographic areas. The results are given in Table 2. The lowest prevalence rates were 51.9% in cadets from the sparsely populated West North Central States of the Plains and 52.9% in the New England area. The highest prevalence rates for EBV antibody were 81.5% in the cadets from the East South Central States and 76.9% in cadets from the West South Central States. Similar results were obtained when the analysis was limited to cadets resident for 12 years in these respective areas. The rates in these different areas might reflect a variety of factors: racial, socioeconomic, population density, hygienic standards, etc. Many of these are obviously interrelated and interdependent. It has already been noted that antibody prevalence rates in the small number of blacks in the class was significantly higher than in whites, probably reflecting differing socioeconomic backgrounds. Crowding in the home as measured by size of the family was not related to antibody prevalence. One indicator that might reflect socioeconomic levels was the income of the cadets' parents. Analyses indicated a high EBV antibody prevalence rate of 77.1% in cadets from families with incomes under $6,000 and a low prevalence rate of 58.6% cadets from families with incomes over $30,000. These differences were significant statistically \( (P = < 0.05) \). However, no clear step-wise correlation

| Area            | States included                                | No. in area | Antibody prevalence |
|-----------------|------------------------------------------------|-------------|---------------------|
| 1. W.N. Central | Minn., Io., Mo., N. Dakota,                    | 77          | 51.9                |
|                 | S. Dakota, Nebraska, Kansas                   |             |                     |
| 2. New England  | Maine, Mass., N.H., Vt., R.I., Conn.          | 68          | 52.9                |
| 3. S. Atlantic  | Del., Md., Va., W. Va., N.C., S.C., Ga., Fla. | 170         | 62.4                |
| 4. Mid Atlantic | N.Y., N. Jersey                                | 196         | 57.1                |
| 5. Pacific      | Wash., Oregon, Cal., Alaska, Hawaii           | 16          | 62.9                |
| 6. E.N. Central | Ohio, Ind., Ill., Mich., Wisc.                | 149         | 63.1                |
| 7. Mountain     | Mont., Ida., Wy., Col., N.M., Ariz.           | 37          | 67.6                |
| 8. W.S. Central | Ark., La., Okla., Tex.                        | 52          | 76.9                |
| 9. E.S. Central | Ky., Tenn., Ala., Miss.                       | 27          | 81.5                |
existed at intermediate levels. No significant difference in EBV antibody prevalence rates was seen between cadets having public (63%) or private (60%) secondary school education.

**Incidence of EBV Infection**

*First year.* All 1401 entering cadets were carefully followed for the occurrence of febrile illnesses suggestive of infectious mononucleosis without knowledge of their initial EBV antibody status. Among the 890 cadets who were later found to possess EBV antibody on entry, two typical cases of infectious mononucleosis occurred. Both individuals were ill at time of the initial bleeding with positive heterophile and elevated EBV antibody tests. Among 437 cadets who lacked EBV antibody on entry and were followed, seroconversion occurred in 54 cadets during the freshman year and a clinical diagnosis of infectious mononucleosis was made in 16 of these.

Fifteen of the 16 cases had elevated sheep cell agglutination titers on sera absorbed with guinea pig kidney, and 14 had elevated ox cell hemolysin titers. The clinical attack rate in susceptible cadets was 15/437 or 3.43 per 100 in the first year. EBV antibody was absent in undiluted preillness sera from each of these 15 cases and consistently appeared during illness with titers of 1:10 to 1:320 (Table 3). The average hospital stay of 13 cases cared for in the station hospital was 22 days with a range of 9–47 days. As cadets must return to full academic and military activity on discharge, hospitalization is longer than in college students.

The 16th case diagnosed as I.M. showed fever, adenopathy, splenomegaly, and atypical lymphocytosis but was persistently heterophile and EBV antibody negative; subsequent serologic testing revealed a rise in adenovirus CF titer from <1:8 in the preillness serum to 1:16 during convalescence. No serologic evidence of cytomegalovirus, herpes simplex or toxoplasma infection was found. This case is regarded as an infectious mononucleosis-like syndrome probably due to adenovirus infection.

