ANTIBODY RESPONSES IN INFECTIOUS MONONUCLEOSIS

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Until recently the importance of heterophile antibodies in clinical medicine was scarcely appreciated. A short time ago, however, these substances became of practical interest when Paul and Bunnell (1) observed the occurrence of heterophile agglutinins and hemolysins in four cases of infectious mononucleosis. Heretofore they had only been found consistently in patients previously treated with horse serum. Confirmation of Paul and Bunnell's report has lately been offered by Rosenthal and Wenkebach (2), Boveri (3), and Bunnell (4).

The purpose of this paper is to report the results of observations on the presence of heterophile antibodies in the sera of patients having infectious mononucleosis and other diseases, with special reference to the value of the test for diagnostic purposes, and also to determine whether or not other unrelated bacterial serological reactions are associated with agglutinins of sheep red blood cells.

MATERIALS AND METHODS

The methods are those described by Davidsohn (5) and, for the sake of uniformity, the results are tabulated according to his arrangement. Previous authors (1) have noted a close parallelism between the titers of agglutinins and hemolysins. In order to simplify the technique, only the agglutinins have been determined in this study. The following materials are required: the patient's serum, a suspension of sheep red cells, and physiological saline solution. The serum, obtained as for any agglutination, is inactivated for 15 minutes at 56° C.; kept in the icebox, its potency, so far as the agglutinins are concerned, remains constant over a period of several months. Dilutions of the serum starting with 1 to 4, are carried out as far as is indicated. Weekly collections of the sheep cells are made. These are washed three times; from them a 2 per cent suspension of packed cells is prepared. To each tube containing 0.5 cc. of diluted serum, 0.5 cc. of 2 per cent sheep cells is added. Finally, the addition of 1 cc. of saline brings the total volume to 2 cc.

The tubes are shaken and placed in a water bath at 37° C. for one hour; then kept overnight in the icebox. The following morning the

tubes are gently inverted three times after which the results are recorded as follows:

+++ a single mass of cells
++ large flakes
+ small flakes
± barely macroscopic agglutination.

HETEROPHILE ANTIBODIES IN SERA FROM CASES OTHER THAN INFECTIOUS MONONUCLEOSIS

In the course of these studies heterophile antibody determinations were carried out in 300 adult patients on the medical service. Many of these tests serve as controls. However, three groups of diseases, which were deemed of particular interest in relation to the problem, were specially included. They were:

(*a*) Conditions with any clinical features similar to those of infectious mononucleosis.
(*b*) Conditions which are associated with bacterial agglutination reactions.
(*c*) Certain blood dyscrasias.

(*a*) *Conditions with any clinical features similar to those of infectious mononucleosis*

Some of the more pertinent examples of these are: diphtheria, secondary syphilis, streptococcus tonsillitis, Vincent's stomatitis, herpetic stomatitis, scarlet fever, mumps, chicken pox, measles, luetic cervical adenitis, miliary tuberculosis, erythema nodosum and multiforme, acute rheumatic fever, subacute bacterial endocarditis, pneumonia, erysipelas, influenza, poliomyelitis, tertian and quartan malaria, yaws, trichiniasis, pregnancy (all stages), obstructive jaundice, hyperthyroidism with lymphocytosis, symptomatic purpura, asthma, angioneurotic edema, and serum disease.

The distribution of titers in the miscellany of diseases studied is in Table I.

TABLE I

Distribution of heterophile antibody titers in 300 hospital patients

Titer	Less than 1 : 4	1 : 4	1 : 8	1 : 16	1 : 32
Per cent.....	29	32	25	14	0

These results conform in general with the data cited by Paul and Bunnell (1). No effort was made to correlate the values with the age group of the patients as these authors have done. The only titers higher than 1 to 16 were encountered in patients who, on further investigation, were found to have had horse serum within a period of less than a year.

The subject of serum therapy merits a word of mention. Davidsohn (6) has demonstrated the eventual appearance of heterophile antibodies in

human serum after administration of horse serum, which contains the heterophile antigen. These results were confirmed. Whatever may be the original titer of the patient's serum, this value will suddenly increase by three or four dilutions, in a period ranging from six to nine days, after the introduction of the serum, but declines once more to the former level over an interval of two to three months. The highest point attained in a series of 18 serum-treated individuals whom we followed, was 1 to 512, this being in a colored woman treated intramuscularly with anti-erysipelas serum. The preliminary titer of her serum had been 1 to 16. For our present purposes this emphasizes the importance of eliminating horse serum as the inciting agent before drawing any conclusions from an increased titer of heterophile antibodies.

