HETEROGENETIC ANTIBODIES IN ACUTE HEPATITIS*

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Preliminary observations (1) on serums from cases of acute hepatitis following yellow fever vaccination have indicated the existence of an "antigen" in acute-phase serums which precipitates with an "antibody" in serums obtained during convalescence.[1] This phenomenon resembles in its general aspects the serum antigen-antibody reaction which was observed by Hughes (2) in yellow fever. Since the latter author attributed the reaction to a product of liver damage rather than to a specific antigenic component of the causative virus, it seemed likely that a similar liver damage reaction might occur in acute infective hepatitis. In view of this possibility, complement fixation tests were done with various samples of liver tissue. The investigation showed that serums from certain cases of acute hepatitis fixed complement with saline extracts of liver from fatal cases of this disease, but that positive reactions of equal intensity and frequency were obtained when the same serums were tested with normal human liver tissue.

Another aspect of serological reactions in acute hepatitis was suggested by the observations that serums from these cases often showed slightly higher titers of agglutinins for sheep cells than did normal serums.[2] The possibility of a heterophile reaction analogous to that found in infectious mononucleosis (3) was considered. Heterogenetic antibodies have also been described in serum sickness (8), and the occurrence of urticaria and arthralgia in certain cases of acute hepatitis suggested the possible association of immunological

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[1] The first of these experiments was performed by Major James H. Gear and Dr. Max Theiler at the Laboratories of the International Health Division of The Rockefeller Foundation in New York.

[2] Colonel Raymond O. Dart (Medical Corps) and Lieutenant Colonel Gerson R. Biskind (Medical Corps), Letterman General Hospital, personal communication.

reactions like those of serum sickness. Recently the existence of heterogenetic antigen-antibody phenomena in primary atypical pneumonia has been suggested by the discovery in this disease of cold agglutinins (4, 5) and associated, but ill defined, heterogenetic antibodies reactive with certain animal tissues (6, 7).

Further studies indicated that the complement fixation with human liver and the agglutination of sheep cells were frequently associated in cases of acute hepatitis, and absorption experiments suggested that a single antigen-antibody system was concerned in these two serological reactions.

The relation of the soluble antigen found in the acute-phase serums from some cases of hepatitis, as previously described (1), to the heterogenetic reactions with liver and sheep cells is not clear at the present time. At first it was thought that this serum antigen was a product of liver damage which might have stimulated the production of antibodies to liver tissue and sheep cells as well as that of the precipitin which was found in the serums of some persons convalescent from hepatitis. Preliminary experiments to test for such a relationship were unsuccessful and indicated, on the contrary, that the serum antigen-antibody system was separate from the other reactions. The serum antibody appeared later in the course of convalescence from hepatitis than did the antibodies to human liver and sheep cells. This antibody could not be absorbed either with human liver or with sheep cells by the methods to be described. It was also found that some serum specimens reacted not only with serum antigen or antibody from cases of jaundice, but also with serums from normal persons.[3] Further investigation is being made of the nature of the immunological reactions between serums from cases of jaundice, but space does not permit the presentation of details at present.

This paper will be limited to a report of preliminary observations on a heterogenetic antibody which occurs in cases of acute infective hepatitis and is characterized by fixation of complement with human liver and agglutination of sheep erythrocytes. Differences from the Forssman antibody, from the Wassermann antibody, and from heterogenetic antibodies occurring in other diseases have been noted.

Materials and Methods

Serums.—Specimens of serum were collected from 154 cases of jaundice attributed to yellow fever vaccination in the United States Army and from 68 cases of so called "catarrhal" jaundice which occurred either in small family or institutional outbreaks among civilians, or as sporadic disease.[4] Blood was collected in some cases as soon

[3] The possibility of immunological reactions between human serums from different individuals has been indicated in various published reports (9–11).

[4] We are indebted to Miss M. Dorthy Beck of the Bureau of Epidemiology, California State Department of Public Health, for obtaining some of the specimens from civilian cases.

as possible after the diagnosis was established and again several weeks later, or during convalescence. A number of single specimens taken from 5 to 150 days after onset of jaundice were also received. As controls, serum specimens from cases of infectious mononucleosis, from cases of primary atypical pneumonia, and from normal persons without history of jaundice, were also available.

Samples of serum were heated at 56°C. for a half-hour immediately before use. A considerable number of the serums from cases of jaundice became anticomplementary after storage for periods of a week to several months. Later it was found that some of the anticomplementary serums could be made usable by heating to 60°C. for 15 minutes.

Antigen for Complement Fixation.—Preliminary experiments showed that jaundice serums gave no stronger fixation with suspension of liver from fatal cases of hepatitis than with suspensions of normal human liver. For this reason suspensions of liver tissue from a fatal case of coronary occlusion without history of jaundice were used in most of the experiments to be described. The specimen of tissue contained a few intestinal bacteria, but these were not present in large enough numbers to account for the observed reactions. Additional tests were done with two other samples of normal human liver with almost identical results.

