TECHNICAL METHODS

A Simple Capillary Viscometer

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The plasma proteins are responsible for the viscosity of plasma being greater than that of distilled water. Lawrence (1950) described a method for estimating the total and the differential viscosity of the plasma proteins. Changes in the total plasma viscosity (T.P.V.) have been considered more indicative of organic disease than of elevation of the erythrocyte sedimentation rate (E.S.R.) (Houston, Harkness, and Whittington, 1945; Benson and Goddard, 1954; and others); Lawrence showed that changes in the viscosity of the fibrinogen and gamma globulin fractions were an even more sensitive indicator than changes in the T.P.V. He reviewed the most reliable methods for determining the T.P.V., and gave a description of his modification of the Ostwald viscometer. He stressed the necessity of maintaining a constant temperature ($\pm 0.3^\circ \text{C.}$) for all viscometry. In practice this has been achieved by means of a thermostatically controlled water-bath. The following instrument was designed to dispense with the need for this costly piece of equipment without significant loss of accuracy.

Description of Viscometer

Principle.—A small water-jacket is used to maintain the viscometer at constant temperature. Temperature regulation of the water in the jacket is achieved very simply by the removal of a small volume of water and by the addition of colder or warmer water as necessary to maintain a temperature of 20°C. ($\pm 0.3^\circ$).

Materials.—The viscometer capillary is of "veridia" glass 7.5 cm. long, internal diameter 0.5 mm. ($\pm 0.01 \text{ mm.}$), external diameter about 4 mm. This is connected by means of rubber pressure tubing to a 1-ml. Ostwald pipette, the end of which is shortened to correspond in external diameter to that of the capillary. The ends are filed smooth and square, and should be touching each other inside the pressure tubing. Marks A and B are etched above and below the bulb (see illustration). A square "perspex" water-jacket ($5 \times 5 \times 16 \text{ cm.}$) is constructed to house the pipette, holes being drilled also for a Van Slyke thermometer reading to 0.2°C. , and for

* Obtainable from Messrs. Chance Bros., Glassworks, Smethwick, Birmingham, 40.

a long wide-bore serum needle. The whole instrument is clamped to a metal stand. Other equipment necessary is an accurate stop-watch, a 20-ml. syringe, a number of 5-ml. "pyrex" beakers, and some small (1-ml.) rubber teats.

Methods.—Plasma is obtained (as described by Lawrence, 1950) from 5 ml. of venous blood, and pipetted into a "pyrex" beaker. The viscometer pipette is filled by means of a teat through the capillary (which is left *in situ* at all times), to a point well above mark A. If necessary the temperature in the water-jacket is adjusted to 20° C. ($\pm 0.3^\circ$) using the 20-ml. syringe either with hot tap water or with water that has been refrigerated. Sufficient air is introduced at this operation to ensure thorough mixing of the water in the jacket. As the external temperature is usually close to 20° C., two minutes are sufficient for the temperature of the plasma to stabilize, though in very warm or cold weather three minutes must be allowed for this operation. At the end of this time, the teat is removed and the time taken for the plasma under test to flow between marks A and B is recorded. The mean of two determinations (three if the sample is abnormal) is taken and compared with the (known) time for distilled water (viscosity=1) under identical conditions. Then, from Poiseuille's law, the relative viscosity of the test solution can be obtained from this comparison:

Time for test solution (in seconds)
Time for distilled water (in seconds) = relative viscosity of the solution
if the specific gravity of the test solution is ignored (for plasma, this introduces an error of less than 1%).

The sample of plasma is then clotted by the addition of 0.015 ml. (measured in a haemoglobin pipette) of 40% calcium chloride, care being taken to avoid excess, as this inhibits clotting (Lawrence, 1950). After incubation for half an hour at 37° C. serum is expressed from the clot (using a glass rod) and its viscosity is then measured in exactly the same way as that described for plasma. The difference between these two measurements gives the "fibrinogen viscosity." (The other plasma proteins may be precipitated with ammonium sulphate as described by Lawrence, but in this study only fibrinogen viscosities were determined.)

To clean the instrument the pipette is filled and emptied forcibly through the capillary with normal saline solution (by means of a teat). The lower end of the capillary is then attached to a suction pump, and distilled water is poured from a beaker into the top of the pipette. In the same way, the instrument is dried with spirit and acetone. (There is no need to remove the instrument from its stand for these operations.) The instrument should always be cleaned after a sample of high viscosity; also when following the measurement of plasma viscosity by that of serum and *vice versa*. It is not necessary to clean the instrument after the measurement of the viscosity of normal samples of plasma (or serum). The time for distilled water (which should be constant, ± 0.1 sec.) should be estimated at the beginning and end of a

series of determinations and also if necessary after abnormal samples have been tested.

Results

Blood was obtained from 45 apparently healthy blood donors and medical students. Using Lawrence's method of expressing results, that is, to multiply by 100 and to calculate viscosity differences (i.e., if plasma viscosity is 1.71, then the plasma viscosity

TABLE I
VALUES FOR PLASMA AND FIBRINOGEN VISCOSITIES
FOUND IN 45 NORMAL SUBJECTS

	Plasma	Fibrinogen
Mean	71	14
Maximum	81	20
Minimum	56	5
Standard deviation	7	3.5

difference is 71), the results set out in Table I were obtained for normal total plasma and fibrinogen viscosities.

