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SOME STUDIES ON INFECTIOUS MONONUCLEOSIS WITH PARTICULAR REFERENCE TO ETIOLOGY

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Some Studies on Infectious Mononucleosis with Particular Reference to Etiology

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Yale University School of Medicine
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Requirements for the Degree
Doctor of Medicine

by Orson R. Dee

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I. Introduction

Much has been written in recent years about infectious mononucleosis, but there remain many aspects of this disease which are quite confusing and not at all understood. This is particularly true in regard to etiology; and indeed it seems almost a misnomer to refer to our "knowledge" of this aspect of the disease, since many aspects of this knowledge are in reality little more than speculation.

This paper is a report of several serological studies done in an attempt to investigate different theories of the causation of the disease, as well as some observations in regard to diagnosis and treatment, drawn from a review of 190 serologically proven (by sheep-cell agglutination tests) cases of infectious mononucleosis treated at the Department of University Health, Yale University, from September, 1958, through December, 1960, the majority of whom were hospitalized at the Yale Infirmary.

For approximately one year, serial serum samples were obtained from patients in various stages of the disease and convalescence and these specimens were frozen at -20 degrees centigrade for later testing. In all, 265 serum samples were obtained on 115 patients. It should be emphasized that all cases included in this

study had positive sheep-cell agglutination tests in addition to the other diagnostic signs. No attempt was made to differentiate between heterophile-positive and heterophile-negative cases, as had been done by some authors. It was felt that if heterophile-negative cases really do exist and are truly the same disease, their diagnosis is subject to too much variation to make it desirable to include them in this study.

II. Diagnosis

A. Symptoms

The common symptoms and objective findings in infectious mononucleosis were classified by Niederman (1) into those seen in the first, second, and third weeks of the disease. I have not attempted such a chronological division, but have summarized the most common symptoms and findings of the early stages in table (1). This summary agrees quite closely with Niederman's except that 70% manifested sore throats while he found only 51%. In his series, the cervical adenopathy category included all those in whom adenopathy was palpable or 58%, while the above figure of 27% covers only those patients who complained of having noticed tender cervical nodes.

It should be noted that three symptoms greatly

outnumber all others: sore throat (70%), fever and or chills (65%), and malaise and or fatigue (41%). If we consider Niederman's figure for cervical adenopathy, which seems like a more realistic figure (58%), we can then characterize the vast majority of all cases of infectious mononucleosis by these four characteristics. Hoagland (2) felt that almost all cases could be grouped into one of two major types: the pharyngeal (80%) and the typhoidal (20%), and believes that all the other symptoms and bizarre presenting findings are really not part of the disease picture, but are in fact complications. He therefore believes that to continue referring to infectious mononucleosis as a "protean disease" makes little sense. He was joined in this view by Bender (3). Mason and Adams (4) also adhere to this in recently surveying 100 cases in which pharyngitis was noted in 91% and lymphadenopathy in 95%.

Paul (5) reported a 10% incidence of palatine petechiae, and even if such a low occurrence rate prevails, this is a significant finding because it is found in relatively few other conditions, most noteworthy being streptococcal sore throat and rubeola.

B. Laboratory data

As has been pointed out, all cases in this series had positive sheep-cell agglutination tests. Recently, Evans (6) reported a series of cases of heterophile-positive and heterophile-negative cases, and felt that the two groups were a manifestation of the same disease process, with the heterophile-negative cases merely representing less severe reactions. He did ox-cell hemolysin tests and felt these to be a slightly more inclusive indicator of infectious mononucleosis than the Paul-Bunnell test. In all cases where the Paul-Bunnell test was positive, the ox-cell test was also positive, but in three "heterophile-negative" cases the ox-cell hemolysin test was positive. The present study includes no evaluation of the ox-cell hemolysin test, but it was felt advisable to follow the lead of Hoagland in regarding a positive heterophile as a *sine qua non* of the diagnosis. It is often found that cases without a positive heterophile titre are really viral pneumonia, rubella, viral hepatitis, etc. It is uncommon to find a case which really convincingly and firmly fits the diagnosis of infectious mononucleosis clinically but in which a positive heterophile does not develop. The group of "heterophile-negative" cases is

in my experience a rather poorly defined group and would better be called "non-specific" or "unidentified virus infections."

It was felt by Mason and Adams (4) that the heterophile test could be sometimes unreliable due to technical reasons, and that the titre must therefore be 112 or higher to be of significance. They observed further than in cases with or without a positive heterophile test a firm diagnosis requires that the peripheral blood differential count must show at least 50% lymphocytes, and that at least 15% must be atypical lymphocytes.

In order to make the determination of sheep-cell agglutination simpler, Evans proposed in 1947 a "Simplified 'qualitative' method for heterophile antibody determination using capillary blood and WBC pipette" (7). This test has apparently not found widespread favor, but at the Yale Department of University Health, it has been used extensively and is considered a very useful abbreviated method of determining the presence of antibodies for rapid diagnostic purposes, and is followed by the Paul-Bunnell test for quantitative comparison of antibody level. Of 168 cases in the

present series who had both tests, 87 (52%) were detected by the Evans test before the regular heterophile test was positive, and indeed many of the heterophile tests were done because of the positive Evans test result. In another 51 cases, the two tests were both positive on blood drawn on the same day, but the results of the Evans test were known immediately whereas there was a delay of 3-6 days for the heterophile test results.