The tissue was ground with alundum and saline to make a 10 per cent suspension, which was then centrifuged at approximately 1,500 R.P.M. for 10 minutes. The resulting suspension was moderately turbid. The suspensions of antigen were stored at −70°C. until used. The antigens were always anticomplementary at 10 per cent concentration and sometimes slightly so at 5 per cent. In most tests the equivalent of a concentration of 2 per cent by weight of wet tissue was used. Alcoholic extracts of human liver were also used in tests to be described in a subsequent section. The antibody which fixed complement with suspensions or extracts of human liver will be referred to in the remainder of this paper as liver antibody.

Method of Performing the Complement Fixation Test.—A hemolytic system consisting of equal parts of 5 per cent sheep red cells and diluted anti-sheep rabbit serum was used. The complement fixation test was set up as follows:—

0.2 cc. serum usually at a single dilution of 1:4.

0.2 cc. complement diluted to contain 2 units.

0.2 cc. antigen diluted as described above.

Controls:

1. Serum diluted 1:4 with complement and saline in place of antigen.
2. Antigen at dilution used in test with complement and saline in place of serum.
3. Normal serums were usually run in parallel with jaundice serums.

All tests were incubated at 37°C. in a water bath for 1 hour. The hemolytic system, consisting of 0.5 cc. of a mixture of equal parts of 5 per cent sheep red cells and amboceptor diluted so as to contain 2 units per 0.25 cc., was then added to each tube. After an additional 30 minutes' incubation the tests were read. Results were recorded as 0, +, ++, +++, or ++++, depending on the estimated degree of hemolysis of the sheep cells, 0 indicating complete hemolysis and ++++ no hemolysis.

Agglutination Tests.—These were performed with the erythrocytes of the sheep, rabbit, horse, and *rhesus* monkey, and with bovine red cells. The cells were washed three times with saline, packed, and resuspended in saline to a concentration of 2 per cent. Fresh suspensions were made on every day when the test was run, and cells

were usually less than 4 days old. To serum dilutions of 1:10, 1:20, 1:40, etc., in Wassermann tubes an equal volume of the erythrocyte suspension was added. The mixture of cells and serum was held at 20°C. for 10 minutes, and the tubes were then centrifuged at 1,700 R.P.M. for 10 minutes in the horizontal head of an International centrifuge. The tests were read by flipping the bottom of the tubes with the index finger to mix the sedimented cells and supernatant. Titers were recorded as the original dilution of serum before addition of red cell suspensions.

Human blood types of the cases of jaundice were determined by using the serums in macroscopic plate tests with known type A and B erythrocytes. Doubtful results were checked by microscopic examination.

Absorption Technique.—Absorption experiments were done on samples of serum from cases of acute hepatitis, infectious mononucleosis, and bronchopneumonia, or from normal persons to determine what materials removed the human liver antibody and the sheep cell agglutinins. For this purpose, suspensions of human liver, washed sheep or rabbit erythrocytes, boiled guinea pig kidney, and boiled beef erythrocytes were used.

Suspensions of human liver were prepared as for complement fixation and used in 10 per cent concentration. One part of serum and 5 parts of the liver suspension were mixed, incubated at 37°C. for 1 hour, and centrifuged at 2,000 R.P.M. for 10 minutes. To the supernatant an additional 2½ parts of liver suspension was added; the mixture was incubated again at 37°C. for 1 hour and then placed in the ice box overnight. The supernatant, after centrifuging at 3,000 R.P.M. for 30 minutes in the angle centrifuge and inactivation at 56°C., was used for complement fixation and agglutination tests. The final dilution of serum after absorption with liver was 1:7.5.

Absorptions with washed fresh sheep red cells and rabbit red cells were done by adding 2½ volumes of 10 per cent red cell and incubating for 1 hour at 37°C. followed by a second absorption with packed red cells overnight in the ice box.

The suspensions of guinea pig kidney and beef cells were prepared in the usual manner. 20 per cent suspensions were heated in a boiling water bath for 1 hour. One part of serum and 5 parts of these suspensions were mixed, allowed to stand overnight in the ice box, and centrifuged at 2,000 R.P.M. for 10 minutes.

Serums, after absorption with these materials, were inactivated at 56°C. for ½ hour, and the dilutions were adjusted to 1:5 for complement fixation and 1:10 for the initial dilution in agglutination tests.

Complement Fixation with Human Liver and Agglutination of Sheep Erythrocytes by Serums from Acute Hepatitis, from Other Diseases, and from Normal Persons

Single or paired serum specimens from cases of jaundice, atypical pneumonia, infectious mononucleosis, and upper respiratory infections, and from other persons before or after inoculation with chick embryo influenza vaccine were tested by complement fixation with the liver antigen and by agglutination of sheep erythrocytes. The purpose of these tests was to determine whether these two reactions are associated in cases of jaundice and whether a similar antibody is found in diseases other than jaundice.

In Table I the results with 4 groups of serums are presented.