(b) Conditions which are associated with bacterial agglutination reactions

For a reason that will become apparent later (*cf.* Case 16), a study was carried out with many of the diseases in which bacterial agglutination reactions are employed.

TABLE II

Heterophile antibody titers in a selected group of infections

Disease	Organism agglutinated	Titer	Sheep cell agglutinins			
			1:4	1:8	1:16	1:32
Typhoid fever	*B. typhosus*	1 : 1280+	+	−	−	−
Typhoid fever	*B. typhosus*	1 : 1280	++	++	−	−
Typhoid fever	*B. typhosus*	1 : 640	++	+	±	−
	B. paratyphosus A	1 : 160				
Typhoid fever	*B. typhosus*	1 : 1280	−	−	−	−
Typhoid fever	*B. typhosus*	1 : 1280	+	±	−	−
Paratyphoid (carrier)	*B. paratyphosus B*	1 : 160	+	+	+	−
Malta fever	*B. melitensis-bovine*	1 : 2560	±	−	−	−
	porcine	1 : 320+				
	caprine	1 : 320+				
Dysentery	*B. dysentery Shiga*	1 : 160	+	−	−	−
Dysentery	*B. dysentery Flexner*	1 : 160	+	−	−	−
B. proteus pyelitis	*B. proteus* X-2 and X-19	1 : 160	−	−	−	−
Tick-bite fever	*B. proteus* X-2 and X-19	1 : 160	±	−	−	−
Tularemia (typhoidal form)	*B. tularense*	1 : 320	−	−	−	−
Suipestifer sepsis	*B. suipestifer*, Group II	1 : 80	++	+	−	−

In not a single one of these cases did sheep cell agglutinins appear in increased titer. It will be recalled that the dysentery Shiga organism contains the heterophile antigen.

(c) Certain blood dyscrasias

One or more of each of the blood dyscrasias detailed in Table III were studied.

TABLE III

Heterophile antibody titers in some diseases of the blood

Disease	Sheep cell agglutinins				Remarks
	1:4	1:8	1:16	1:32	
Pernicious anemia	+	±	−	−	One week after treatment begun. R.B.C. 1,400,000
Erythremia	+	−	−	−	No therapy. R.B.C. 7,500,000
Paroxysmal hemoglobinuria	+	±	−	−	
Chronic aplastic anemia (benzol)	++	++	+	−	R.B.C. 2,500,000; Hb 45 per cent; platelets 80,000; W.B.C. 3,000
Sickle cell anemia	+++	++	+	−	R.B.C. 2,000,000; Hb 40 per cent; W.B.C. 12,000
Hemophilia	−	−	−	−	Clotting time 3 hours
Purpura hemorrhagica	+++	++	±	−	Platelets 100,000. *Cf.* Case 17, however
Hodgkin's disease	+	−	−	−	
Leukopenic infectious monocytosis (7)	++	+	±	−	W.B.C. 2,300; lymphocytes 37 per cent; monocytes 63 per cent. Recovered after pentnucleotide
Agranulocytic angina	−	−	−	−	W.B.C. 500, all plasma cells
Acute myeloblastic leukemia	++	±	−	−	W.B.C. 25,000; 40 per cent myeloblasts
Chronic myeloid leukemia	−	−	−	−	W.B.C. 37,000 (after irradiation)
Chronic monocytic leukemia	−	−	−	−	W.B.C. 10,800; 46 per cent monocytes
Lymphosarcoma	−	−	−	−	W.B.C. 7,000; 57 per cent lymphocytes
Acute lymphatic leukemia	−	−	−	−	W.B.C. 12,000; 95 per cent lymphocytes
Chronic lymphatic leukemia	−	−	−	−	W.B.C. 150,000; 95 per cent lymphocytes

The titers tabulated are representative values from individual cases. In none of these were the agglutinins found above normal levels.

HETEROPHILE ANTIBODIES IN SERA FROM CASES OF INFECTIOUS MONONUCLEOSIS

Fifteen cases fulfilling the clinical requisites of infectious mononucleosis, have been studied in some detail.[1]

In each case recorded in Table IV the agglutinin titer is the highest value obtained in the course of the disease, during the period of observa-

TABLE IV

Concentration of sheep cell agglutinins in the acute stage of fifteen cases of infectious mononucleosis

