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Research Article

THE HETEROPHILE ANTIBODIES IN INFECTIOUS MONONUCLEOSIS AND AFTER THE INJECTION OF SERUM [1]

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Recent investigations have shown that the so-called heterophile antibodies found in cases of infectious mononucleosis and after injection of horse serum have distinguishing serological characteristics. These findings may prove helpful for differential diagnosis in certain cases with borderline titers or high titers of sheep cell agglutinins, particularly in children and adults with a history of previous serum injection. The present paper deals with a study of the heterophile antibodies in sera from normal persons, from cases of infectious mononucleosis and from individuals who have received injections of serum. The purpose of this investigation was to define more clearly the differences among these sera and to determine the possibilities for using them in differential diagnosis.

The clinician, in a case of infectious mononucleosis would be especially interested in knowing whether a borderline titer (1:40 or 1:80) of the sheep agglutinins [2] is due to a high normal antibody content or to a low titer due to infectious mononucleosis (1). The clinician may also encounter cases, especially in children, in which a diagnosis of infectious mononucleosis or glandular fever is suspected and in which serum had been administered for active or passive immunization before the disease was contracted. In such cases, the heterophile antibodies can be due either to the injection of serum or to infectious mononucleosis. Cases are sometimes encountered which are injected with antitoxic serum because of a tonsillitis simulating diphtheria or because of a rash similar to that of scarlet fever and in which the later course suggests a diagnosis of infectious mononucleosis. Under such conditions a confirmation of the clinical and hematological findings may be desirable. This is possible only if a serological difference can be found between the antibodies in infectious mononucleosis and those found after the injection of serum. There are also cases in which some of the features of serum sickness resemble those of infectious mononucleosis, namely, the general adenopathy and the enlargement of spleen and liver.

Various animal blood cells and tissues have been used for the absorption of heterophile antibodies and the results of these absorptions recommended for differential diagnosis. Stuart, Tallman and Brintzenhoff (2) used rabbit erythrocytes. Bailey and Raffel (3) used beef blood corpuscles and also found that horse kidney absorbs part of the antibody from cases of infectious mononucleosis. Davidsohn and Walker (4) used guinea pig and rabbit kidney. Stuart and coworkers (2) found high titers of rabbit agglutinins in serum sickness as compared with infectious mononucleosis. Bailey and Raffel (3) found high hemolysin titers for the beef cells in the latter disease.

In the present study, the blood corpuscles of the horse, goat, guinea pig, pig, dog and chicken were used for absorption in addition to the ones employed by the previous investigators, and the titers for all these agglutinins were determined. The horse blood corpuscles were used for absorption because the titer of agglutinins with these cells was found to be high in infectious mononucleosis, a finding noted by Stuart, Griffin, Wheeler and Battey (5) since this work was completed. The pig cells were used for absorption because they were found by Deicher (6) to absorb the sheep agglutinins after injections of serum. Goat erythrocytes were employed because of their antigenic relationship to sheep cells.

METHODS

Titration of agglutinins was carried out approximately after the method used by Stuart et al. (7) for the reasons which he outlined. Five tenths cc. of serum dilutions, beginning with 1:2.5 or 1:5, were made with saline and 0.5 cc. of 1 per cent suspension of blood corpuscles was added, the tubes shaken, incubated at 37° C. for about

[1] This study was carried out under a special grant from the Friedsam Foundation.

[2] The terms sheep agglutinins, beef agglutinins, etc., are frequently used in this article as a substitute for more cumbersome, though perhaps more exact terms, such as, sheep red blood cell agglutinins, beef red blood cell agglutinins, etc.

2 hours and read after storage in the ice box over night. The titer was read as the "final dilution" of serum in which agglutination was observed with the naked eye.

Hemolysins for beef cells were determined only in a few cases, after the method of Bailey and Raffel (3), but with half the amounts of all components: 0.5 cc. of serum dilution, 0.25 cc. of 2 per cent suspension of beef blood corpuscles and 0.25 cc. of guinea pig serum 1:10 for complement. Due to the presence of hemolysins in the dilution of the guinea pig serum used, a control tube containing complement and beef cells alone was used. A 1:30 or 1:60 dilution of the complement, which did not hemolyze by itself, occasionally did not seem to provide sufficient complement.

