

Technical methods

Automatic determination of euglobulin lysis time

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Despite the criticisms of substrate variability, the euglobulin lysis time remains a most useful assay of plasma plasminogen activator. It is simple to execute and correlates well with the radioactive clot assay (Sawyer, Fletcher, Alkjaersig, and Sherry, 1960), dilute blood clot lysis time (Fearnley, Balmforth, and Fearnley, 1957), and fibrin plate method (Kekwick and Mackay, 1954). It continues to be used in physiological studies of the fibrinolytic system and remains an important activator assay in thrombolytic therapy (Douglas and McNicol, 1964).

There are two important sources of error in this method which may account for the variation in resting levels between different laboratories. The first are those procedures which lead to the formation of the euglobulin clot. Blix (1961) has shown that reliable conclusions cannot be made unless blood collection, euglobulin precipitation, and clot formation are carefully standardized. The second source of error lies in the determination of the end-point of clot lysis, which is normally done visually. This subjective assessment of clot lysis has proved to be unsatisfactory on the grounds of accuracy and the uneconomic use of the laboratory worker's time. An automatic device, capable of determining the end-point of euglobulin clot lysis time with good reproducibility and accuracy, would prove to be a useful instrument to workers in this field.

Lackner and Goosen (1959) developed an automatic cine-photographic apparatus which while suitable for whole blood clots was not so for euglobulin clots nor for purified standard clot systems. Nanninga, Zeller, and Maynes (1964) and Newman (1964), using photoelectric systems, described an instrument for investigating purified standard clot systems, but because they required a critical pre-set optical density at which a relay circuit was activated to stop a timing device, these instruments proved to be unsuitable for euglobulin clots.

Figure 1 demonstrates the range of optical density, measured in the instrument described below, obtained after complete lysis of 19 different euglobulin clots, prepared simultaneously under identical conditions by the method of Nilsson and Olow (1962). (The zero optical density represents that of distilled water.) These results show that automatic recording of euglobulin

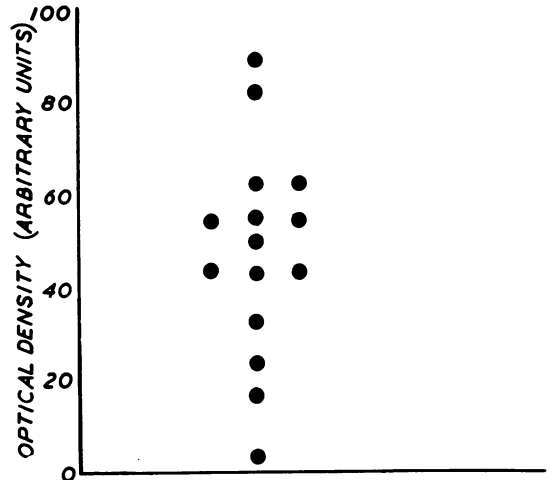


FIG. 1. *The range of optical density after complete lysis of 19 different euglobulin clots.*

lysis time is not possible if a pre-set optical density is required at which a relay circuit is activated. Baumgarten, Ambrus, McCall, and Pennell (1960) and Kuhnke, Gill, and Brilla (1960) have suggested that this problem might be solved by arranging a continuous recording of the optical density. The following communication describes such an instrument which is inexpensive to build and simple to operate.