One criticism of the Evans test which has been made is that so often gives negative results early in the disease. This is quite true, and often the test will have to be repeated several times before it turns positive. But the same criticism could be made of the heterophile test since neither test will be positive until the patient develops sufficient agglutinating antibodies. The average length of time from onset of symptoms to the development of a positive Evans test was 6 to 7 days in this series, although several cases required up to 14 days. Another criticism of the Evans test is that it will be positive only if the patient's titre is 1:160 or higher, and it may be desirable to detect lower levels of antibody titre. As pointed out by Evans, however, the dilution of 1:160 was selected as being that level which is ordinarily considered

above the level which may be found in normal individuals, and if desired a titre of half that may be detected by merely drawing up twice the amount of blood into the pipette.

In 1953, Liebowitz (8) published a comprehensive survey of the literature on this disease to that date, including an excellent bibliography and picture of the disease as understood then, as well as a careful survey of his own 25 cases. He stressed the frequency of hepatic involvement and considered abnormal liver function tests as almost a diagnostic requirement, being found in 96% of his cases. In this series, liver function tests were not done on all cases and included are many mild cases whose tests would not be expected to be abnormal, while Leibowitz was dealing only with hospitalized patients. In those of our patients who had function studies of any sort done, only 48% had abnormal function, shown by elevation of bromsulphalein retention (greater than 6%), total bilirubin (1 mg.% or higher), or elevation of cephalin flocculation. None of the other liver function studies were done in sufficient number to warrant comment.

III. Etiology

A. Virus etiology

It has been assumed by most authors that the etiological agent of infectious mononucleosis is a filterable virus, yet innumerable attempts by workers in this laboratory and elsewhere have been unsuccessful in isolating a virus in tissue culture, using varying techniques and cell types.

Attempts to transmit the disease experimentally from patients to animals and to human volunteers have also been notably unsuccessful. Direct blood transfusions, throat washings, and stool samples have all been tried (9-12). There have also been experiments consisting of injecting serum from patients with infectious mononucleosis intramuscularly into subjects who had acute leukemia (6) (13), on the theory that the resistance of these subjects to viral infections is decreased. A febrile illness somewhat resembling infectious mononucleosis resulted, but all diagnostic criteria were not met. These results cast some doubt on the idea of virus etiology.

Hoagland (14) has advanced the theory that the virus of infectious mononucleosis is a labile one which must be transmitted by close personal contact, such as the actual exchange of saliva in kissing, and that there is a

prolonged incubation period of 32 to 49 days before onset of symptoms. This would perhaps account for the fact that we have been unable to isolate the virus from cases which are in the symptomatic stage. It would also explain the age distribution of the disease and the fact that roommates in dormitories almost never have the disease simultaneously. In this series of cases, no active questioning was done concerning past contacts, but it was noted in two instances that two roommates were ill with the disease simultaneously; in one instance, it was noted that the two patients had indeed both kissed each of the same two girls during the previous three months, and in the other instance, the two patients had separately kissed a pair of sisters and the last exposure had been approximately one month prior to onset of symptoms in one of the two cases. One of the sisters exclaimed "The last three boys I've dated steadily have developed infectious mononucleosis." Evans (6) distributed questionaires to everyone admitted to the University of Wisconsin infirmary asking about intimate oral contact in the months preceding onset of their illness. It was found that in the period 31 to 60 days before admission, 68% of the infectious mononucleosis patients and 41% of the patients with other diagnoses gave a history of intimate oral contact.

Hoagland's theory was partially based on the observation that the incidence of the disease was highest at West Point during the two months period after the cadets had been on leave, and he attributed this to the fact that with the exception of leave periods, there is little opportunity for this type of exposure. Evans and others have also noted a similar increase in cases following the summer, Christmas and spring vacations in students. The monthly distribution of cases in this series is shown in figure (2). Although there were no striking seasonal differences in the number of cases, it can be seen that there is in fact a relatively high incidence in September and October, another high during February, and the highest point in April.

It has been proposed that the virus may be related to the group of myxoviruses because of the ability of serum from patients with infectious mononucleosis to agglutinate human red cells midified by Newcastle disease virus, reported first by Burnet and Anderson (15). Accordingly, hemagglutination titres were determined in 181 serum samples from 70 patients, and the results are summarized in table (3). Sixty-one patients, or 87%, had elevated titres. The method used was that described by Evans and Curnen (16,17), using type O human RBC's incubated with

high concentrations of Newcastle disease virus and then washed in saline repeatedly to elute the virus. The result in this series compares closely with their finding of 74.5% with elevated titres. When the NDV titres were compared with the heterophile titres performed on the same dates, it was found that the rise and fall of the two titres were roughly parallel in most cases. The interpretation of this phenomenon is not complete since it is not the virus which has been agglutinated by the immune serum but rather the normal red cells which have been modified in some way by the virus.

B. Bacterial sensitivity

1. Concept

Many attempts have been made to explain infectious mononucleosis as a bacterial infection with staphylococci (18, 19), E. coli (20), and Listeria monocytogenes (21-23), but these efforts have not been successful. As has often been noted in the past, the number of infectious mononucleosis patients who have positive throat cultures for beta hemolytic streptococci is high, and it would appear that there may be some causative relationship between streptococcal infection and infectious mononucleosis. Could it be that infectious mononucleosis is a delayed hypersensitivity phenomenon following streptococcal sore throat, similar

