STUDIES IN HAEMOLYSIS WITH SPECIAL REFERENCE
TO THE PROPERTIES OF THE BLOOD AND BODY
FLUIDS OF HUMAN BEINGS.

By H. T. MARSHALL, M. D., FELLOW OF THE ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH.¹

(From the Royal Institute for Experimental Therapy, Frankfort-on-the-Main. Geh. R.
Prof. Dr. P. Ehrlich, Director.)

A. COMPARISON OF THE RECEPTORS OF THE RED BLOOD-CORPUSCLES OF MAN AND OF THE MONKEY (*Makakus rhesus*, AND *M. cynomolgus*).

B. BEHAVIOR OF HUMAN BLOOD-CORPUSCLES IN THE PRESENCE OF SERUM FROM VARIOUS ANIMALS, AND THE HAEMOLYTIC PROPERTIES OF NORMAL HUMAN SERUM.

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A. COMPARISON OF THE RECEPTORS OF THE RED BLOOD-CORPUSCLES OF MAN AND OF THE MONKEY (*Makakus rhesus*, AND *M. cynomolgus*.)

The comparative study of the body cells of man and of those animals that are zoologically nearly related is not only of great theoretical interest, but also depends upon important practical considerations. Attention has already been called to this point by Ehrlich,² who said in his address at Hamburg: "I think it probable that the isotoxins will play a great rôle in diagnosis and pathology. Metschnikoff found that, when he produced a chronic acid nephritis in dogs, an isonephrotoxin developed in their blood serum, as was

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² Ehrlich, die Schutzstoffe des Blutes. Vortrag auf der 73. Versammlung deutscher Naturforscher und Aertzte, Hamburg, 1901. *Deutsche med. Wochenschrift*, 1901, xxvii, 865, 888, 913.

shown by the fact that this serum produced a nephritis when injected into normal dogs. It is more than probable that the most diverse isotoxins may occur in human beings—as has already been demonstrated with certainty in the case of the blood by various authors, among whom are Landsteiner and Ascoli.³ “. . . . With the exception of experiments with the red blood-corpuscles, we can, of course, make no experiments upon the isotoxins of the body cells of man, but there are many indications that it will be possible to perform these experiments upon monkeys and thus to win a new basis for the pathology and therapy also of man.”

As the isotoxins are acquiring an ever increasing interest in the study of pathological processes, it seemed profitable to investigate the relations between the receptors of man and monkey, and as the red blood-corpuscles are the cells most readily obtained, they were chosen for the study.

In this investigation the same methods were applicable that were employed by Ehrlich and Morgenroth⁴ to demonstrate that there are certain receptors common to the red blood-corpuscles of goat, sheep and ox.

It was to be expected that investigation would reveal a partial correspondence between the receptors of the red blood-corpuscles of man and of the monkey, for it had already been demonstrated by means of the precipitin reaction that monkey serum and human serum contain certain specific substances in common. Thus Wassermann, Uhlenhuth, Stern, and Nuttall, all found that when rabbits were injected with human serum, their serum acquired the property of giving a precipitate when added to human serum. This precipitation was a specific reaction for human serum and occurred with the serum of no other animals except monkeys, and even in the last case the reaction was only slight. This fact shows that a part of the specific substance affected by this precipitin is common to the serum of man and monkey.

³ See, for example, the recent work of Ascoli and Figari upon nephrollysins, *Berlin. klin. Wochenschr.*, 1902, xxxix, 560, 634.

⁴ *Berliner klinische Wochenschrift*, 1900, xxxvii, 453; 1901, xxxviii, 569, 598.

Hitherto there have been no reports of investigations of other haptines of these species. In my experiments the blood and serum were obtained from two monkeys; one, *Makakus cynomolgus*, the other, *Makakus rhesus*. The human blood employed was infant's blood obtained from the maternal end of the umbilical cord. In all cases the amount employed in experiments was 1.0 cubic centimeter of a 5% suspension.

I. Action on the Blood-Corpuscles of Monkey and of Man, of Serum from an Animal Previously Treated with Injections of Human Blood.

The action of sera specific for human blood was tried upon monkey blood. It was found that the inactivated serum of a goat previously treated with human blood contains amboceptors for monkey blood and is reactivated by rabbit serum-complement better than by the complements of guinea-pig serum, human serum or monkey serum. The inactivated serum of a rabbit treated with human blood also contains amboceptors for monkey blood, and is reactivated by the complements of guinea-pig serum better than by rabbit serum, human serum or monkey serum.⁵

Interesting results were obtained from experiments to determine the smallest dissolving dose of amboceptor and of complement for human blood and monkey blood.

In the amboceptor tests, decreasing amounts of inactivated human-blood-rabbit serum were employed with a fixed amount of guinea-pig serum complement, and a fixed amount of monkey blood or human blood: 0.5 cubic centimeter guinea-pig serum was used as complement in the monkey blood experiment; 0.1 cubic centimeter guinea-pig serum as complement in the human blood experiment.

In each case 0.075 cubic centimeter of amboceptor serum was the smallest dose that effected complete solution of the blood-corpuscles.

⁵ For convenience the serum of a goat previously treated with human blood corpuscles will be called "human-blood-goat serum." In the same way, specific rabbit's serum will be called "human-blood-rabbit serum," and so in other cases; the name of the material injected will be placed first in the compound word, the name of the immunised animal second.

Complement determinations were then made, using 0.1 cubic centimeter amboceptor serum for each specimen of blood with decreasing amounts of guinea-pig serum complement. The result in this case was that 0.075 cubic centimeter of complement reactivated the human blood and amboceptor, while 0.3 cubic centimeter of complement was required for the reactivation of the amboceptor with the monkey blood.

A similar result was obtained when inactivated human-blood-goat serum with rabbit serum complement was used as haemolysin, the dose of amboceptor being the same for monkey blood and human blood, the dose of complement being twice as great in the case of monkey blood as in the case of human blood.

