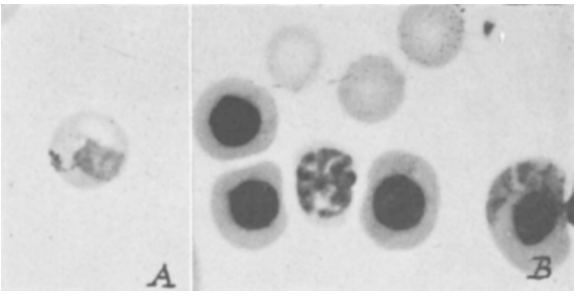

FIG. 1a. A medium trophozoite of *P. lophurae* in a mature mouse erythrocyte. ×1770.

FIG. 1b. A segmenting parasite of *P. lophurae* in a slightly enlarged mouse erythrocyte, from a smear made 36 hours following introduction of mammalian cells. ×1600.

which times avian cell infections averaged about 8000 per 10,000 red blood cells. Five to 11 merozoites, smaller than those in avian erythrocytes, were produced, generally in a rosette form. No gametocytes were found.

Cultures prepared according to the formula of Trager[4] using whole mouse blood in conjunction with infected chick embryo blood indicated a certain amount of invasion of mouse erythrocytes *in vitro*.

In view of the high degree of host specificity of avian malarias, it is surprising that cells of hosts so distantly related as to be placed in another phylum are invaded. Parasitism of mouse cells might, therefore, demonstrate that in certain cases the resistant principle lies in the serum constituents, rather than in the erythrocyte. *P. lophurae* is transmitted readily by *Anopheles quadrimaculatus* and this fact, along with the successful infection of mouse erythrocytes, might indicate a closer relationship between *P. lophurae* and mammalian malaria parasites than was previously supposed.

[4] Trager, W., *J. Parasitol.*, 1947, **33**, 345.

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17094. A Falling Sphere Method for Studying Clotting in Systems of Purified Blood Components.

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(Introduced by Francis O. Schmitt.)

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Although the conversion by thrombin of fibrinogen to fibrin is generally used as an indicator of reactions occurring earlier in the process of blood coagulation, methods for the study of this conversion, with very few exceptions, measure only a single end-point in fibrin formation.[1] It is apparent that a method which measures quantitative changes in the coagulation system up to some definite end-point might be of value in following the kinetics of the system. An involved technic has been reported[2] for measuring early alterations in clotting systems by recording changes in intensity of transmitted light as coagulation proceeds. Methods based upon changes in viscosity of a clotting solution have

[1] Quick, A. J., The Hemorrhagic Diseases and the Physiology of Hemostasis, Charles C. Thomas, Springfield, 1942.

[2] Nygaard, K. K., Hemorrhagic Disease. Photoelectric Study of Blood Coagulation, C. V. Mosby Company, St. Louis, 1941.

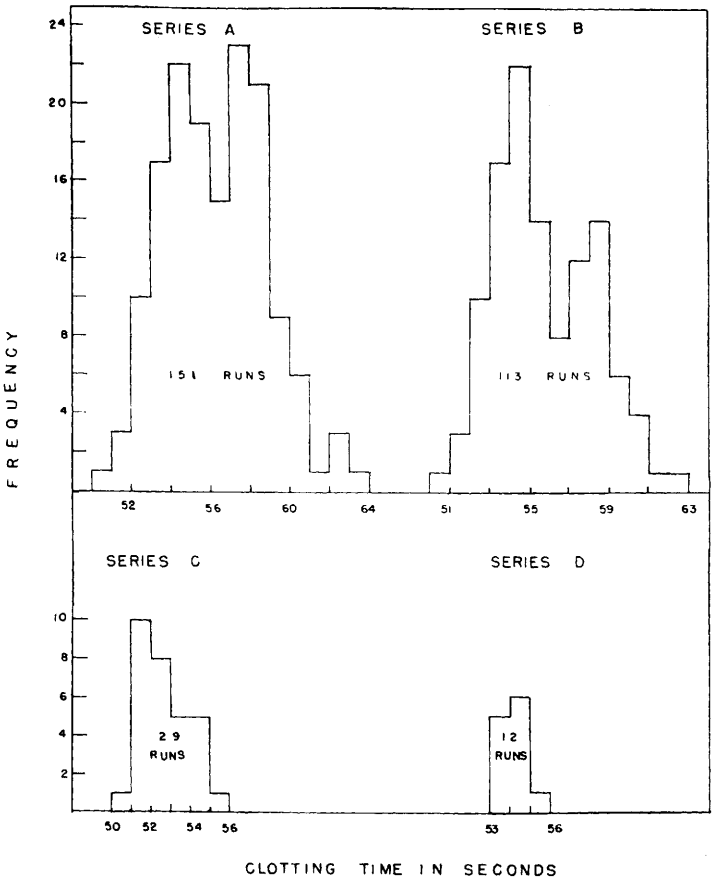

FIG. 1.

Frequency distribution curves of coagulation times, as measured by the falling sphere method, of many different experiments on the thrombin-fibrinogen system. For the experimental conditions of each series of runs, see text.

been described but not in quantitative terms.[3] The purpose of this study was to develop and standardize a quantitative technic for measuring coagulation employing purified thrombin and fibrinogen. This method is based upon the measurement of gelation which occurs in a system during the conversion of fibrinogen to fibrin and utilizes Stoke's law for a falling sphere in the measurement of viscosity.