to rheumatic fever or glomerulonephritis? In this series, four patients had sore throats with positive beta streptococcal cultures approximately two weeks before the onset of the symptomatic phase of infectious mononucleosis. Three of these were treated with penicillin and one was not. Powers and Boisvert (30) formulated the concept that the reaction to streptococcal infection "expresses itself in different clinical patterns at different age periods," for example suppurative infections (otitis, rhinopharyngitis, impetigo, erysipelas, and infected eczema) in childhood, scarlet fever in the intermediate age group (6-10 years), and acute tonsillitis in adults. The non-suppurative sequelae (rheumatic fever and glomerulonephritis) are found to be most common in the intermediate and early adult age groups. In 1954, Hunt (24) noted the similarity of infectious mononucleosis to the serum-sickness type diseases, shown by the radiculitis and Guillian-Barre syndrome following the acute phase of the disease. He reported a case of a young female who had infectious mononucleosis and a positive beta streptococcal throat culture while two older members of the family simultaneously had streptococcal tonsillitis. As a result of these observations, he postulated that infectious mononucleosis might be an atypical reaction to streptococcus or any one of several

bacterial infections. This concept of bacterial hypersensitivity was felt to be improbable by Spritzer (25), but he did agree that infectious mononucleosis could be not a single disease but a reaction to multiple stimuli. It is noted that German clinicians about the turn of the century were interested in the streptococcus as a possible etiologic agent, but in 1934, Tidy sharply disagreed in The Lancet (26), stating that "Foreign authorities went astray from the start. They became obsessed with the idea that the development of lymphocytosis was the result of a constitutional tendency in the individual which was called into activity by the angina."

Campbell (18) performed throat cultures on 66 infectious mononucleosis patients and noted that 53% showed pathogenic staphylococci, 28.8% streptococci, and 42.1% Vincent's organisms. In throats of 469 patients with pharyngitis due to other causes, he found 13.9% positive streptococcal cultures. Tidy and Morley (27) cultured lymph nodes in a "few" cases and always found streptococci. Others have reported the incidence of positive streptococcal throat cultures as 46% (28), 14.3% (29), 45.5% (30), and 21% (31).

2. Methods and results

In the present series, throat cultures for beta

hemolytic streptococci were performed on 125 patients and a total of 41.6% were found to be positive, but about one third of these were of groups other than group A. The significance of beta streptococci of other groups is not definite but Dingle (32), summarizing the literature, states "On the basis of the clinical picture, isolation of the streptococcus from the throat, and the development of antibodies to streptolysin O and to streptokinase, it seems reasonably certain that strains of groups C and G can cause human respiratory infections. Clinical and epidemiological data suggest that streptococci of groups B and F may have similar capacity, but serological methods of confirmation are not available for infection due to organisms of these groups."

Throat cultures at the Yale Department of University Health are generally performed by the Connecticut State Department of Health laboratories in Hartford, Connecticut. The throat swab is placed in a tube of sterile gelatin carrying medium and mailed to the laboratory. Utilizing this culture technique, throat cultures from 115 patients with upper respiratory infections, some of whom had clinically diagnosed streptococcal pharyngitis, and from 27 normal controls were analysed. These results are shown in table (4). Only limited comparison can be made, however, between the

infectious mononucleosis patients and the control groups, because the infectious mononucleosis patients' cultures were performed over a period of 2½ years, whereas the control groups were performed during two periods of two months each in the spring of 1960 and 1961. Comparing the control patients cultured in spring of 1960 with the infectious mononucleosis patients cultured during the same period gives an extremely small sample and is therefore of little statistical value. This comparison is shown in table (5). Although the control groups of throat cultures are not statistically comparable to the figures for infectious mononucleosis patients, they may nevertheless serve to give some indication of the validity of the culture technique.

To investigate further the implication of streptococcal infection in infectious mononucleosis, antistreptolysin O titres were determined in a group of 19 patients. These patients were not selected on the basis of having positive or negative throat cultures, severity of their disease, etc., but were merely those from whom adequate sera happened to be available at the time. An attempt was made to test a serum sample from the acute phase and at different times during the recovery phase of the disease. In all, 34 titres were determined on 19 different patients,

using the standard method of Rantz and Randall (33). The results are outlined below:

Only two patients, or 10.5% had significantly elevated titres:

#1; titre of 1:333 twice during convalescence. Patient had three negative throat cultures.

#2; titre of 1:333 on acute phase serum. One negative throat culture.

Three patients had small rises in titre, but not to really significant levels:

#1; titre rose from 1:100 to 1:166 over period of a month. Cultures positive for beta strep, not group A. Treated with penicillin.

#2; titre rose from 1:100 to 1:125 in period of three weeks. Culture positive for group A strep. Treated with penicillin.

#3; titre rose from 1:100 to 1:166 in 12 days. Culture negative. Treated with prednisone.

One patient showed a fall in titre, again at very low levels:

#1; titre fell from 1:100 to less than 1:50 over a two month period. Culture negative. No treatment.

These results contrast with those of Lagercrantz (30), who found an antistreptolysin titre of at least 1:200 in 8 of 11 patients, or 72.7%. Bennike (31) found titres of 1:200 or greater on discharge in 14% of infectious mononucleosis patients receiving no treatment.

3. Discussion and conclusions

It was found that 41.6% of all infectious mononucleosis patients in this series who had throat cultures had beta-hemolytic streptococci of all groups present in their throats

during or very near the acute phase of their disease. The real question, then, is whether these organisms act as causal factor in the disease, as secondary invaders, or merely as saprophytes. This figure of 41.6% is not unreasonable when compared to the results of other series. It almost certainly does represent a substantially greater proportion of positive cultures than would be found in the population at large (6.9% in this sample) or in patients with other upper respiratory infections (12.2% in this sample), although it is recognized that these percentages are not strictly comparable because of the difference in time of sampling.

However this data does show that only 10.5% of unselected infectious mononucleosis patients have elevated ASLO titres, and since there is no reason to believe that hypersensitivity to the streptococcus could be the basis of this disease without the simultaneous development of elevated ASLO titres, we must conclude that this high occurrence of beta streptococci represents either harmless flora or, more likely, a secondary invader.

