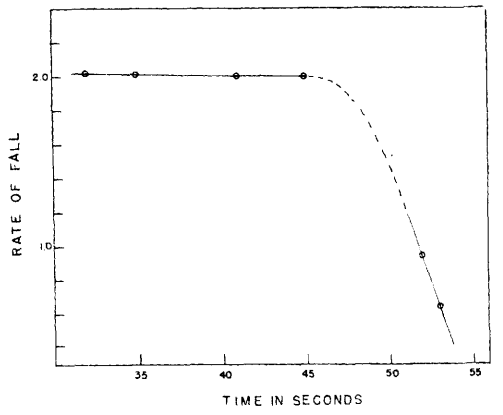

FIG. 4.

The rate of fall of plexiglass beads, expressed in centimeters per second, is plotted as a function of the time elapsing between mixing fibrinogen with thrombin and inserting a bead into the solution. Beads were allowed to fall into the solution 30 seconds after mixing the thrombin and fibrinogen and at 5-10 second intervals after that until gelation had occurred.

for approximately the first 46 seconds of the reaction. Then followed a rapid increase in viscosity leading to gelation and a cessation of falling of the bead. The reproducible clotting times that we have been able to obtain in the statistical study find a ready explanation in the abrupt change in the curve associated with coagulation.

Using 0.3, 0.4, 0.5 and 0.6 ml of thrombin solution (concentration 10 units per ml) average clotting times of 106, 84, 69, and 60 seconds respectively were obtained. (At each dilution the range of readings was ± 3% of the average clotting time). These results indicate a roughly linear relationship between thrombin concentration and rate of clotting.

Three different commercial lots of fibrinogen gave different absolute clotting times under standard conditions owing partially at least to variation in solubility and "percent of clottable protein" in the plasma fraction I. However, different samples of the same lot gave consistent results. The thrombin showed much less variation of activity between lots.

Summary. 1. To study the coagulation of the fibrinogen-thrombin system, a simple rapid method has been developed which measures the time required for the developing coagulum to stop the fall of a plastic bead in a glass tube containing the fibrinogen-thrombin mixture.

2. The "clotting time" thus obtained, increases somewhat with the age of the thrombin and fibrinogen solutions.

3. Kinetic studies of the coagulation process indicate that there is no change in viscosity for a considerable time after the mixing of thrombin and fibrinogen, but that this phase is followed by an abrupt increase in viscosity signifying coagulation of the system.

4. The rate of clotting varies with thrombin concentration in a roughly linear fashion.

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17095. A New Serological Test (Inhibition Test) for Human Serum Globulin.

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Moreschi[1] described a special serological technic by which it is possible to demonstrate the presence in immune sera of antibodies for red cells or bacteria in higher titers than by the classic agglutination technic. His technic consisted in first titrating the antiserum against the test blood or bacterial suspension in saline media and subsequently adding a precipitin serum against the serum of the animal providing the antiserum for the erythrocytes or bacteria. The following precautions were necessary in order that this test might work: (1) The precipitin serum had to be absorbed with the washed erythrocytes or bacteria used as antigen, in order to remove any natural hemagglutinins or bacterial ag-

[1] Moreschi, C., *Centralbl. f. Bakteriol.*, 1908, **46**, 49; *ibid.*, 1908, **46**, 456.

glutinins which might be present in the serum. (2) After the first stage of the reaction was completed and before the precipitin serum was added, the sensitized cells were washed several times with large volumes of saline in order to remove the supernatant serum proteins which could inhibit the reaction. Moreschi thought that his test served to accentuate the reaction of immune *agglutinins*. Actually, we now know, principally as result of the recent work on Rh antisera, that the test is specific for so-called *univalent antibodies* or *glutinins*.

Following the demonstration by one of us[2,3] that Rh-negative individuals sensitized to the Rh factor may produce two sorts of Rh antibodies, namely, Rh agglutinins (bivalent antibodies) and Rh blocking antibodies or agglutinins (univalent antibodies), Coombs *et al.*[4] suggested the application of the anti-globulin test as a means of detecting univalent Rh antibodies. The purpose of the present communication is to describe a modification of the anti-globulin test by which it is possible to identify human serum globulin specifically and estimate its concentration.