Absence of agglutinins or hemolysins in the first dilution used is recorded as "0" in the tables.

Absorption tests were also carried out after the method of Stuart et al. (2). The third absorption was found to make no appreciable difference in titers as compared with the second. Therefore, only 2 absorptions were carried out in the later part of this study. The sera of approximately the same number of cases of infectious mononucleosis, and after the injection of serum, were studied with 2 and with 3 absorptions, made with blood cells from 5 different species, namely, those of sheep, rabbit, beef, horse and pig. The sera were titrated for agglutinins with each of the different kinds of blood corpuscles before and after each absorption. Control sera were absorbed only twice, and the serum was used in a dilution of 1:2 because of the low titers.

SUBJECTS

Sera were obtained from 9 cases of infectious mononucleosis in different stages of the illness.[3]

The sera of 24 patients were studied after the injection of therapeutic serum. The sera injected were: in 13 cases, antitoxic scarlet fever serum; in 4 cases, diphtheria antitoxin; in 5 cases, antimeningococcus serum and in 2 cases, antigas gangrene and antitetanus serum combined. The patients' sera were chosen without regard to the presence or absence of serum sickness.

Sera from normal healthy individuals and from persons who were suffering from conditions other than infectious mononucleosis were studied for comparison. None of these subjects gave a history of previous serum injection.

RESULTS

Titration in control sera (Table I)

In the different sera, the agglutination for the same kind of blood corpuscles varied to a certain extent; there was, however, a certain sequence in the average titer of agglutinin for the various kinds of blood corpuscles, and the same sequence was usually observed in the individual sera. The agglutinins for beef blood corpuscles showed the lowest titers and were usually absent in the dilution 1:5 or 1:10. The sheep agglutinins were present in somewhat higher titer. The highest titers were obtained with rabbit and pig corpuscles. The approximate sequence of the agglutinability was as follows: beef, sheep, goat, horse, guinea pig, dog, rabbit, pig.

TABLE I

Control sera from cases without infectious mononucleosis and not previously injected with serum

Number of control sample	Agglutination titer with blood corpuscles of *								Hemolysin titers for beef blood corpuscles
	Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	0	0	10	80	40	40	20	20	0
2	10			0	40	40	40	20	40
3	0	0	10	5	40	20	40	20	0
4	0	0	10	0	20	20	10	0	0
5	0	0		0	40	20	20	0	20
6	10	0		0	20	10	0	0	0
7	0	0	0	10	20	20	?	0	0
8	0	0	0	10	20	20	?		0
9	5			20	20	80	20	20	
10	20			20	80	160	40	40	
11	20	5		10	80	40	40	40	80
12	10	0		10	40	20	40	20	40
13	10	0		10	40	20	20	20	20
14	0	0		40	40	40			
15	10	0		20	40	40			
16	10	0		10	40	80			
17	0	0	10	10	20	20			
18	20	0	40	20	80	40			20
19	20			10	40	40			
20	0	0		10	20	20			
21	20	10?		20	80	40			
22	0	0		40	20	20			
23	10	0		20	80	40			
24	0	0		0	20				
25	0	0		0	0	10			
26	0	5		10	20	20			
27	10	5		10	20	20			0
28	0	0		0	20	20			0
29	0	0		0	40	20			
30	0	0		20	20	40			
Average titer	6.8	1.0	11.4	13.8	36	35.2	22.3	16.7	15.7

* Figures 5, 10, 20, etc., to indicate a titer of 1:5, 1:10, 1:20, etc.

Titration in cases injected with serum (Table II)

There was a marked increase in the beef hemolysins in most of the sera. The sequence of the average agglutinin titers for the different kinds of

[3] The author is indebted to Dr. Arlie V. Bock, Dr. Morris N. Davidow, Dr. Joseph H. Fay, Dr. Alfred Kranes and Dr. Hyman Morrison of Boston and Dr. Doran J. Stephens, Strong Memorial Hospital, Rochester, N. Y., for supplying the sera of cases of infectious mononucleosis.