C. Relationship between infectious mononucleosis and toxoplasmosis

1. Concept

A form of acquired toxoplasmosis in the adult has been previously described (34,35), characterized by lymphadeno-

pathy, lymphocytosis with atypical lymphocytes and a negative Paul-Bunnell reaction, with or without fever, which cannot be distinguished clinically from infectious mononucleosis. Beverley (36) stated that about 7% of cases of "glandular fever" with negative heterophile reactions are really the adult acquired form of toxoplasmosis. The question then arises regarding the relationship between heterophile-positive cases and toxoplasmosis. Could there be a causal relationship between the two diseases?

2. Methods

Sabin and Feldman (37) described a toxoplasma dye-fixation test which could be used on serial dilutions of a patient's serum to detect the presence of antibodies against the protozoa and measure their titre. Briefly, the principle of the test is as follows: fresh extracellular toxoplasma organisms growing in the peritoneal fluid of an infected mouse will take up methylene blue stain. Incubation of the organisms with serum from normal people will have no effect on their ability to take up the stain, but incubation with immune serum containing antibodies against the toxoplasma will affect the organisms in such a way as to prevent them from staining with the methylene blue. The titre is defined as that dilution of serum which affects 50% of the organisms and allows the other 50% to take up the stain.

This test is complex and technically exacting in that it requires the preparation of special reagents which must meet precise specifications and must be prepared fresh every few days. It is sometimes difficult to propagate the toxoplasma in mice and obtain the required amount of peritoneal exudate available on a specified day. Reading of the results is difficult and time-consuming. In performing this dye-fixation test, three major unforseen difficulties were encountered:

(1) Differentiation between those organisms which are stained and those which are not is difficult when the observer does not know from previous experience what to anticipate. Some of the organisms become rounded and deeply stained with prominent granules, while others remain totally colorless, almost completely transparent, and retain their original crescentic shape. Differentiation between these is not difficult; but there are gradations between the two forms. Many of the organisms retain their original shape and show no granular structure, but take on a grayish-blue tint. These are counted as "stained."

(2) Considerable time was spent trying to establish growth of the toxoplasma in mice from a frozen sample. Weinman (38) and Eyles, Coleman & Cavanaugh (39) described preservation of the organisms by freezing them slowly in 10%

glycerol and storing at -70 degrees centigrade. After thawing and inoculation into mouse peritoneal cavity, the organisms should grow but do not always do so. It is sometimes more successful to inject them subcutaneously and then recover them from the spleen, liver and brain for further culture in the peritoneum. Blind passage may be necessary.

(3) In order to inactivate non-specific inhibitors, the serum to be tested is first inactivated at 56 degrees centigrade for 30 minutes. This also destroys a "labile factor" which has been shown to be a properdin fraction and which must be replaced by the addition of some fresh normal human "activator serum". The activator serum must be one which has no effect of its own on the toxoplasma organisms. Unfortunately, several of the sera which were initially used as activator sera did in fact contain some antibody or non-specific inhibitors, thus, causing considerable confusion and delay.

Other investigators have also had difficulty with this dye test and have attempted to standardize and simplify the test (40-42). It is not a popular test among many of the people who have performed it.

3. Results

A total of 138 dye tests were run on serial sera from 69 infectious mononucleosis patients. Of these, 14 people,

or 20.3%, had measurable titres (1:8 or greater). Of these, 9 (13%) were of levels of 1:64 or greater, which is the level considered significant by most investigators. This data compares with Weinman's figure of 21.3% positive tests among normal adults in the New Haven area (43). None of the sera tested showed any significant rise in titre, although several showed minor changes which were probably within the range of experimental error. Only one patient had a really markedly elevated titre (1:2048). This fell to zero in less than three weeks, which makes the high titre unacceptable in view of the fact that toxoplasma antibodies tend to remain at high levels for a period of years after infections and then decline very gradually.

4. Conclusions

The prevalence of detectable toxoplasma antibodies was essentially the same in the cases of infectious mononucleosis as would be expected in the general population of New Haven. In the absence of evidence to the contrary, it must be concluded that in this age group, there appears to be no apparent causative role by toxoplasma in heterophile-positive cases of infectious mononucleosis.

D. Other serological studies

1. Blood groups

Following the reasoning that this disease may represent

an autoimmune phenomenon, it was decided to analyze the blood group distribution of those patients in this series on whom the data was available. Data on 58 patients is tabulated in table (6). It will be seen that the distribution of blood groups in the infectious mononucleosis patients conforms to the normal distribution in the population at large.

2. Latex fixation tests

At the suggestion of Dr. Paul Boisvert, latex fixation tests were performed on a small group of serial sera from infectious mononucleosis patients. Since the latex agglutination test apparently depends upon the presence of an abnormal serum protein or imbalance of protein fractions, it was felt worthwhile to investigate the latex agglutination phenomenon in this disease. Surprisingly, a large fraction of these tests gave positive results, so the test was continued until in all, 170 sera, representing 77 patients were tested. It was found that 38 patients (49.3%) were positive at some time during the course of their infectious mononucleosis. However, after inactivation of the sera at 56 degrees centigrade for 30 minutes, only 17 (22%) remained positive.

This data is at variance with the expected change after inactivation, according to the work of Schubart, Cohen and

Calkins (44) who found that many sera contain a "prozone inhibitor" which will prevent the latex fixation reaction in the raw serum but which can be removed by heating at 56 degrees centigrade for 30 minutes. This inhibitor was later found by them to have the characteristics of the first component of serum complement. Obviously, the problem in this instance was not one of inhibition, but rather one of destroying or partially decreasing the factor which caused the agglutination, by the inactivation process. It seems quite possible that this factor could be the heterophile antibody or something closely related to it, since the heterophile is also destroyed by inactivation at that temperature.