Six other cadets in whom a clinical diagnosis of “possible infectious mononucleosis” was made were all heterophile antibody negative, failed to meet the hematological criteria of infectious mononucleosis, and showed no change in EBV antibody status.

EBV infection without full-blown illness was detected by seroconversion in 39 cadets and the antibody titers found were similar to those clinically ill. Careful retrospective tests for heterophile antibody on sera from this group revealed 12 cadets with a fourfold or greater rise in sheep cell and/or beef cell hemolysin tests over preillness values. This was accompanied by changes in total IgM levels in

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**TABLE 3**

LEVELS OF EBV ANTIBODY IN FRESHMAN CADETS BEFORE, DURING AND AFTER CLINICAL INFECTIOUS MONONUCLEOSIS

| Time              | <U | 10 | 20 | 40 | 80 | 160 | 320 | G.M.T. |
|-------------------|----|----|----|----|----|-----|-----|--------|
| Pre-illness (July 1969) | 15 |    |    |    |    |     |     |        |
| During illness*   | 2  | 4  | 4  | 3  | 1  | 62.5|     |        |
| After illness (June 1970) | 3  | 5  | 6  | 1  |    | 101.0|     |        |

* No sera was available in one cadet during his acute illness. G.M.T. = Geometric mean titer.

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*Performed at the Center for Disease Control, Atlanta, GA.*
five subjects. Review of these hospital records indicated that an episode of pharyngitis had occurred in eight of these 12 cadets with rises in heterophile antibody titers but they had been inadequately studied to differentiate infectious mononucleosis.

Thus during the first year there were 54 EBV antibody seroconversions in 437 antibody negative cadets, an infection rate of 12.4%. Among the 54 cadets infected, definite clinical I.M. occurred in 15 cadets (27.8%) and retrospective analysis revealed suspected I.M. in another 12 (22.2%), for a total of 50%. The other 50% presumably had mild or asymptomatic infections for which they did not seek medical care. The apparent (definite + suspected):inapparent infection ratio was 1:1. With the exception of two cadets who were ill with infectious mononucleosis at the time of initial bleeding, no clinical case fulfilling the diagnostic criteria of infectious mononucleosis occurred in the 890 cadets possessing EBV antibody on entry. Figure 1 summarizes the findings of the first year.

Results of the second, third, and fourth years of the cohort group were similar to the first year and are therefore more briefly discussed.

**Second year.** Three-hundred-fifty-six cadets who lacked EBV antibody at the start of the second year were available for follow-up. During the second year 87 of these susceptible cadets developed EBV antibody, a seroconversion or infection rate of 24.4 per 100. Of these seroconverters definite clinical infectious mononucleosis was recognized in 21 (24.1%), suspected I.M. in three (3.4%), and no known illness in 63 (72.4%). This was an apparent:inapparent infection ratio of 24:63 or 1:2.6. Of those without known I.M. nine showed a rise in heterophile antibody titer.

**Third year.** Follow-up of 186 cadets lacking EBV antibody at the start of the year revealed 28 seroconversions during the third year, an infection rate of 15.1%. This included seven cadets with definite infectious mononucleosis, one with suspected I.M., and 20 with no known illness. EBV infection thus was clinically definite in 25.0% and the apparent:inapparent ratio was 8:20 or 1:2.5.

**Fourth year.** The last serum collection possible was in October 1972 of the senior year. On the basis of cadets lacking antibody in the preceding serum sample (April, 1972) a seroconversion rate of 30.8 per 100 was found between the two bleedings 6 mo apart. Clinical surveillance carried out in the entire group throughout the senior year revealed ten cadets with clinical infectious mononucleosis. All of these had lacked EBV antibody in the preceding bleeding.