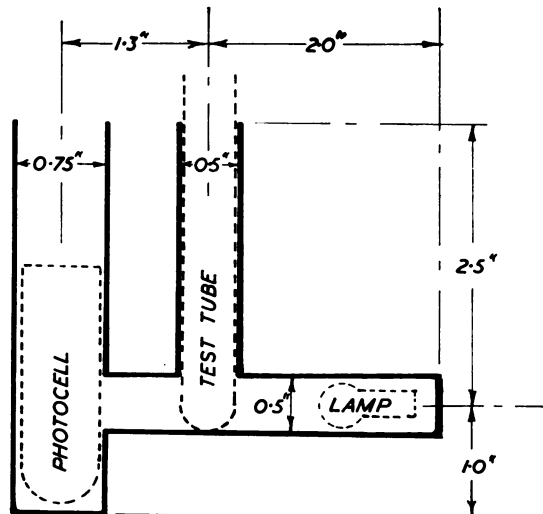


FIG. 2. *Layout and dimensions of the lysis chamber.*

DESCRIPTION OF INSTRUMENT

Light, from a 12 v. 0.1A bulb passes through a 1 ml. clot in a standard laboratory $3 \times \frac{1}{2}$ inch test tube. As the clot lyses more light passes through onto the photoelectric cell and reaches a maximum when lysis is complete. The layout and dimensions of the lysis chamber are shown in Figure 2. It is constructed of brass tubing and coated inside with a black matt paint to prevent light scatter. The lamp is fitted with a watertight seal as the whole lysis chamber is three-quarters immersed in a standard laboratory water-bath at 37°C.

The circuit diagram is shown in Figure 3. The photoelectric cell is used in a bridge circuit thus enabling the

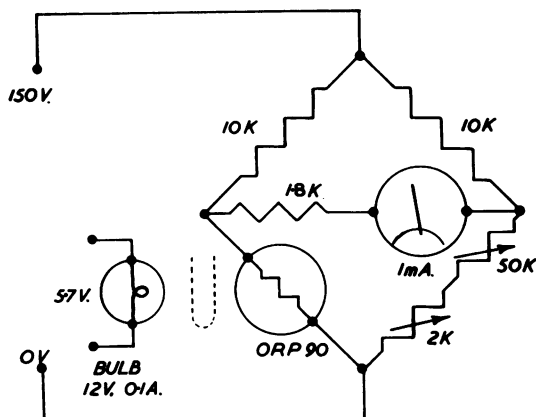


FIG. 3. Circuit diagram.

initial current to be set at a level which permits the recorder to follow the complete lysis cycle. This arrangement allows the recorded current to be set at the same

level whatever the initial density of the clot. Sensitivity of the circuit may be altered by varying the value of the resistor in series with the recorder, but we have found that a 1.8K resistor, with the type of recorder used, was satisfactory for the wide range of euglobulin clots studied. The bridge and lamp are powered by a 150 v. and 5.7 v. stabilized D.C. supply, respectively. The chart recorder for this prototype was an Everett, Edgcombe two-channel recorder, type M.R.S., with an internal resistance of 3,000 ohms and a F.S.D. of 1mA. The paper speed was 3 inches per hour.

RESULTS

Figure 4 shows an actual recording of duplicate clots recorded simultaneously: an increase in amplitude on the chart represents a rise in turbidity. There is an initial decrease in turbidity on adding the thrombin which is followed by an increase as clotting takes place. There then appears to be an optical steady state until lysis occurs which is represented by a fall in turbidity to a new steady level. The euglobulin lysis time is taken as the time from the addition of the thrombin to the point following lysis when a new steady state has been reached.

Figure 5 is a photographic record of euglobulin clots taken at the times indicated on the lower tracings of Figure 4. The contrast between the clot and extraneous fluid was obtained by removal of this fluid and replacing it with the same volume of distilled water. These photographs demonstrate good agreement between the chart record and visualization.

Figure 6 is a record, from the same subject, of euglobulin clot lysis at rest (record A) and one after venous occlusion (record B), showing the expected marked reduction in euglobulin lysis time following venous occlusion.

The success of this prototype has encouraged us to build an instrument which can record the lysis of 12

