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Technical methods

Multichannel system for the automatic recording of clot lysis

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Following the successful trials of a prototype two-channel automatic euglobulin lysis time recorder (Cash and Leask, 1965), we were encouraged to build a machine capable of recording the lysis of 12 clots simultaneously. Although the basic principles of the system have remained the same, the problems of observing the lysis of 12 clots simultaneously necessitated fundamental alterations in design.

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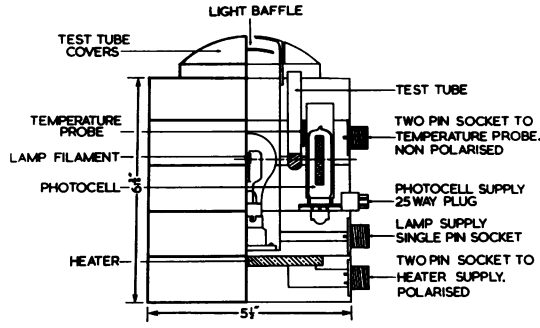
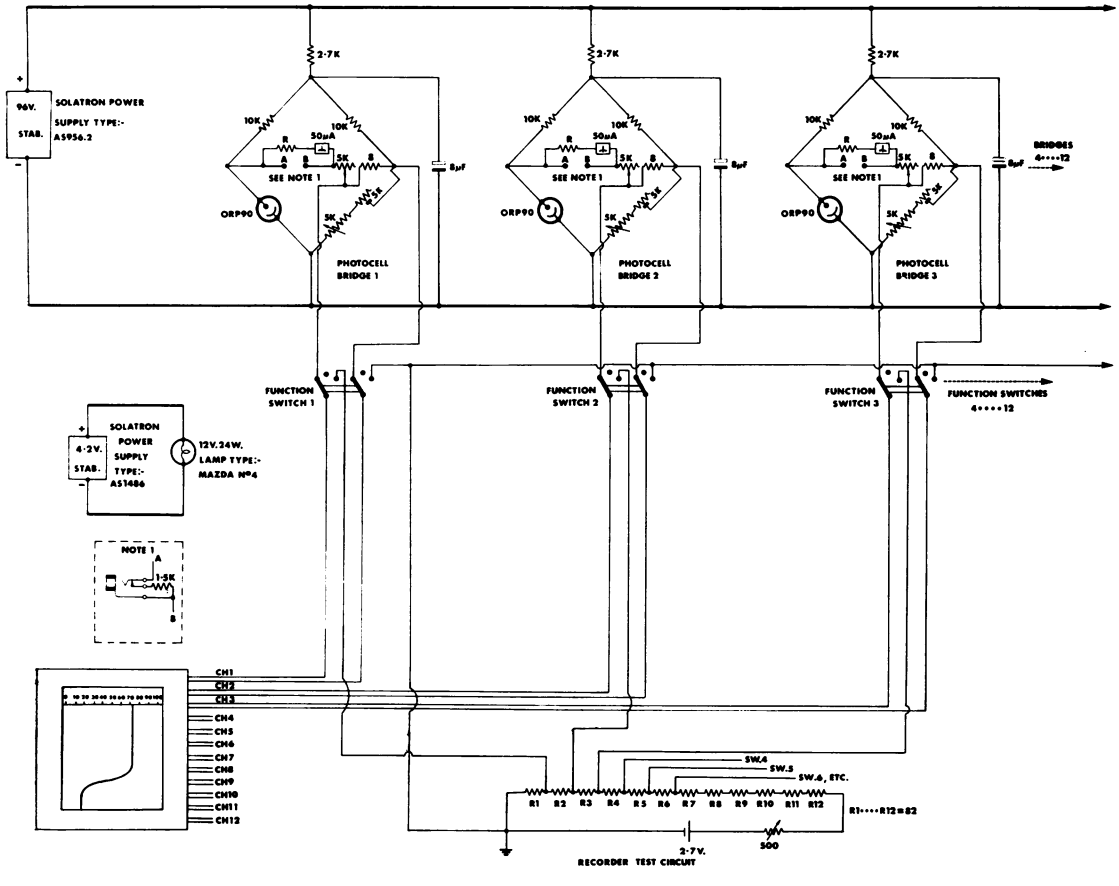