In this and subsequent tables the term "postvaccinal hepatitis" is used to designate the disease apparently resulting from inoculation with yellow fever vaccine. "Infective hepatitis" designates those cases which occurred in civilians and were not connected with yellow fever vaccine. These terms are used without prejudice as to the identity of the two diseases or the possible diverse etiology of acute hepatitis. The first 2 groups in Table I included specimens from cases of hepatitis. The third group was composed of specimens from cases of primary atypical pneumonia or pneumonitis of which a large number were available. The last, or "normal," group also included

TABLE I

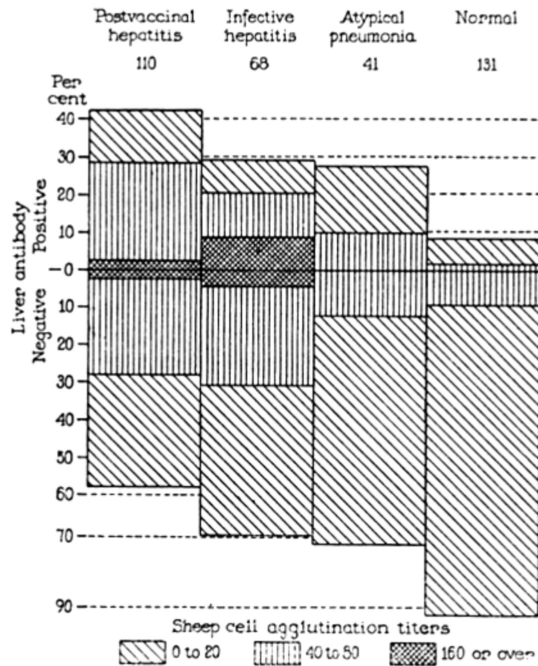
Complement Fixation Reactions with Human Liver and Sheep Cell Agglutination in Cases of Jaundice, Primary Atypical Pneumonia, and Normal Control Serums

Serum specimen from	Complement fixation with liver		Per cent with sheep cell titers of		
	Reaction	Number	0 to 20	40 to 80	160 or over
Postvaccinal hepatitis	Positive	47	32	62	6
	Negative	63	52	43	5
	Anticomplementary	44	36	48	16
	Total	154	42	50	8
Infective hepatitis	Positive	20	30	40	30
	Negative	48	57	37	6
	Total	68	49	38	13
Atypical pneumonia	Positive	11	64	36	0
	Negative	30	83	17	0
	Anticomplementary	15	80	20	0
	Total	56	79	21	0
Normal controls	Positive	11	82	18	0
	Negative	120	90	10	0
	Anticomplementary	11	82	18	0
	Total	142	89	11	0

persons with upper respiratory infections and those who had received influenza vaccine. The data were assembled without selection as to date of onset, but duplicate results for paired serum specimens have been eliminated. Where different results were obtained with 2 serums of a pair, the positive complement fixation reaction and the highest sheep cell agglutination titer were counted. Further details of the results with these paired specimens will be presented in the next section. In 8 cases of jaundice and 5 cases of atypical pneumonia the serums were slightly anticomplementary, while the reaction with liver antigen was +++ or ++++. These were counted as positive, with the reservation that the result was somewhat in doubt.

When the over-all results for sheep cell agglutination, given under totals for each group in Table I, are considered it is evident that titers over 20 occur in a higher proportion of cases of hepatitis (51 to 58 per cent) than in cases of atypi-

cal pneumonia (21 per cent), or in normal serums (11 per cent). From the data presented in the third column of the table it will be seen that positive complement fixation reactions varying from + to ++++ with human liver antigen were obtained in about one-third of the cases of hepatitis, one-fourth of the cases of atypical pneumonia, and one-eleventh of the normal controls.



TEXT-FIG. 1. Sheep cell agglutination titers in serums with positive and negative liver antibodies from 110 cases of postvaccinal hepatitis, 68 cases of infective hepatitis, 41 cases of atypical pneumonia, and 131 normal persons.

In those serums from cases of hepatitis with positive liver antibody, sheep cell titers over 20 were more frequent (68 to 70 per cent) than in serums from the same disease with negative liver complement fixation, in which the proportion with titers over 20 was 43 to 48 per cent.

The same data are presented graphically in Text-fig. 1.

The length of each column above the solid line in the middle of the graph represents the percentage of positive complement fixation reactions with human liver antigen, while that below the line indicates the negative reactions. Each column is divided by cross-hatching according to the percentage of the total group (liver positive plus liver negative) showing the sheep cell titers indicated. Comparison of the 4 columns

shows that liver antibody associated with elevated sheep cell agglutination titers was found much more frequently in cases of jaundice than in atypical pneumonia or in normal individuals. In the serums negative for liver antibody elevated sheep cell titers also occurred with somewhat greater frequency in the hepatitis group than in the control group. It is likely that more of the jaundice serums would have given positive complement fixation with liver antigen if they had been tested at lower dilutions, and this might have brought about a closer correspondence between the liver antibodies and sheep cell agglutinins.

