Anticoagulants

Heparin, which inhibits the formation of thrombin from prothrombin, is the most satisfactory anticoagulant since it produces no change in the composition of the blood. About 2 mg. of the sodium, lithium or calcium salt per 10 ml. of blood is used. Nevertheless, because they are cheaper, oxalates and citrate have been most used. It is important not to use more of these salts than is necessary, otherwise appreciable changes in the distribution of water between cells and plasma may result, and they may interfere with some determinations.

Potassium or Sodium Oxalate. These act by precipitating the calcium. Potassium oxalate has been most commonly used of the oxalates since it is the most soluble. Ten to 20 mg. per 10 ml. blood are required to prevent clotting, but 20 to 30 mg. should be used. Technicians making up tubes should become familiar with this amount. The salt is used finely powdered. Peters and Van Slyke (1932) recommended preparing a 30 per cent. solution of neutral potassium oxalate, recrystallized if necessary, and adjusted to a *p*H of 7·4 ± 0·2 by adding potassium hydroxide or oxalic acid solution. An appropriate amount, 0·1 ml. for 10 ml. of blood, is measured into the tube which is to hold the blood, is spread on the walls as a film by rotating the tube, and then dried in a stream of air. The blood then mixes easily and rapidly with the oxalate and very little further shaking is necessary. This is specially useful for specimens to be taken under oil.

Ammonium Oxalate (3 parts) + Potassium Oxalate (2 parts) has been used (Heller and Paul, 1934) in certain hæmatological investigations since it does not affect the red cell volume. One mg. per ml. has been suggested, but 2 mg. per ml. may be necessary to ensure that clotting does not occur. Ammonium oxalate should not be included in the anticoagulant for any determination in which ammonia is being produced, for example, in urease urea estimations, Kjeldahl protein and non-protein nitrogen determinations.

Sodium Citrate. Citrate does not precipitate the calcium but converts it into a non-ionized form. Nevertheless citrated plasma is not as satisfactory as serum for the determination of calcium. About 30 mg. of sodium citrate per 10 ml. of blood will prevent clotting. About twice this amount should be used. Appreciable withdrawal of water from the cells results.

Ethylenediaminetetra-acetic acid and its salts act by chelating calcium ions. The dipotassium and dilithium salts are most used, the former being preferred to the disodium because of its greater solubility ; 10–20 mg. per ml. is satisfactory.

Sodium Fluoride also acts as an anticoagulant, but larger amounts are required than of either citrate or oxalate, so that it is chiefly used as a preservative, when it is usually mixed with potassium oxalate (see p. 7). It is not recommended as an anticoagulant for as much as 100 mg. per 10 ml. of blood is necessary. Fluoride is an enzyme poison.

substances is convenient, particularly if it is desired to extract some blood constituent into the organic solvent. Thus, for example, ethanol ether mixtures are used to precipitate proteins and extract fats and cholesterol.

Measuring Blood

Although ordinary pipettes can be used to measure plasma or serum, they cannot be used with whole blood because of its greater viscosity, without introducing appreciable error. For this reason, pipettes used for measuring whole blood should be calibrated to contain the amount of blood which they are designed to measure, and the blood should be washed out of the pipette. With pipettes measuring small volumes of blood, such as 0·1 or 0·2 ml., the blood is usually delivered into a measured amount of a solution and the pipette washed out by sucking the liquid up into it, and blowing it out, until all the blood is washed out. If the blood can be run into one part of the fluid and another part be used for washing out the pipette before the whole is mixed, it is even less likely that a small amount of blood will be left behind in the pipette.

Small blood pipettes should be calibrated by weighing the amount of mercury they can contain. The specific gravity of mercury is 13·57 at 10° C., 13·56 at 14° C., 13·55 at 18° C. Because of the difference in the shape of the meniscus a slightly smaller volume of mercury is weighed than the volume for which the pipette is being calibrated. This varies with the diameter of the bore at the graduation mark. Examples are : 0·0005 ml. for a bore of 1·0 mm., 0·0015 ml. for 1·5 mm., 0·0025 ml. for 2·0 mm., and 0·0040 ml. for 2·5 mm. A correction curve is given by Peters and Van Slyke (1932). Once the required amount of mercury has been weighed out in a small beaker, it can be used to calibrate a whole series of pipettes by drawing up the mercury or transferring it from one pipette to another. The mercury should be clean and dry.

When using pipettes delivering larger volumes, the blood should be washed out by passing water, or some solution used in the test, through the pipette. In calibrating such pipettes a suitable volume of water is weighed, the pipette filled to a provisional mark from this, and the remaining water reweighed. The mark is adjusted and the process repeated until an accurate calibration is achieved.

In pipetting whole blood, care should be taken to see that the cells are evenly distributed through the plasma. This is particularly important if the blood is being used for the determination of some constituent which is present in different concentrations in the cells and in the plasma.