FIG. 1. *Lysis chamber (castle).*

Fig. 2. *Flow diagram of the control unit.*



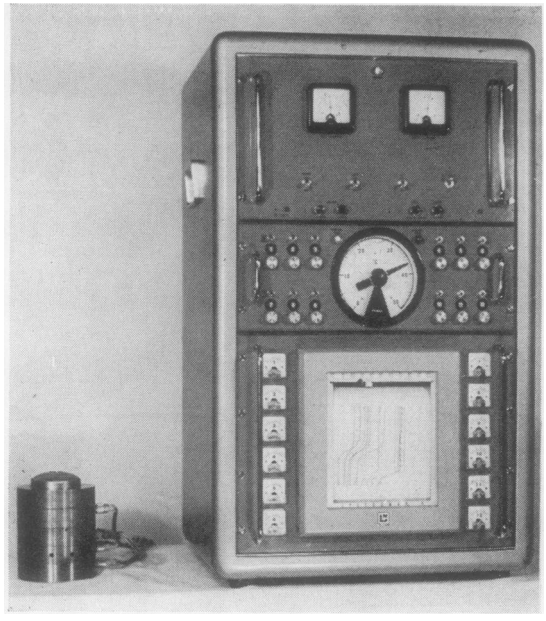


FIG. 3. *The complete assembly.*

DESIGN

LYSIS CHAMBER (CASTLE) (Fig. 1) Temperature control of the prototype lysis chamber was obtained by immersion in a water bath at 37°C. This produced inherent problems of leakage and corrosion. A new design was developed using a solid copper block (thermal conductivity 0·918 CAL/sq. cm./°C) into which holes were machined to accommodate the various components and light paths. A heating element housed in the block maintained the temperature at 37°C. by means of a temperature sensing and controlling system. Insulation of the castle was found to be unnecessary: standing on a bench at room temperature (20°C.) its temperature was maintained at 37°C. with a differential of only 0·2°C.

A common light source was used to illuminate all sample tubes, the photocells being located radially about the single source. The provision of a single light source for 12 clots assured electrical uniformity of the 12 channels.

CONTROL UNIT (Fig. 2) Each photocell was fed to its appropriate bridge network and a 'set position' control using a meter in each bridge enabled the trace for each clot to be set to a different position on the chart paper. This arrangement made the apparatus easy to operate.

RECORDER The outputs from the bridges were coupled

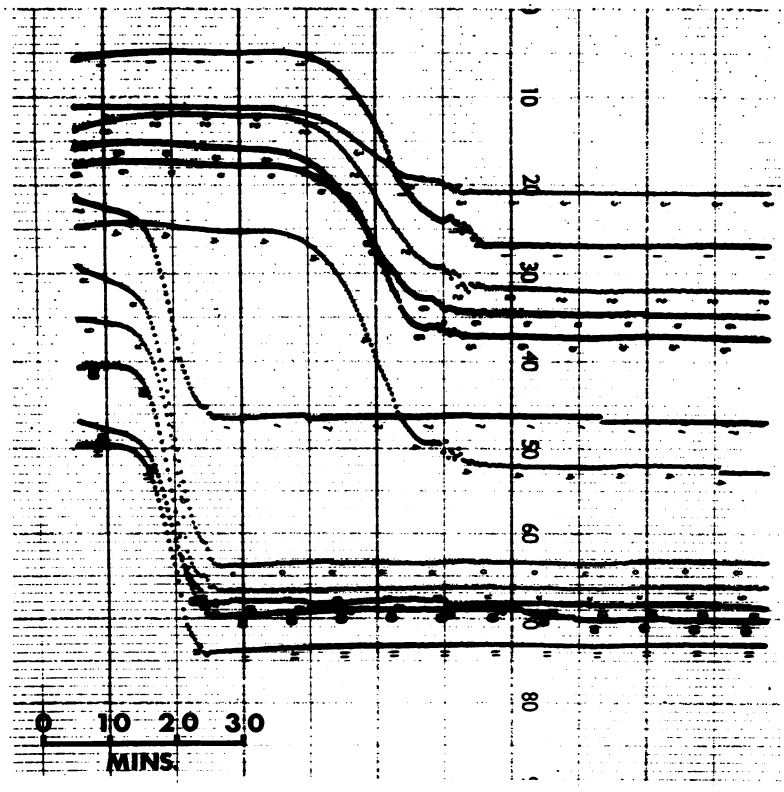


FIG. 4. *Reproduction of an actual record of two sets of six euglobulin clots.*

to a Leeds and Northrup Cleretrend 12 point recorder, provision being made for matching the photocell outputs. The recorder selects each channel in turn and prints a dot corresponding to the deflection from that channel. At the paper speed of 3 in. per hour the resultant trace is a continuous line. Channel identification is by colour and number. This type of recorder was chosen after careful consideration of those available and has proved satisfactory.