Serums from 15 cases of infectious mononucleosis with sheep cell agglutination titers ranging from 40 to 1,280 were also tested by complement fixation with the human liver antigen. One of these gave a positive reaction. In addition, 6 other serums with low agglutination titers from cases with a doubtful or tentative clinical diagnosis of infectious mononucleosis were also tested. Of these, 3 gave weakly positive complement fixation reactions with human liver. A number of the serums which gave positive reactions with liver were found to be Wassermann negative.

Time Relation of Serological Reactions to Onset of Icterus

Paired serum specimens collected from 46 cases of infective hepatitis were available. The first serum of each pair had been collected 1 to 27 days after onset of icterus and the second specimen 20 to 150 days after onset. It was not possible to do complete serological studies on all of the paired specimens because of an insufficient quantity of serum and, in several cases, one or both serums in the pair became anticomplementary after several tests had been run.

The results with paired specimens from 22 cases are presented in Table II. The complement fixation reaction with human liver was not consistent, 7 cases showing an increase in these reactions between the first and second specimens, while 7 others showed a decrease. It must be noted, also, that the intensities of the reactions with liver and sheep erythrocytes sometimes rose and fell in parallel (serums 6 and 7), but sometimes showed opposite tendencies (serums 17 and 21). With 11 other pairs of serums no positive reactions for liver antibody and no significant changes in the sheep cell agglutination titers were observed. On the remaining specimens the data were incomplete.

Control tests were run on paired serum specimens from 9 cases of bronchopneumonia, 9 cases of infectious mononucleosis, and from 20 persons before and after inoculation with influenza vaccine. In 1 case diagnosed clinically as infectious mononucleosis, but having no significant sheep cell agglutination titer, a weak reaction with liver antigen occurred in the second specimen. In 1 case of bronchopneumonia a one-plus complement fixation with liver antigen was found in the serum specimens collected on the 4th day, but was absent in specimens collected on the 8th and 20th days. In 1 person receiving influenza vaccine a weak reaction for liver antibody appeared in the postvaccination

serum. Thus, in a total of 3 out of 38 persons not having hepatitis, weak reactions with human liver seemed to appear or disappear during the interval of time between the collection of the 2 serum specimens.

TABLE II

Results of Serological Tests on Paired Serum Specimens from Cases of Hepatitis Following Yellow Fever Vaccination and from Cases of Hepatitis Not Associated with Vaccination

Serum No.	Disease	Time after onset		Liver antibody		Sheep cell agglutination titer	
		First specimen	Second specimen	First specimen	Second specimen	First specimen	Second specimen
		days	*days*				
1	Postvaccinal	9	43	±	0	5	20
2	hepatitis	?	?	0	+	5	5
3		9	45	+	++	20	40
4		6	42		++	10	20
5		3	34	+	++	40	10
6		14	36	0	+++	10	40
7		5	30	0	0	20	80
8		1	31	+		5	20
9		12	32	0	+	80	160
10		10	24	++	0	40	20
11		9	21	0	++	160	160
12	Infective	7	120	+	0	10	10
13	hepatitis	7	120	++		10	10
14		4	120	0	0	40	10
15		9	62	0	0	160	40
16		12	66	++	0	40	160
17		21	75	++	0	160	40
18		13	65	+++	0	40	40
19		16	68	0	0	160	40
20		14	55	0	++	20	5
21		3	22	0	0*	10	40
22		16	70	++	0	80	40

* Specimen taken on 14th day after onset + liver antibody.

The relation of the serological reactions to the time of onset of icterus is shown in Table III. These results were obtained with all the specimens, both single and paired, from cases in which the date of onset was known with reasonable accuracy. The data for complement-fixing antibodies to human liver indicate that this reaction was present in a considerable proportion of cases in the earliest phases of the disease, and a large proportion of the cases also showed sheep cell agglutination titers of 1:40 or over during the first 10 days. Comparison with Table I shows that 11 out of 132 normal persons,

or 8.3 per cent, had liver antibodies, while in cases of hepatitis during the first 10 days after onset of icterus positive reactions were obtained in 17 out of 49, or about 35 per cent. Similar comparisons for the sheep cell agglutinins give 11 per cent of normal persons with titers of 40 or over and 34 per cent with similar titers among those in the acute phase of the disease.

TABLE III

Relation of Serological Reactions to Time of Appearance of Icterus

Serum tested for	Kind of hepatitis	Days after onset							
		1 to 10		11 to 30		31 to 60		61 to 150	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Liver antibody	Postvaccinal	13	17	18	19	9	13	6	14
	Infective	4	15	13	9	4	20	4	12
	Totals	17	32	31	28	13	33	10	26
Sheep cell agglutination*	Postvaccinal	16	30	29	18	22	21	12	8
	Infective	9	9	13	8	15	9	4	13
	Totals	25	39	42	26	37	30	16	21

* In this table, serums with sheep cell titers of 20 or less are called "negative;" those with titers of 40 or more are called "positive." This division is based on tests with normal serums described in Table I.