A summary of the 4 yr experience is shown in Table 4. There were 201 demonstrable EBV infections among the 437 cadets who lacked antibody on entry into the academy, an infection rate of 45.9% over the 4 yr period. The actual rate

![Figure 1](image-url)

**Fig. 1.** EBV antibody status and occurrence of infectious mononucleosis in 1327 cadets on entry into the United States Military Academy and at end of the first year.
TABLE 4
EBV Infection Rates in the Cadet Class of 1973 U.S. Military Academy During Four Undergraduate Years

| Yr                | No. without EBV antibody | With definite clinical IM | IM suspected | No known IM* | Infections | EBV infection rate per 100 susceptibles per year† | % of those infected with definite IM. |
|-------------------|--------------------------|---------------------------|--------------|--------------|------------|-----------------------------------------------|-------------------------------------|
| First (1969-1970) | 437                      | 15                        | 12           | 27           | 54         | 12.4                                          | 27.8                                |
| Second (1970-1971)| 336                      | 21                        | 3            | 63           | 87         | 24.4                                          | 24.1                                |
| Third (1971-1972)| 186                      | 7                         | 1            | 90           | 28         | 15.1                                          | 25.0                                |
| Fourth* (1972-1973) | 208                     | 10                        | 0            | 22           | 32         | 30.8                                          | 12.5†                               |
| **Total**         | **53**                   | **16**                    | **132**      | **201**      | **401**    | **20.7**                                     | **26.4**                            |

* Seroconversion in fourth year based on serum collection in April and October 1973.
† Based on elapsed months between bleedings converted to rate per year (see Table 1).
‡ Based on no. cases occurring between April and October 1973.

would be higher as some of this initial group resigned from the Academy for personal or academic reasons. Based on those cadets available for study, the average annual EBV infection rate per 100 susceptibles was 20.7. Clinically recognized and serologically confirmed infectious mononucleosis occurred in 53 cadets or 26.4% of those with EBV infections. No cases developed among 890 healthy cadets who possessed EBV antibody or entry (except two who were ill at the time of initial bleeding).

*Season.* No clearcut February peak of cases occurred in the present study in contrast to the preceding 5 yr. This may have been related to increased social privileges on weekends rather than just during Christmas vacation.

*Contagiousness*

No clustering of cases in particular dormitory or military units was apparent. However, two cases occurred in the same company 30 days apart after they had been housed in the same barracks together on entry into the preacademic summer training program at Camp Buckner. The index case had arrived there with infectious mononucleosis and was ill at the time of the initial bleeding. The degree of contact between the two cadets was unknown.

Special attention was directed at close clinical and serologic follow-up of roommates of clinical cases for at least 2 mo, using the absence of EBV antibody as a marker of susceptibility in the exposed roommate and EBV seroconversion as evidence of EBV infection. Each cadet usually had two to three roommates during a year with whom he was closely associated in the dormitory, in the classroom, on the drill field, in the mess hall, and in off-duty activities. Such close and prolonged exposure would be conducive to the spread of most diseases transmitted by close contact. This type of surveillance was carried out during the first 2 yr of the study.

Evidence of clearcut clinical infectious mononucleosis occurred in only two roommates of index cases. In one instance the 70 day interval between onset of illness in two cadets makes it unlikely that this represented a direct spread secondary case because the usual incubation period is taken to be 30–50 days (13).
In the other instance I.M. in one roommate was followed 39 days later by a positive mono-spot test and the appearance of EBV antibody but without known clinical illness; this may have reflected secondary infection based on personal contact.

The rate of EBV infection in susceptible and exposed roommates of index clinical cases of infectious mononucleosis was compared to the rate in susceptible but unexposed persons with healthy roommates. During the first year two of 13 exposed and susceptible exposed roommates had serologic evidence of infection (15.4%) without clinical illness as compared to an infection rate of 52/424 or 12.2% in nonexposed roommates. In the second year the infection rates were 18.2% in 11 exposed and 24.6% in 345 nonexposed and susceptible cadets. There was therefore no evidence of a higher risk of infection in exposed roommates of index cases. This was further substantiated by analyzing data for the 2 yr on the basis of observed and expected EBV infection rates. These results are shown in Table 5 in which the number of susceptibles has been adjusted to represent the changing pattern over 2 yr. There was no significant difference in infection rates in the exposed and nonexposed roommates. Indeed, the observed number of infections in the exposed group was half as many as would have been expected; but the difference was not statistically significant.