Dresner and Trombly (45) reported the results of latex fixation tests on a group of normals and on patients with diseases other than rheumatoid arthritis. They found three groups who had a relatively high incidence of positive tests: (1) patients with acute viral infections, (2) patients with syphilis, and (3) patients with hepatocellular diseases. The test was not positive in bacterial or mycotic infections. These authors reported that 83% of 35 patients with acute viral infections had positive latex fixation tests. However, in studying their results, it is apparent that when the regular latex fixation test was performed with whole sera, only 17% were positive. The 83% which was quoted represents the patients

who were positive using their euglobulin-inhibition technique. They also stated that "In two cases of infectious mononucleosis studied, latex agglutinator preceded the appearance of heterophile antibody agglutinin in the serum and could be differentiated from it by absorption with guinea-pig kidney, which preferentially removed the heterophile antibody."

The results in this series, on the other hand, shows that the latex fixation test was often negative during the first 2-3 days of illness and became positive in most cases toward the end of the first week, just about the time the heterophile antibody is also appearing. Of those patients who had positive latex fixation tests at any time, all but four were positive by the end of the first week and all but five had again reverted to negative by the end of one month. As can be seen from table (7), the highest relative number of tests which were positive occurred during the period from 16-20 days after onset of symptoms.

It would seem, therefore, that the latex agglutinating antibody which is found in infectious mononucleosis is heat labile, appears about the end of the first week of clinical symptoms and disappears about one month later and is very likely closely related to the heterophile antibody. Since it is positive in about the same percentage of infectious

mononucleosis patients as in miscellaneous acute viral infections, it could possibly be due to a hyper-gammaglobulinemia. It would also tend to lend some support to the theory of a virus etiology for infectious mononucleosis.

IV. Treatment--the use of steroids

No specific therapy has been found for infectious mononucleosis. Antibiotics have proved to be of no value except in the case of superimposed bacterial infections. However, due to their well established anti-inflammatory effects, the use of steroids or ACTH has been tried by many clinicians, and there have been several reports of their results (4), (6), (46-52). Generally, all agree that there is a marked regression of clinical symptoms and improved general condition of the patient, but there have been no figures to quantitate these clinical impressions. Mason and Adams (4) found the duration of illness to be 18 days on the average, whether treated with steroids or not. They found an improvement in the patient's clinical state but no change in blood count, liver function tests, or heterophile titres. Evans (6) has reported a double-blind study in which he compared 9 patients treated with prednisolone with 15 controls treated with aspirin alone. He found no significant difference in cases treated with prednisolone and those treated with aspirin, although he feels steroids would be of value to "tide a patient

over a critical period" if a "life-endangering complication arose." It should be pointed out that his survey was conducted on relatively mild cases, and that only 77% of the treated and 60% of the control patients had positive heterophile tests during their illness. It might also be justified to comment that sore throat persisted after starting therapy for 2.2 days in treated patients and for 4.9 days in controls. The only other difference which might assume some significance is that fever persisted for 3.0 days in treated patients and only 0.5 days in controls.

The evaluation of treatment with steroids in this disease is difficult and is complicated in this series by the lack of randomization of patients, variability of observer reporting and lack of sufficient data for comparison of laboratory values before and after the start of treatment.

Clinically, the administration of steroids in severe cases of infectious mononucleosis results in prompt defervesence of fever, disappearance of tonsillar and pharyngeal exudate, subsidance of symptoms of malaise and sore throat, and rapid disappearance of splenomegaly and lymphadenopathy. It has been impressive to note the rapidity with which the patient is able to resume activity and ceases complaining of his very sore throat. In this series, steroids were not prescribed until the diagnosis of infectious mononucleosis was confirmed on serological grounds (heterophile or Evans

test), and this medication has been used primarily in those patients who presented an unusually toxic picture. The drug used was prednisone, usually in an initial dose of 40 mg. a day followed by gradual daily reduction to 5 mg and then withdrawl. The average length of administration was 5.7 days.

The present series of 190 patients includes 42 who were treated with steroids, of whom 31 patients, or 71.4% had prompt subsidance of temperature to normal levels within 12-24 hours and whose temperature remained within normal range. Four (9.5%) remained febrile for 48 hours or greater, and one fell to normal promptly but then developed fever again briefly on two succeeding days. Seven had normal temperatures before treatment was started.

It will be seen in table (8) that the average number of febrile days after admission to the infirmary, for those who were febrile at all, was 4.3 days for untreated cases and 4.2 days for steroid treated cases.

Of 123 patients receiving no steroid therapy, 46 were not ill enough to be admitted to the infirmary. Of the 77 admitted, the average length of stay was 6.8 days. This compares with the 42 treated patients whose average length of stay in the infirmary was 7.9 days.

It is thus seen that the number of febrile days, in

those who were febrile at all, was the same in those patients treated with steroids and those untreated; and the average length of stay in the infirmary was actually a day longer in treated patients. From this, it might be concluded that the drug had no effect on these aspects of the disease whatever. However, as noted previously, the patients who received treatment were those who presented the most toxic picture. Therefore, the fact that duration of fever and length of infirmary stay were the same in the two groups is important. In an attempt to document the fact that the treated group consisted of the more toxic individuals, the highest temperatures recorded on each patient were tabulated and are shown in table (9). It can be readily seen that those patients treated with prednisone as a group had markedly higher temperatures than the controls at the onset of the disease. It should also be pointed out that in compiling these percentage figures, all patients who were afebrile to start with were omitted in order to make the statistics more meaningful, and had they been included, the results would be even more striking.