Agglutination with Erythrocytes of Other Species

Agglutination tests with the erythrocytes of rabbits, *rhesus* monkeys, horses, and cattle were done with serums from cases of acute hepatitis and from normal persons, using the same technique as for the sheep cell agglutination. The results are presented in Table IV. In the agglutination of rabbit erythrocytes the titers of serums from 45 cases of jaundice tended to be higher than the titers of normal serums. There was no significant difference between hepatitis and normal serums in the agglutination titers with the erythrocytes of the other 3 species tested.

Absence of Relation of the Heterogenetic Antibody in Hepatitis to the Principal Human Blood Groups

Determination of blood group was done on 193 cases of jaundice. The results, arranged according to complement fixation reaction with human liver and agglutination of sheep erythrocytes, are presented in Table V. It is doubtful that much significance can be assigned to the slight variations from normal such as the lower proportion of group B individuals among those having positive liver and sheep cell reactions and the increased over-all percentage in group

AB. These results do not include tests on 9 cases of jaundice in American Indians, all of whom were group 0.

TABLE IV

Agglutination of Erythrocytes of Various Species with Jaundice and Normal Serums

Erythrocytes used in tests	Serum from	No. tested	Per cent with titers of			
			100 or less	200	400	800 or over
Rabbit	Hepatitis	45*	9	11	44	36
	Normal	30	30	43	27	0
			Per cent with titers of			
			10 or less	20	40	80 or over
Rhesus	Hepatitis	18	72	22	6	0
	Normal	20	55	25	20	0
Horse	Hepatitis	19	26	37	26	11
	Normal	16	12	38	25	25
Cattle	Hepatitis	17	47	47	6	0
	Normal	17	41	53	6	0

* By complement fixation with human liver—11 positive, 12 negative, 22 anticomplementary.

TABLE V

Human Blood Groups of Cases of Hepatitis

	No. tested	Per cent of blood group			
		0	A	B	AB
Liver positive..................	56	48	41	2	9
Liver negative.................	93	38	41	15	6
Sheep cell titer over 20..........	105	47	42	6	5
Sheep cell titer less than 20......	88	40	35	14	11
Normal distribution*............	—	45	41	10	4

* In the white population of the United States as reported by W. C. Boyd, Fundamentals of immunology, New York, Interscience Publishers, Inc., 1943, 151.

Absorption Experiments

The two principal purposes of the absorption experiments were to demonstrate the relation between the liver antibody and sheep cell agglutinin in cases of hepatitis and to differentiate these reacting substances from other heterogenetic antigen-antibody systems. The absorptions were done with unheated human liver, boiled guinea pig kidney, boiled beef erythrocytes, and unheated

rabbit and sheep erythrocytes, as described in the section on Materials and methods.

The results of absorption experiments with serums from 20 cases of infective hepatitis, which were selected because of elevated sheep cell agglutinins, and

TABLE VI

Absorption of Agglutinins for Sheep Erythrocytes in Serums from Cases of Hepatitis

Serum No.	Disease	Liver antibody titer	Sheep cell agglutination titer after absorption with					
			Nil	Human liver	Guinea pig kidney	Rabbit erythrocytes	Boiled beef erythrocytes	Sheep cells
115	Postvaccinal hepatitis	++++ 1:4	40	0	0	10	0	0
120		0 1:4	80	10	0	0		
121		0 1:4	40	0	0	10		
124		0 1:4	160	0	0	0		
126		0 1:4	80	0	0	0		
139		++ 1:4	40	20	0	0		
153			80	10		10		
103		+++ 1:4	160	40				0
50			40	0				
16	Infective hepatitis	++ 1:4	40	20	0	20	10	0
17		++ 1:4	40	10	0	0		0
192			40	10	0	0		0
193		+++ 1:4	160	0	0		10	0
227		+ 1:4	80	40				10
228		++ 1:4	80	20	0		10	0
256		+ 1:4	10	10	0	0		
257		0 1:4	160	40	0	10		
258		+ 1:4	160	10	0	10		
268		+ 1:4	40	10	0	0		
259		+++ 1:4	20	0	0	0		0
287		++ 1:4	40	10	0	0		0
260	Hepatic cirrhosis	0 1:4	40	20				
268		++++ 1:4	40	10				

0, titer less than 10 except in cases noted.

2 cases of chronic hepatic cirrhosis with jaundice are presented in Table VI. In 18 cases of jaundice and 1 case of chronic hepatic cirrhosis, the titer of sheep cell agglutinins was significantly reduced (over twofold) by absorption with human liver. The agglutinins were also removed with equal or greater completeness by absorption with guinea pig kidney and rabbit erythrocytes. Five serums (Nos. 16, 50, 192, 193, and 256) from cases of acute infective hepatitis were collected between the 9th and 12th days of icterus. In 2 of these the sheep

cell agglutinins were not absorbed by liver. Of the remaining 13 serums collected after the 12th day, the absorption was successful in all except 2 (Nos.