Abundant experimental proof was obtained showing that the serum of untreated goats and rabbits possessed no such amboceptor for monkey blood as was present in the human-blood-goat and human-blood-rabbit. It was also shown that the human-blood-goat serum and human-blood-rabbit serum had no unusual action upon the blood-corpuscles from animals of other species. *In other words, it was shown that these two sera were specific for human blood, and at the same time had a specially strong action upon the blood of the monkeys employed.*

II. Action on the Blood of Monkey and of Man of Serum from an Animal Previously Treated with Injections of Monkey Blood.

Two rabbits were immunized by treatment with blood-corpuscles of *Makakus rhesus*. As only small amounts of monkey blood were available, the degree of immunity reached was not high. With each rabbit 0.025 cubic centimeter of the inactivated serum exactly sufficed to dissolve 1.0 cubic centimeter of 5 per cent suspension of monkey blood (*M. cynomolgus*), when reactivated with guinea-pig serum. These two monkey-blood-rabbit sera acted upon human blood-corpuscles only slightly more markedly than did the serum from a normal unimmunized rabbit; 0.5 cubic centimeter of the inactivated

serum producing only a "strong"⁶ degree of solution of the human blood-corpuscles, when guinea-pig serum was used as complement.

Experiments showed that the haemolytic action of monkey-blood-rabbit serum upon monkey blood was destroyed by subjecting the serum to a temperature of 52° C. for one-half hour, while after one-half hour at 50° C. there was still evidence of haemolytic action.

The serum was inactivated at 52° C. and tested for amboceptor for human blood-corpuscles with a variety of sera as complement. It was found that ox serum reactivated best, that guinea-pig serum and human serum acted fairly well, rabbit serum acted very slightly, and goat serum, sheep serum and horse serum almost not at all. With ox serum as complement, 0.8 cubic centimeter of inactive monkey-blood-rabbit serum was sufficient to dissolve 1.0 cubic centimeter of human blood.

III. Relative Affinity of Human Blood and Monkey Blood for Specific Amboceptors.

An elective absorption experiment was made after the method described by Ehrlich and Morgenroth.⁷

Preliminary experiments were carried out by the method of union in order to determine the simple dissolving dose of amboceptor for human blood-corpuscles and monkey blood-corpuscles (*Makakus rhesus*). Decreasing amounts of inactivated human-blood-goat serum were employed as amboceptor; 0.3 cubic centimeter guinea-pig serum was used as complement in each case.

Amboceptor.	Human blood.	Monkey blood.
0.15 ccm.	complete	complete
0.1 "	"	almost complete
0.05 "	filmy	moderate
0.0 (complement alone)	slight	minimal

⁶ The degree of haemolysis that has been effected is judged of by the color of the fluid and the amount of sediment remaining. The following terms will be used to designate the degree of haemolysis. Complete, filmy, translucent, strong, moderate, slight, trace, minimal, 0.

⁷ *Berliner klin. Wochenschrift*, 1901, xxxviii, 569.

Four parallel series of tubes were then arranged with graduated amounts of inactivated human-blood-goat serum.

Two series, *A* and *B*, were treated with 1.0 cubic centimeter of 5 per cent suspension of human blood, the other two, *C* and *D*, with 1.0 cubic centimeter 5 per cent suspension of monkey blood.

After staying for one hour at 37.5° C. the corpuscles were removed by centrifugalization and the fluids tested for amboceptor for human blood and monkey blood.

Menstruums *A* and *C* were treated with the sediment of corpuscles obtained by centrifugalizing 1.0 cubic centimeter of 5 per cent suspension of human blood; menstruums *B* and *D* were treated with sediment from monkey blood. In each tube 0.3 cubic centimeter of guinea-pig serum was added as complement.

TABLE I.

Amount of human-blood-goat serum.	<i>A.</i> Solution of human blood by human-blood-goat serum, previously treated with human blood.	<i>B.</i> Solution of monkey blood by human-blood-goat serum, previously treated with human blood.	<i>C.</i> Solution of human blood by human-blood-goat serum, previously treated with monkey blood	<i>D.</i> Solution of monkey blood by human-blood-goat serum, previously treated with monkey blood
1.5 ccm.	almost complete	strong	complete	slight
1.0 "	filmy	moderate	"	"
0.5 "	strong	slight	"	trace
0.35 "	moderate	trace	almost complete	"
0.25 "	"	"	filmy	minimal
0.15 "	slight	minimal	moderate	"
0.1 "	"	"	"	"
0.05 "	"	"	slight	"
0. "	"	"	"	"
(only complement.)				

It is seen that human corpuscles absorb much more amboceptor than is needed to produce haemolysis, 15 times the simple fatal dose of amboceptor not being quite enough to dissolve two separate doses of human blood. It is also seen that the human red corpuscles pick up amboceptor otherwise available for monkey blood, since ten times the fatal dose of amboceptor serum after previous treatment with human blood, produced only "strong" solution of the monkey blood.

It is also evident that the monkey blood absorbs much more than the fatal dose of amboceptor, as ten times the simple fatal dose produced only "slight" solution of a second supply of monkey blood. The monkey blood absorbs more amboceptor available for monkey blood than is absorbed by human blood, for in the one case the solution of monkey blood is "slight," in the other "strong." This is merely an indication that this specimen of human blood has not receptors for all the amboceptors that have been set free in the serum of the goat as the result of a long continued course of immunization with different supplies of human blood. The monkey blood possesses receptors which the human blood specimens lack, showing affinities for certain amboceptors present in the immune serum. On the other hand, the monkey blood possesses to only a slight degree the power of removing from the immune serum amboceptor available for the human blood-corpuscles, since three and a half times the complete dose of amboceptor is almost sufficient to supply first the receptors of monkey blood, next those of human blood.

These experiments may now be summarized.

