A SIMPLE METHOD FOR THE ESTIMATION OF TOTAL PROTEIN CONTENT OF PLASMA AND SERUM.[1] I. A FALLING DROP METHOD FOR THE DETERMINATION OF SPECIFIC GRAVITY

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In 1926, Barbour and Hamilton described a falling drop method for determining specific gravity (1). They used a mixture of xylene and bromobenzene in a tube 30 cm. in length. With the use of standard solutions and nomograms, they obtained an accuracy within 1×10^{-4} in specific gravity. Guthrie and Kruse in their unpublished work, found that a mixture of methyl salicylate and mineral oil permitted the use of a shorter tube (2). The present method is an extension of their work.

A mixture of methyl salicylate and mineral oil was chosen because such a mixture has a higher viscosity so the tube length can be shortened and a more uniform temperature obtained throughout the oil. In addition, it is less volatile than the xylene-bromobenzene mixture. (Methyl salicylate B. P. 222.2° C., mineral oil 190.6° C.; m-xylene 138.8° C., bromobenzene 156.2° C.).

The ultimate aim was to obtain the specific gravity of blood serum at 25.0° C. referred to water at 25.0° C., *i.e.*, 25°/25°. This temperature was chosen because it was most convenient when the determinations were done at room temperature.

THEORY

Stokes' law indicates that the rate of fall of a small solid sphere in a viscous fluid is a function of the radius and specific gravity of the sphere, the specific gravity and viscosity of the fluid, and the acceleration due to gravity. This method provides that a drop of fluid may be timed as it falls through an oil with which it is not miscible. The radius of the drops is kept constant by the use of a calibrated pipette to deliver a definite volume. The specific gravity and viscosity of the oil being kept constant, the specific gravity of the drop may be determined from its rate of fall in the oil.

MATERIALS

A mixture of synthetic methyl salicylate and heavy California mineral oil is used. The proportions vary according to the range which is desired. For serum and plasma, we have found a mixture of specific gravity 1.0130, 25°/25° C. most useful.

This mixture is placed in a glass tube 15 to 16 cm. long, having a uniform inside diameter of 14 mm. This tube is etched with two rings which are exactly 10 cm. apart, the lower one being 15 mm. above the bottom of the tube. The tube is held upright in a glass water jacket which is equipped with a stirring rod and a thermometer to read 0.1° C. between 20° and 30° C. This tube

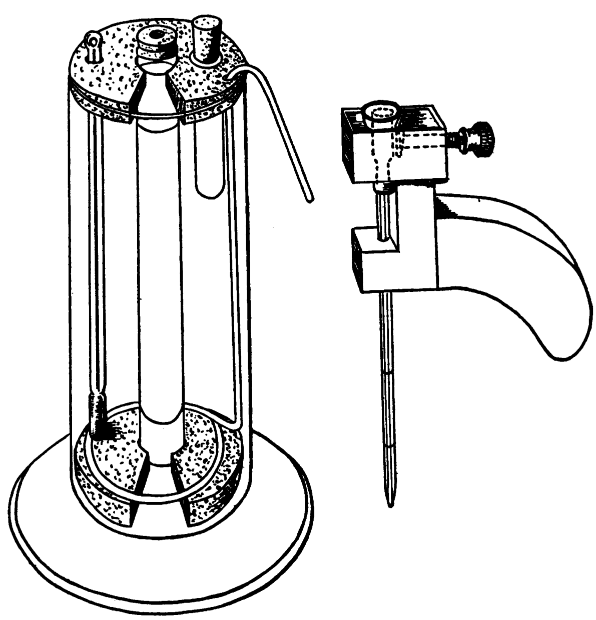

FIG. 1. APPARATUS FOR DETERMINATION OF SPECIFIC GRAVITY BY FALLING DROP METHOD

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is filled to 15 mm. from the top with the oil and provided with a one-holed disc to center the pipette when the drop is released. Within the water jacket, there is a small test tube in which the serum may obtain the same temperature as the oil (Figure 1).

The pipette is made from unconstricted capillary tubing calibrated to deliver 0.015 cc. (15 c.mm.) between two marks. A wooden block is provided which contains a rubber bulb and a metal screw. The pipette fits into the bulb. When the screw is turned, it presses upon or releases the bulb and this permits fluid to be drawn into or discharged accurately from the pipette.

A stop watch which reads in tenths of a second is used.

METHOD

The fluid to be tested is placed in the small test tube until it comes to the temperature of the water bath. It is then drawn into the pipette to the level of the first ring, and the tip of the pipette is placed under the surface of the oil in the tube. A drop of 15 c.mm. is delivered beneath the surface of the oil, and as the pipette is withdrawn, the drop remains in the oil and falls. The time required by the drop to pass between the two rings on the tube (10 cm.) is determined with the stop watch.

