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

(7) Deproteinizing solutions (serum)

A. 12.5 g. $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, 125 ml. of 0.25 N. sulphuric acid, water to 1,000 ml.

B. 0.75 N. sodium hydroxide

(8) Standard solution of ethylene diamine tetra-acetic acid (1 mg./ml.): 0.1476 g. of $[\text{CH}_2\text{N}(\text{CH}_2\text{COOH})\text{CH}_2\text{COONa}]_2 \cdot \text{H}_2\text{O}$ is dissolved in water and the volume made up to 100 ml.

Procedure

Urine.—To 10 ml. of urine is added 1.5 ml. of 1.33% nickel sulphate solution and the mixture allowed to stand at room temperature for 10 minutes, then 0.5 ml. of ammonia (sp. gr. 0.88), is added, followed by 1.5 ml. of a 1.5% ethanolic solution of dimethylglyoxime. After standing for 10 minutes at room temperature, the mixture is filtered (Whatman No. 42 filter paper).

One millilitre of the filtrate is measured into a round-bottomed digestion flask followed by 6 drops of concentrated sulphuric acid. Organic matter is destroyed by heating and the addition of perhydrol.

The clear digest is diluted to 10 ml. with water, 5 drops of saturated bromine water added, and the solution well mixed. Four drops of ammonia (sp. gr. 0.88) are added, followed by 0.5 ml. of a 1.5% ethanolic solution of dimethylglyoxime, the solution being shaken after each addition.

A blank (10 ml. water) and a standard (2 mg. E.D.T.A. in 10 ml. water) are prepared at the same time, the complete procedure being carried out. After standing for 15 minutes at room temperature, the colours developed in the standard and unknown are read against the blank at 515 $\text{m}\mu$.

Serum.—To 1 vol. serum is added 8 vol. of reagent (7A) followed by 1 volume of 0.75 N. sodium hydroxide. The mixture is allowed to stand at room temperature for 15 minutes and then filtered (Whatman No. 42 filter paper).

Sixteen millilitres of the filtrate are digested with sulphuric acid and perhydrol as described for urine. The nickel content of the digest is similarly determined.

A blank and a standard (200 μg . E.D.T.A.) are prepared at the same time.

Discussion

The results shown in the Table indicate that satisfactory recoveries of added E.D.T.A. could be made from urine and serum.

TABLE
RECOVERY OF E.D.T.A. ADDED TO URINE AND SERUM

	E.D.T.A. Added (mg.)	E.D.T.A. Recovered (mg.)	Recovery (%)
<i>A. Recovery Added to Urine</i>			
1	0.25	0.22	88
2	0.50	0.47	94
3	0.75	0.69	92
4	1.00	0.95	95
5	1.50	1.52	101
6	2.00	2.08	104

Volume of urine used, 10 ml.

Initial content of E.D.T.A. in urine, nil.

	E.D.T.A. Added (μg .)	E.D.T.A. Recovered (μg .)	Recovery (%)
<i>B. Recovery Added to Serum</i>			
1	100	92	92
2	200	208	104
3	250	240	96
4	300	320	107
5	400	390	98
6	600	590	98

Volume of serum used, 4 ml.

Initial content of E.D.T.A. in serum, nil.

Substances in urine which may simulate the chelating property of E.D.T.A. were found never to amount to more than 27 mg./litre.

In the examination of the E.D.T.A. content of urine by the above procedure, Beer's law is followed up to concentrations of 0.5 g./litre urine. When concentrations above this figure are encountered, suitable dilutions must, as a preliminary, be made. This presents no difficulty in the examination of 24-hour collections of urine, since such should, under most conditions, contain practically all the administered dose, provided an intravenous infusion has been employed. In other cases some degree of trial and error will be necessary.

This problem will not arise in the case of serum examinations, since concentrations seldom exceed 10 mg./100 ml.

Summary

A method is described for the determination of E.D.T.A. in urine and serum.

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