Case number	Name	Age	Day of disease	Temperature	W.B.C.	Differential			Heterophile antibody titer
						Pmn.	Lym.	Mon.	
		years	*days*	*° F.*	*per c.mm.*	*per cent*	*per cent*	*per cent*	
1	J. M.	19	5	103.0	10,500	30	70		1 : 256
2	R. H.	23	22(?)	101.0	10,700	40	60		1 : 1024
3	B. W.	23	6	102.4	5,680	46	51	3	1 : 1024
4	W. D.	16	12	100.8	17,500	29	71		1 : 2048
5	B. C.	6	13	100.0	12,000	45	47	8	1 : 4
6	L. W.	24	14	101.0	12,000	23	76	1	1 : 2048
7	E. M.	21	23	102.6	12,300	40	53	7	1 : 128
8	J. A.	14	26	99.0	8,050	30	66	4	1 : 32
9	M. G.	29	10	100.0	7,600	37	60	3	1 : 1024
10	B. K.	10	12	102.0	46,000	11	89		1 : 2048
11	M. B.	19	14	99.0	9,700	23	66	11	1 : 32
12	E. W.	6	13	101.0	12,000	52	40	8	1 : 16
13	R. B.	20	19	102.0	8,750	26	61	13	1 : 2048
14	F. C.	23	11	101.0	7,550	41	59		1 : 4096
15	H. C.	17	12	100.0	27,000	15	83	2	1 : 512

tion. In thirteen of the fifteen examples, heterophile antibodies were found present in the patient's serum to a titer of 1 to 32 or higher.

As soon as there exists any clinical basis for suspicion of the disease, sheep cell agglutinins will be found in abnormal concentrations in those instances where any increase is ultimately observed. This concentration rapidly attains a maximum after which there is a gradual decline, lasting between 6 weeks and 9 months, to normal levels even though this may be temporarily coincident with an aggravation of the symptoms of the disease. From observations on patients who have received horse serum, and, hence,

[1] Data and sera in six of these cases were supplied through the courtesy of Doctors Louis P. Hamburger, Sydney R. Miller, Benjamin H. Rutledge, A. A. Silver, T. P. Sprunt and J. N. Zierler, to whom the author wishes to express his appreciation.

in whom the exact time of introduction of the heterophile antigen is known, the latent period, before the antibodies appear, is found to be six days or more. By analogy, then, the incubation period of infectious mononucleosis would be expected to be at least six days, which is in accord with clinical estimates.

The highest titer encountered in the group of fifteen cases was 1 to 4,096. This value seemed to bear no closer parallelism with the severity of the disease than do the titers, after serum therapy, conform with the degree or, indeed, even the presence of serum sickness. The greatest concentration of antibodies in a group of 18 serum-treated individuals occurred in a case in which the only evidence of serum sickness was a rise of temperature to 101.6° F. on the sixth day after institution of serum therapy. The fever was unaccompanied by any of the usual symptoms of serum sickness.

Brief summaries will be given of five of the cases of infectious mononucleosis, each one illustrating a point of interest.

Case 1. Antibodies preceding abnormal cells in the blood.

J. M., a white, male, medical student, aged 19, was admitted to the Johns Hopkins Hospital on October 4, 1932, complaining of a sore throat.

Six days before admission, enlarged cervical glands were noted. Two days later, sore throat and anorexia developed. Two days before entry, the patient had chills and fever.

On admission, the temperature was 100.6° F. The pharynx appeared dusky red; there was one hemorrhage on the soft palate. Marked general grandular enlargement was present and the spleen was palpable. The white blood cell count was 7,400 of which 47 per cent were polymorphonuclears, 45 per cent normal large lymphocytes and 8 per cent small lymphocytes. It was not until two days later that the characteristic abnormal large cells with irregular foamy nuclei made their appearance in the blood stream. Fever persisted for almost a week, temperature attaining a normal level on October 10. The leukocyte count rose to 14,240, falling within normal limits after October 14.

On the day after entry the agglutinin titer was 1 to 256. Subsequent determinations are presented in Table V, from which it may be seen that the heterophile antibodies were present in high titer for ten days. Subsequent tests, at intervals, showed a progressive diminution in the reactivity which reached a normal value 3½ months after the onset of the disease.

This case illustrates the typical behavior of the antibodies and also indicates that these may precede the appearance of abnormal white cells in the peripheral blood.

Case 2. Antibodies present in abnormal titer during long prodromal period.

R. H., a white, male, medical student, aged 23, was admitted to the Johns Hopkins Hospital on November 1, 1932, complaining of general malaise.

Onset of present illness occurred one week before admission, with a cold and malaise followed by an unproductive cough. Two days before entry, fever, prostration and anorexia put in their appearance.

