A "Coagulometer" for the Thrombin Generation Test on Plasma*)

From "The Old People's Town", Copenhagen (Chief: Dr. Torben Geill, M.D.)

René Dybkær

The thrombin generation test has of late attracted some interest as a valuable tool in the investigation and diagnosis of blood coagulation disorders. The procedure as described by Biggs and Macfarlane (1953) presents difficulties even to the experienced technician who will be very pressed for time at critical points of the test. The result may be some inaccuracy in the recording of coagulation times and consequently distorsion of the curve obtained.

With the apparatus to be described fewer manipulations are required and, therefore, more numerous and possibly more exact determinations may be made in each test.

The principle in the procedure described by Biggs and Macfarlane (1953) is as follows: The citrated plasma sample is placed in a water bath at 37° C and recalcified at zero time. Whenever a coagulum is formed it is removed by rolling it on the end of a glass rod while expressing the serum from the clot. Immediately and every minute following recalcification a small sample is removed and mixed with a fibrinogen solution in a test tube placed in the water bath. At the mixing of each new sample a stop-watch is started and afterwards the tube is repeatedly turned approximately 110 degrees to record the coagulation at the instant when a gelatinous mass is visible.

As the coagulation times in the first two tubes are usually one to several minutes and in the later samples 15—20 seconds it will be seen that about three to five minutes after recalcification the technician must at the same time remove the coagulum from the original coagulating mixture, measure new samples and mix at the appropriate times, turn and examine three or four small tubes repeatedly, start and stop several stop watches. For one person to manage this requires sure hands and nerves of steel. Even with practice the coagulation times recorded in this most important period may well be five or more seconds too long.

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The apparatus constructed is shown in figures 1, 2 and 3. The whole structure is immersed in a glass-walled thermo-regulated water-bath, e. g. an aquarium, with a stirer. The rack is filled with small test tubes (7—8 mm in diameter), each containing 400 µl fibrinogen solution (1 g per liter). At the ends of the rack larger holes are provided for tubes with calcium chloride solution and for the tube with the plasma sample. After recalcification 100 µl aliquots are taken

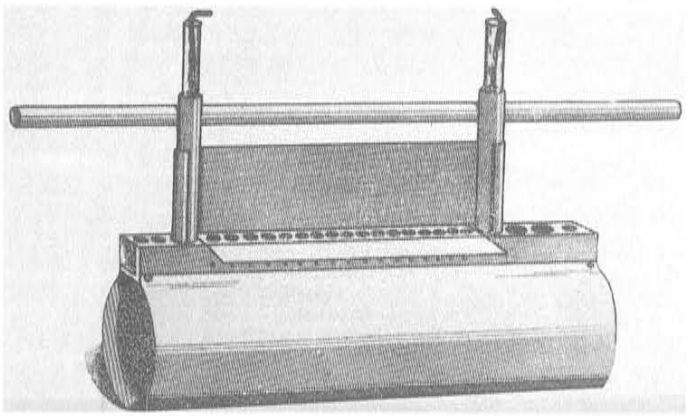

Fig. 1: Perspective of assembled apparatus which would be immersed in the water bath up to a few millimeters over the upper row of holes for the test tubes. The superstructure is partly used for clamping and partly for protection of the tubelike extensions of glass (with electric wire). The vertical brass sheet is proctection against "false" light. The "awning" shading over the row of "portholes" should be tipped a little more downwards. Total length and height of "hull" are 400 mm and 90 mm respectively.

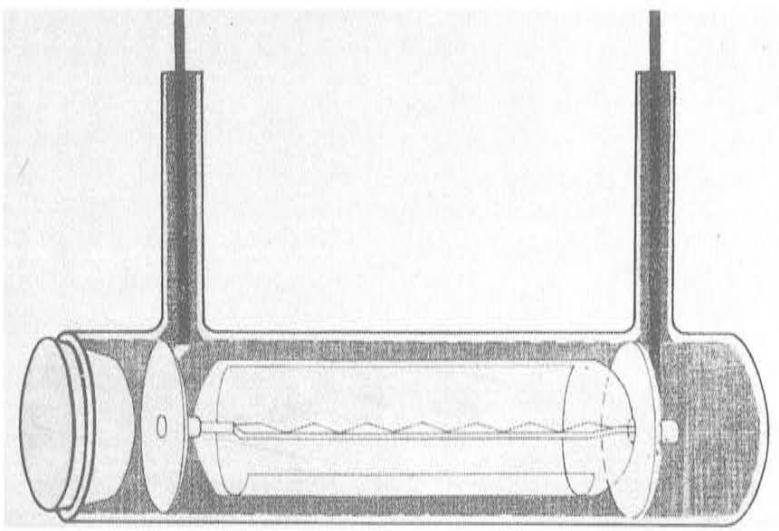

Fig. 2: A semidiagrammatic drawing of rubber-stoppered pyrex glass structure (to be placed inside the brass case), showing the placing of the 220 V 40 W cylindrical bulb (Philips) with halfmirror (for greater light intensity). The bulb is fixed by two circular tightly fitting fiber plates.

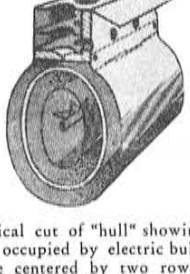

Fig. 3: Perspective of transversal, vertical cut of "hull" showing brass sheet case screwed on 25×25 mm angle-brass. The lower cylindrical part is occupied by electric bulb and its pyrex glass container. The upper part is the rack for test tubes which are centered by two rows of holes, 11 mm in diameter and with 14 mm between centers. The bottoms of the tubes are supported in a row of conical depressions continuing into light-transmitting short canals, 1 mm in diameter. The "portholes" in the vertical part of the angle-brass are 5 mm in diameter.

at suitable intervals and transferred to the small test tubes and mixed by air bubbling through the constriction pipette.

The thin beams of light coming through the fine canals in the bottom of the rack, one beam traversing each test tube vertically, are barely visible when observed at right angles through the small "portholes". This is only true, however, as long as the fibrinogen-plasma mixture is clear. When fibrin is beginning to form the beam will suddenly appear white against a dark background because of the Tyndall phenomenon.

In a dim room and by adjustment of the intensity of the light in the bottom of the apparatus (through a variable resistance) the coagulation is easily determined with short practice.

As no test tubes have to be turned the procedure is rather easy and unhurried, and shorter intervals than one minute between samples may be achieved without difficulty. This is sometimes of interest, especially during the first four or five minutes of the test when changes in the rate of thrombin generation are most pronounced.

The apparatus has proved very satisfactory in practice and a comparison between results obtained by this method and by conventional procedure will appear in a separate paper. (Dybkær and Geill, 1959).

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References

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Author's address: René Dybkær, M.D. Institut f. almindelig patologi Juliane Maries vej 22, Ø Danmark.