1. Serum specifically haemolytic for the human blood acts with remarkable power upon the blood-corpuscles of *Makakus rhesus* and *Makakus cynomolgus*.
2. In each case the amboceptor doses for the monkey blood and the human blood was the same, or very nearly the same.
3. Rabbit serum specifically haemolytic for monkey blood acts only feebly upon human blood. There is a great difference in its action, according to the complementing serum employed.
4. Human blood-corpuscles readily pick up from the serum specific for human blood-corpuscles amboceptors available for monkey blood, but the human corpuscles do not pick up these amboceptors as completely as the monkey blood-corpuscles themselves do.
5. Monkey blood-corpuscles pick up from the serum specific for human blood some of the specific amboceptors. This power is, however, only moderate.

IV. Action of Normal Sera upon Blood-Corpuscles of Man and Monkey.

The action of various active sera upon monkey's corpuscles (*M. rhesus*) was compared with the action of the same sera upon human corpuscles.

I. SERUM FROM GOAT.

Serum.	Monkey.	Human
1.0 ccm.	complete	complete
0.5 "	"	"
0.25 "	"	"
0.15 "	translucent	filmy
0.1 "	trace	strong
0.0 "	0	0

II. SERUM FROM SHEEP.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	translucent	slight
0.25 "	moderate	trace
0.15 "	slight	minimal
0.1 "	trace	0
0.0 "	0	0

III. SERUM FROM OX.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	minimal	slight
0.25 "	0	minimal
0.15 "	0	"
0.1 "	0	0
0.0 "	0	0

IV. SERUM FROM GOOSE.

Serum.	Monkey.	Human.
1.0 ccm.	strong	translucent
0.5 "	slight	moderate
0.25 "	0	slight
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

V. SERUM FROM RABBIT.

Serum.	Monkey	Human
1.0 ccm.	0	moderate
0.5 "	0	slight
0.25 "	0	trace
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

Neither monkey's corpuscles nor human corpuscles were dissolved by serum from man, horse, or guinea-pig.

Summary.

1. Goat, sheep, ox, goose and rabbit serum affect monkey blood to almost the same extent that they do human blood.
2. Human, rabbit and guinea-pig serum fail to produce haemolysis of monkey blood or human blood.
3. Monkey blood and human blood behave very similarly in the presence of normal active haemolysins.

These experiments indicate that there is quite a close relationship between the corpuscles of the human being and those of the two varieties of monkey examined. The conclusion is justified that there is a similarity of the receptor groups of the blood-corpuscles of the human being, *Makakus rhesus* and *Makakus cynomolgus*.

While there is this similarity, however, there are receptor groups in the corpuscles of the monkey which are not present in human corpuscles, and likewise the human corpuscles contain receptor groups not shared with the monkey corpuscles.

The following figure (taken from Ehrlich and Morgenroth) illustrates the relation between the receptors of human blood and monkey blood, as it is shown by the elective absorption experiment.

β represents the receptors common to the corpuscles of monkey and man.

α and γ represent the receptors peculiar to man and monkey respectively.

The partial correspondence between the receptors of the blood-corpuscles of man and monkey makes it probable that a similar rela-

tion exists between the other body cells of these animals, and, as Ehrlich has suggested, we may now be able to study the isotoxins of man by means of animal experimentation upon monkeys.

B. BEHAVIOR OF HUMAN BLOOD-CORPUSCLES IN THE PRESENCE OF
SERUM FROM VARIOUS ANIMALS, AND HAEMOLYTIC PROPERTIES OF NORMAL HUMAN SERUM.

Attention is already being turned to the application of cytolytic methods in the investigation of various questions of general medical and biological importance. In view of the developments that have taken place in our ideas of immunity, it seems very important to determine the cytolytic properties of human blood according to the newer methods.

The following study of the haemolytic properties of human serum was made under the influence of this idea. Such a research should give a general idea of the cytolytic action of the serum, and furnishes a small part of the preliminary work that must form the basis of an attempt to apply the haemolytic properties of the serum to diagnosis and to questions of pathological interest.

In the recent literature there have appeared reports of several investigations along the lines here indicated.

E. Neisser and Doering^{*} studied the blood from twenty persons in hospital wards, including cases of pneumonia, nephritis, tuberculosis, syphilis and emphysema. The action of the human serum upon rabbit blood was quite constant, 0.1 cubic centimeter to 0.15 cubic centimeter of serum completely dissolving 1.0 cubic centimeter of 5 per cent suspension of rabbit blood, and 0.01 cubic centimeter producing a trace of solution.

The human serum acted with decreasing strength upon suspensions of rabbit, goat, guinea-pig and pigeon blood, in the last case causing only a trace of solution. The serum from these animals showed a similar gradation in the lytic action upon human corpuscles, pigeon serum giving barely a trace of haemolysis, and 1.0 cubic centi-

^{*} *Berlin. klin. Wochenschrift*, 1901, xxxviii, 593-595.

meter of rabbit serum completely dissolving the specimen of human blood.

Tests made at 0° C. and by centrifugalization showed that the haemolytic action of human serum on rabbit blood is caused by amboceptor and complement. It was possible to inactivate the human serum by heat (56° C.) and reactivate it with active rabbit or horse serum.

A specimen of human serum passed through the bacterial filter retained its complement undiminished for guinea-pig blood, but suffered loss of strength for rabbit blood, hence human serum must contain more than one kind of complement. By suitable experiments it could be shown that human serum contains separate amboceptors for rabbit and guinea-pig blood.

It was possible partly or completely to destroy the amboceptor by prolonged heat—namely, 56° C. for 45 minutes to one hour.

In contrast to E. Neisser and Doering may be cited the results published by Camus and Pagniez.⁹ These investigators examined the serum from over one hundred persons and found that the action of the serum on rabbit blood varied considerably. They concluded from their results that the varying haemolytic action is an expression merely of individual differences and has no pathological value.

The preliminary report of Resinelli¹⁰ is of interest in this connection. He investigated the differences in haemolytic power between the serum of the mother and that of the foetus. He found that the latter has distinctly less haemolytic action than the former upon the blood-corpuscles of rabbit, ox, frog and chicken.