On admission the temperature was 101.4° F. The pharynx was diffusely injected; the axillary, epitrochlear, cervical, and inguinal glands were slightly en-

TABLE V

Relationship between heterophile antibody titer and the clinical course of a mild case of infectious mononucleosis (Case 1)

Date	Sheep cell agglutinins								W.B.C.	Differential				Temperature
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512		Pmn.	Ll.	Sl.	Mono.	
									per c. mm.	*per cent*	*per cent*	*per cent*	*per cent*	*° F.*
October 5, 1932	+++	+++	+++	++	++	++	++	−	10,500	30	66	4		103.0
October 7, 1932									14,240	37	59	4		101.0
October 11, 1932	+++	+++	+++	+++	+++	++	++	−	8,160	33	60	7		99.0
October 14, 1932	+++	+++	+++	+++	++	++	+	−	9,920	35	55	10		99.0
November 16, 1932	++	++	++	++	+	±	−	−	7,280	62	7	24	7	normal
January 13, 1933	++	+	+	−	−	−	−	−						normal
March 25, 1933	++	+	±	−	−	−	−	−						normal

larged and the spleen was palpable. The leukocyte count was 6,900 of which 81 per cent were polymorphonuclear neutrophilic leukocytes, 6 per cent eosinophiles, and 13 per cent lymphocytes. Four days later, the patient's temperature became normal. At this time the blood count was 10,200, polymorphonuclear neutrophils 79 per cent, eosinophils 1 per cent, lymphocytes 20 per cent. The patient was discharged on November 9, after being afebrile for five days, although a sore throat, which had appeared while he was in the hospital, persisted.

The diagnosis of this patient's condition was, at first, in doubt. The usual laboratory tests for enteric fever were negative. However, with serum obtained on November 2, agglutinins for sheep red blood cells were found to be present in a titer of 1 to 32. Even though this degree of serological activity is higher than the average normal, it was not deemed sufficiently significant to justify a diagnosis of infectious mononucleosis.

Three days after discharge his throat became more sore, fever returned and he was readmitted on November 14, three weeks after the onset of the initial symptoms. Temperature was 101° F., and glandular and splenic enlargement, noted on the previous admission, were still present. In addition there was a characteristic follicular tonsillitis as well as a mottled scarlatinal-like erythema over the body. However, the true diagnosis was now revealed by the hematological examination which showed a leukocyte count of 10,700, 48 per cent large and 12 per cent small lymphocytes, many of the cells typical of infectious mononucleosis being present. His course was a mild one. After four days of rehospitalization, the fever abated and remained normal. On November 25 with a blood count of 9,300 there were still 60 per cent lymphocytes, some of these abnormal in type.

On the day of the second admission serum was obtained in which the heterophile agglutinin titer was found to be 1 to 1,024. The hematological diagnosis was therefore confirmed by the serological test. Additional observations with samples of serum, obtained at intervals during convalescence, demonstrated a progressive decline in antibodies. The last test, made ten months after recovery, reacted only in a dilution of 1 to 8.

Interest in this case centers around the fact that the final outcome emphasized the significance of the early, slightly increased, heterophile antibody titer contained in the patient's blood. The serological titration made with the patient's serum at the time of the first undiagnosed stay in the hospital indicated, in all probability, the true nature of the illness. Additional experience with sera from other cases of infectious mononucleosis suggests that a titer of sheep cell agglutinins no higher than 1 to 32 is adequate, in most instances, to establish a diagnosis.

Case 3. Diagnostic significance of antibodies in a case without conspicuous adenopathy.

B. W., a white, female, student nurse, aged 23, was admitted to the Johns Hopkins Hospital on May 1, 1933, complaining of headache of five days' duration.

The illness began one day after the first inoculation with typhoid vaccine. It was characterized by fever, sweats, headache, dizziness and weakness. Her throat was not sore.

On admission, the temperature registered 102.4° F., the pulse, 84. She appeared prostrated. Small post-cervical, axillary and inguinal glands were palpable. The tip of the spleen could be felt. The leukocyte count was 5,680, polymorphonuclears 46 per cent, small lymphocytes 32 per cent, large lymphocytes 19 per cent, monocytes 3 per cent.

With blood obtained on the day of admission, the heterophile antibody titer was 1 to 1,024. Within five days the leukocyte count rose to 14,640 of which polymorphonuclears composed only 17 per cent. Normal temperature was attained on the eleventh day of the disease. On discharge one week later, the leukocyte count was 8,000 of which 60 per cent were lymphocytes and monocytes. During the stay in hospital, several cervical lymph glands became slightly more enlarged, but at no time were sufficiently conspicuous to indicate an acute adenopathy.

The results obtained with the blood of this patient are a striking example of the value of the heterophile antibody determination. The serological test was strongly positive four days before conclusive hematological changes were demonstrable.

Case 4. Association of the antibodies with a false positive Wassermanan reaction.

