A STUDY OF THE PROPERDIN SYSTEM

COMMUNICATION I. A SIMPLIFIED METHOD OF ESTIMATION OF PROPERDIN

K. M. Rozental' and G. B. Savel'vol'f

From the Division of Microbiology (Head — Prof. V. I. Ioffe)
of the Institute of Experimental Medicine of the AMN SSSR, Leningrad

(Received March 21, 1958. Presented by Active Member AMN SSSR
N. N. Anichkov)

The properdin (from the Latin word perdere — to destroy) system was first described by Pillemer and his co-workers [2] in 1954. It is a protein fraction of the serum which, in the authors' opinion, determines the general (nonspecific) resistance of the body to infection, by bringing about lysis of bacteria and inactivation of viruses. This property, and the therapeutic action of properdin in experimental radiation sickness, have attracted considerable attention to this substance.

The action of properdin is shown only in the presence of the other components of the system, namely all four fractions of complement and magnesium ions. The titration of properdin in the serum is based on its power of inactivating the third complement fraction C'_3 in prescribed conditions and in the presence of zymosan (at 37°). It may therefore be estimated by two methods: by the ability of the test serum to lower the bactericidal titer of complement, and by its ability to lower its hemolytic activity. In these experiments the ordinary complement must not be used, but complement from which its own properdin has been removed (the so-called Rp, obtained by keeping complement with a suspension of zymosan for 1 hour at 1°).

Estimation of properdin by the first method, in spite of the apparent lucidity of the scheme of the experiment, in fact proved very complicated. Pillemer therefore considered it necessary to develop and recommend the second method. The essence of this method is as follows. Into a series of test tubes containing diminishing volumes of test serum (from 0.25 to 0.15 ml) are added Rp (0.25 ml by volume), a suspension of zymosan (0.1 ml) and veronal buffer (to 0.75 ml). After standing for 1 hour at 37°, the content of the third fraction of complement in the supernatant fluid of each tube is estimated. For this purpose, to 0.2 ml of supernatant fluid is added R_3 (i.e., complement without third fraction) in a dose of 2 units (in a volume of 0.05 ml), veronal buffer (to 0.5 ml) and hemolytic system (1 ml). The onset of hemolysis demonstrates the presence of C'_3 in the titrated fluid, and absence of hemolysis indicates total inactivation of this fraction of complement. The properdin titer in the test samples is determined by the amount of inactivated C'_3.

Pillemer uses preparations of Rp from human serum and considers that preparations containing not less than 120 units C'_3/ml* are suitable for the experiments.

* According to Pillemer, the unit of C'_3 is that quantity which, in the presence of 2 units of R_3, will cause hemolysis in standard experimental conditions (a volume of 1.5 ml, of which 1 ml is hemolytic system). The unit of R_3 is the minimum dose of active complement (before removal of third fraction from it) causing 100% hemolysis in the same standard experimental conditions. The unit of properdin is the amount which will inactivate (in the presence of zymosan) 120 units of third fraction of complement in 1 ml of Rp.

TABLE 1

Scheme of the Experiment for Using Preparations of Rp from Guinea Pig Serum

I

Selection of doses of Rp and C'_3 for Making Up Working Mixtures

Dilution of C'_3	Dilution of Rp					
	1 : 10	1 : 15	1 : 20	1 : 30	1 : 40	1 : 60
1 : 10	-	-	-	-	-	-
1 : 15	-	-	-	-	-	+
1 : 20	-	-	-	-	±	++
1 : 25	-	-	-	-	+	+++
Veronal buffer	-	±	++	+++	++++	++++

II

Main experiment Rp + C'_3 (working mixture . . . . . 0.2 ml
Suspension of zymosan . . . . 0.1 ml
Test serum (in different dilutions) . . . . 0.2 ml
Veronal buffer . . . . 0.1 ml

1 hour at 37°; centrifugation . . . . .
To the supernatant fluid from each tube (0.3 ml) is added 0.2 ml of hemolytic system.

Legend to this and subsequent tables: - hemolysis; +, ++, +++ and ++++ various degrees of inhibition of hemolysis.

He himself points out, however, that such preparations of Rp can be obtained from human sera in not more than 20% of experiments. Nor was he able to obtain satisfactory preparations of R_3 from human sera.

The difficulties which arise in connection with the use of preparations from human serum and with the complexity of the experiment itself, have made it imperative to seek other possible methods: 1) to find an infallible method of obtaining Rp with a higher content of third fraction; 2) to dispense, if possible, with the use of the preparation of Rp and to replace it with crude serum with a low properdin content, which could be ignored, and 3) to simplify the plan of the experiment. This was the subject of our research, the results of which are described below.