Materials and methods. Bovine thrombin (Upjohn) was dissolved in 0.85% NaCl solution in a concentration of 20 units per milliliter immediately before use. Fraction I of bovine plasma (Armour) was employed as fibrinogen in a concentration of 0.2% in 0.85% NaCl solution; the fresh solution was filtered through analytical filter paper before using. After adjusting all preparations to 25 ± 0.3°C by immersing in a thermostatically controlled water bath, three-tenths milliliter of fresh thrombin solution was added to fifteen milliliters of fibrinogen solution at zero time and rapidly mixed by a rotary movement of the beaker. A pyrex glass tube eight millimeters in diameter, forty centimeters long and stoppered at one end was filled to the brim with the mixture and placed vertically in the water bath with about 3 cm of the upper end of the tube above the surface. As the solu-

[3] Hedenius, P., *Acta med. Scand.*, 1936, **88**, 440.

tion became opaque a plexiglass bead* (moistened with saline) was inserted in the upper end of the tube and allowed to fall to an abrupt stop. The elapsed time from mixing of solutions to this sharp end-point was taken as the clotting time. Fibrin or air bubbles clinging to the bead disturbed its fall and rendered the end-point uncertain. The pH was 6.2 in both clotted and unclotted preparations.

Results. Series A (Fig. 1) shows the statistical variation in 151 runs before any standardization of the technic as described above was made. The arithmetical mean is 56.2 seconds and the mode is the same indicating a normal distribution curve. The standard deviation is 2.87%.

Series B shows the scatter of experimental data obtained in 113 runs after standardization of the technic but employing fibrinogen solutions varying in age from only a few minutes to several hours. The arithmetical mean is 55.7 seconds, the mode is 55.2 seconds and the standard deviation is 2.5%.

Series C includes data of 29 runs in which the variation caused by deterioration of the fibrinogen solution was reduced by using a freshly prepared solution every 10 runs (the solution was always less than one and a half hours old when used). The arithmetical mean is 53.5 seconds, the mode is 55.3 seconds and the standard deviation is only 0.98%.

Series D shows the accuracy obtained when the errors of weighing, diluting, etc. of different samples are minimized by using the same solutions of fibrinogen and thrombin throughout 12 runs. The runs were made in less than one and a half hours in order to minimize the effect of aging on the thrombin and the fibrinogen. The arithmetical mean is 54.1 seconds, the mode is 54.1 seconds and the standard deviation is 0.49%.

Deterioration of fibrinogen and thrombin in solutions standing at 25°C was manifested by a gradual prolongation of the clotting time. Fig. 2 illustrates the rapid increase in clotting time of a fibrinogen solution as demonstrated by coagulating aliquots of it at successive intervals over a period of six hours using a fresh thrombin solution. The activity of thrombin solutions also changed on standing, but at a very low rate as illustrated in Fig. 3.

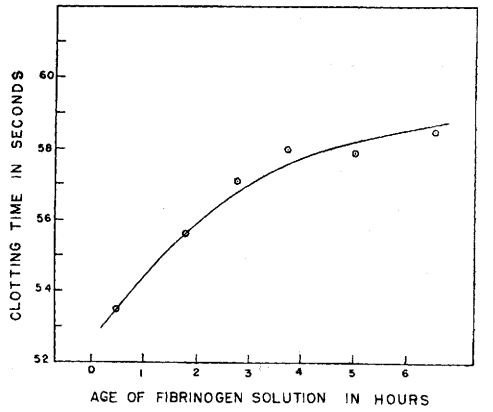

FIG. 2.

The clotting time is shown to increase with the age of a fibrinogen solution allowed to stand over 6 hours at 25°C.

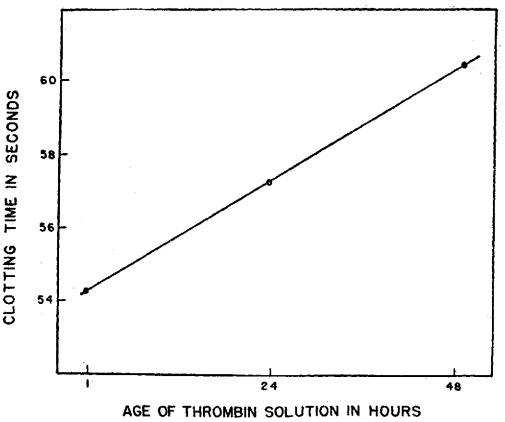

FIG. 3.

Clotting time is shown to increase with the age of a thrombin solution allowed to stand over 48 hours at 4°C.

By inserting successive beads at various intervals before gelation was complete and timing the rate of fall of the beads over a measured length of tube, the data for the kinetics curve of Fig. 4 were obtained. The rate of fall of the bead is plotted as a function of the duration of the action of the thrombin upon the fibrinogen. There was no apparent change in the viscosity of the mixture

* Spherical beads were made of plexiglass (lucite), 3.25 ± 0.1 mm in diameter and weighing 21.6 mg. They were standardized for uniformity by timing their fall over a definite distance in a tube filled with fibrinogen solution.

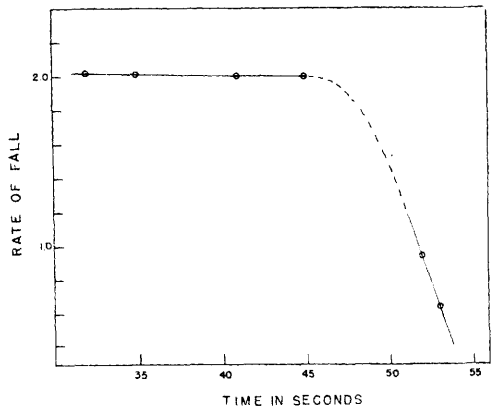

FIG. 4.

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.