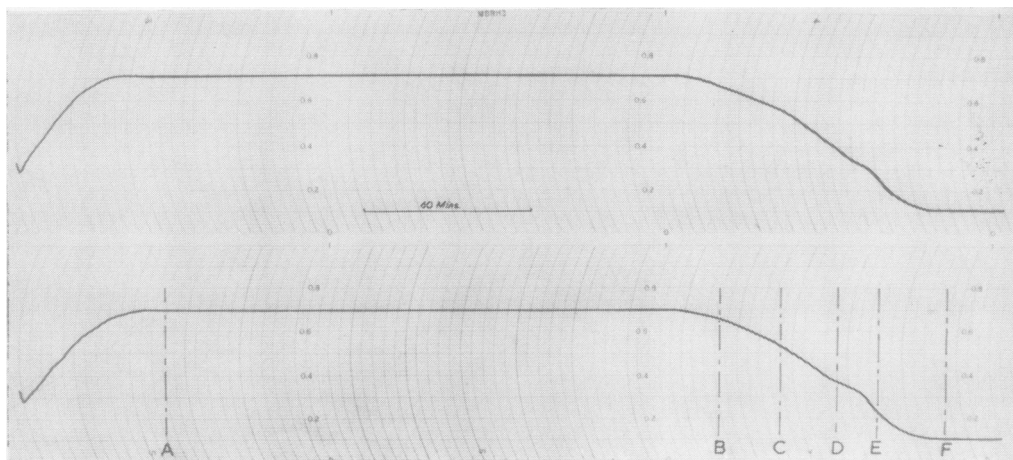


FIG. 4. Recording of duplicate clots done simultaneously.

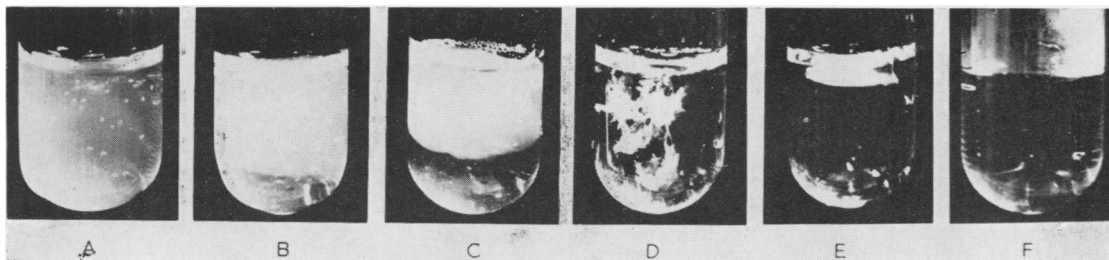


FIG. 5. Photographic record of euglobulin clots in Figure 4.

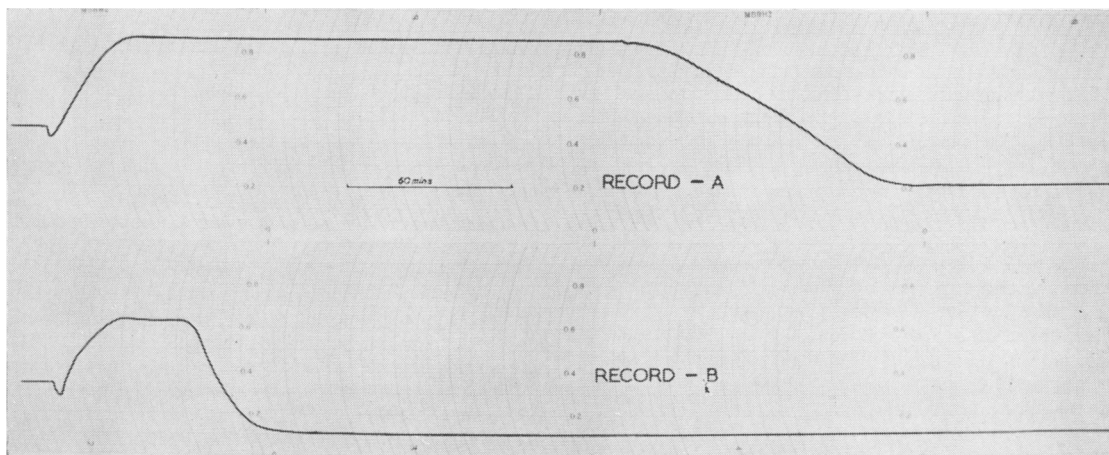


FIG. 6. Record of euglobulin clot lysis at rest.

euglobulin clots simultaneously. It will be welcomed as a most useful tool in our further studies of euglobulin clot lysis.

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