Figures (10) and (11) show graphically the total leukocyte counts in the peripheral blood and the absolute number of lymphocytes, respectively, comparing these statistics in the control and in the steroid treated groups. It will be seen that in both groups, the total WBC count has a peak

at about the 10th to 11th day, and the peaks are both at about the same level. The return to normal levels takes place at about 30-33 days in both groups. This same relationship holds for the graphs of the absolute number of lymphocytes (total WBC X % lymphocytes).

There have been no serious complications which could be attributed to the use of prednisone therapy. The question of whether or not there was a higher incidence of positive throat cultures in those treated with prednisone is considered in table (12), comparing the number of steroid treated patients in each group having positive cultures with the overall percentages for the group. It can be seen that there is in fact a somewhat higher incidence of positive cultures among treated patients than the average for each group.

V. Summary and conclusions

Some general observations concerning diagnosis and therapy with steroids in infectious mononucleosis have been presented, from clinical review of 190 cases treated at the Department of University Health of Yale University from September, 1958, through December, 1960. It was concluded that the vast majority of cases of this disease manifest sore throat, cervical lymphadenopathy, fever, and malaise, so the disease is really not protean in its sympto-

matology. For the purposes of this survey, the Paul-Bunnell heterophile test is a <u>sine qua non</u> of the diagnosis, and the Evans test is felt to be a useful abbreviated method for quickly determining the presence of heterophile antibodies.

The use of steroids in severe cases is felt to be of great benefit in symptomatic improvement and reduction of morbidity, although no effect was observed on total leukocyte counts or number of lymphocytes in the peripheral blood, the heterophile titre, or the NDV-treated RBC hemagglutinating titre. The total duration of pyrexia and length of infirmary stay was found to be the same in the treated and control groups, but this is felt to be a significant finding in view of the fact that the treated group is demonstrated to have a more serious and toxic illness than those not receiving the medication. A slightly higher incidence of positive beta streptococcal throat cultures was noted in the people receiving prednisone, which would indicate that perhaps patients being so treated should be studied for the presence of bacterial infection.

Serological data relating to several possible theories of etiology have been presented. In a general way, support is lent to the theory that the disease may be caused by a virus related to the myxovirus group by the high percentage

of patients whose serum in high dilutions agglutinated human RBC's treated with Newcastle disease virus. This theory is also supported by the fact that infectious mononucleosis sera show positive latex fixation tests in about the same percentage as sera from people with miscellaneous acute viral infections.

No relationship between serologically proven cases of infectious mononucleosis and toxoplasmosis was found. The distribution of blood groups among patients with infectious mononucleosis was found to be the same as that in the general population.

The concept that infectious mononucleosis may represent an atypical delayed hypersensitivity reaction to streptococcosis has been explored. It is true that a high percentage of infectious mononucleosis patients have positive throat cultures for beta hemolytic streptococci, but there was a low incidence of elevated ASLO titres in these patients. Hence, it must be concluded that the streptococci found in the throats probably represent secondary invaders in throats predisposed to infection rather than an immediate cause of the infectious mononucleosis.

Table (1)

Common symptoms and signs in early stages of infectious mononucleosis

Symptom	Incidence
Sore Throat	70%
Fever and or Chills	65%
Malaise and or Fatigue	41%
Cervical Adenopathy	27%
Headache	24%
Splenomegaly	23%
Rhinitis	8%
Nausea, vomiting	8%
Palpable liver	5%
Anorexia	5%
Earache	5%
Backache or generalized aches	5%
Cough	4%
Syncope	2%
Chest Pain	2%
Rash	2%
Jaundice or dark urine	1%

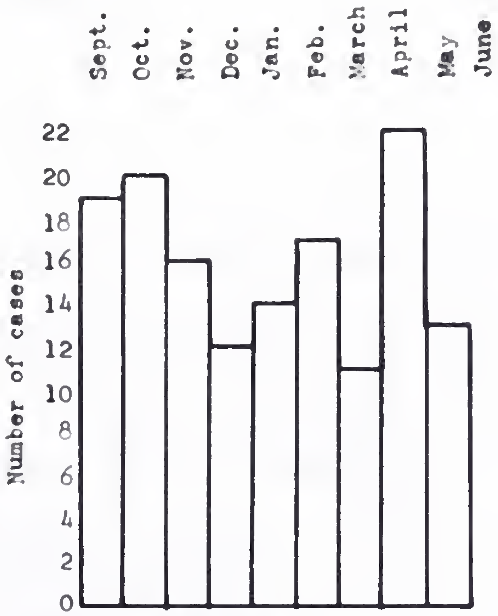

Figure (2)
Monthly incidence of infectious mononucleosis during two school years (1958-59 and 1959-60).

TABLE (3)

NDV-treated human RBC hemagglutination titres on infectious mononucleosis patients.

	No. patients	Per cent
Patients with normal titres (1:64 or less)........	9	.....13%
Patients with titres 1:128 to 1:1024.............	32	.....46%
Patients with titres 1:2048 or higher............	29	.....41%
Total	70	

TABLE (4)

Beta-hemolytic streptococcus throat cultures on infectious mononucleosis patients and controls.