W. D., a white, male, student, aged 16, was admitted to the Union Memorial Hospital on May 17, 1933, complaining of stiffness of the neck.

Ten days before admission the patient developed a stiffness of the left side of his neck which was followed soon after by generalized cervical aching. There were night sweats and sore throat for two days preceding entry.

On admission, the temperature was 100.8° F. The cervical, axillary, and inguinal lymph glands were palpable; the left cervical glands were markedly enlarged. The spleen was readily felt. There was a follicular inflammation of tonsillar stumps. The leukocyte count was 10,920 with a differential formula of polymorphonuclears 32 per cent, small lymphocytes 65 per cent, large lymphocytes 3 per cent.

For a week the patient ran moderate fever and suffered from a continued sore throat. Subsequent convalescence was uneventful.

A Wassermann test on May 19 was positive. This finding was verified several times thereafter, until, by July 6, the Wassermann reaction had become completely negative. Heterophile antibodies were present on the first occasion to a titer of 1 to 2,048. The titer gradually declined, as indicated in Table VI, until it fell within normal limits on August 10. By this time, the leukocyte count had fallen to normal, while there was still a slight lymphocytosis of 49 per cent.

The chief feature of interest here was the occurrence of a positive Wassermann reaction in a serum which also contained sheep cell agglutinins. Among others, Parkes Weber (8) has reported the occasional presence of a false positive Wassermann reaction in infectious mononucleosis.

Case 5. No increase in heterophile antibodies in a child of six with infectious mononucleosis.

B. C., a white, male child, aged 6, was admitted to the Sinai Hospital on October 8, 1933, complaining of cough.

This was the twelfth day of an illness characterized by fever and moderate cough. The child did not suffer from sore throat.

On admission the temperature was 100° F. In addition to a general glandular enlargement and a palpable spleen, there were signs of a localized pneumonic process at the right base. The leukocyte count was 12,000; 45 per cent polymorphonuclears, 47 per cent lymphocytes, 8 per cent monocytes. Many of the lymphocytes were of the type characteristic of infectious mononucleosis. Sheep cell agglutinins on October 9, were present in the patient's serum to a titer of 1 to 4.

TABLE VI

Heterophile antibody titer, leukocyte count, differential and titered Wassermann in Case 4

Date	Sheep cell agglutinins										Wassermann reaction	W.B.C.	Lympho-cytes
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048			
												per c. mm.	*per cent*
May 19	+++	+++	+++	++	++	++	++	++	+	+	Positive 1 : 4+	17,500	71
May 25	+++	+++	++	++	++	+	+	±	−	−	Positive 1 : 150	6,200	86
June 23	Not done										Doubtful	6,800	66
July 6	+++	++	++	+	±	−	−	−	−	−	Negative	7,080	35
August 10	+	−	−	−	−	−	−	−	−	−	Negative	5,700	49

The boy recovered rapidly so that he was discharged a week later.

On November 20, 1933, he returned to the Hospital once more with fever and signs of pneumonia, which had reappeared. The glandular enlargement was no longer present while the spleen was barely palpable. Temperature and physical signs were again normal within three days. The leukocyte count on this admission was 18,250; 49 per cent polymorphonuclears, 24 per cent small lymphocytes, 19 per cent large lymphocytes, 8 per cent monocytes. A week after entry his serum gave a plus minus agglutination in a dilution of 1 to 4 with sheep cells, or essentially the same value as was found seven weeks previously.

This was one of the two cases clinically considered infectious mononucleosis, in which heterophile agglutinins were not found to exist above normal limits. The second instance was also a boy of six with characteristic physical and hematological findings. At the height of his course, his serum agglutinated sheep cells to a titer of 1 to 16. Neither of the children, it should be noted, had sore throats at any stage of their illnesses.

Other cases associated with heterophile antibodies

Two other cases of especial interest will now be presented.

Case 16. Probable infectious mononucleosis accompanied by agglutinations with multiple bacterial antigens.

Dr. C. C. B., a white, female member of the House Staff, aged 26, was admitted to the Johns Hopkins Hospital on August 18, 1932, complaining of fever and headache. Two days previously, she had developed headache, pains in the eyes, chills, fever and sweats. Her leukocyte count was found to be 3,000. On the following day, when a rhinitis had appeared, the leukocytes were 3,600.