TABLE II

Sera from cases injected with serum

Case number	Days after injection	Agglutination titer with blood corpuscles of								Hemolysin titer for beef blood corpuscles
		Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	11	320	10		40	640	320			
2	11	40 to 80	0		80	320	80			
3	17	160 to 320	5		40	320	80			
4	12	160	10		160	640	160			
5	12	80	5		40	160	80			
6	10	40 to 80	10		160	320	320			
7	13	80	5				320			
8	9	320	10	320	640	1280	640	160		
9	11 and 15	160	20		5120	640	640	640	640	2560
10	15	80	20		1280	60	320	320		
11	19	40	0		320	80	160	80		
12	11	40 to 80	5	400	640	320	640	320		
13	9	160	0	160	640	320	320			1280
14	13	80	0		80	160	160			
15	9	160	40	320	1280	320	640	320	160	160
16	9	160	40		640	80	160			
17	29	160	40		160	640	160	640	160	640
18	14	160	80		320	1280	1280	640	320	640
19	11	80	40		80	1280	1280	640	320	1280
20	8	160	80		160	2560	1280	1280	1280	1280
21	20	20	10		10	40	40			
22	12	5	0	20	20	20	40	20	20	0
23	20	40	10		80	80	40			
24	14	20	10		40	20	40			
Average titer		113.5	18.8	244	523	507.8	383.3	460	414.3	980

blood corpuscles is as follows: beef, sheep, goat, rabbit, guinea pig, dog, pig, horse.

With two exceptions, namely for horse and rabbit, the sequence of the agglutinins for the different kinds of blood corpuscles is similar to that recorded in normal sera. The increase in agglutinins for 5 of the 8 kinds of cells used, namely those for sheep, beef, goat, guinea pig and dog ranged about 20 times that of the normal value. The increase for pig and rabbit agglutinins is not as great. The horse agglutinins showed the greatest increase—about 40 times normal. This greater increase in horse agglutinins occurred only in certain cases, while in others the increase was similar to that noted with other cells.

The smaller increase in the pig and rabbit agglutinins may be due to the normally higher titers of these antibodies as compared with agglutinins for other cells.

These results together with the observations on the absorption tests, to be mentioned later, suggest that the agglutinins found after the injection of serum may be due to an increase in normal agglutinins for most cells (8) and that the increase in the horse agglutinins may be a more specific reaction.

Titration in infectious mononucleosis (Table III)

A marked increase in sheep agglutinins in the serum of cases of infectious mononucleosis was first noted by Paul and Bunnell (9) and later by other investigators (8, 10), and this is now used routinely as an aid in diagnosis. Increases in beef hemolysins (3) and more recently in horse agglutinins (5) have also been noted in this condition. These findings are here confirmed. In addition, an increase in goat agglutinins was observed in 5 of our cases.

TABLE III

Sera from cases with infectious mononucleosis

Case number	Agglutination titer with blood corpuscles of								Hemolysin titer for beef blood corpuscles
	Sheep	Beef	Goat	Horse	Pig	Rabbit	Dog	Guinea pig	
1	1280	0		2560	160	160	80		
2	320	0		640	40 to 80	80	40		
3	160	0	160	160	40 to 80	80			1280
4	640	0	1280	2560	40	40	20	20	1280
5	320	0	320	800	40	40	20	10	640
6	320	10	320	640	40	40			
7	2560	0	2560	2560	80	40	40	40	1280
8	640	0		640	40	40	Under 50	Under 50	
9	160/320	5		640	20	20			
Average titer	711	1.07	928	1254	55.5	60	41.7	30	1536

The titers for all of these agglutinins are about 80 to 100 times the normal value and about 2 to 6 times the value found after injection of serum. Occasional cases show high titers after serum injection which are comparable with the low titers seen in some cases of infectious mononucleosis. The difference in the titer alone, therefore, cannot be used for differential diagnosis.