TABLE VII

Absorption of Agglutinins for Sheep Erythrocytes in Control Serums Not from Hepatitis

Serum No.	Disease	Liver antibody titer	Sheep cell agglutination titer after absorption with					
			Nil	Human liver	Guinea pig kidney	Rabbit erythrocytes	Boiled beef erythrocytes	Sheep cells
818	Serum sickness		160	160	0		10	
795	Mononucleosis		80	40	40		0	
796			80	80	80		0	
262			320	320	320	320	0	
272		0 1:4	320	160	160	320	0	
039		0 1:4	320	320	320		10	0
105		+ 1:4	320	320	320	320	10	10
058		0	80	80				
020			80	80				
742			80	80	80	80	0	
031			1280	1280		640		
500			1280	1280				
469			1280	1280	1280	1280	0	
637	Pneumonitis		40	40	0	0	0	
607		++++ 1:4	40	40	0	0	0	
608		0 1:4	40	40	0	40	0	
609		+++ 1:4	+++ 1:5	+ 1:5	0 1:5	0 1:5		
432	Normal		80	0	40			
922		0 1:4	40	40	10	0		
993		0 1:4	20	20	0			
042		+++ 1:4	++ 1:5	+ 1:5	0 1:5	+ 1:5		
686			40	40	0	0	0	
697		0 1:4	40	40	0	0	10	
216	Contact to jaundice	0 1:4	80	40	0			0
242		0 1:4	40	40	0	10		
243		+ 1:4	160	160	0	20		
247		± 1:4	80	80	0	0		

139 and 227). It is also evident that the absorption of sheep cell agglutinins by liver occurred irrespective of whether the complement fixation reaction for liver antibody was positive or negative.

Table VII sets forth the results of similar experiments with serums from 1

case of serum sickness, 12 cases of infectious mononucleosis, 4 cases of pneumonitis, and 10 normal persons, 4 of whom were contacts to cases of infective hepatitis in one of the outbreaks among civilians. In these 27 serums the agglutinins for sheep erythrocytes were significantly absorbed by human liver in only 1 case, normal serum 432.

TABLE VIII

Absorption in Serum from Infective Hepatitis of Agglutinins for Sheep Erythrocytes by Various Human Tissues

Serum No.	Sheep cell agglutination titer after absorption with							
	Nil	Liver heated to 100°C.	Jaundice liver 1	Jaundice liver 2	Spleen	Kidney	Lung	Brain
120	40	10	10	10	20	10	40	40
121	40	10	0	0	20	0	40	40
139	40	10	0	0	10	20	40	40

TABLE IX

Absorption of Liver Antibody with Various Materials

Serum No.	Disease	Complement fixation with human liver antigen after absorption with*				
		Nil	Sheep erythrocytes	Rabbit erythrocytes	Guinea pig kidney	Beef erythrocytes
191	Hepatitis	+	0	0	+	0
193	Hepatitis	+++	0		++	0
278	Hepatitis	+	0	0	+	
105	Mononucleosis	+	0		0	0
609	Pneumonitis	++	++	±	++	
042	Normal	++	0	0	0	
216	Normal	+++	+++			

* Serum dilutions 1:5 after absorption.

The mononucleosis antibody differed from that found in jaundice in not being absorbed by human liver, guinea pig kidney, or rabbit erythrocytes. In the serums from cases of pneumonitis and from normal individuals positive absorptions were obtained with guinea pig kidney and rabbit erythrocytes with one or two exceptions. Absorptions with these materials are, therefore, of no value in differentiating jaundice serums from normal ones.

Other human tissues were tested for their ability to absorb the sheep cell agglutinins from jaundice sera. The results in Table VIII indicate that liver heated to 100°C. for 60 minutes retains the ability to absorb the agglutinins. Specimens of liver other than those used in the previous absorption experiments

also had this property. The agglutinins were absorbed less constantly by spleen and kidney than by liver. Absorptions with lung and brain did not reduce the titers appreciably.

Reciprocal absorptions of the liver antibody using guinea pig kidney and the erythrocytes of sheep, rabbits, and cattle were also done. This study was hindered by shortages of serum and by technical difficulties, principally those due to anticomplementary effects. The results of a few of the more successful experiments are presented in Table IX. The liver antibody was apparently absorbed out with sheep cells, beef cells, and rabbit cells, but not by guinea pig kidney. This negative result with guinea pig kidney is difficult to explain in view of the fact that this material absorbed the agglutinins for sheep cells. The results with 4 control serums not from cases of hepatitis were variable, but none behaved in exactly the same way as the jaundice sera. It is noteworthy that in 2 of the control serums the complement-fixing antibodies for human liver were not absorbed by sheep cells.

Nature of the Reacting Antigen

Heat Stability.—When suspensions of liver were heated to temperatures of 56°C. or higher for 1 hour, a heavy coagulum formed. No fixation of complement occurred when the supernatant was mixed with serum containing liver antibody. However, the resuspended sediment from suspensions heated to 90°C. for 1 hour fixed complement with some jaundice serums and, as reported in the previous section, absorbed the agglutinins for sheep erythrocytes. These rather incomplete data indicate that the antigen in liver which is concerned in complement fixation and inhibition of agglutination is heat stable, but is carried down in the particles of coagulated protein when aqueous suspensions of liver are heated.