Future systems would be improved by the addition of a multipoint selector, now available for this particular recorder, which allows those channels not in use to be kept clear of the chart. In the present machine, a built-in test circuit, when switched on, applies different millivolt potentials to each channel not in use, allowing those channels to trace out steady lines and thus avoid confusion with channels carrying clot lysis information.

A photograph of the complete assembly is shown in Figure 3.

RESULTS

Figure 4 shows the good reproducibility from an actual record of two sets of six identical clots. (The clots were transferred from a water bath at 37°C. to the lysis chamber 80 min. after the addition of thrombin).

Preliminary investigations have shown that the basic instrument can be modified to record the lysis times of the clot system described by Hawkey and Stafford (1964), Nanninga, Zeller, and Maynes (1964), and Mann (1966). Provision has also been made in the circuit to allow a faster paper speed recorder to be connected to any channel to observe clotting times.

CONCLUSION

The instrument described permits the automatic simultaneous recording of the lysis time of up to 12 euglobulin clots. It can also be used for other clot lysis systems. Its usefulness is based upon its objectivity, simplicity of operation, and the economic use of the laboratory worker's time. This instrument is now commercially available from Carmanan Instrumentation Ltd, 2, Hamilton Road, Larkhall, Lanarkshire.

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CORRECTION

The legends to Figures 2 and 3 have been interchanged with their figures in the paper 'Electrophoresis using a constant potential across the separation field', by Dike and Bew (*J. clin. Path.*, **20**, 97) in the January issue of the Journal.

Determination of methaemalbumin in plasma

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Methaemalbumin has a characteristic absorption spectrum which changes on the addition of reducing agents such as dithionite (Fig. 1). This change, which is due to the formation of haemalbumin (Fairley, 1941), can be used as a basis for the measurement of methaemalbumin.

To 2 ml. of plasma (or serum) is added 1·0 ml. of phosphate buffer (1M, *p*H 7·4). The mixture is centrifuged for 5 min. to remove traces of fibrin and any remaining cells and the absorbance measured at 569 mμ in a spectrophotometer such as the Unicam SP500. The solution is then returned to a test-tube and a small amount (about 5 mg.) of solid sodium dithionite added. The tube is shaken gently to dissolve the dithionite and left for 5 min. to allow complete reduction of methaemalbumin. The absorbance at 569 mμ is again determined and the increase computed.

The concentration of methaemalbumin is obtained from a calibration graph. This is constructed from readings obtained with methaemalbumin solutions of known concentration (1 to 10 mg./100 ml., as haemin) prepared by dissolving haemin (British Drug Houses) in a minimum volume of 1 M NaOH and adding this immediately to a solution (4% w/v) of human serum albumin.

Measurement of absorbance before and after addition of dithionite avoids interference due to background colour or to turbidity which invalidate the spectro-

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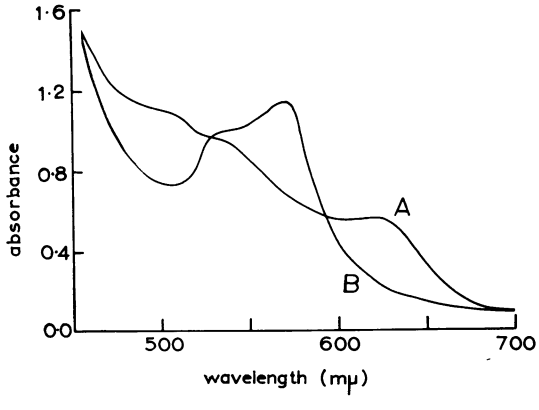

FIG. 1. *Absorption spectra of methaemalbumin (10 mg./100 ml., as haemin): A, before, and, B, after treatment with sodium dithionite.*