Calibration. Standard solutions of sodium chloride are made so that they cover the range 1.0140 to 1.0370, 25°/25° as determined by pyknometry (4). A large pyknometer (about 65 cc.) enables one more easily to obtain accuracy of the order of 10^{-5}. Ten determinations of the falling time are then done on each solution and the average falling time plotted against the specific gravity of the standard as in Figure 2. The temperature must be kept at 25.0° C. during the standardization. Owing to difference in viscosity, particularly in various samples of mineral oil, slight differences in results are obtained unless each new preparation is standardized in this manner.

Temperature correction factor. As the temperature rises, the viscosity and specific gravity of the oil decrease. The specific gravity of the fluid to be tested also decreases as the temperature rises. If the temperature of the fluid to be tested is kept at the same temperature as the oil, all the variables can be accounted for at one time. In

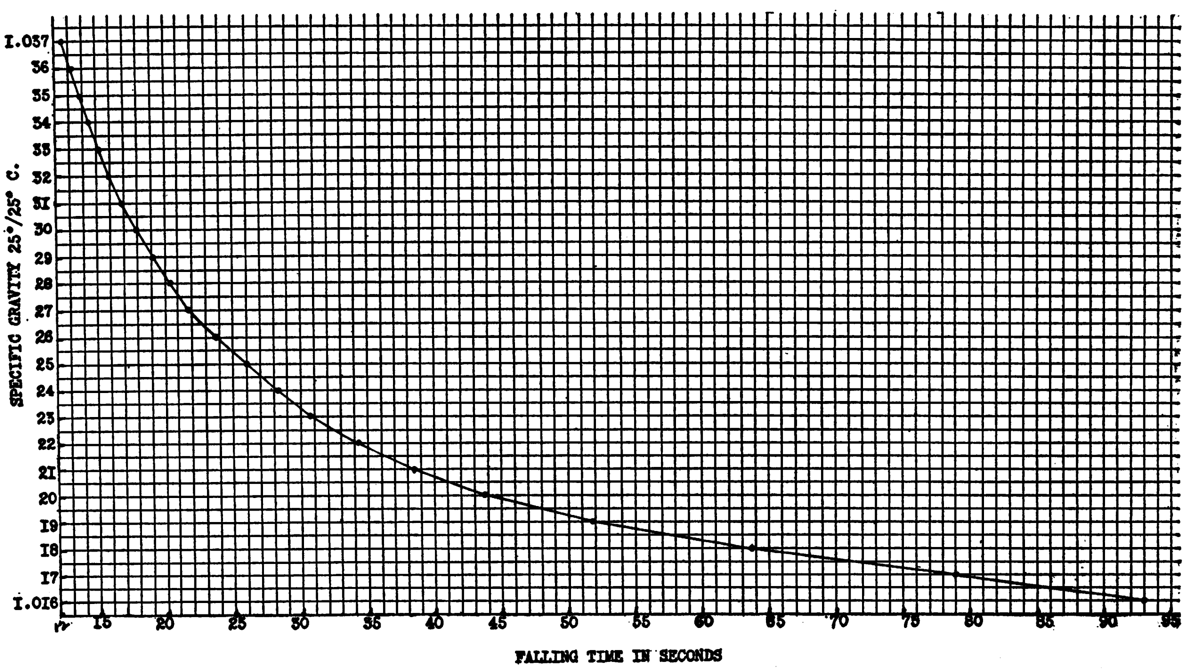

FIG. 2. RELATION OF FALLING TIME TO SPECIFIC GRAVITY

Graph paper must be used which will permit the reading of tenths of a second and specific gravity to 1×10^{-4}.

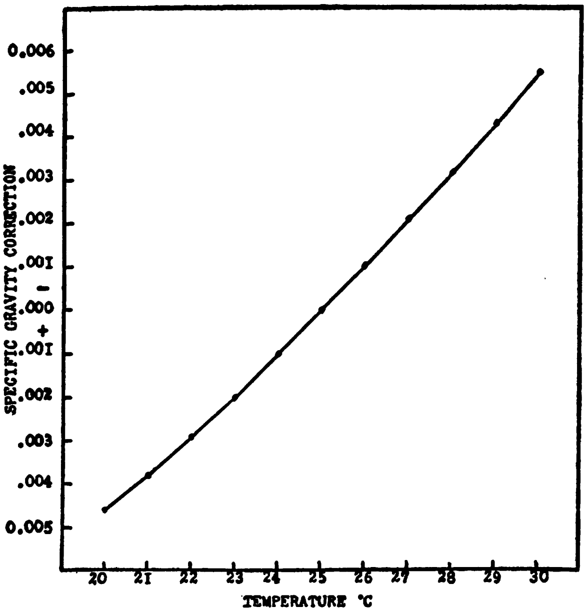

FIG. 3. SPECIFIC GRAVITY CORRECTION

The ordinate gives the value which must be added (+) or subtracted (—) from the specific gravity read from Figure 2 for the falling time determined at any temperature represented in the abscissa.