URINE

Collection of Urine Specimens

Single specimens of urine are used for ward examinations and for most qualitative tests, but for quantitative work twenty-four-hour specimens are best employed, except when collecting specimens as

Schmidt (see, for example, Cammidge, 1914). This contains approximately 102 grams of protein, 111 grams of fat, 190 grams of carbohydrate, and has a calorie value of 2,230. The diet should be a bland one with a low residue. A number of rather similar diets have been used. Thus Wollaeger, Comfort and Osterberg (1947) used a diet containing 102 grams fat, 117 grams protein, and 270 grams of carbohydrate, with a calorie value of 2,532. Nothman (1951) gave a modified Schmidt diet which contained 105 grams of protein, 135 grams fat, 180 grams carbohydrate, calories 2,424.

COLORIMETRY

Owing to a number of factors such as the small amount of many of the substances which have to be determined in clinical biochemistry, and the difficulty of isolating them, colorimetric methods are frequently used. In the space available here it is not possible to give a full account of the theory of the subject and of the instruments used, but only to deal briefly with the more immediately relevant points. For more detailed accounts see Müller (1939), Snell and Snell (1948), Lothian (1958), Vogel (1961), Willard, Merritt and Dean (1965) and Edisbury (1966). In addition, full descriptions of the instruments referred to below, with accounts of the method of using them, are usually avilable from the manufacturers.

Visual Comparison

One of the simplest methods, which can be used when no instrument is available, is to match the unknown against a set of standards, using test tubes of similar diameter. Values intermediate between successive standards can be assessed. The accuracy clearly depends on the number of standards used. If a series of standards has to be prepared each time, the method is more troublesome than the use of an instrument, but if a set of standards can be kept, the method is convenient and can give results which are sufficiently accurate for clinical purposes in many cases. This principle is used in the *Lovibond Comparator* in which the coloured solution is compared with permanent standard discs comprising a series of coloured glasses. These are available for most of the commoner methods used in the hospital laboratory. For a full list of these see, for example, Messrs. British Drug Houses' catalogue.

Another simple method is the *dilution method.* In this case a standard is prepared and the standard or the unknown diluted until they match in intensity when observed in similar test tubes by the naked eye. The concentration of the unknown can be easily calculated from the amount of dilution required. This is less accurate than the previous method since dilution may alter some of the conditions which influence the intensity of the colour, and this will only affect one of the colours which are being compared.

Visual comparison was employed in the visual colorimeters so widely used for many years. These have now been superseded by photoelectric instruments.

Filters. Ilford spectrum filters (603–609) and bright spectrum filters (621–626) are widely used. These are of gelatin containing suitable dyes. Curves which show the percentage transmission at different wave-lengths for these filters are given in Fig. 9. It will be seen that maximum transmission of the spectrum filters is as follows:

600	Spectrum	deep violet	420 millimicrons
601	,,	violet	430 ,,
602	,,	blue	470 ,,
603	,,	blue green	490 ,,
604	,,	green	520 ,,
605	,,	yellow green	550 ,,
606	,,	yellow	580 ,,
607	,,	orange	600 ,,
608	,,	red	680 ,,
609	,,	deep red	700 ,,

The curves also show that these filters have narrow transmission bands and so transmit approximately monochromatic light.

Sometimes it is necessary in order to get full scale deflection of the galvanometer to use filters with a broader wave band so that they transmit more light. Examples among Ilford filters are : Minus blue, 110 ; minus red, 302 ; minus green, 503 ; tricolour red, 204 ; tricolour blue, 304 ; tricolour green, 404. This latter and narrow cut tricolour red, 205, and a blue filter micro 2, No. 303, are supplied with the EEL Portable Colorimeter. The per cent. transmission curves for these filters are shown in Fig. 10. Tables giving the per cent. transmission of Ilford filters for all wave-lengths from 350–720 millimicrons are given in the booklet " Ilford Filters," published by Ilford Ltd.

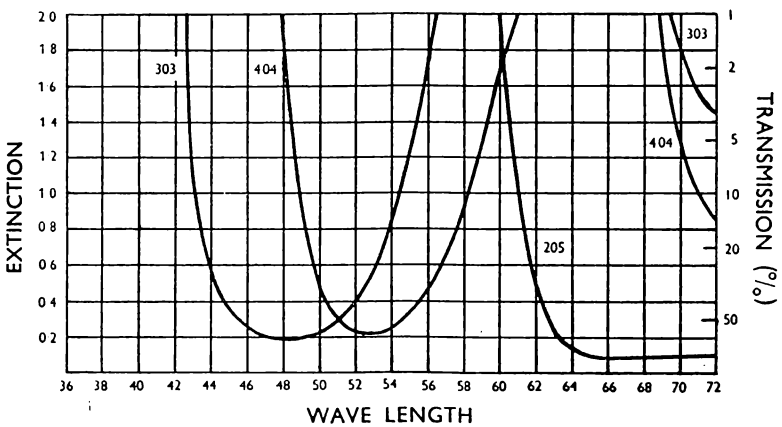

FIG. 10. Transmission Curves of Ilford Filters used with the EEL Portable Colorimeter.

(Abscissæ × 10 = millimicrons.)