Materials and method. The principle of the absorbed, diluted anti-human-globulin is as follows. The solution to be tested for the presence of serum globulin is mixed with the absorbed, diluted anti-human-globulin serum and the mixture allowed to react in a water bath at 37°C for one hour. Then a suspension of Rh-positive cells, coated with Rh_o univalent antibody, is added and the mixture allowed to stand for a second period of incubation, after which the reaction is read. Failure of clumping to occur indicates the presence of human serum globulin in the material being tested; the occurrence of clumping indicates the absence of human serum globulin.

The sensitized Rh-positive cells used in the test were prepared as follows. A fresh, 2% suspension of Rh_o-positive red cells in saline was prepared and washed once with saline solution. This suspension was mixed with an equal volume of an Rh_o antiserum containing univalent antibodies with a titer of approximately 100 units by the albumin-plasma method.[5] This mixture was allowed to react in the incubator at 37°C for 45 minutes. The tube was then centrifuged at moderate speed, the supernatant fluid discarded, and the sedimented cells washed four times with large volumes of saline solution. The sensitized cells were then resuspended in sufficient saline to make a concentration of approximately 2%.

The antiglobulin serum used in these experiments had been prepared in the usual way and was kindly provided by Drs. Peter Vogel and Richard Rosenfield.

In the experiments to be described the following materials were tested: normal human adult and umbilical cord serum; human spinal fluid, saliva, semen and urine; and normal animal serum from horse, ox, rabbit, and rhesus monkey. The tests were carried out quantitatively by preparing progressively doubled dilutions in saline solution of the material to be tested and mixing a drop of each dilution, in a corresponding series of small test tubes, with a drop of the anti-human globulin serum. After 45 minutes incubation at 37°C, a drop of the suspension of Rh-positive, sensitized cells was added to each tube; the tubes were shaken and reincubated for 60 to 90 minutes. The tubes are then gently shaken and the reactions read with the naked eye and under the low power of the microscope.

Results. Table I contains a selection of the results obtained in the various experiments. The tests shown in the table were not all done on the same day but are combined for purpose of comparison.

With the technic used, normal adult human serum completely inhibits the agglutination reaction of the anti-human-globulin serum in dilutions as high as 1:500, on the average. It is necessary to emphasize at the onset that the method shares the limitations of all other serological titrations, namely, a lack of precise reproducibility. In tests made on different days or even on the same day, variations in titer as great as two serum dilutions

[2] Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 173.

[3] Wiener, A. S., *J. Lab. and Clin. Med.*, 1945, **30**, 662.

[4] Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Brit. J. Exp. Path.*, 1945, **26**, 255.

[5] Wiener, A. S., and Hurst, J. G., *Exp. Med. and Surg.*, 1947, **5**, 285.

TABLE I.

Results of Inhibition Tests, Using Rabbit Anti-Human-Globulin Serum and Rh-Positive Erythrocytes Coated with Anti-Rh_o Glutinin.

Material tested	Dilution of material tested											
	Undil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:5096	1:10,192
Normal human serum (adult)	—	—	—	—	—	—	—	—	—	—	±	++	++	++
" " (umbilical cord)	—	—	—	—	—	—	—	—	—	—	tr.	++	++	++
10% purified human globulin	—	—	—	—	—	—	—	—	—	—	—	—	—	±
25% " albumin	+	+	+	+	+	+								
Normal human spinal fluid	—	—	±	+	+	+	+	++						
Spinal fluid (4+ Wassermann)	—	—	—	—	±	+	+	+						
Normal human urine	+	±												
" " saliva	—	—	+	±	±	+	+	+						
" " semen	+	±												
Normal horse serum	+	+	+	+	+	+	±	+	+	+	+	±	+	+
" ox "	+	+	+	+	+	+								
" rabbit "	+	+	+	+	+	+								
" rhesus "	+	+	+	+	+	+								

were not unusual, so that only a tolerable degree of accuracy was possible by averaging the results of several titrations. In addition the end points of the inhibition titrations were often difficult to determine due to the irregularity of the degree of clumping in successive tubes in many of the experiments.

In an attempt to determine which serum fraction is responsible for the inhibition of the anti-globulin serum, titrations were carried out with purified human serum albumin and purified human serum globulin. The serum albumin used was a 25 percent salt-poor solution purchased from Cutter Laboratories. The serum globulin was a 10 percent solution of Squibb's Red Cross Gamma Globulin, kindly provided by Dr. P. Vogel. As shown in the table, the serum albumin failed to inhibit the anti-globulin serum, while the serum globulin inhibited the serum strongly, in accordance with expectation. These results therefore provide additional proof that the antibody coating the sensitized red cells consists of serum globulin. It is to be noted that the inhibition titer of the purified human globulin is higher than that of unprocessed serum, in proportion to the concentration of globulin.