Extraction with Alcohol.—The human liver tissue was thoroughly broken up by grinding in a mortar, then dried at about 100°C. in an oven. The dried material was then pulverized and extracted for several days with a volume of absolute ethyl alcohol equal to 10 times the weight of wet liver. The alcoholic extract was evaporated to a small volume, about ½ to 1 cc., and a volume of physiological saline equal to that of the alcohol used in extraction was added rapidly. After thorough shaking the suspension was filtered through lens paper or cheese cloth to remove large clumps. The resulting milky suspension was used in complement fixation, flocculation, and agglutination-inhibition tests.

The results of complement fixation tests with about 30 serum specimens suggested that the complement-fixing antigen in the human liver was extractable with alcohol. Serums from cases of hepatitis which fixed complement with saline suspensions of whole liver also fixed complement with the alcoholic extracts of liver. Normal serums and jaundice serums negative for liver antibody did not react with these extracts.

Preliminary experiments also indicated that the alcoholic extracts were flocculated by serums from cases of jaundice, but these results were not readily reproducible. Three syphilitic serums strongly positive by the Kline, Kahn, and Kolmer tests gave no flocculation of an alcoholic extract of human liver which did flocculate with jaundice serums.

Inhibition of sheep erythrocyte agglutination was obtained by mixing the alcoholic extract and serum before addition of the cells. The antigen diluted 1:2 and 1:8 was added to appropriate serum dilutions and the mixture incubated for 1 hour. 2 per cent sheep cells were then added and the experiment completed in the same way as for the sample agglutination test. The results with serums from 4 cases of hepatitis and 2 cases of infectious mononucleosis are presented in Table X. Although the inhibition of agglutination by alcoholic extracts was not as definite as the absorption of the agglutinin by whole liver, the results suggest that at least part of the absorbing antigen was extracted by alcohol.

TABLE X

Inhibition of Sheep Cell Agglutination by Alcoholic Extracts of Human Liver

Serum No.	Disease	Agglutination titers		
		With saline	With antigen 1:8	With antigen 1:2
140	Hepatitis	160	80	40
151	Hepatitis	80	40	20
154	Hepatitis	80	10	10
159	Hepatitis	160	40	40
2500	Mononucleosis	640	640	640
262	Mononucleosis	160	160	160

Cephalin-Cholesterol Flocculation.—The possibility was considered that the reactions observed with the alcoholic extracts of liver might be related to the cephalin-cholesterol flocculation phenomenon which has been proposed as a test for liver damage (12, 13). Various jaundice and normal serums were tested with a cephalin-cholesterol preparation purchased from the Wilson Laboratories, Chicago, Illinois. Although flocculation was obtained at various dilutions from 1:10 to 1:1,000 with most of the serums tested including the normal controls, no parallelism between the cephalin-cholesterol reaction and the presence of serum antigen, serum antibody, liver antibody, or sheep cell agglutinins could be detected.

DISCUSSION

The reagin found in cases of hepatitis as described in this paper seems to fit the definition of a heterogenetic antibody because it gives immunological reactions with an antigen found in human liver and in the erythrocytes of a variety

of species. It cannot be claimed on the basis of the available data that the presence of this antibody is indicative of one particular form of hepatitis. It is possible that a similar antibody may be found in other diseases where damage to the liver parenchyma is a prominent feature. Although an antibody fixing complement with human liver was found occasionally in primary atypical pneumonia and in other diseases, elevated titers of sheep cell agglutinins did not accompany this reaction as commonly as in hepatitis and the agglutinins were not absorbed by human liver tissue. The occasional complement fixation reactions between normal human liver and normal serums may have been due to natural antibodies resembling those found in the rabbit by Kidd and Friedewald (24). Our results are in disagreement with those of Fox *et al.* (26) and Oliphant, Gilliam, and Larson (27), who reported no fixation of complement by liver tissue from cases of hepatitis with serum from this disease. This discrepancy may be due to differences in the complement fixation technique or the preparation of antigen.

The heterogenetic antibody in acute hepatitis can be distinguished from other hetero-antibodies in human serum primarily by its absorbability by human liver tissue. Forssman antigen is not present in rabbit or bovine erythrocytes, but these seem capable of absorbing the liver antibody and the agglutinins for sheep erythrocytes in the serums from hepatitis. The mononucleosis antibody differs definitely from that found in jaundice by its non-absorbability with human liver, guinea pig kidney, and rabbit erythrocytes, and by its insolubility in alcohol. The heterogenetic antigen common to human blood group A cells and sheep erythrocytes (23) apparently did not play a part in the reactions because the distribution of the principal human blood groups among serums showing significant reactions with liver and sheep cells was essentially normal. The fixation of complement with human liver could not be definitely attributed to the Wassermann reagin because this reaction occurred with serums that were shown to be Wassermann negative and because Wassermann positive serums failed to flocculate alcoholic extracts of human liver when tested by a method that gave positive flocculation with jaundice serums. The differentiation of the hepatitis antibody from the serum sickness antibody (8) is less clear. Only one serum from a known case of serum sickness was available, but the sheep cell agglutinins in this serum were not absorbed by human liver. Because of many resemblances in other respects a relationship between the heterogenetic antibodies found in jaundice and serum sickness cannot be excluded.