Patient Classification	% + Group A	%+ Other Groups	%+ Total
49 infectious mononucleosis patients, in school year, 1958-59	47%	2%	49%
50 infectious mononucleosis patients, in school year, 1959-60	8%	20%	28%
26 infectious mononucleosis patients, in school year, 1960-61	23%	31%	54%
Total of above 125 infectious mononucleosis patients	26.4%	15.2%	41.6%
115 patients with other URI's (some clinically strep pharyngitis), spring 1960 and 1961	3.5%	8.7%	12.2%
27 normal controls without any throat infection, spring 1960 and 1961	0	6.9%	6.9%

TABLE (5)

Comparison of throat cultures of infectious mononucleosis patients and control groups in the spring, 1960

PATIENT CLASSIFICATION	%+ Group A	%+ Other Groups	%+ Total
10 cultures from infectious mononucleosis patients	10%	10%	20%
10 cultures from patients with other URI's (some clinically strep pharyngitis)	0	0	0
10 cultures from normal controls without any apparent throat infection	0	10%	10%

TABLE (6)

Blood group distribution of 58 patients with infectious mononucleosis

Blood group	No. patients	% of patients	% normal distribution
A	24	41.3%	42.7%
B	9	15.5%	12.1%
AB	5	8.6%	3.5%
O	20	34.5%	41.7%

TABLE (7)

Percent of positive latex fixation tests in infectious mononucleosis patients at varying periods after onset of symptoms

Time interval	No. of tests	% Positive
Days 1-5	20	20%
Days 6-10	28	25%
Days 11-15	16	31%
Days 16-20	13	38%
Days 21-25	10	20%
Days 26 and up	45	11%

TABLE (8)

Total number of febrile days after admission to Infirmary

No. of days	No.Untreated patients	No. patients treated with steroids
0	26	1
1	8	9
2	5	3
3	6	7
4	5	5
5	5	5
6	2	3
7	2	2
8	2	2
9	3	0
10	0	1
11	0	1
12	0	0
13	0	0
14	0	0
15	0	0
16	1	0
Total	65	39
Total febrile	39	38
Average days febrile	4.3	4.2

TABLE (9)

Highest temperatures of infectious mononucleosis cases recorded during Infirmary admission

Temperature range	Patients from control group		Patients from treated group	
	No.	%	No.	%
Normal (up to 99.4)	29	-	1	-
99.5 to 100.9	21	38%	5	12%
101 to 101.9	18	33%	15	36%
102 to 102.9	10	18%	8	19%
103 to 103.9	3	5.5%	11	26%
104 or above	3	5.5%	2	5%
Total febrile	55		42	
Temperatures of 101 or above		62%		90%
Temperatures of 102 or above		29%		50%
Temperatures of 103 or above		11%		31%

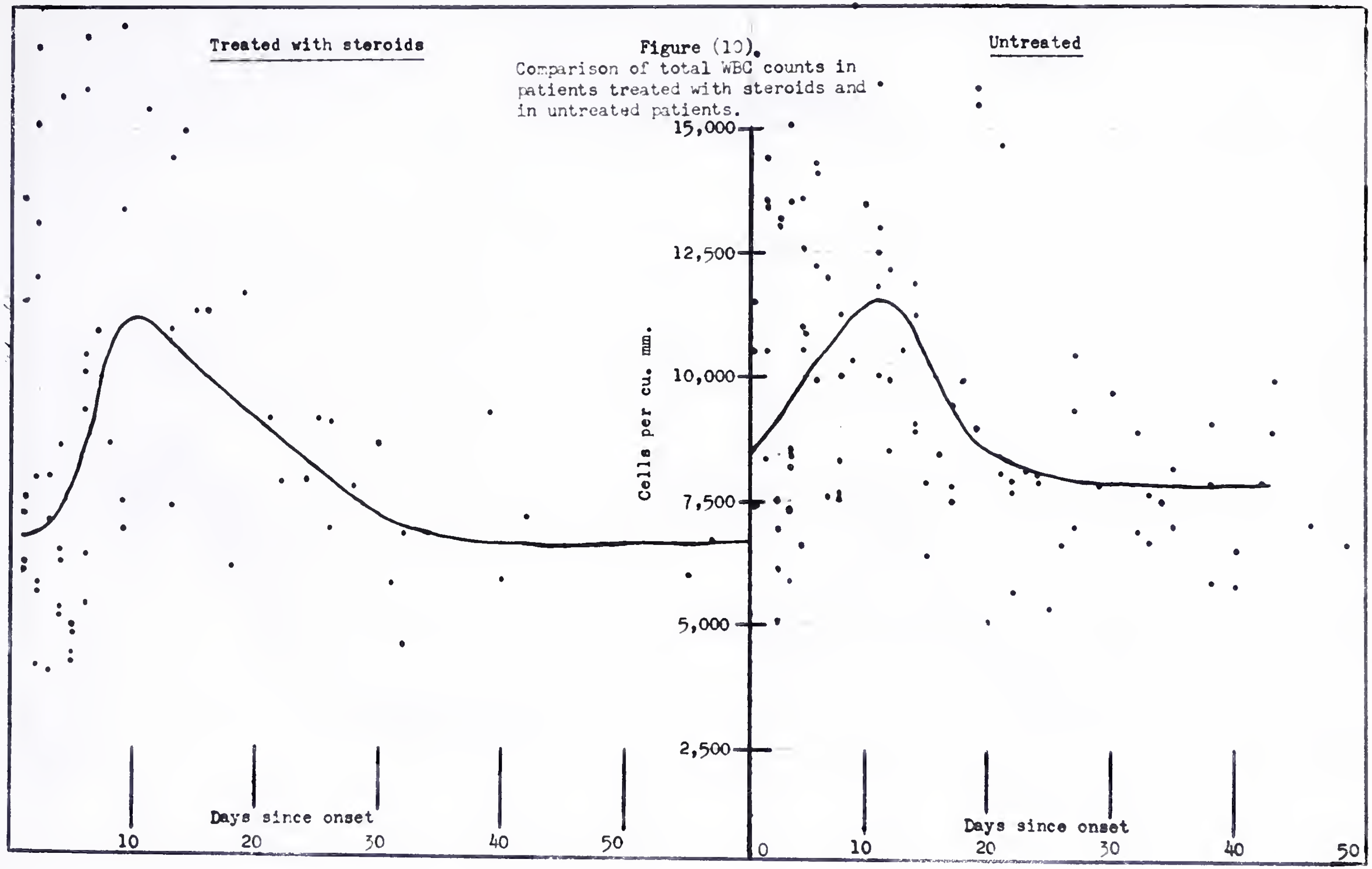

Figure (10).
Comparison of total WBC counts in patients treated with steroids and in untreated patients.