An effort was made to determine the contagiousness of an EBV infection without known infectious mononucleosis to susceptible roommates. Of 203 roommates of 39 “subclinical” cases tested in the first year, 88 lacked EBV antibody. Of these, eight or 9.1% seroconverted to positive, and two of these had clinical infectious mononucleosis. However, in the absence of clinical features to mark the time of occurrence of the index case, it is not possible to determine which roommate was infected first or whether both were infected at the same time. The difficulty was further compounded by one to three changes of roommates within a year. All that can be said is that when subclinical cases in roommates were studied the infection rate did not seem higher than in the total group of susceptibles.

**Immunoglobulins**

**Normal values.** As a reference baseline, immunoglobulin levels were determined on fresh sera from 100 cadets on their initial bleeding. These results are listed in Table 6.

**During clinical I.M.** The mean immunoglobulin levels observed in 15 cadets before, during and after clinical infectious mononucleosis are shown in relation to...
TABLE 6
IMMUNOGLOBULIN LEVELS

| Subjects | No. tested | Immunoglobulin in mg/100 |
|----------|------------|--------------------------|
|          |            | IgG | IgA | IgM |
| I. Healthy cadets* | 100 | 1269.0 | 229.8 | 92.8 |
| SD       |            | ±27 | ±28.9 | ±29.8 |
| II. Clinical I.M. |       |       |       |     |
| Preillness | 15 | 1363.3 | 202.0 | 96.3 |
| 0-30 days | 14 | 1303.3 | 266.3 | 182.8 |
| 31-60 days | 9 | 1438.9 | 278.3 | 148.0 |
| 61-90 days | 5 | 1400.0 | 244.0 | 113.6 |
| 91-316 days | 12 | 1342.1 | 244.6 | 96.8 |
| III. Subclinical I.M. |       |       |       |     |
| July 1969 | 35 | 1368.8 | 201.6 | 93.7 |
| June 1970 | 35 | 1338.6 | 216.6 | 95.6 |

* These values were obtained on fresh sera in duplicate determinations.

normal levels in Table 6 and Fig. 2. The mean IgM level increased from 96.3 mg% in preillness specimens to 182.8 mg% during acute illness, decreased to 148.0 mg% in the second month after onset and then returned to normal. Increases in IgG and IgA values also occurred but developed somewhat later and persisted for longer periods. Correlations between the degree of IgM increases and individual peak titers of heterophile or of EBV antibody titers were not found even though all increased at about the same time.

During subclinical infections. Immunoglobulin values in the initial sera and in those obtained at the end of the year were also measured in 35 cadets with subclinical EBV seroconversion to determine whether elevated IgM levels reflected asymptomatic or mild infection. No such changes were demonstrated (Table 6). The mean IgM value in preilness sera was 93.7 mg% and in postillness sera was 95.6 mg%. As the date of infection in relation to serum sampling could not be determined, it is not clear whether the second serum sample may have been too late to reflect IgM increases or whether such increases in IgM do not occur in mild or asymptomatic infections.

![Fig. 2. Immunoglobulin levels in relation to pre-illness values in 15 cases of infectious mononucleosis.](image)
EBV-specific IgM. The indirect immunofluorescence test of Schmitz and Scherer (11) was used to demonstrate EBV-specific IgM. To date, 185 sera from 35 cases before and during infectious mononucleosis have been tested. Seventy-two sera taken prior to illness lacked EBV-specific IgM antibody. It was demonstrated in 84% of 25 sera obtained during the first month after onset and with decreasing frequency after that. Forty-seven sera tested 12 mo or more after onset lacked this antibody. These preliminary results will be reported in more detail when completed.

**ABO Blood Groups and EBV Infections**

The risk of EBV infection in persons of different blood groups was analyzed. Of 330 lacking EBV antibody on the first bleeding (July 1969) and rebled in October, 1972, 157 or 47.6% had developed EBV antibody in this 40 mo period. As shown in Table 7 no significant difference in susceptibility to EBV infection was noted in persons of different blood groups.