The agglutinin titers for sheep, goat and horse blood corpuscles together with the beef hemolysin titers are very high in contrast to the low titers of agglutinins for rabbit, pig, dog, and guinea pig. The average titer of the latter agglutinins is not more than twice the normal. The difference between the agglutination in infectious mononucleosis and after the injection of serum is striking. After the injection of serum, there is an increased titer for all these kinds of blood corpuscles, whereas, in infectious mononucleosis, the increase is limited to antibodies against certain blood cor-

puscles, but not against others. One observation may be emphasized. There is an increase in the beef hemolysins, but not in the beef agglutinins in cases of infectious mononucleosis (3). After the injection of serum, on the other hand, the beef hemolysins and the beef agglutinins are both increased. These findings point to a possible difference in the character of the various antibodies for the same species of blood corpuscles.

The results of the titrations are sufficient to be applied for a differential diagnosis in a case where the titer is high. The method is very simple because one needs only 2 kinds of blood corpuscles, one of the type increased in infectious mononucleosis and another of the type not increased in this condition. In cases where the differences in titers between these 2 kinds of cells are not marked, possibly in cases with low titers, the absorption method gives a more certain differentiation.

Absorption in control sera (Table IVa)

The results may be summarized briefly.

The sheep agglutinins are more readily absorbed than the rabbit and horse agglutinins. This is true for any kind of cells used for absorption. They are especially well absorbed by pig cells.

Rabbit agglutinins are only slightly absorbed by sheep and horse blood corpuscles, but more so by beef and pig blood corpuscles.

Horse agglutinins are little absorbed by sheep and beef blood corpuscles, but more by rabbit and pig blood corpuscles.

Pig agglutinins are almost completely absorbed by rabbit and beef blood corpuscles, but only partially by sheep blood corpuscles.

These findings suggest, that, among other things, rabbit blood corpuscles have a receptor for both rabbit and pig agglutinins, which absorbs both, while the pig blood corpuscles lack the receptor for rabbit agglutinins. That is one of

TABLE IV

Absorption experiments

Agglutinins for blood corpuscles of	Percentage of original agglutinin titer retained after absorption with blood corpuscles of																								
	Sheep			Rabbit			Beef			Horse			Pig			Goat			Guinea pig			Dog		
	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained	Cases with >10 per cent retained	Cases with <10 per cent retained	Average percentage retained
(a) CONTROL SERA																								
Sheep				6	1	41	6	1	30	6	1	46	4	3	21									
Rabbit	2	0	75				8	0	48	8	0	75	8	0	45									
Horse	2	0	75	7	1	45	8	0	63				8	0	47									
Pig	2	0	38	0	8	0.4	1	7	3	8	0	47												
(b) SERA OBTAINED AFTER INJECTIONS OF SERUM																								
Sheep				14	7	21	15	6	20	14	2	31	6	10	13	0	2	0	1	0	33	1	0	67
Rabbit	6	0	79				19	2	40	10	0	73	8	2	31	2	0	50	1	0	25	1	0	50
Horse	6	0	53	5	5	19	10	0	83				15	1	66	2	0	100	1	0	50	1	0	100
Pig	6	0	30	2	8	6	0	10	0	16	0	57				1	1	9	1	0	100	1	0	100
Beef	5	0	50	1	15	3				7	0	50	1	6	7	0	2	0	1	0	50	1	0	50
Goat	0	1	0	1	0	25	0	1	6	1	0	13	0	1	6									
Guinea pig				2	0	38	2	0	25	2	0	75	2	0	19							1	0	25
Dog				0	2	2	0	2	2	0	2	38	0	2	0				1	0	25			
(c) SERA FROM CASES OF INFECTIOUS MONONUCLEOSIS																								
Sheep				9	0	88	0	9	0	0	9	1	9	0	84	0	4	0.3	1	0	66	1	0	66
Rabbit	5	0	60				9	0	33	8	0	75	8	0	42	4	0	50	1	0	100	1	0	13
Horse	0	5	4	8	0	94	0	9	0.6				9	0	100	1	3	6	1	0	50	1	0	50
Pig	5	0	50	2	6	5	0	9	0	9	0	67				3	1	45	1	0	100	1	0	50
Beef	0	1	0	0	2	0				?1	0	?50	0	1	0	0	1	0						
Goat	0	1	0	2	0	100	0	2	0.6	0	1	1	2	0	44									
Guinea pig				0	?1	?0	1	0	50	1	0	50	0	1	0							0	?1	?0
Dog				0	?1	?0	0	?1	?0	1	0	50	0	1	0				1	0	100			

several examples of partial receptors (11) common to 2 species of blood corpuscles.