My experiments were made with specimens of blood obtained from a maternity hospital.¹¹

Upon cutting the umbilical cord, the blood flowing from the placental end was caught in a clean glass flask, defibrinated by shaking with clean iron filings, and brought to the laboratory, where the spec-

⁹ *Société de Biologie*, 1901, 242.

¹⁰ *Congresso della Società italiana di Ostetricia e Ginecologia*. Rome, 1901.

¹¹ I wish in this place to express my thanks to Dr. Vömel, director of the maternity hospital at Frankfort-on-the-Main, for his kindness in supplying the necessary blood.

imen was centrifugalized and the clear or blood-tinged serum was utilized as soon as possible, the sediment of blood-corpuscles being suspended in a volume of 0.85 per cent sodium chloride solution equal to the amount of serum removed, and kept on ice till needed. From this suspension, a 5 per cent suspension was made with 0.85 per cent salt solution, and 1.0 cubic centimeter of this 5 per cent suspension was the standard amount of blood used in all experiments.

The haemolytic action of various normal sera upon human blood-corpuscles was investigated. The result is recorded in Table II.

Test-tubes were arranged with a constant amount (1.0 cubic centimeter) of 5 per cent suspension of human red blood-corpuscles and decreasing amounts of haemolytic serum (from 1.0 to 0.1 cubic centimeter), and an amount of 0.85 per cent salt solution sufficient to bring the total volume of fluid up to 2.0 cubic centimeters. The several tests were made simultaneously and upon the same specimen of human blood. The test-tubes were kept in a thermostat at 37.5° C. for two hours, being shaken from time to time. They then remained in ice chests (7½° C.) over night. The results were recorded in the morning.

TABLE II.

Amount.	Goat.	Sheep.	Ox.	Horse.	Rabbit.	Guinea-pig.	Goose.	Man.
1.0 ccm.	complete	moderate	moderate	0	moderate	trace	translucent	0
0.5 "	"	slight	slight	0	slight	0	moderate	0
0.25 "	"	trace	minimal	0	trace	0	slight	0
0.15 "	filmy	minimal	minimal	0	minimal	0	minimal	0
0.1 "	strong	0	minimal	0	minimal	0	"	0
0.0 "	0	0	0	0	0	0	0	0

Different specimens of serum from the same species exhibit variations in haemolytic activity; for example, it happened several times that guinea-pig serum was found which exerted a distinct, though weak action upon human blood. Hence many repetitions would be necessary in order to determine the relative haemolytic strength of the sera included in Table II. It is seen, however, that goat, goose, rabbit, ox and sheep serum gave positive lytic action, while human, guinea-

pig and horse serum were negative. In other experiments it was found that eel serum is strongly haemolytic and pig serum negative.

The serum of an animal inoculated with human blood-corpuscles acquires strong lytic powers: 0.06 cubic centimeter of the inactivated specific serum of a rabbit and 0.1 cubic centimeter of the inactivated specific serum of a goat containing sufficient amboceptor to dissolve 1.0 cubic centimeter of human blood suspension; while it proved difficult to produce haemolysis of human blood with inactivated normal goat serum as amboceptor. Of the latter 0.8 cubic centimeter, using guinea-pig or rabbit complement, gave only slight or moderate solution.¹²

No special endeavor has been made to find variations in the behavior of different specimens of human blood-corpuscles. Judging from the numerous tests made with specific haemolysins and anti-haemolysins, the action of the human red blood-corpuscles appears to be remarkably constant.

The haemolytic action of normal, active human serum was determined. 1.0 cubic centimeter of a 5 per cent suspension of the blood of each animal tested was treated with decreasing amounts (0.8 to 0.1 cubic centimeter) of a specimen of human serum, the quantity of fluid in the test tubes being kept constant. The experiments were made simultaneously. There was no solution of the blood-corpuscles of man, ox, goat, sheep, guinea-pig, rat or goose. Rabbit blood was completely dissolved by 0.8 cubic centimeter of serum, while 0.2 cubic centimeter showed no action.

In the course of other experiments controls were made in many cases to determine the action of other specimens of human serum upon human, ox and sheep blood. In all cases the human serum failed to show any lytic action.

In one instance that came under observation, there was a very interesting difference between the action of infant's serum and serum from the mother upon guinea-pig blood. The infant's serum in doses

¹² For the reactivation of normal inactive sera see Sachs. *Berliner klin. Wochenschrift*, 1902, xxxix, 182, 216.

up to 1.0 cubic centimeter failed to dissolve this blood, while 0.25 cubic centimeter of the mother's serum dissolved it readily. Apparently, the adult serum contains a haemolysin that is absent from the infant's serum, a condition hitherto unrecorded. It will require further investigation to determine whether this condition is due to absence of both amboceptor and complement from the infant's serum, or of only one of these constituents.

Max Neisser¹³ observed a similar difference between serum from old and young horses, the former normally containing antitetanolysin and antistaphylolysin, substances which are completely or nearly completely absent from the serum of young horses, and G. Müller¹⁴ describes the same condition in regard to the agglutinins of the serum of cattle.

From the experiments above recorded it is seen:

1. That the red blood-corpuscles of the infant are dissolved by the serum of various animals, and that the action of one kind of animal serum differs from that of another kind.

2. That infant's serum possesses but feeble haemolytic properties, acting as it does upon only one species of animal blood, namely, the rabbit's. The lytic action in the instance observed by me was weak— weaker than the action found by Neisser and Doering to be constant. This result may be due to individual differences or it may be the result of a peculiarity in infant's serum corresponding to the condition described by Resinelli. (Op. cit.)

In further experiments the complement strength of human, ox and horse serum was compared.

Preliminary tests showed that horse serum exerted no lytic action on human blood-corpuscles, while only 0.2 cubic centimeter of ox serum could be used, as 0.25 cubic centimeter brought about partial solution of the human blood-corpuscles. In one series of tests 1.0 cubic centimeter of human blood suspension and 0.8 cubic centimeter of inactivated specific goat serum were treated with the three

¹³ *Deutsche med. Wochenschr.*, 1900, xxvi, 790.