**DISCUSSION**

Sera from 63.5% of 1401 entering cadets at United States Military Academy (USMA) had antibody to EB virus indicative of prior infection; only 5.6% of these gave a history of prior infectious mononucleosis. This antibody prevalence is higher than that of 51% found in a recent analysis of entering freshmen of the same age at Yale University (6) and that of 57% among first year students in English colleges and universities in 1969 (7). On the other hand, Marine recruits of similar age have an entering antibody frequency of 85.7% (9). EBV antibody prevalence varied with the geographic area in which the cadet had previously lived over the past 6–12 yr or more. The highest prevalence rates of over 70% occurred in cadets who lived in East and West South Central States (Arkansas, Louisiana, Oklahoma, Texas, Kentucky, Tennessee, Alabama, Mississippi) and the lowest rates of about 50% or less in those from West North Central States (Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, Kansas) and the New England States. Social and economic levels may be the determining factors in EBV infection rates in childhood rather than climate or population density per se. The occurrence of the highest antibody rates in cadets from low income families and the lowest prevalence rates in cadets with parents in high income brackets supported this concept. On the other hand, possible crowding in the home as reflected by the number

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**Table 7**

RELATION OF EBV INFECTION TO ABO BLOOD GROUPS IN 330 CADETS LACKING EBV ANTIBODY IN JUNE 1969 AND FOLLOWED TO OCTOBER 1972

| Blood group | Total | Antibody negative | Antibody positive |
|-------------|-------|-------------------|-------------------|
|             |       | Observed  | Expected | Observed | Expected |
| A           | 145   | 68       | 76.0     | 77       | 69.0     |
| B           | 26    | 16       | 13.6     | 10       | 12.4     |
| O           | 147   | 85       | 77.1     | 62       | 69.9     |
| AB          | 12    | 4        | 6.3      | 8        | 5.7      |
|             | 330   | 173      |          | 157      |          |

*a Based on seroconversion rate of 157/330 = 47.6%. \( \chi^2 = 6.12 \) = No significant difference between blood groups.

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**Notes:**

- EBV: Epstein-Barr Virus
- ABO: American National Blood Groups
- antibody: antibody response
- EBV-specific: EBV-specific antibody
- IgM: Immunoglobulin M
- ABO Blood Groups: Blood groups based on the ABO system
- EBV Infections: Epstein-Barr virus infections
- Discussion: Further analysis and implications of the data

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of children in the family or the population density of the community could not be correlated with prevalence rates. The most important determinant of early infection is probably the opportunity for close and prolonged contact in early childhood under circumstances which promote oral or oral/fecal spread. We have no direct measure of this.

This prospective study of EBV infection confirms prior investigations in Yale University students (3–6) on the relationship of EB virus and infectious mononucleosis. Similar findings have also been reported in 2 other prospective studies:—one in England (7) and one in the U.S. (8). In the English study 1457 freshmen in five universities and colleges were bled on entry and at the end of the first year with careful clinical and laboratory surveillance during the intervening 7 mo. In the American investigation, published as yet only in abstract form, 800 Cornell University students were bled on entry and followed over a 2 yr period (8); 15 cases of infectious mononucleosis occurred in 200 students lacking EBV antibody, a rate of 7.5%.

These prospective surveys support the concept that EB virus is the sole cause of heterophile-positive infectious mononucleosis and that this clinical picture is the predominant host response to EBV infection in young adults. The presence or absence of antibody to EBV at a 1:5 serum dilution has provided a reliable indicator of immunity and susceptibility to clinical disease in all three studies. No cases of clinical infectious mononucleosis occurred in students with EBV antibody on their entry into college. In those lacking antibody on entry the EBV infection rate in freshman college students has been remarkably similar in different settings: 12.4% in the cadets of the current report, 12.0% in first year students in 5 English colleges and universities (7), and 13.1% in Yale College freshmen (6). However, the frequency with which these infections have been expressed as clinical illness has varied in the different groups. In the military cadets, 26.4% of 201 EBV infections were accompanied by definite evidence of heterophile-positive infectious mononucleosis. In English college students 45% of those infected with EB virus had suspected or definite infectious mononucleosis, and in EBV-infected Yale college students 74% had clinical episodes compatible with the disease (14). These differences may reflect various patterns of motivation in students to seek medical care, differences in the intensity of clinical surveillance, or certain host factors. The excellent physical condition of U.S. military cadets may contribute to their low illness rates; another factor may be their concern of the effect of a relatively long period of hospitalization on their academic, military, and athletic performance.

