Technical methods

An instrument for recording the lysis time of fibrin clots

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In the course of work on fibrinolysis it became evident that no satisfactory method was available for determining with reproducible accuracy the lysis time of fibrin clots *in vitro*. The technique in use in our investigations involved a visual determination of the point of lysis, and as lysis times varied from a few minutes to several hours, constant vigilance was necessary and a large personal factor was present, different observers obtaining widely different results for the same test.

Various methods of overcoming these difficulties were tried involving mechanical devices, but all suffered from a lack of sensitivity and most were technically difficult to set up. An automatic cine-photographic apparatus has been described by Lackner and Goosen (1959) which takes serial photographs of clots undergoing lysis at predetermined intervals, and while very suitable for use with whole blood clots, this was not considered the best method for recording lysis in purified systems. A method was devised, therefore, which determines the end-point of lysis photoelectrically and with good reproducibility. It also has the advantages that the clot is not subject to mechanical interference and that visual observation is not required. The principle of operation is as follows:

A light beam projected upwards through the bottom of a test tube containing the clot is diffused by the clot and falls on a photo cell. When lysis occurs, the light beam is

no longer diffused and the light collected by the cell is substantially reduced. The change in light falling on the cell is relatively greater at the point of complete lysis than during the time while lysis is proceeding, and the sensitivity of the system is arranged so that this large change brings about an alteration in the circuit conditions causing a relay to operate, thus stopping a clock or other recording mechanism.

DESCRIPTION OF INSTRUMENT

A standardized clot of 2 ml. volume is made in a 5 in. $\times$ $\frac{1}{2}$ in. test tube which is placed in a holder in the instrument. The holder consists of a block with a hole in its centre to take the tube and a small hole at the base through which a light beam passes upwards through the tube. One half of the tube holder is lined by a reflecting surface and on the opposite side a photo cell is mounted parallel to the tube wall. A lightproof tubular cover is placed over the top of the tube to exclude extraneous light. A small thermostatically controlled heating element is applied to the side of the block so that tests can be carried out at 37°C. if required. Light from a 6v. 6w. bulb fed from a stabilized source is directed upwards through the tube. Diffused light falls on the photo cell, the output from which is taken via a 'sensitivity' control to a special micro-ammeter (supplied by Evans Electroselenium Ltd.). This meter has a small vane attached to the hidden end of the pointer and in approximately the mid-scale position this vane interrupts a light path from a small 3v. instrument bulb on to a photo-sensitive transistor. The collector current of this is amplified by a second transistor, the collector current of which is used to operate a sensitive relay. Interruption of the light beam to the photo-transistor thus causes the collector current of the amplifier to rise and pull in the relay. The relay contacts are of single-pole.

FIG. 1. Circuit diagram of instrument for determination of clot lysis times.

change-over type, the 'normally closed' contacts being part of an external counting circuit which may be an electric clock or, as in the present design, a counter operated by contacts which close once every minute.

The 'sensitivity' control is set with a tube of water in the holder (representing a fully lysed clot) so that the meter pointer is opposite a mark on the scale in which position the photo-transistor light beam is interrupted. The clot is then inserted in the holder in place of the water and the scale reading increases above the setting mark. The counting circuit is then started. As lysis occurs the meter reading representing the output from the photo cell gradually falls until, at the point of complete lysis, the vane again interrupts the light path of the photo-transistor, the relay operates and breaks the counting circuit. The second ('normally open') contact on the relay is included in a holding circuit so that once operated the relay cannot be de-energized should the vane move slightly beyond the position where it cuts off the light beam. The holding circuit is broken by a switch for setting purposes. The circuit diagram is shown in the accompanying figure.

Power requirements are very small, the amplifier unit requiring only 10 mA at 6v., and may be supplied from a single 6v. battery or from a simple mains unit. Stabilization is probably not essential for the main light source unless wide mains voltage variations are expected. Test tubes need not be specially selected provided they are of reasonably consistent diameter and gross irregularities are not present in the rounded ends.

Tests carried out with the instrument showed good agreement with results obtained previously with visual tests. Clots were prepared from fibrinogen clotted with thrombin and containing known amounts of streptokinase and plasminogen, the expected lysis times of which were known, and results showed excellent reproducibility.

The instrument has been developed mainly for use in a method for detection and estimation of small amounts of plasminogen, details of which are to be published, and is well suited to work on purified systems. It could equally well be used with plasma clots which may not become completely clear after lysis, but is not applicable to systems using whole blood.

SUMMARY

An instrument is described for photoelectric determination and automatic recording of clot lysis times of use in the investigation of fibrinolytic systems.

The work for which this instrument was devised is part of a research programme into fibrinolysis being carried out at Queen Mary's Hospital for Children, Carshalton, under the direction of Dr. G. T. Stewart, to whom I am grateful for the original suggestion and for much helpful advice. I should also like to thank Evans Electroelenium Ltd. for the loan of a component used in the early stages of development.

REFERENCE

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The preservation of agar gel electropherograms

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The use of microscope slides as supports for the medium during electrophoresis on agar gels makes the preservation of the gel, by drying it on the slide, a simple matter. The following method has been found suitable for preserving larger preparations of agar on which electrophoretically separated proteins have been stained; in the work during which the method was developed the gels measured $90 \times 50 \times 2.5$ mm. and contained 1% agar.

The stained and washed gel is placed between two sheets of cellulose acetate membrane previously wetted in distilled water, and kept overnight in an incubator at 37°C. In this way the gel dries evenly and without cracking. The cellulose acetate electrophoresis strips supplied by Messrs. Oxo Ltd. have been found to be satisfactory. When the agar is dry the sandwich is immersed in distilled water whereupon the cellulose acetate membranes become detached from the agar. The gel is then floated onto the emulsion side of a piece of unexposed, double weight, glossy, bromide, photographic paper which has previously been fixed and washed. The paper and agar are then placed on the polished plate of a photographic glazing machine with the gel in contact with the plate. The normal photographic glazing operation is then carried out and the agar thereby becomes incorporated in the gelatine of the bromide paper so that the final product has the appearance of a photographic print.

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