On admission her temperature was 102° F. She had a slightly red throat, a patch of urticaria in the right axilla and small lymph glands at the angles of the jaw. There were no other palpable nodes and no demonstrable splenic enlargement. The leukocyte count was again 3,000; 65 per cent polymorphonuclears, 30 per cent small lymphocytes, 5 per cent monocytes. Fever persisted for one week. A white blood cell count on the seventh day of the disease was 8,350; another two days later was 9,000; 38 per cent polymorphonuclears, 50 per cent lymphocytes, 12 per cent monocytes. Many of the lymphocytes are described in the record as "young forms." Unfortunately, the smear was not available for subsequent study. Cultures of the blood, stools, urine and throat gave no further information. The patient was discharged on August 24, but felt below par for some time thereafter, being troubled by a mild sore throat and a dry conjunctivitis. The diagnosis was recorded as rhinopharyngitis.

Shortly after admission when the patient's serum was found to agglutinate the paratyphoid B antigen to a titer of 1 to 160, the behavior with other antigens was investigated, with the results indicated in Table VII. It was the clinical impression that the patient did not have paratyphoid fever, although her serum contained agglutinins for *B. paratyphosus B* as well as for several other organisms. Heterophile antibodies were first measured two months after the onset of the disease. At that time their titer was 1 to 256. Subsequently all the agglutinins gradually decreased to approach a normal level.

Several other antigens were tested in addition to those tabulated above, but with negative results. Among these were *B. suipestifer I., B. proteus X–19,* and the three strains of *B. melitensis.*

Whatever interpretation one chooses to apply to these findings, one statement would seem warranted; namely, that the group of heterologous antibodies must have appeared in the course of the acute illness. (The patient had received typhoid vaccine over a year previously.)

Two points worthy of comment emerge from this case. In the first place, it indicates the value of determining sheep cell agglutinins even after a delayed period following the acute stage of a disease, for it was not until

TABLE VII

Values of agglutination titers with various antigens in Case 16

	Agglutination titer			
Date	August 24, 1932	November 11, 1932	January 25, 1933	May 30, 1933
Antigen				
Sheep cells	Not done	1 : 256	1 : 64	1 : 16
B. typhosus	1 : 40	1 : 40	1 : 20	0
B. paratyphosus A	1 : 80	1 : 80	1 : 40	0
B. paratyphosus B	1 : 320	1 : 320	1 : 80	1 : 20
B. aertrycke	0	1 : 160	0	0
B. suipestifer II	1 : 320	1 : 80	1 : 40	1 : 40
B. enteriditis	1 : 160	0	0	0

the heterophile antibody test was found positive two months later, that the history records were scanned for confirmatory evidence which the previously neglected differential count seemed to provide. No definite assertion can be made, but it seems highly probable that the diagnosis should have been infectious mononucleosis.[2] In the second place, here is an instance of human blood serum containing a number of heterologous bacterial antibodies in addition to those against sheep cells.

The frequency of bacterial agglutinins in infectious mononucleosis was investigated. In 14 cases, excluding the present ones, found in the hospital records, one or more agglutinations had been done in six instances. A Widal was found to be negative in all six; melitensis agglutinations negative in two, but a third showed significant findings: an agglutination for the porcine strain of *B. melitensis* 1 to 640. This was a medical student about whom there was no collateral evidence to suggest Malta fever. After running the typical course of a moderately severe infectious mononucleosis,

[2] A patient (Case 14, Table IV) subsequently observed, during his acute illness, ran a course similar in essential details to the present one. The serum of this patient, too, agglutinated *B. typhosus*, *paratyphosus B*, and *suipestifer*. At first he was considered to be suffering from typhoid fever, but in the course of the first week of the disease his blood developed the characteristic changes of infectious mononucleosis. During this time, the leukocyte count varied between 3,000 and 5,000. His serum eventually agglutinated sheep red cells in a dilution of 1 to 4,096.

he recovered completely. In five cases of the present group, agglutinations were carried out for *B. typhosus, paratyphosus A* and *B,* as well as for two strains of *B. suipestifer.* For the typhoid group of organisms, agglutinins in 1 to 40 dilution or higher were found in two instances. For the suipestifer group they were found in three, and all of these in 1 to 80 dilution or above. Nothing of note was unearthed in the remaining cases with the exception of the nurse (Case 3) who had recently received typhoid vaccine. Her serum agglutinated the typhoid group in low titer but with *B. suipestifer* gave negative results. Kuttner (9) has observed positive suipestifer agglutinations only in patients from whom the organism has been isolated, and occasionally in other members of the same families when there has been reason to suspect, from the history, that the latter may have recently been afflicted with the same disease. From this one may conclude that, not uncommonly, unrelated bacterial agglutinins are present in infectious mononucleosis.