¹⁴ Müller, *Über Agglutinine Normaler Thiersera*, Inaug. Dissert., 1901.

different complements. In a second series, made simultaneously, inactivated normal goat serum, human blood and complement were brought together. After two hours in the thermostat, the specimens were kept on ice over night and the results were noted in the morning.

TABLE III.

AMBOCEPTOR.	COMPLEMENT.		
	0.2 ccm. Ox.	0.75 ccm. Horse.	0.8 ccm. Man.
0.8 ccm.			
1. human-blood-goat serum:	complete	complete	moderate
0.8 ccm.			
2. normal goat serum:	moderate	0	slight
3. control: no amboceptor	trace	0	0

About three times the completely dissolving dose of inactivated human-blood-rabbit serum and of inactivated human-blood-goat serum were treated with two specimens of fresh human serum as complement.

0.8 ccm. Human serum.	Human-blood-rabbit.	Human-blood-goat.
I	complete	filmy
II	minimal	translucent

The two sera reactivated the goat serum almost equally well, but there was a great difference in the power of the first and the second to reactivate the rabbit serum.

The complement action of several sera was compared, a constant amount of complement being used with decreasing amounts of amboceptor (inactivated human-blood-rabbit serum).

TABLE IV.

Amboceptor	0.3 ccm. Rabbit.	0.2 ccm. Guinea-pig.	0.75 ccm. Horse.	0.8 ccm. Man.
0.2 ccm.	complete	complete	complete	complete
0.15 "	"	"	moderate	"
0.1 "	"	"	slight	translucent
0.06 "	filmy	"	trace	slight
0.04 "	"	filmy	0	trace
0.02 "	translucent	translucent	0	0
0.01 "	moderate	moderate	0	0
0.0 "	0	0	0	0

In this case the reactivating power of human serum was weaker than that of rabbit or guinea-pig serum and was approximately as strong as the serum of the horse.

From the experiments just recorded it is seen:

1. That human serum contains complement which will reactivate an amboceptor specifically haemolytic for human blood-corpuscles.
2. That the amount of complement varies with different specimens of serum.
3. That the complement available for one amboceptor and that available for another amboceptor may vary independently of each other.

The following further experiments were made with human serum complement:

Three fresh specimens of human serum were tested as complement for ox-blood-rabbit serum. A preliminary test with ox-blood-rabbit serum reactivated by guinea-pig serum showed that 0.0005 cubic centimeter sufficed to dissolve completely 1.0 cubic centimeter of 5 per cent suspension of ox blood. With three times this amount complement determinations were made, using decreasing amounts of the three specimens of human serum.

TABLE V.

Amount.	Serum I.	Serum II.	Serum III.
0.5 ccm.	moderate	slight	filmy
0.25 "	slight	trace	translucent
0.1 "	trace	minimal	moderate
0.05 "	minimal	"	slight
0.035 "	"	"	trace

Controls with 0.75 cubic centimeter complement without amboceptor were negative.

The complement action of the first of these specimens was also tried with other amboceptors.

Preliminary determinations, using goat serum as complement, showed that 0.075 cubic centimeter of ox-blood-goat serum, and 0.3 cubic centimeter of sheep-blood-goat serum sufficed to dissolve 1.0 cubic centimeter of ox blood and sheep blood respectively. With guinea-pig serum as complement it was found that 0.075 cubic centimeter of human-blood-goat serum was the simple lytic dose.

TABLE VI.

Amount of complement.	0.1 ccm. Ox-blood-goat serum.	0.5 ccm. Sheep-blood-goat serum.	0.3 ccm. Human-blood-goat serum.
1.0 ccm.	moderate	complete	complete
0.75 "	"	"	"
0.5 "	"	"	"
0.35 "	"	"	filmy
0.25 "	"	"	translucent
0.2 "	slight	"	slight
0.17 "	"	"	trace
0.13 "	"	"	0
0.1 "	"	filmy	0

Controls showed that 1.0 cubic centimeter of complement alone produced no haemolysis.

A similar experiment was made with another specimen of human serum, an excess of amboceptor being employed with decreasing amounts of complement.

TABLE VII.

Human serum.	0.5 ccm. Human-blood-goat serum.	0.2 ccm. Human-blood-rabbit serum.	0.012 ccm. Ox-blood-rabbit serum.	0.3 ccm. Sheep-blood-goat serum.
0.8 ccm.	0	strong	moderate	complete
0.4 "	0	slight	slight	filmy
0.2 "	0	trace	trace	translucent
0.1 "	0	0	minimal	moderate
0 "	0	0	0	0

As experiment with human serum as complement for normal haemolysis resulted as follows:

Preliminary determinations showed that 1.0 cubic centimeter of 5 per cent suspension of guinea-pig blood was completely dissolved by:

- 0.1 ccm. of active dog serum,
- 0.25 ccm. of active sheep serum,
- 1.0 ccm. of active rabbit serum,
- 0.1 ccm. of active ox serum.

The four sera were inactivated at the lowest inactivating temperature (see Sachs, loc. cit.); and used as amboceptor for guinea-pig blood.

TABLE VIII.

Complement.	0.25 ccm. Dog serum.	0.35 ccm. Sheep serum.	1.0 ccm. Rabbit serum.	0.25 ccm. Ox serum.
0.5 ccm. Human serum	complete	complete	complete	complete

The control with 0.5 cubic centimeter human serum alone had no effect upon guinea-pig blood.

All of the experiments with complement reported above were performed in the usual manner, the amboceptor, complement and blood being mixed in test tubes and kept at 37.5° C. for two hours. The following experiments indicate that this is not a reliable test of the actual complement strength of human serum.

Two amboceptor determinations were made in the usual manner, with fixed quantities of two specimens of human serum as complement, and decreasing amounts of amboceptor (inactive human-blood-goat serum). The result was as follows:

TABLE IX.