Absorption in sera from cases injected with serum (Table IVb)

Absorption was carried out for sheep agglutinins with beef and rabbit cells in 21 cases. Absorption of the 3 other agglutinins investigated in the control cases was performed with sheep, rabbit, horse, beef and pig blood corpuscles in a number of cases. Only a few experiments were carried out with goat, guinea pig and dog blood corpuscles. The results may be summarized in the same order as noted in the controls.

Sheep agglutinins are absorbed in about the same manner, but to a greater degree than in controls.

Rabbit agglutinins are absorbed by different corpuscles in the same manner as from control sera.

As in the controls, horse agglutinins are little absorbed by sheep and beef blood corpuscles, but there is a distinct difference from the controls in the absorption with pig and with rabbit cells. The former absorb less and the latter more of the horse agglutinins. In half of the cases more than 90 per cent of the horse agglutinins were absorbed by rabbit cells.

The absorption of the pig agglutinins by rabbit and beef blood corpuscles is the same as in controls.

The results obtained with the absorption of the beef agglutinins by different blood corpuscles are not so valuable because of the low titer of these agglutinins. The goat and sheep blood corpuscles absorb the agglutinins for each other (both blood corpuscles have the same receptors in contrast to rabbit and pig blood corpuscles). The goat blood corpuscles do not remove the horse agglutinins. Guinea pig and dog blood in a few experiments did not show any striking features. The same was true for chicken blood which is not recorded here.

Absorption in sera from cases of infectious mononucleosis (Table IVc)

Absorption was done with sheep blood corpuscles in 5 cases, and with the other 4 kinds of blood corpuscles used in the controls in 9 cases. The result is less complicated than in normals and in cases injected with serum.

In general, the agglutinins for each of the cells that are increased in titer are absorbed by any of the cells in the same group. For example, beef blood corpuscles absorb the sheep agglutinins completely, but they also absorb the horse and goat blood agglutinins almost completely. The beef hemolysins were not examined. Blood corpuscles of all these species seem to possess one common receptor for the increased agglutinins and hemolysins. In this respect, the antibodies in infectious mononucleosis differ from those in controls and in cases after injection of serum. The absorptions of sheep and goat agglutinins by their blood corpuscles are similar in infectious mononucleosis and after injection of serum, and are probably the same as in controls.

The blood corpuscles of rabbit, pig, guinea pig, dog and chicken (not recorded in the table), for which the antibodies are not increased, react quite differently. They do not absorb any appreciable amount of the increased agglutinins for the sheep, horse and goat cells. The amount of agglutinin absorbed in the "increased group" is often less than in normal sera and in sera from cases injected with serum. The sheep agglutinins, which in normal sera and in sera after injection of serum are absorbed to a great extent by the different kinds of blood corpuscles, are very little absorbed by rabbit and pig blood corpuscles in infectious mononucleosis. This difference was previously noted by Stuart et al. (2) for rabbit blood. This fact may be explained by the higher resistance of the increased antibodies to absorption by nonspecific antigens.

The absorption of the agglutinins which are not increased, such as rabbit and pig agglutinins, is similar to that found in the controls and after injection of serum.

Application of results for differential diagnosis

In applying the absorption tests for differential diagnosis, the specific and mutual absorption of the increased antibodies by their antigens makes it possible to differentiate sera of cases of infectious mononucleosis from those of both normal cases and those injected with serum. Each of these antigens may be used. In making a

choice between the antibodies to be used for this purpose, it is necessary to select an antibody which is not absorbed to a great extent in normals and in cases injected with serum. The sheep agglutinins, for example, are not useful because they are largely absorbed by all blood corpuscles, and especially by beef blood corpuscles, in normals and cases injected with serum (cf. Table IV*a* and *b* and Stuart (12)). Among 21 cases injected with serum, the sheep agglutinins were completely removed in 3 and over 90 per cent removed in 3 others. Absorption with boiled beef blood corpuscles in 3 of our cases showed about the same amount of absorption as with raw blood corpuscles. Hence, Bailey and Raffel's method (3) of determining the sheep agglutinins after absorption with beef cells is not useful for the differential diagnosis.