Multiple bacterial agglutinins were observed more frequently in that form of infectious mononucleosis that started with leukopenia than in the more usual type characterized by leukocytosis. The leukopenic variety is the one in which heterophile antibody determinations are of especial value. In two of the present fifteen cases (Cases 8 and 11, Table IV) pentnucleotide was administered before the true nature of the disease was recognized. In each instance, the white blood cell counts were at first below 5,000, which aroused a suspicion of agranulocytosis.

Case 17. Thrombocytopenic purpura accompanied by heterophile antibodies and a false positive Wassermann reaction.

I. W., a white, female student, aged 16, was admitted to the Johns Hopkins Hospital on March 7, 1933, complaining of nose-bleeds. There was a history of some familial hemorrhagic diathesis. Starting at the age of six years the patient had suffered episodes of bleeding from various sites. Six days before entry a spontaneous epistaxis set in. Following packing of the nose a pharyngitis and otitis media appeared.

On admission, there were the typical findings of purpura hemorrhagica complicated by a draining right ear: fever, purpuric lesions on the skin, a palpable liver and spleen. At no time was there generalized adenopathy or jaundice. Examination of the blood showed a mild secondary anemia, a leukocyte count of 10,000, 54 per cent polymorphonuclears, 11 per cent large lymphocytes, 29 per cent small lymphocytes, 6 per cent monocytes. The platelet count was 70,000; bleeding time 25 minutes; clotting time normal. Coincident with a transfusion the bleeding gradually subsided so that the patient was ready for discharge one month later. Within three weeks the bleeding time had become normal although the platelets continued about 100,000. Subsequent differential counts were not noteworthy.

A Wassermann reaction on the day of admission was positive while a flocculation test, after the Eagle technique (10), was negative. False positive Wassermann reactions are known to occur occasionally in this disease. Three days later heterophile agglutinins were first sought for and found to exist up to a titer of 1 to 1,024. Subsequent findings are outlined in Table VIII.

TABLE VIII

Relation of heterophile antibodies to titered Wassermann reactions in a case of purpura hemorrhagica (Case 17)

Date	Sheep cell agglutinins									Wassermann
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	titered
1933										
March 7	Not done									Positive 1 : 4+
March 10	+++	+++	+++	++	++	++	+	±	±	Positive 1 : 4+
March 16	Not done									Positive 1 : 150
March 20	+++	+++	++	++	++	++	+	±	−	Not done
March 24	+++	+++	+++	++	++	++	+	−	−	Negative
March 28	+++	+++	++	++	+	±	−	−	−	Anticomplimentary
April 6	+++	+++	++	++	±	−	−	−	−	Negative
April 26	++	++	+	−	−	−	−	−	−	Not done
August 2	−	−	−	−	−	−	−	−	−	Negative

Note: Flocculation test always negative.

The only moot point is whether infectious mononucleosis co-existed with the purpura. The findings were all compatible with the single disease, purpura, from which the patient unquestionably suffered, but the possibility of an atypical form of infectious mononucleosis can not be dismissed.

The nature of the false positive Wassermann reaction in infectious mononucleosis

It has been possible to analyze the character of the false positive Wassermann reaction which occurred in the one case of infectious mononucleosis and in the instance of purpura. The term " false positive " is used to denote the situation wherein the Wassermann reaction becomes transiently positive in the absence of syphilitic infection. One type of such reaction—termed by Eagle the " anomalous false positive reaction "—was observed in these two cases when the complement fixation test was temporarily positive, while a flocculation test, after the Eagle modification, was consistently negative.

That two diseases, unrelated so far as prevailing knowledge reveals, both of which on occasions are known to be associated with false positive Wassermann reactions, also may present heterophile antibodies, would seem to be more than a coincidence. It appears that the positive Wassermann reaction exists *in spite of* the antibodies, for it is apparent that sheep cell hemolysins in serum would upset the hemolytic system in the Wassermann set-up and tend to make a positive reaction negative. Titered Wassermann reactions carried out on serum from the case of infectious mononucleosis, before and after absorbing the hemolysins with sheep cells, supported this conclusion. Starting with 0.2 cc. of serum the values for successive dilutions were 2–2–4–4–4–4–2 and 4–4–4–4–4–4–2 respectively;

more strongly positive in the lower dilutions, therefore, after the removal of the antibodies. (The degree of positivity is represented as ranging from 1 up to 4.)

Heterophile antibodies were found lacking in the sera of several patients who, without any stigmata of syphilis, had intermittently positive Wassermann reactions but negative flocculation tests. Again, the antibodies were not demonstrated in some of the other conditions in which false positive Wassermann reactions are reputed to occur, such as jaundice, pneumonia and scarlet fever.