Human-blood-goat serum.	0.2 ccm. Human serum "A."	0.3 ccm. Human serum "B."
1.0 ccm.	0	0
0.75 "	0	0
0.5 "	0	complete
0.35 "	trace	"
0.3 "	complete	"
0.25 "	filmy	"
0.2 "	strong	moderate
0.0 "	0	0

In each case haemolysis was absent with large amounts of amboceptor; as the amount of amboceptor decreased the reaction reached a maximum and again decreased with insufficient amboceptor.

In order to determine the complement strength when the inhibiting action of the amboceptor fluid was eliminated, the following experiment was made on the same day and with the same materials: Amboceptor and blood-corpuscles were mixed and after one-half to one hour the fluid was removed by centrifugalizing. The corpuscles now laden with amboceptor were suspended afresh in salt solution. Complement determinations now showed that 0.035 cubic centimeter of either specimen of human serum produced complete solution. Smaller amounts of complement were not tried. 0.3 and 0.35 cubic centimeter of amboceptor were used for the first and second complements respectively.

The following day, using the same specific serum, the same blood and the second complement serum, an amboceptor determination

was made by the same method. Corpuscles and amboceptor were united, freed from serum and suspended in salt solution, as described above: 0.15 cubic centimeter of human serum was used as complement.

TABLE X.

Amboceptor.	Degree of solution.
0.14 ccm.	complete
0.12 "	filmy
0.075 "	very slight
0.05 "	0

From this table it will be seen that the presence of the serum of the immunized animal exerts an inhibiting action upon the complement, and, what is rather remarkable, seems to interfere with its own amboceptor.

The goat from which this serum was derived has been frequently injected with human blood, not completely free from serum, and the specific serum therefore contains, in addition to the specific amboceptor, an anticomplement for human serum. Whether the inhibitive action was caused by an excess of amboceptor these experiments do not show.¹⁵

Summary.

1. Human serum has complement available for a variety of normal and specific amboceptors.
2. The complement differs in amount in various specimens of human serum.
3. The complement strength of different specimens of human serum for different amboceptors has no definite ratio, and the complements for different amboceptors may vary independently.
4. Inactivated human-blood-goat serum inhibits the reactivation of its own specific amboceptor by human serum.

C. HAEMOLYTIC PROPERTIES OF HUMAN TRANSUDATES AND EXUDATES.

A study was made of the haemolytic properties of several ascitic and pleuritic fluids. In the recent literature but little has appeared

¹⁵ Neisser and Wechsberg, "*Ueber die Wirkungsart bactericider Sera.*" *Münchener med. Wochenschr.*, 1901, xlviii, 697.

on this subject. Strauss and Wolff¹⁶ studied the haemolytic action of a series of transudates and exudates, taking as a standard the power of the fluid to dissolve rabbit blood. They tried to correlate the haemolytic action and the physical-chemical condition of the fluid, and concluded that the haemolytic properties of a fluid depend in part upon the complex structure (albumen content) of the fluid. Their experiments indicated that the complexity of the fluid was only one of the factors upon which the haemolytic power depends.

Seven different fluids were examined:

Fluids I and II (ascites) were obtained from a case of cirrhosis of the liver.

Fluids III and IV (ascites) from cases in which the clinical diagnosis was unknown.

Fluid V (ascites) from a patient with myocarditis.

Fluid VI (ascites) from a patient with carcinoma of the ovaries.

Fluid VII (pleuritis) from a case diagnosed as "tuberculous pleurisy."

Haemolytic Action.

The haemolytic activity of the fluids was tested in the same manner as the human serum, 1.0 cubic centimeter of 5 per cent suspensions of blood from various animals being employed with graduated amounts of the fluid to be examined. Without detailing the experiments, the results are grouped in Table XI, the maximal and minimal degree of solution being noted. The experiments with the various fluids were made at different times.

From a consideration of Table XI it is seen that:

1. All the specimens act as haemolysins for blood from animals of several species.
2. The haemolytic strength of the various fluids differs.
3. The action of several fluids upon the several species of blood can not be expressed by a constant ratio: Fluid "A" dissolves each of two species of blood; fluid "B" dissolves the first species, but not the second.

¹⁶ *Fortschritte der Medizin*, xx, 1, 1902.

TABLE XI.

1 ccm. of 5 per cent suspension of blood.	Fluid I.	Fluid II.	Fluid III.	Fluid VI.	Fluid VII.
Ox.	{ 0.5 ccm. complete. 0.15 " slight.	1.0 ccm. strong. 0.5 " slight.	1.0 ccm. trace. 0.5 " minimal.	1.0 ccm. 0	1.0 ccm. slight. 0.35 " minimal.
Sheep.	{ 0.5 " complete. 0.1 " minimal.	1.0 " moderate. 0.35 " minimal.	0.25 " complete. 0.1 " slight.	1.0 " 0	0.5 " complete. 0.1 " trace.
Guinea-pig.	{ 0.5 " complete. 0.15 " slight.	1.0 " moderate. 0.25 " trace.	0.5 " complete. 0.1 " trace.	0.35 " complete. 0.1 " minimal.	0.35 " complete. 0.1 " slight.
Rabbit.	{ 0.35 " complete. 0.15 " slight.	1.0 " complete. 0.5 " trace.	0.35 " complete. 0.15 " trace.	1.0 " complete. 0.1 " trace.	1.0 " filmy. 0.35 " slight.
Rat.	{ 1.5 " trace. 0.25 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " 0	1.0 " slight. 0.5 " 0
Goat.	{	1.0 " filmy. 0.25 " trace.	0.25 " complete. 0.1 " slight.	1.0 " trace. 0.15 " minimal.	0.25 " complete. 0.1 " moderate.
Goose.	{ 1.5 " minimal. 0.35 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " minimal. 0.35 " minimal. 0.25 " 0	1.0 " 0
Chicken.	{	1.0 " trace. 0.25 " minimal.	1.0 " strong. 0.1 " minimal.	1.0 " slight. 0.15 " minimal.	1.0 " strong. 0.1 " minimal.
Pigeon.	{	1.0 " moderate. 0.15 " minimal.	1.0 " translucent. 0.1 " minimal.	1.0 " strong. 0.1 " slight.	1.0 " translucent. 0.1 " trace.
Man.	{ 1.5 " 0	1.0 " 0	2.0 " minimal. 1.0 " 0	1.0 " 0	1.0 " 0
Horse.	{	1.0 " filmy. 0.25 " minimal.	1.0 " filmy. 0.1 " trace.	1.0 " complete. 0.1 " trace.	0.25 " complete. 0.1 " slight.
Pig.	{	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " 0	1.0 " 0	1.0 " 0

4. In general, the action of the various fluids, except fluid VI, which acts exceptionally with ox and sheep blood, is very similar.

