A New Improved Method for Determination of Prothrombin Levels in Blood[1]

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A previous communication by one of the authors (*1*) described a simplified and accurate procedure for blood cell counts and hemoglobin determinations which had been developed for experiments on mice. Its advantages over previous methods, namely, greater precision and increased saving of time, effort, and expense, have been stressed in a recent publication in which its clinical application was described (*4*). It differed from similar procedures in that it used calibrated capillary tubes instead of the conventional pipettes. The use of these tubes, in addition to some technical adjustments, resulted in a reliable, practical method for the estimation of prothrombin activity.

The procedure is as follows: Capillary tubes, prepared as described previously (*1*), are filled with a 2% solution of potassium oxalate which is evaporated to dryness in the oven. A suspension of rabbit lung thromboplastin[2] is prepared according to the Link-Shapiro modification of Quick's method (*2, 3*), except that a stronger calcium chloride solution (0.125M), and whole blood, instead of plasma, are used. The blood is taken into tubes with dry rather than dissolved oxalate. Then 15 cu mm of the thromboplastin solution is placed on a clean glass slide.

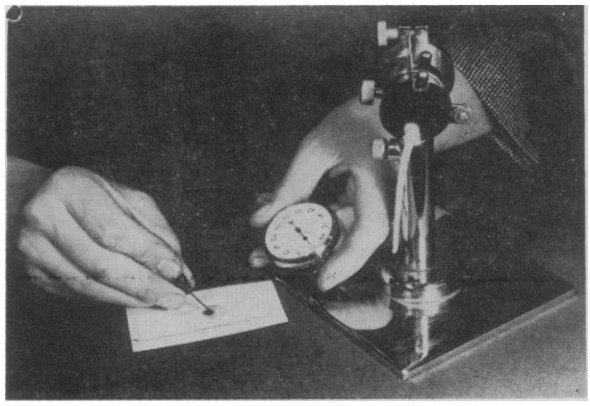

FIG. 1. Mouse holder, 5-cu-mm capillary tube for blood samples, minute rubber bulb of the type used for smallpox vaccinations, stop watch, and glass slide on which the blood clot can be noted.

A 5-cu-mm oxalated capillary tube is filled with blood from the mouse tail (see Fig. 1). The oxalated blood is quickly pressed out into the thromboplastin on the glass slide with the aid of a minute rubber bulb, of the type used for smallpox vaccinations (Fig. 1), and the blood and thromboplastin are mixed gently. The stop watch is started at the same instant that the blood is added to the thromboplastin. The mixture on the glass slide is drawn in and out of the capillary tube by gently pressing the rubber bulb, avoiding air bubbles. At each filling, the capillary tube is lifted up slightly to permit observation of the formation of the fibrin strand. The latter can be noticed suddenly, giving a clearly defined end point of blood clotting. When it appears, the stop watch is instantly stopped and the time is noted. Table 1 summarizes the results obtained by the use of this method on normal Swiss white mice.

TABLE 1

PROTHROMBIN LEVELS OF WHITE MICE, SWISS STRAIN

No. of Mice	Determinations	Temp. in °C	Prothrombin time in sec: lowest	highest	mean	Deviation	Remarks
40	40	23	19	31	28.4	2.7	Single determinations
9	20	26.5	20	32	24.9	2.5	Repeated determinations
7	18	28.5	18	24	22.9	3.7	within 3 days, at 24-hr intervals

The method has proved to be simple, reproducible, and economical. A major advantage is that the test can be performed promptly and with small volumes (5 cu mm) of blood, thus avoiding the necessity for venipuncture. In small species, such as mice, it permits repeated determinations on the same animal with only negligible trauma to the tip of the mouse tail and loss of only insignificant volumes of blood. In man, the method permits the bedside determination of prothrombin time from blood obtained by finger puncture. However, the method introduces two new variables which warrant consideration. 1) Since the method uses whole blood, subjects with low hematocrit values may have greater plasma volumes per aliquot of whole blood. 2) The test is performed at room temperature rather than in a constant temperature water bath. These factors may cause small but significant variations, which may be corrected or taken into account in problems calling for greater accuracy. The relatively narrow range of average deviation indicates that the technique is sensitive and reliable.

[1] This method was developed during a tenure of grants obtained by the senior author from the National Cancer Institute, U. S. Public Health Service, and from the Damon Runyon Fund.

[2] A satisfactory commercial preparation is made by the Maltine Co.

References

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