If the sheep cells are to be used for differential diagnosis, the antigen selected for absorption must be of the group in which antibodies are not increased in infectious mononucleosis, such as rabbit, guinea pig, pig and dog corpuscles. When this is done, the titer of sheep agglutinins from cases of infectious mononucleosis will be found almost unaltered, whereas in normals or after the injection of serum it will be reduced after absorption. Stuart et al. (2) used rabbit blood corpuscles as absorbing antigen. Davidsohn and Walker (4) used rabbit kidney and guinea pig kidney, but he had a high degree of nonspecific absorption which obscures the results. The results of the absorption with rabbit blood corpuscles in infectious mononucleosis agree with those of Stuart et al. (2), but there are a few cases injected with serum in which the sheep agglutinins are not absorbed as well by rabbit blood corpuscles. In Table V*a* are shown examples of cases in which absorption tests gave doubtful results.

Since, as shown in Table IV*c*, the horse agglutinins are almost completely absorbed by beef blood corpuscles in infectious mononucleosis and are particularly high in infectious mononucleosis and after injection of serum, beef blood corpuscles were selected for absorption and the horse agglutinin titer was determined. Though applied in only 10 cases, this method appears to be somewhat more practical than the others. Cases illustrating the use of this test in differential diagnosis are recorded in Table V*b*. The beef-horse method has the additional advantage that only one absorption seems to be necessary.

TABLE V

Results of absorption experiments in certain cases illustrating their use in differential diagnosis

(a)				(b)			
Case number*	*Sheep* agglutinins present after absorption with blood corpuscles of *rabbit*			Case number	*Horse* agglutinins present after absorption with blood corpuscles of *beef*		
	After 1st absorption	After 2nd absorption	After 3rd absorption		After 1st absorption	After 2nd absorption	After 3rd absorption
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
SERA FROM INFECTIOUS MONONUCLEOSIS							
1	75 or more	75 or more	75 or more		In no case was more than 3 per cent of the agglutinin present after the first absorption		
2	100	100	50				
3	100	75					
	Cases 4 to 9 no absorption						
SERA FROM SUBJECTS INJECTED WITH SERUM							
5	50	50		13	100	50	50
7	66	33		15	100	50	50
8	66	66		18	25	25	
15	75	50	50	20	50	50	
	In the remaining sera there was less agglutinin present after absorption than in these sera				In the remaining sera there was more agglutinin present than in in any of these sera		
CONTROL SERA							
21		100			More than 50 per cent of agglutinin present in every instance		
18		50					
12		50					
	In the remaining sera there was less agglutinin present after absorption than in these sera						

* The case numbers in the three groups refer to case numbers in Tables III, II, and I respectively.

Interference of heterophile antibodies in patients with infectious mononucleosis injected with serum

Stuart et al. (2) report only one case of infectious mononucleosis where they found an increase of the rabbit agglutinins from 1 : 160 to 1 : 1280. This case had been injected previously with 0.1 cc. of horse serum. He points out, that "it is questionable, whether this amount of serum could have initiated the rise."

We had an opportunity to observe a case of the same kind.

Case 1. An 18 year old girl showed a typical picture of infectious mononucleosis; slight fever, ulcerative tonsillitis, general enlargement of the lymph nodes and the spleen just palpable. The white blood count after admission to the hospital was 10,200, with 18 per cent polymorphonuclear leukocytes, 14 per cent large and 61 per cent small lymphocytes, 1 per cent eosinophiles, 1 per cent basophiles and 5 per cent monocytes.