A few tests were performed with human umbilical cord serum, which gave titers approximately equal to those of normal adult serum. Even assuming that the fetus *in utero* is incapable of producing serum globulin, this finding was to be expected from the fact that univalent maternal antibodies readily traverse the placenta into the fetal circulation until the titers in the maternal and fetal circulation are equal.[6,7]

The normal human spinal fluid inhibited the anti-globulin serum but only up to the dilutions of approximately 1:2 or 1:4. Since normal serum contains approximately 7 g of protein per 100 cc while normal spinal fluid contains only about 30 mg per 100 cc, the inhibition titer of approximately 3 units obtained for spinal fluid corresponds satisfactorily to the expectation, on the basis of an inhibition titer of approximately 500 units for

[6] Wiener, A. S., and Sonn, E. B., *J. Lab. and Clin. Med.*, 1946, **31**, 1016.

[7] Wiener, A. S., *Ann. Allergy*, 1948, **6**, 293.

blood serum. With abnormal spinal fluid such as those obtained from syphilitic patients, higher inhibition titers were obtained, as to be expected. Saliva gave definite inhibition though only in low titer, in conformity with the known presence in this secretion of small amounts of serum globulin. Only doubtful reactions were obtained with normal human semen and urine.

Experiments were also conducted to determine the ability of animal sera to inhibit the anti-human-globulin serum. As shown in the table, serum from horse, ox, and rabbit had no apparent inhibiting action. Rhesus serum weakened the clumping, even when highly diluted, but did not inhibit the reaction completely. This indicates the presence in rhesus serum of a serum globulin chemically related to, though qualitatively different from the globulins in human serum.

Summary and conclusions. 1. A new serological method of demonstrating human serum globulin has been described, based on its ability to inhibit the agglutinating action of anti-human-globulin serum for Rh-positive human red cells coated with Rh_0 univalent antibody.

2. Using this technic, further evidence was obtained that the Rh antibody coating sensitized Rh-positive cells is a serum globulin.

3. With the new technic it is possible to demonstrate the presence in normal spinal fluid, and in saliva of small amounts of serum globulin, in proportion to the known concentration in these fluids. Normal human semen and urine failed to inhibit the antiglobulin serum.

4. Umbilical cord serum inhibited anti-globulin serum to the same titer as adult serum.

5. Serum from horse, ox and rabbit did not inhibit the anti-human-globulin serum, while rhesus monkey serum merely weakened its reactions.

6. The new technic may find application not only in clinical medicine, but also in forensic work for the examination of blood stains, and in studies on biochemical evolution.

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17096. Relationship of Catalase Activity to Virulence in *Pasteurella pestis*.

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Huddleson[1] and Merz[2] observed that virulent *Brucella* organisms showed greater catalase activity than avirulent strains. This investigation was undertaken to determine whether a similar relationship exists for *Pasteurella pestis*. It was felt that such findings might be applied to the development of a rapid, inexpensive, *in vitro* method for virulence titration. Furthermore, such a relationship would indicate a basis for approaching the problem of virulence along the lines suggested by Jawetz and Meyer[3] who state, "It appears probable that the final criterion of virulence will have to rest on a biological and chemical identification of the bacterial enzyme systems which are responsible for the multiplication of the plague bacilli in the animal host."

Procedure. Huddleson's[1] method was modified as follows to measure the catalase activity of virulent and avirulent strains of *P. pestis*. Standard bacterial suspensions were prepared from twice-washed, 24 hour, heart infusion broth cultures suspended in phosphate buffer and adjusted to a turbidity of 100 ± 5 on the scale of the Klett-Summerson photoelectric

[1] Huddleson, I. F., Univ. Mich. Agri. Exp. Station, Technical Bull. 182, January, 1942.

[2] Merz, P., Uber die Katalasen der Brucellen, Inaugural Dissertation for the Degree of Doctor of Veterinary Medicine, University of Zurich, 1938.

[3] Jawetz, E., and Meyer, K. F., *Am. J. Path.*, 1944, **20**, 457.