DISCUSSION

The data presented herein relative to the occurrence of heterophile antibodies in infectious mononucleosis are in accord, in most respects, with the original findings of Paul and Bunnell (1), and the subsequent report of Bunnell (4). In a variety of clinical conditions, sheep cell agglutinins were found to be present in the patients' sera in concentrations now recognized as falling within normal limits. In thirteen out of fifteen cases of infectious mononucleosis, the agglutinin titers of the blood sera were elevated. Two instances of the disease, however, gave perfectly normal figures in so far as sheep cell agglutinins were concerned.

Rosenthal and Wenkebach (2) recounted the histories of ten patients in whom the only detail whereby their illnesses could be differentiated from infectious mononucleosis was the normal concentration of heterophile agglutinins in their blood sera. On the basis of this finding they suggested that in those instances where the agglutination test was positive, the patient had infectious mononucleosis, while in those where the test was negative, the disease process was glandular fever. Such an interpretation must be cautiously made, however, both because the cause of the increase of heterophile antibody is unknown and because of the wide range of antibody concentration in normal people. One must remember that if the increased titer of agglutinins in infectious mononucleosis merely represents an enhancement of the concentration of antibodies already present, then there is just as much increase in a patient's serum with a normal agglutination titer of 1 to 1, rising to 1 to 16 as there would be in one starting at 1 to 8 and rising to 1 to 128. Yet the former would be considered a negative heterophile antibody test and the latter a positive one. In this sense, no such sharp distinction can be made here as is possible between a positive and negative Wassermann reaction.

The phenomenon of heterophile antibody production in infectious mononucleosis may indicate some valuable guides pointing towards the etiology of the disease. After the injection of horse serum, which contains the heterophile antigen, the appearance of the antibodies is comprehensible. The source of the antigen in infectious mononucleosis may be extrinsic or intrinsic. If the former, it must be found in the organism or virus which

causes the malady; if the latter, it must be associated with the tissues of the patient. Paul and Bunnell were unable to find supportive evidence to incriminate Vincent's fusiform bacilli as the offenders. In connection with their experiments, it is pertinent to recall the fact, that although the dysentery Shiga bacillus contains heterophile antigen, two human carriers of this organism did not possess sheep cell agglutinins in more than normal concentrations.

Of all the human tissues that have been tested, only the red cells from type A subjects (Moss Groups I and II), seem to contain heterophile antigen. Normally, such persons do not show a significantly higher titer of sheep cell agglutinins than the other two groups. One might hypothecate that in infectious mononucleosis red cells are, for some reason, broken down with the liberation of the antigen which subsequently produces the antibodies. There is ample evidence that such is not the case. Sheep cell agglutinins appeared in Moss Group IV patients with just as great regularity as in Group II. Furthermore, several patients receiving multiple transfusions with Group II blood did not develop an increased antibody titer.

It is plain that the substance responsible for the positive Wassermann reaction in infectious mononucleosis and purpura hemorrhagica is not reagin, which appears in the serum of syphilitics. If it were, the flocculation test should be positive as well as the complement fixation reaction. Nor is it the agent which agglutinates sheep cells. While this latter substance is present in infectious mononucleosis with greater consistency than either the complement-fixing material for the Wassermann reaction or the various bacterial agglutinins, the significance of the appearance of these three agents would seem to be the same: i.e., evidence of the versatility of antibody responses in infectious mononucleosis.

CONCLUSIONS

Blood sera of 300 adult hospital patients have been tested for the presence of agglutinins for sheep's red cells. In this control group the agglutinin titer never exceeded 1 to 16. Thirteen out of fifteen accepted cases of infectious mononucleosis, studied in the acute stage of the disease, showed an increased titer of agglutinins for sheep's red cells attaining dilutions as high as 1 to 4,096. Except in these subjects, titers above normal levels were encountered only under the following circumstances: (1) in individuals who had received injections of horse serum, which contains heterophile antigen; (2) in one patient whose serum contained agglutinins for several bacterial antigens, the exact nature of whose disease was never definitely determined, but who probably suffered from infectious mononucleosis; (3) in a patient with purpura hemorrhagica who presented scant evidence of a concomitant infectious mononucleosis. The blood serum from several cases of infectious mononucleosis contained agglutinins for a num-

ber of bacterial antigens. However, in none of the diseases in which bacterial agglutinations occur were heterophile antibodies unusually increased. Similar observations were made in a study of a number of the blood diseases. One case of infectious mononucleosis is detailed in which the Wassermann reaction became temporarily positive. A similar state of affairs was met with in a patient with purpura hemorrhagica. Of the other clinical conditions in which a false positive Wassermann may occur, none with an increased heterophile antibody titer was encountered.

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