The titration of the serum with the different kinds of blood corpuscles showed:

	Blood corpuscles				
Agglutinins for...	Sheep	Beef	Horse	Pig	Rabbit
Titer.....	640	<10	2560	20	40

The titration value did not change during the normal course of the illness, blood samples being taken on the 4th and 9th day after admission. The patient was discharged the 13th day after admission. On this day 0.1 cc. of diphtheria antitoxin was injected intradermally to investigate a possible sensitivity to horse serum caused by the infectious mononucleosis antibody. There was only a slight cutaneous reaction to the serum. Seven days after the injection, the patient had an unusually severe local serum exanthem at the site of the injection followed by a severe serum sickness with high fever, so that she was again admitted to the hospital. The titration of the serum at this time showed:

	Blood corpuscles				
Agglutinins for...	Sheep	Beef	Horse	Pig	Rabbit
Titer.....	640	<10	2560	20	160
8 days later or 15 days after the injection:					
	320	<10	2560	40	640

The only difference in the titers was the marked increase of the rabbit agglutinins. All other agglutinins, including those for pig cells which are usually high after the injection of serum, remained practically unchanged. The absorption tests showed but a slight change in the sheep agglutinins after the absorption with rabbit blood corpuscles; in this respect the sheep agglutinins retained the characteristic observed in infectious mononucleosis. Similarly, beef blood completely absorbed the sheep agglutinins, as was found before the injection of horse serum. Distinct changes occurred in the absorbability of horse agglutinins after the injection of serum. As seen in Table VI they became less absorbable by sheep blood corpuscles and non-absorbable by beef blood corpuscles.

TABLE VI

Absorption tests in a case of infectious mononucleosis injected with horse serum

	Horse agglutinins present after absorption with blood corpuscles of	
	Sheep	Beef
	per cent	*per cent*
a. 4 days before injection of serum.....	6.2 *	1.6†
b. 7 days after the injection....		12.5†
c. 15 days after the injection....	50†	100†

* After second absorption.
† After third absorption.

Case 2. This patient received an injection of horse serum (antitoxic scarlet fever serum) because of a severe scarlatiniform rash. The rash turned out to be a symptom of infectious mononucleosis. The serum of this patient examined 11 days after the injection did not show any change in the titer or absorbability of the different agglutinins. Possibly, in the first period of the illness the antibody forming system does not allow the formation of a second antibody.

Case 3. A 24 year old nurse suffered from a sore throat which looked like a streptococcic sore throat, but diphtheria bacilli were cultured from the throat. She was, therefore, injected with 40,000 units of diphtheria-antitoxin. Twelve days later she developed a serum sickness, consisting of urticaria and enlarged lymph nodes, especially in the neck. About 20 days after the injection the adenopathy persisted and, in addition, 37 per cent mononuclear cells were found in her blood smear. Twenty-eight days after the injection of serum the white blood count was 9,500, with 56 per cent lymphocytes, partially large, and 4 per cent monocytes. Later, the patient contracted a subacute nephritis. Titrations of her serum are shown in Table VII.

TABLE VII

Titrations of serum in Case 3

Days after serum injection	Titer of agglutinins for blood corpuscles of				
	Sheep	Beef	Horse	Pig	Rabbit
26	40		160	160	80
44	20		320	80	40
52	80	5	80	40	40

Since all the titers were low and the horse or sheep agglutinins not distinctly higher, the result was not particularly useful in differential diagnosis. The absorption tests were also inconclusive. Absorption of the sheep agglutinins by rabbit blood corpuscles was doubtful and only 50 per cent of the horse agglutinins were removed

by the beef blood corpuscles. The stability of the horse agglutinins is not evidence against the diagnosis of infectious mononucleosis when serum has been previously administered (cf. Case 1).

It appears, therefore, that when the two reactions interfere with one another the results of these tests may not be conclusive for differential diagnosis. Further observations may point to some other difference which will be useful in such cases.

DISCUSSION

The results of this investigation indicate that the antigen in infectious mononucleosis which reacts with the increased antibodies, is present in goat blood corpuscles as well as in horse, beef and sheep blood corpuscles. The interrelationship of goat, sheep and beef blood corpuscles was shown by Ehrlich and Morgenroth (11). The antibodies in infectious mononucleosis are probably not the same as those of Weil (13), as suggested by Stuart et al. (5), since they react with horse blood corpuscles, as shown in this study, are absorbed by horse tissue and since the antibodies described by Weil are not absorbable by horse kidney. Our absorption experiments with the different kinds of blood corpuscles show that each species of blood corpuscles containing this antigen removed almost completely the agglutinins for each of the other blood corpuscles in this group. The conclusion seems to be justified that we are dealing with one specific antibody and also with one antigen which is contained in all these blood corpuscles. While this antibody has certain characteristics in common with the Forssman antibody, it has other properties which are quite different (3, 14). The source of this antibody remains unknown. It is probably related to the etiologic agent in infectious mononucleosis.