Figure 3 is given the correction which must be made on the specific gravity as read from Figure 2 for the falling time at any temperature between 20° and 30° C. It is not necessary to reconstruct this chart for each new preparation of oil.

Figure 4 illustrates the effect of temperature on the specific gravity of serum alone. The weight of pooled serum which filled a large pyknometer at various temperatures ($X°$ C.) was determined and referred to the weight of the same volume of water at 25.0° C. The resultant specific gravity $X°/25°$ was plotted against the temperature at which the serum was weighed. Thus, it may be seen that the temperature of the serum need be only within 0.5° C. of that of the oil in order to obtain accuracy within 1×10^{-4} in specific gravity.

Error of the single drop. In the application of this method, we have used an average of 3 determinations. The accuracy is, however, not appreciably affected if only one determination is done with care. Thus the timing of drops in the range of 13 to 30 seconds checks within 0.2 second. When drops take longer than 30 seconds to fall the variations in time of fall of individual drops is greater. At this point, however, the curve becomes more flattened. In this range, the difference in timing of separate drops does not mean as much in specific gravity so that the accuracy over the entire range of specific gravity remains about the same.

Application of the method to human serum and plasma. The specific gravity of 28 specimens of human serum and oxalated and heparinized

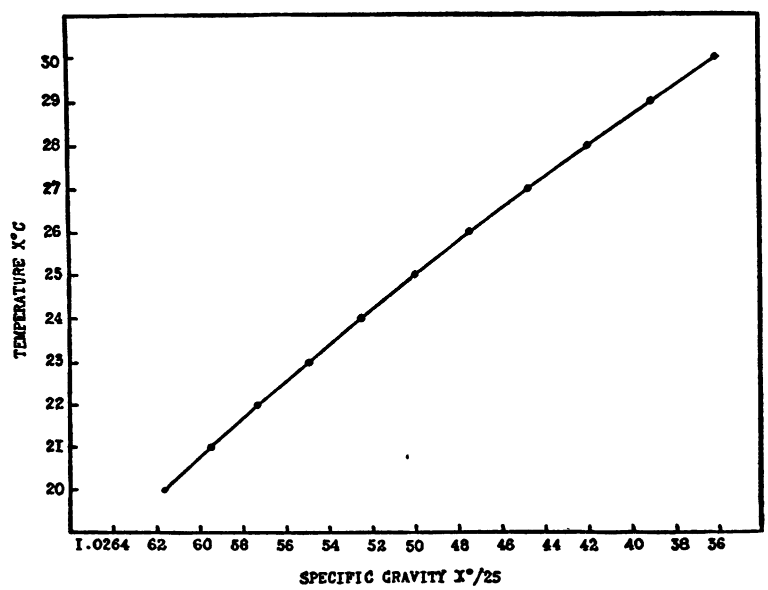

FIG. 4. EFFECT OF TEMPERATURE ON THE SPECIFIC GRAVITY OF SERUM

TABLE I

A comparison between the specific gravity of serum and plasma as obtained by use of a 2 cc. pyknometer and the drop method

Material	Specific gravity		Difference
	Drop method	2 cc. pyknometer	
Serum	1.0285	1.0288	−0.0003
	1.0256	1.0254	+0.0002
	1.0222	1.0222	0.0000
	1.0281	1.0283	−0.0002
	1.0283	1.0283	0.0000
	1.0306	1.0304	+0.0002
	1.0280	1.0280	0.0000
	1.0286	1.0290	−0.0004
	1.0280	1.0283	−0.0003
	1.0289	1.0292	−0.0003
	1.0303	1.0302	+0.0001
	1.0283	1.0279	+0.0004
	1.0209	1.0210	−0.0001
	1.0277	1.0279	−0.0002
	1.0285	1.0286	−0.0001
	1.0278	1.0274	+0.0004
	1.0297	1.0295	+0.0002
	1.0220	1.0220	0.0000
	1.0287	1.0284	+0.0003
	1.0261	1.0262	−0.0001
Oxalated plasma			
	1.0234	1.0233	+0.0001
	1.0310	1.0312	−0.0002
	1.0268	1.0268	0.0000
	1.0330	1.0328	+0.0002
	1.0234	1.0232	+0.0002
Heparinized plasma			
	1.0272	1.0274	−0.0002
	1.0297	1.0298	−0.0001
	1.0221	1.0223	−0.0002

plasma was determined by the use of a 2 cc. pyknometer according to the method described by Moore and Van Slyke (3). A comparison of the results obtained by the two methods is shown in Table I. The greatest difference was 4×10^{-4}. The falling time was taken at various temperatures and the correction factor used as indicated above.

SUMMARY

A simple, rapid, and easy method for the determination of specific gravity is presented which is based upon Stokes' law for the velocity of falling bodies. The materials and methods are described in detail. In ordinary usage, the method will yield the specific gravity of serum or plasma with a maximum difference from that determined by a 2 cc. pyknometer of 4×10^{-4} and has a range of 1.0150 to 1.0370.

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