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A Simple Volumetric Method for Determination of Fat in Blood Plasma*

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Investigations of changes in the fat content of the blood have been handicapped greatly by the tedious methods available for determination of blood lipids. The method described in this paper is easily and quickly carried out and is thus adapted to work requiring the analysis of large numbers of samples. Duplicate samples check closely, and composite samples agree well with calculated values, indicating that the method is satisfactory for comparative studies.

The design and approximate dimensions of the test bottle used for

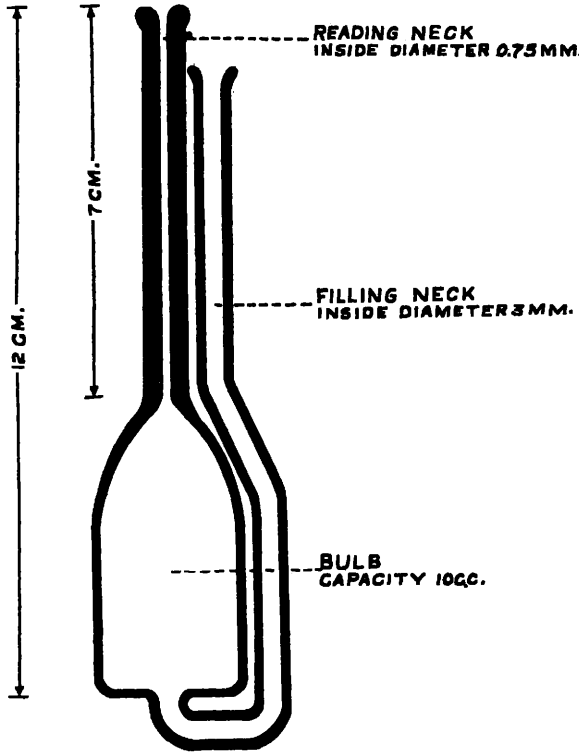

FIG. 1.

Cross section of test bottle used for determination of fat in blood plasma.

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the determination are shown in Fig. 1. An alkali reagent developed by Petersen and Herreid[1] for the purpose of testing buttermilk by a modified Babcock procedure is employed to free the fat from the blood plasma. This reagent consists of 110 gm. of sodium carbonate and 200 gm. of sodium salicylate dissolved in water and made up to a volume of one liter, to which are then added successively 30 cc. of 50% sodium hydroxide and 100 cc. of normal butyl alcohol. The samples should be tested in duplicate if the amount of blood plasma available permits.

Three cc. of blood plasma are measured into the bottle by means of a pipette. A very gentle suction applied to the opening of the reading neck will cause the sample to flow into the bottle readily. Five cc. of reagent are then added in the same manner and thoroughly mixed with the plasma by allowing air to be drawn through the mixture by the suction used in filling the bottle. The filling neck is then closed tightly by means of a small stopper, the bottle is placed in a water bath, and digested for 20 minutes at 180° to 190° F., with occasional shaking.

The stopper is then removed and the bottle is centrifuged for 5 minutes. A speed of 800 revolutions per minute in a centrifuge with a diameter of 18 inches has been found to be sufficient. Water at 160° to 180 F. is then added until the bottle is filled almost to the base of the reading neck. After centrifuging for about 2 minutes water is again added until the filling neck overflows. Since the reading neck is about 1 cm. longer than the filling neck, this automatically brings the fat column to the proper level for reading. The addition of water to the sample is greatly facilitated by use of a long slender cannula, such as a hypodermic needle of large diameter, connected to an elevated container of hot water by means of rubber tubing equipped with a pinchcock. The cannula is inserted well into the filling neck when water is being added, to avoid forcing bubbles of air into the bottle. The sample is again centrifuged for 2 minutes and removed to a water bath at 140°F. for 5 minutes before reading. Before placing in the water bath, a small wooden plug is inserted tightly into the opening of the reading neck to prevent any shifting of the position of the fat column.

The length of the fat column is measured by means of calipers, and a calibration factor is applied to convert the reading to mg. per 100 cc. The reading neck is calibrated by means of mercury and the factor for a 3 cc. sample is secured by the formula:

[1] Petersen, W. E., and Herreid, E. O., *Minn. Agr. Exp. Sta. Tech. Bul.*, 1929, 63.

$$\text{Calibration Factor} = \frac{\text{Vol. of neck in cc. per mm.} \times \text{specific gravity of fat} \times 1000 \times 100}{\text{size of sample in cc.}}$$

$$= \frac{\text{Vol. of neck in cc. per mm.} \times 0.93 \times 1000 \times 100}{3}$$

$$= \text{Vol. of neck in cc. per mm.} \times 31{,}000$$

The length of the fat column in millimeters is multiplied by this calibration factor to convert the reading directly to milligrams per 100 cc. The figure used for specific gravity, 0.93, is the average specific gravity of the fat recovered from bovine blood by the method described in this paper, at a temperature of 140° F., as found by the pycnometer method for 13 different samples. By merely changing the specifications of the test bottle illustrated in Fig. 1, a bottle of similar design but smaller dimensions has been constructed for testing samples of only 1 cc. of plasma.

In addition to the speed and ease with which this method is carried out, it has the further advantage of requiring no filtrations or extractions which might result in a loss of material. The fat as recovered is presumably in the same chemical state as when it is in the blood plasma, the only difference being that it is freed from the emulsifying agents. The only factor necessary for securing the final reading on the sample is that necessary for converting the volumetric measurement to terms of weight.

The work indicates that phospholipids and free fatty acids are not recovered with the fat. The cholesterol of the blood plasma is apparently recovered with the fat, as shown by applying tests for cholesterol to the fat and to the non-fat residue of the test. This probably is largely responsible for the relatively high specific gravity of the blood fat. More extensive work is necessary to completely establish these facts in connection with the applicability of the method for absolute determinations.