In the present investigation 1 case of typical heterophile-negative mononucleosis occurred. While both EBV (4) and cytomegalovirus (15–17) have clearly caused this syndrome, neither could be implicated in this patient. EBV antibody remained persistently negative and no rise in CMV antibody occurred. However, a rise in adenovirus complement-fixing antibody developed from $<1:8$–$1:16$.

Wahren et al. (17) reported adenovirus rises in 11% of paired sera from heterophile-negative cases suspected of infectious mononucleosis but did not attach much significance to this. Among English students 49 heterophile-negative mono-like illnesses occurred (7). Only six of these could be associated with antibody rises to known viruses: four to rubella virus and one each to CMV and herpes simplex virus (7). This problem of heterophile-negative cases deserves more careful study (18).

*We are indebted to the Center for Disease Control for carrying out tests for herpes simplex, CMV, toxoplasmosis, and adenovirus in this patient.*
The low infectiousness of infectious mononucleosis even among exposed and susceptible roommates has been further confirmed in this investigation and is in accord with similar observations in other college settings (6, 7). Of 24 exposed and susceptible roommates of clinical cases only one developed definite clinical infectious mononucleosis with an onset compatible with the incubation period of the disease. The seroconversion rate in those exposed to clinical cases was even lower than that of roommates not so exposed. This lack of spread in cadets is especially impressive because of the prolonged and close contact between roommates and the known presence of EB virus in the oropharynx over extended periods (19). Transmission from the opposite sex during kissing as first proposed by Hoagland in 1955 (9) while studying cadets at the U.S. Military Academy and later supported in a controlled study at the University of Wisconsin (20) appears increasingly likely as a primary source of infection in young adults. While transmission of infection via inanimate objects such as bottles or eating utensils, or toys in the case of children, seems another plausible route of transmission, this has not yet been demonstrated nor has survival of EB virus been tested under these circumstances.

Elevations of immunoglobulins, especially IgM, found during the course of infectious mononucleosis in the present investigation confirm the observations of Wollheim et al. (21, 22). The availability of preillness sera in the present study permitted calculation of the increase in immunoglobulin levels in infectious mononucleosis above each individual’s own baseline level. Increases in IgM were the most impressive. The mean IgM level rose from preillness values of 96.3 mg% to 182.8 mg% during acute illness, an increase of 90%. It then declined sharply by the second month of illness, and returned to baseline values at the end of the academic year. The mean IgG and IgA values also increased but not as much as the IgM; these increases occurred later than IgM rises and declined more slowly. Determination of immunoglobulin values in patients’ sera collected 1 yr apart gave no significant clue that the individual had experienced clinical infectious mononucleosis during the interval. This being so, it was not surprising that immunoglobulin values in cadets with EBV antibody seroconversion during the year but without known illness also failed to show significant differences in the paired sera one year apart. Preliminary results with EBV-specific IgM have also shown increases during acute illness with absence of this antibody in sera taken before or 1 yr after clinical infectious mononucleosis. Persistence of EBV-specific IgM was found up to 6 mo in a few instances.

No difference in susceptibility to EBV infection was found in persons of different blood groups. This is in agreement with prospective studies in Yale University freshmen students (6). We have previously reported that the prevalence of EBV antibody in the current group of cadets bore no relation to ABO blood type (23).

The major observations of this prospective study of 1401 cadets over their college experience are: (1) about half of the cadets who lack EBV antibody on arrival are infected with EB virus over a 4 yr period; (2) only 26.4% of 201 EBV-infected cadets had a clinically recognized episode of infectious mononucleosis (3) EBV infections were not more common in exposed and susceptible roommates of clinical cases than in the susceptible cadet population not so exposed; (4) except for two cadets already ill at the time of bleeding, infectious mononucleosis did not develop in 890 cadets possessing EBV antibody on entry.
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