The antigen in human liver which fixes complement and inhibits the agglutination of sheep erythrocytes is apparently heat stable and alcohol soluble. This indicates that it is probably either a lipoid or a carbohydrate. Apparently a closely related or identical antigen is present in the erythrocytes of sheep, rabbits, and other species. It is not unlikely that this antigen will be found in the livers of species other than man, but tests with heterologous liver tissue

were not done in the present study because of the complications that might be expected from various unrelated antigen-antibody reactions.

In certain diseases, heterogenetic antibody is produced in response to an antigen which is actually a component of the infecting organism as, for example, the appearance of Forssman antibodies in pneumococcal pneumonia (14) and of agglutinins for *Proteus vulgaris* in typhus (15). In other diseases, heterogenetic antibodies presumably may be stimulated by some antigenic product of tissue damage. To this category probably belong the Wassermann reagin in syphilis, the yellow fever precipitin described by Hughes (2), and possibly also the heterogenetic antibody of infectious mononucleosis (3). It is not possible to determine with certainty in which class to place the heterogenetic antibody found in hepatitis, but some evidence favors the tissue damage hypothesis.

In 1843 Robert J. Graves[5] noted the association of urticaria and arthralgia with attacks of acute hepatitis. During the present studies these manifestations of hypersensitiveness have been seen in a small proportion of cases of acute infective hepatitis. They have also been found in about 10 to 20 per cent of cases of hepatitis following yellow fever vaccination (1) and in jaundice following the use of convalescent serum (25). These symptoms usually preceded by several days the appearance of icterus. The presence of these signs of hypersensitiveness and the production of a heterogenetic antibody to a constituent of liver suggest the possibility that *in vivo* antigen-antibody reactions may occur in some cases of acute hepatitis. It is conceivable that an antigenic complex liberated from the liver tissue in the acute phase of hepatitis, preceding the appearance of icterus, gives rise to an antibody which in turn reacts with more antigen *in vivo*. This consideration would by no means disprove the infectious nature of hepatitis because the observed phenomena may be only secondary reactions to parenchymal liver damage produced by a virus or other self-propagating agent.

An additional interesting speculation is the possibility that the liver antibody once produced combines with additional antigen *in situ* in the liver cells and causes further liver damage after the acute infectious process has subsided. This might be the explanation for some of the fatal cases in which the disease was greatly prolonged, with findings at autopsy resembling those of subacute yellow atrophy of the liver (16). Analogous examples of homologous and autogenous antibody production can be cited in the work of several investigators. Schwentker and Comploier (28) produced antibodies to kidney in rabbits by simultaneous injection of bacterial toxins and homologous kidney tissue. These authors also reported finding antibodies to rabbit kidney in 92 per cent of scarlet fever patients and 10 per cent of normal individuals, and in scarlet fever

[5] A system of clinical medicine, Dublin, Fannen and Co., 1843, 564.

the antibodies were presumed to be associated with kidney damage. Previously Schwentker and Rivers (19) had produced antibodies to brain in rabbits with suspensions of homologous brain autolyzed or infected with vaccine virus. Other examples of the production of homologous organ-specific antibodies may be found in work with the crystalline lens (17, 18) and with skin (21).

Other investigators have produced tissue damage with anti-organ heterologous serums or antigens. For example, Smadel (22) has described the production of kidney damage in rats with antiserum to rat kidney produced in rabbits. A review of the earlier literature will be found in Smadel's article. Rivers and Schwentker (20) produced pathological changes accompanied by myelin destruction in monkeys by repeated injections of aqueous emulsions and alcohol-ether extracts of normal rabbit brains. Although these experiments indicated the participation of a lipoid, Smadel (22) was unable to absorb the "nephrotoxin" from anti-kidney sera with alcohol-ether extracts of kidney.

The serological procedures described in this paper are not particularly satisfactory as a diagnostic test for hepatitis. Further investigations will be undertaken to devise a more delicate and specific method of detecting the heterogenetic antibodies of jaundice with red cell agglutination and inhibition of this reaction by purified alcohol-soluble antigen from liver.

SUMMARY

A heterogenetic antibody showing fixation of complement with human liver and agglutination of sheep erythrocytes was found in certain cases of acute infective hepatitis.

The antigen concerned in these reactions was apparently heat stable and alcohol soluble.

Differences from other heterogenetic antigen-antibody systems have been noted.

The possible relation of the heterogenetic antibody to liver damage was considered.

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