5. Haemolysis was absent, or of minimal degree, with the blood of man, pig, rat and goose.

6. The lytic action of the fluids is much stronger than the lytic action of the infant human serum examined.

Complete records of the agglutinating action of the fluids were not made. Agglutination was very frequently observed, however. For example, fluid VI agglutinated the blood of the ox, sheep, rabbit, guinea-pig, rat and goose and did not agglutinate the blood of man, chicken, pigeon or goat.

The several fluids exhibited unequal agglutinating powers.

Complement Content.

Complement tests were conducted with the fluids in the manner described when considering human serum, and the results of the tests are grouped in Table XII.

Constant amounts of amboceptor and blood suspension were employed with graduated amounts of the fluid used as complement.

In all cases where the body fluids were tried upon human blood infant's blood was employed.

Experiments made with fluid III gave results like those with human serum complement.

When amboceptor, 0.5 cubic centimeter inactive human-blood-goat serum, suspensions of human blood and complement (ascitic fluid III) were mixed, the complement determination gave the following result:

Complement (ascitic fluid III).	Result.
0.5 ccm.	complete solution
0.35 "	trace
0.3 "	minimal
0.25 "	0

The following day, with the same materials, an amboceptor determination was made. Amboceptor and corpuscles were mixed and after one-half hour the laden corpuscles were recovered by centri-

TABLE XII.

Complement.	Human blood-goat serum ambo- ceptor with human blood corpuscles.	Ox blood-goat serum ambo- ceptor with ox blood cor- puscles.	Sheep blood-goat serum am- boceptor with sheep blood corpuscles.	Ox blood-rabbit serum am- boceptor with ox blood corpuscles.	Horse blood-goat serum with horse blood corpuscles.
Fluid I {	0.35 ccm. complete. 0.1 " trace. Fluid I alone, 0.		No reactivation.	No reactivation.	
Fluid II {		1.0 ccm. translucent. 0.1 " slight. Fluid II alone, 0.	No reactivation.	No reactivation.	
Fluid III {	0.1 ccm. complete. 0.02 " slight. Fluid III alone, 0.	No reactivation.		0.1 ccm. complete. 0.012 " 0. 1.0 " (alone) 0	
Fluid VI {	0.5 ccm. moderate. 0.35 " 0. Fluid VI alone, 0.	0.2 ccm. moderate. 0.5 " 0. Fluid VI alone, 0.	0.5 ccm. complete. 0.05 " moderate Fluid VI alone, 0.	0.15 " complete. 0.035 " slight. Fluid VI alone, 0.	No reactivation.
Fluid VII {	0.5 ccm. filmy. 0.25 " 0. Fluid VII alone, 0.	0.5 ccm. filmy. 0.75 " 0. Fluid VII alone, 0.	0.35 ccm. complete. 0.035 " moderate. 0.5 " fluid VII alone, 0.	0.25 ccm. complete. 0.035 " trace. Fluid VII alone, 0.	No reactivation.

It was found in addition that Fluid II reactivated sheep-blood-rabbit serum and rabbit-blood-goat serum, and did not reactivate inactivated normal dog, sheep or ox serum for rabbit blood. Fluid VI did not reactivate goat or ox serum for rabbit blood, and reactivated both sera very slightly for guinea-pig blood.

fugalization, suspended afresh in salt solution and treated with complement, consisting of 0.5 cubic centimeter of ascitic fluid III.

AMBOCEPTOR.	
Human-blood-goat serum.	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.02 "	minimal
control (complement alone)	0

Finding that 0.1 cubic centimeter was the simple completely lytic dose of amboceptor a complement determination was made in the same manner.

Human corpuscles were treated with 0.1 cubic centimeter of amboceptor, the excess of fluid was removed by the centrifugalization, and the laden corpuscles, freshly suspended, were treated with graduated amounts of complement.

Complement (ascitic fluid III).	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.03-0.02 "	slight
0.0 "	0

It is seen here that the presence of the specific serum throughout the reaction makes it necessary to employ five times as much amboceptor and five times as much complement as would be needed were the inhibitive action of the specific serum eliminated.

Experiments described in another article¹⁷ proved that the ascitic fluids exert but very feeble antiamboceptor action upon the human-blood-goat serum, and, therefore, all or very nearly all of the inhibitive action depends upon the amboceptor serum, as in the case considered when dealing with complement of human serum.

Summary.

1. The body-fluids examined contain complements capable of reactivating various amboceptors.
2. The reactivating power is not equal in the several specimens.

¹⁷ Marshall and Morgenroth, *Centralblatt f. Bakteriologie und Parasitenkunde, erste Abtheilung*, 1902, xxxi, 570.

3. The complement strength for a given lysin may vary in several fluids independently of the complement strength for other lysins.

4. In order to determine the actual complement strength of the fluid any inhibiting action of the amboceptor fluid must be eliminated. The same precaution is necessary in order to determine the simple completely lytic dose of amboceptor.

5. The complement action of the body fluids seems more pronounced than that of the human serum examined.

Amboceptor Content.

The several fluids were inactivated by heating to 56° C. for half an hour and examined for amboceptor. No complement action remained after this procedure.

Fluid I was devoid of amboceptor for human red corpuscles.

When ascitic fluid and complement (goat serum or guinea-pig serum) and blood were mixed no amboceptor appeared for ox blood or sheep blood. By the method of union, however, amboceptor was found. Corpuscles and ascitic fluid I were mixed, and after one-half hour the corpuscles were recovered by centrifugalization, washed with salt solution, again centrifugalized, suspended afresh in salt solution and treated with 0.15 cubic centimeter guinea-pig complement.