Figure (11)
Comparison of total lymphocytes
(WBC X % lymphocytes) in patients
treated with steroids and in untreated.

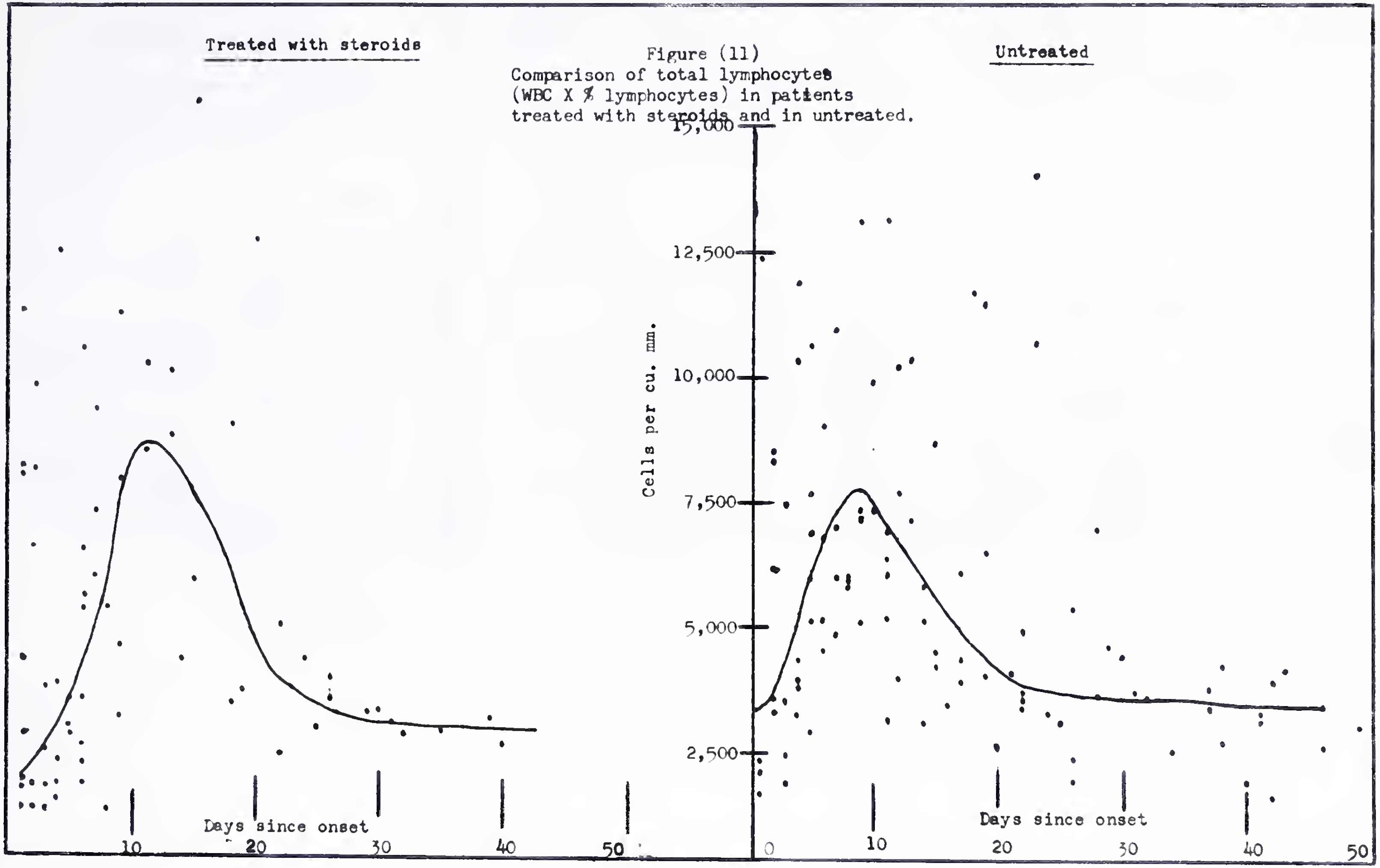

TABLE (12)

Incidence of positive streptococcal throat cultures in patients treated with steroids, compared to the incidence in all infectious mononucleosis patients.

Positive, group A			Positive, not group A			Total positive		
No. pos.	% pos.	Overall %	No. pos.	% pos.	Overall %	No. pos.	% pos.	Overall %
13 patients treated with steroids in school year 1958, 59								
8	61.5%	47%	0	0	2%	8	61.5%	49%
15 patients treated with steroids in school year 1959, 60								
2	13.3%	~~8~~ 8%	4	26.6%	20%	6	40%	28%
5 patients treated with steroids in school year 1960, 61								
0	0	23%	2	40%	31%	2	40%	54%
Totals for above $2\frac{1}{2}$ years:								
10	30.3%	26.4%	6	18.2%	15.2%	16	48.5%	41.6%

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