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, pH 7.4). The mixture is centrifuged for 5 min. to remove traces of fibrin and any remaining cells and the absorbance measured at 569 m μ 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 m μ 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.

scopic method proposed by Shinowara and Walters (1963). Oxyhaemoglobin, which sometimes accompanies methaemalbumin in plasma, *e.g.*, after recent haemolysis, is converted by dithionite to haemoglobin, with change in absorption spectrum but oxyhaemoglobin does not interfere in this method because, at the chosen wavelength, oxyhaemoglobin and haemoglobin have identical absorption coefficients. However, the change in absorbance of both oxyhaemoglobin and haemoglobin with change in wavelength at this point is considerable and a check should be made, using a solution of oxyhaemoglobin, that the wavelength setting on the spectrophotometer is correct in this respect.

The validity of the method proposed here depends on the absence of any other substance in plasma which is affected by dithionite with significant change in absorbance at 569 m μ . We have examined in this respect a number of normal and pathological plasmas, including some from jaundiced patients, and have found no evidence for such interference.

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Rapid method for the preparation of replicate microslide tissue cultures to facilitate immunofluorescent identification of unknown virus isolates

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Undoubtedly the slowest part of the identification of virus isolates obtained from clinical specimens is their precise identification by the conventional neutralization test in tissue cultures. The problem lies in the wide range of possible antisera which, particularly in the case of entero-viruses, have to be tested before the homologous, infectivity neutralizing serum is found. Hambling, Davis, and Macrae (1963) and Lim and Benyesh-Melnick (1960) have both reported schemes for the production of entero-virus serum pools to facilitate identification of entero-virus isolates. Both systems have the disadvantage of requiring tedious neutralization tests in tissue culture both at the serum 'pool' stage, and subsequently to distinguish the specific reacting serum within the pool.

The immunofluorescent technique offers a potential speed-up in identification, wherein the same enterovirus pools of rabbit antisera may be used in an indirect, two-stage test with fluorescein-isothiocyanate conjugated anti-rabbit serum in the second layer of the fluorescent 'sandwich'. This technique is well known as a method of tracing viruses growing in tissue culture monolayers, invariably on cover slips placed in Leighton tubes. The Leighton tube cover slip method has several important disadvantages in practice for the identification of unknown agents.

First the cover slips are extremely fragile, and are consequently difficult to manipulate in numbers. Secondly, the problem of identification of an unknown agent by immunofluorescent methods can best be tackled by constant reference to control monolayers, infected but treated with normal rabbit serum in the middle layer of the fluorescent 'sandwich'. Frequent comparison is also necessary with the results obtained with other antiserum pools, in the middle layer, for the criteria of positivity are best established by a process of comparison for any unknown agent.

The cover slip method necessitates frequent changes of microslide for cross reference. These are time consuming and comparison from one slide to another may give misleading results because of batch variations from tube to tube.

The method to be briefly described here makes use of tissue culture monolayers grown in glass rings on microslides. These are easy to manipulate, simplify immunofluorescent staining, and greatly facilitate comparison with control spots for comparative purposes. The method

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