EXPERIMENTAL METHOD AND RESULTS

The comparative study of the complement in man and the guinea pig, in respect to their content of the individual components, gave us the idea in the first place, of preparing Rp from a mixture of the corresponding sera, and later, from the serum of the guinea pig alone. In the first case the optimal mixture was one of 4 volumes of guinea pig serum and 1 volume of human serum. The Rp preparations obtained from such a mixture had a much higher hemolytic activity than analogous preparations from human serum. Nevertheless their titer was only half that of the crude mixture from which they were obtained. The same thing was observed in relation to preparations of Rp obtained directly from guinea pig's complement. We therefore added to the preparation of Rp a small quantity of C'_3 in the form of complement heated to 52°. We usually had to add 1 part of C'_3 to 2 parts of Rp. This completely restored the hemolytic activity of the preparation.

The properdin of the test sera was estimated as follows. As a preliminary step the optimal concentrations of Rp and C'_3 were titrated, as shown in Table 1. We selected a dilution of Rp which itself would give almost total hemolysis (in this particular case 1:12), and C'_3 was added in a concentration sufficient to increase the Rp activity two- or threefold (in this particular experiment 1:16). To 0.2 ml of the preparation was added 0.2 ml of

TABLE 2

Inactivation of Guinea Pig Complement by Rat Serum in the Presence of Zymosan

Dilution of rat serum	Dilution of complement			Control (addition of rat serum without zymosan to 1:30 complement)
	1:20	1:25	1:30	
1:100	++++	++++	++++	—
1:150	++++	++++	++++	—
1:200	+++	++++	++++	—
1:300	++	++++	++++	—
1:400	—	+	+++	—
1:600		—	++	
Veronal buffer	—	—	—	—

the test serum in different dilutions, 0.1 ml of a suspension of zymosan* and 0.1 ml of veronal buffer.** After allowing the mixture to react at 37°, the contents of the tube were centrifuged, 0.3 ml of the supernatant fluid was withdrawn and to it was added 0.2 ml of hemolytic system. The result of the test was read after 20 minutes at 37°. Absence of hemolysis showed inactivation of the C'_3. The properdin content could be judged by the minimum dose of test serum in which hemolysis was observed to be inhibited.

For all its simplicity, however, this method had certain essential drawbacks: the Rp preparation cannot be preserved under ordinary conditions but must be obtained ex tempore; titration has to be carried out immediately, followed by the main experiment. This was very time consuming and very laborious. We therefore tried to do without a special preparation of Rp, by substituting guinea pig complement untreated in any way.

The properdin content in guinea pig complement is in fact negligible, and after dilution it is still further reduced, so that this amount of properdin can be ignored. At the same time, guinea pig complement has characteristically high values of C'_3 and other fractions.

The suitability of crude guinea pig complement to replace the Rp preparation was tested in experiments in which rat serum was used as a source of properdin. We chose this serum because, as reported in the literature, it is distinguished by a high properdin content which, in ordinary conditions, does not undergo any very considerable variation.

Table 2 shows the results of one of the experiments in which we tested the ability of rat serum to inactivate complement in the presence of zymosan.

Rat serum was used in dilutions from 1:100 to 1:600 and was added in equal volumes (0.2 ml) to complement, diluted from 1:20 to 1:30. To each tube was added a suspension of zymosan in a volume of 0.1 ml (containing 0.5 mg of the preparation) and veronal buffer (0.1 ml). The mixture was kept for 1 hour at 37°. Veronal buffer was added to the control tubes instead of rat serum. A second control series was also used in which complement, diluted 1:30, was mixed with the above doses of rat serum, but veronal buffer was added instead of the sus-

* Zymosan was prepared by Pillemer's formula [2] from yeast of strain No. 7 from the Leningrad yeast factory. The working dose of each batch of zymosan was titrated and the dose chosen which gave the most active Rp preparation by treatment of complement. In these experiments we used a suspension containing 0.5 mg zymosan in 0.1 ml.

** Composition of the veronal buffer: sodium chloride 8.5 g, veronal 0.575 g, medinal 0.375 g, magnesium chloride 0.5 ml, calcium chloride (M/1) 0.15 ml, distilled water to 1 liter.