Amboceptor (ascitic fluid I).	Degree of Solution.	
	Ox blood.	Sheep's blood.
1.0 ccm.	complete	complete
0.5 "	"	moderate
0.25 "	"	slight
0.1 "	filmy	trace
0.0 "	0	"
(complement alone)		

On the same day and with the same materials the following experiment was performed:

Amboceptor for ox blood was removed by treating ascitic fluid I with ox blood-corpuscles freed from serum by several washings with salt solution.

Ascitic fluid thus prepared was recovered by centrifugalization and tested for amboceptor for sheep blood, controls showing that this fluid no longer contained amboceptor for ox blood.

As complement 0.15 cubic centimeter of guinea-pig serum was employed.

Ascitic fluid.	Degree of solution of sheep's blood.
1.0 ccm.	moderate
0.5 "	slight
0.25 "	trace
0.1 "	"
0.0 "	"
(complement alone).	

Summary.

1. Ascitic fluid I contains no amboceptor available for human blood-corpuscles.

2. Ascitic fluid I contains amboceptor for sheep's blood and ox blood.

3. The inhibitive action of ascitic fluid I conceals the presence of the amboceptor when guinea-pig serum or goat serum is used as complement.¹⁸

4. There are at least two groups of amboceptors in ascitic fluid I. One with affinity for ox blood and one for sheep blood. (In removing the amboceptor for ox blood the ascitic fluid is somewhat diluted with salt solution. The weakened amboceptor action seen in the second sheep blood experiment may be due to this dilution alone; or it may be due in part to a third amboceptor group with affinities for both ox blood and sheep blood.)

An experiment made with ascitic fluid III may be recorded at this point.

Tests were made to learn at what temperature the haemolytic action of fresh active ascitic fluid III occurs; and to determine whether there is union of corpuscle and haemolysin at temperatures too low for haemolysis to occur.

Tubes containing ascitic fluid III and rabbit blood were kept at various temperatures, and after two hours the degree of solution was noted. The corpuscles and fluid were separated by centrifugalization, the corpuscles suspended afresh in salt solution and to the

¹⁸ Marshall and Morgenroth, loc. cit.

menstruum was added the sediment obtained by centrifugalizing 1.0 cubic centimeter of rabbit blood suspension. The tubes were then kept for two hours in the thermostat, and remained on ice over night. The results were recorded in the morning.

Preliminary determinations showed that 0.35 cubic centimeter exactly dissolves 1.0 cubic centimeter of 5 per cent suspension of rabbit blood. 2.0 cubic centimeters of ascitic fluid III and 1.0 cubic centimeter rabbit blood were employed.

TABLE XIII.

Temperature.	After 2 hours.	Menstruum.	Sediment.
5° C.	0	complete	0
10° C.	0	"	0
15° C.	trace	"	0
20° to 38° C.	complete		

This experiment shows that:

1. With ascitic fluid III haemolysis of rabbit blood occurs between the temperature 15° and 20° C.

2. Both amboceptor and complement of ascitic fluid III require a minimal temperature of from 15° to 20° C. in order for union to occur with rabbit blood.

3. Amboceptor and complement of ascitic fluid VII can not be separated by this procedure.

For the sake of brevity the experiments with the various fluids upon blood of man, ox and sheep are tabulated.

The experiments were made by the method of union, using guinea-pig serum as complement. The amount of ascitic fluid used is noted in the table.

TABLE XIV.

Fluid.	Degree of solution of human blood.	Degree of solution of ox blood.	Degree of solution of sheep's blood.
I	1.0 ccm. = 0	0.25 ccm. complete	1.0 ccm. complete
II	1.0 " = 0	1.0 " slight	1.0 " translucent
III		1.0 " trace	0.1 " complete
IV	2.0 " = 0		1.0 " moderate
VI	2.0 " = 0	2.0 " complete	1.0 " complete
VII	2.0 " = 0	0.5 " "	0.25 " "
			lowest complete dose not reached

Controls made without removing the ascitic fluids by centrifugalization gave, for the most part, no solution, as was described for fluid I; but with fluids II, III, V and VII an amboceptor for sheep blood appeared when the fluid, sheep's blood and guinea-pig complement were simply mixed and allowed to stand in the thermostat. The same result occurred with fluid VII and ox blood. In all of these cases much stronger amboceptor action was obtained by the method of union.

Other amboceptor experiments will be given briefly:

Fluid II failed to show amboceptor for human blood when reactivated by fresh ox, goat, sheep or rabbit serum. With fluid II and ox blood the rabbit complement acted slightly. The other three sera gave negative results.

Fluid III showed no amboceptor for ox or rabbit blood when ox, goat, or rabbit serum were used as complement.

Fluid V showed no amboceptor for human or ox blood with human serum as complement.

Fluid VI showed no amboceptor for chicken or goose blood with guinea-pig, ox, goat or rabbit serum as complement.

Fluids VI and VII were tried as amboceptor for several bloods, with guinea-pig serum as complement.

TABLE XV.

Blood.	Fluid VI.		Fluid VII.	
Rabbit	1.0 ccm.	minimal	1.0 ccm.	filmy
Goat	0.25 "	complete	0.25 "	complete
Horse	2.0 "	0	2.0 "	translucent
Guinea-pig	2.0 "	0	2.0 "	slight

Smaller doses of fluid were not employed.

In all cases examined (fluids II, III, IV, VI) it was found that amboceptor for sheep blood was not removed when amboceptor available for ox blood was removed.

From these experiments it is seen that:

1. The pathologic body fluids contain amboceptor for a variety of bloods.

2. The amboceptor content of several fluids for a given variety of blood may vary independently of the amboceptor content for another variety.
3. There is more than one group of amboceptors in the fluids.
4. Different fresh sera act in different degrees as complements for the fluids.
5. No amboceptor was found available for human blood.