The antibodies after the injection of serum are more difficult to explain. Our findings suggest that they represent an increase in the normal agglutinins. Therefore, the Forssman character of the sheep antibodies after the injection of serum is not surprising, for the normal sheep antibodies are of Forssman character (14, 15). The antibodies consist of agglutinins and hemolysins for a large variety of blood corpuscles, many of which can be separated by absorption. They do not react with any common antigen, as is the case in infectious mononucleosis. There is nothing to suggest that some are "major" and others "minor" agglutinins, as in the case of certain bacterial species. The antigen which is agglutinated to the highest titer, namely, horse blood corpuscles, absorbs less of the rest of the agglutinins than any other antigen. Unless an antigen is found which absorbs or neutralizes all the increased antibodies, the increase after the injection of serum must be considered as nonspecific.

This assumption does not exclude the fact that some of the antigens with which the antibodies after the injection of serum react, contain a receptor for one or more different antibodies. Thus, rabbit and beef blood corpuscles absorb and, therefore, contain a receptor for pig agglutinins; rabbit blood corpuscles may contain a receptor for horse agglutinins and the pig blood corpuscles contain one for sheep agglutinins.

A more specific nature of the antibodies after the injection of serum was recently suggested by Stuart et al. (5). Because of the absorption of sheep lysins by raw and boiled beef and by rabbit blood corpuscles and because of the inhibition of the beef lysins by horse serum, Stuart concluded that there is a thermostable antigen common to these three which may cause the increase of beef and of some other agglutinins. This is, of course, different from the antigen in infectious mononucleosis for it is contained in rabbit blood corpuscles. We suppose that Stuart assumed that the reaction after the injection of serum is due to the action of several antigens of this kind (as for instance the Forssman antigen) which are supposedly contained in horse serum. The above mentioned antigen cannot be responsible alone for the numerous antibodies which are not absorbed by this one antigen. In contrast to this theory, we consider the reaction after the injection of serum as nonspecific, with certain restrictions mentioned above, because there is no mutual absorption of the antibodies and because of the resemblance to the normal antibodies.

SUMMARY

1. In infectious mononucleosis, the antigen with which the so-called heterophile antibodies react, is

found in horse and goat blood corpuscles as well as in sheep and beef blood corpuscles. The agglutinins for 3 of these species of blood corpuscles, namely, those of sheep, horse and goat are markedly increased. In the case of beef blood corpuscles the hemolysins are increased, but the agglutinins are not. Each of these kinds of blood corpuscles can absorb almost completely the agglutinins for the blood cells of each of these species. On the other hand, these antibodies are almost non-absorbable by any antigen for which the corresponding agglutinins are not increased.

2. After injection of serum, the increased agglutinins for many kinds of mammalian blood corpuscles show about the same sequence in their titer as is found in sera of persons without a history of injections of serum.

3. Serum from cases with infectious mononucleosis can be differentiated from normal sera and from those obtained after the injection of serum in one of 2 ways: (*a*) by absorption of the sheep, horse and goat agglutinins with one of the following species of blood corpuscles: sheep, beef, horse and goat; (*b*) by the resistance of these increased agglutinins to absorption by any of the blood corpuscles the agglutinins for which are not increased in infectious mononucleosis, namely, rabbit, pig, dog and guinea pig.

The most reliable method besides the absorption of sheep agglutinins by rabbit blood corpuscles (Stuart) is the absorption of horse agglutinins by beef blood corpuscles. By the first method, one obtains almost no absorption of the agglutinins in infectious mononucleosis; by the second, almost complete absorption. In normal sera and in sera after the injection of serum the reverse is true.

4. The sera in two cases of infectious mononucleosis were studied after injection with horse serum. In one of these cases the interference of the antibodies characteristic of infectious mononucleosis and those usually found after the injection of serum is shown. A third case is described which illustrates that after the injection of serum the differential diagnosis of infectious mononucleosis cannot always be made serologically.

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