TABLE 3

Comparative Evaluation of the Properdin Content of Different Sera (Typical Experimemt)

Concentration of test sera (in %)	Rat serum (initial concentration 1:200)		Donor's serum		Sera from patients			
					1		2	
			initial concentration 1:20					
	experiment	control (without zymosan)	experiment	control (without zymosan)	experiment	control (without zymosan)	experiment	control (without zymosan)
100	++++	—	++++	—	++++	—	++	—
90	++++	—	++++	—	++++	—	++	—
80	++++	—	++++	—	+++	—	—	—
70	++++	—	++++	—	++	—	—	—
60	++++	—	++++	—	—	—	—	—
50	++++	—	++++	—	—	—	—	—
40	++++	—	++++	—	—	—	—	—
33	+++	—	+++	—	—	—	—	—
25	+	—	++	—	—	—	—	—
20	—	—	+	—	—	—	—	—
Control (complement, zymosan, buffer)	—	—	—	—	—	—	—	—

pension of zymosan. After all the tubes had been kept for 1 hour at 37°, 0.3 ml was withdrawn from each, and to it was added 0.2 ml of hemolytic system; all the tubes were then placed in a water bath at 37°. The results of the experiment were read when complete hemolysis took place in the control tubes. Complement, when treated with small doses of rat serum in the presence of zymosan, lost its hemolytic activity. At the same time, the addition of rat serum itself, without zymosan, did not affect the complement titer, nor did treatment with zymosan in the presence of buffer. Rat serum, inactivated by heat, also had no action whatsoever in the presence of zymosan, for it was deprived of its properdin. It might be mentioned that, with a higher concentration of complement, a larger dose of rat serum was required for its inactivation. It was therefore necessary to titrate the optimal dilution of complement for the main experiment. In accordance with the results shown in Table 2, this dilution must be considered to be 1:27–1:30, for this dose itself produced complete hemolysis and, at the same time, when treated with small doses of rat serum in the presence of zymosan, it lost not less than half its activity.

Some doubt may arise whether a sufficient quantity of all 4 fractions required for manifestation of the action of properdin is present in the dilution of complement chosen. This was tested as follows. An experiment was carried out on one series of tubes in the ordinary way, and in another series complement was not diluted with buffer but with a preparation of R_3. In the second case, therefore, the amount of the first, second and fourth fractions of complement was greatly increased. It was found that the action of the properdin of the rat serum was the same in both experiments. From this it follows that all the components of complement were present in the first series too, in sufficient quantity to demonstrate the action of properdin.

The results obtained enabled the properdin content of different sera to be compared. An example of such an experiment is given in Table 3.

A rat serum, a healthy donor's serum and two sera from patients were tested simultaneously. The initial dilution of the rat serum was 1:200 and of the others 1:20. In each successive dilution the concentration of the test serum diminished by 10%. The experiment was carried out by the scheme described above. From each dilution of the test sera 0.2 ml was poured into a test tube, and to it was added an equal volume of complement in a titrated dilution (1:25) and 0.1 ml each of a suspension of zymosan and veronal buffer. The necessary controls were set up at the same time. The tubes were kept for 1 hour at 37° and centrifuged; to 0.3 ml of the supernatant fluid was added 0.2 ml of hemolytic system and the mixture was kept on a water bath at 37° until the onset of hemolysis in the controls.

It can be seen from Table 3 that 50% hemolysis (inhibition of hemolysis ++) developed if the rat serum was in dilutions of 1:600-1:700 (>25%<33% of the original dilution). The donor's serum showed the same action in a dilution of 1:80, the first patient's serum in a dilution of 1:33 and the second – 1:20. This gave grounds for considering that the properdin content of the donor's serum was one-eighth that of the rat serum, that of the first patient one-twelfth to one-fourteenth, and that of the second patient one-thirtieth that of the rat serum.

It was thus possible to express the properdin content as quantitative values by comparing the action of the test serum with that of a standard properdin preparation. We considered that rat serum fulfilled this purpose. According to Pillemer, rat sera contain 25-30 units of properdin per ml. The method described enabled the conventional unit of properdin to be considerably reduced. Since the final effective dilution of rat serum was 1:600, it could be accepted that 1 ml contains 600 conventional units of properdin. In accordance with this definition, the properdin content was calculated in the test sera. The establishment of a smaller unit of properdin widened the range of titration, which had definite advantages.

It must be pointed out that the properdin titer is maintained for a sufficiently long time in rats serum if kept in a frozen state (-20°). It is therefore desirable to use as a standard a mixture of several rat sera, kept under the above conditions. In order to obtain comparable results, each new mixture must be titrated in the same experiment with the previous mixture. It is also possible to use the same complement for 7-10 days, if it is kept in the frozen state. The method described enabled a considerable number of sera from healthy persons and patients and also from experimental animals to be investigated without undue difficulty. The results of these investigations will be described in the next communication.

SUMMARY

This work deals with an experimental analysis of the two possible methods of properdin determination and their disadvantages. The authors suggest a new simple method which consists of the substitution of Rp by a guinea pig complement with the utilization of normal rat serum (characterized by high and stable properdin titer) as a standard. The optimal dilution of the complement, which loses its hemolytic activity after treatment with rat serum in 1:600 – 1:800 dilution in presence of zymosan, is determined in a preliminary experiment. The amount of the serum under investigation capable of giving the same result with the titrated dose of complement, is determined in the main experiment.

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* In Russian.