Discussion

These results are in agreement with those of other workers determining the plasma viscosity at 20° C. (Miller and Whittington, 1942; Woodmansey and Wilson, 1948). It should be emphasized, however, that such results cannot be directly compared, for each capillary has its own characteristic "shear" for colloidal solutions. Nor is it possible to calibrate this instrument described against Lawrence's viscometer "O," for his measurements are made at 25° C. with correspondingly faster times. If Lawrence's results for plasma are increased by 12.5% (Table II) the

TABLE II
THE CORRELATION WITH LAWRENCE'S VALUES

	Lawrence's Values for Plasma	Corrected to 20° C.	Lawrence's Values for Fibrinogen
Mean	58	65	13
Maximum	71	80	19
Minimum	44	50	5
Standard deviation	10		2

factor which he states represents the approximate increase of the values at 20° C. compared with those at 25° C. (the values for fibrinogen are very similar at the two temperatures), it can be seen that they bear a close relationship to the values given in Table I.

The results recorded here apply to this one instrument only, but similar instruments will give similar though not necessarily identical results, the upper limits of normal being obtained from the study of a sufficient number of normal plasmas.

The regulation of the temperature in the water-jacket by hand represents the simplest arrangement that is compatible with accurate work. If many determinations were undertaken, the "perspex" bath could be enlarged and a small thermostat added. The

abolition of the small, though constant, dead space between the end of the Ostwald pipette and the capillary is another possible refinement. However, the experimental error for the instrument as shown by repeat experiments does not exceed $\pm 0.7\%$ (cf. Lawrence's method where the error is $\pm 0.4\%$). No difficulties were found in maintaining a constant temperature in the water-jacket for the period of the test under conditions of high (or low) external temperature. Owing to the rapid rate of flow of fluid through the capillary, no significant error is introduced because the capillary itself is not surrounded by the water-jacket.

The instrument described has the advantage that, being straight, it is not subject to the filling errors that may occur in U-type viscometers. It is of course liable to such sources of error as air bubbles and particulate matter in the fluid under test, and to timing errors, but these are avoided by good technique as are those caused by haemostasis at the time that the blood is collected. Other types of straight viscometer have been described. Repeat determinations are impossible with the instrument invented by Mann (1948), as the sample is lost in a water-bath. Woodmansey and Wilson (1948) prevented this occurrence by enclosing their viscometer in a test-tube before putting it into a water-bath, but such an arrangement does not dispense with the need for a water-bath. It can be seen that the advantages of employing a water-jacket as opposed to a water-bath are real.

Lawrence (1950) has demonstrated the value of the measurement of the total and differential viscosities of the plasma proteins, and with the capillary viscometer described such observations are possible without requiring elaborate equipment.

Summary

A simple capillary viscometer is described, and the values for total plasma and fibrinogen viscosity found in 45 healthy subjects are recorded.

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The Determination of Ethylene Diamine Tetra-acetic Acid (E.D.T.A.) in Urine and Serum

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It is now generally recognized that intravenous infusions of ethylene diamine tetra-acetic acid (E.D.T.A.) as the calcium disodium salt readily remove excess lead from the body in plumbism. Up to 4 g. of the salt may be employed at each session. Leckie and Tompsett (1958) have made a study of the use of this substance in plumbism both for therapeutic and diagnostic purposes. It appeared desirable to carry out simultaneous determinations of E.D.T.A. and lead in the urine, since it was found that the excretion of the former may be delayed in the presence of the renal disease which may accompany plumbism. Similar studies would be of value in the assessment of the use of E.D.T.A. orally, concerning which there is little information.

Darbey (1952) has described a method for the determination of E.D.T.A. in urine. Excess of a nickel salt is added, a very stable nickel chelate compound with E.D.T.A. being thereby formed. Excess nickel is removed by precipitation with dimethylglyoxime, the nickel chelate compound remaining in solution. The nickel content of the filtrate, which is a function of the E.D.T.A. content, is then measured. The reagent employed by Darbey for the determination of nickel, which he used directly, is, however, unobtainable. The following is a modification of the method in which the nickel content of the filtrate is determined by the conventional dimethylglyoxime method after destruction of organic matter (cf. Sandell, 1944).

It was considered desirable to measure the E.D.T.A. content of serum. The question of the deproteinization of serum appeared at first to create a problem, but this was overcome by using one of the well-known metal precipitating techniques, nickel being used in place of zinc sulphate. The filtrates usually contain sufficient nickel to combine with the E.D.T.A.

Reagents

- (1) 1.33% (w/v) solution of nickel sulphate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) in water
- (2) Ammonia, sp. gr. 0.88
- (3) 1.5% (w/v) dimethylglyoxime in ethanol
- (4) Concentrated sulphuric acid, Analar
- (5) Perhydrol, Analar
- (6) Saturated bromine water